Major Patterns of Body Size Variation within Arthropod Species: Exploring the Impact of Habitat, Temperature, Latitude, Seasonality and Altitude

Submitted in partial fulfilment of the requirements of the

Degree of Doctor of Philosophy

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I, Curtis Robert Horne, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

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Details of collaboration and publications

Author contributions and additional collaborators are listed below for each chapter, as well as details of publications where applicable. This work was supported by the Natural Environment Research Council (NE/L501797/1). I use the term ‘we’ throughout the thesis to acknowledge the contribution of others.

- **Chapter 1:** Curtis R. Horne wrote the chapter.
- **Chapter 2:** Curtis R. Horne, Andrew G. Hirst and David Atkinson designed the study and wrote the paper. CRH collected the data and performed the statistical analyses. **This chapter has been utilized in publication** (see Appendix 7.1).
- **Chapter 3:** Curtis R. Horne, Andrew G. Hirst and David Atkinson designed the study and wrote the paper. CRH collected the data and performed the statistical analyses. **This chapter has been utilized in publication** (see Appendix 7.2).
- **Chapter 4:** Curtis R. Horne, Andrew G. Hirst, David Atkinson, Aitana Neves and Thomas Kiørboe designed the study and wrote the paper. CRH collected the data and performed the statistical analyses. **This chapter has been utilized in publication** (see Appendix 7.3).
- **Chapter 5:** Curtis R. Horne, Andrew G. Hirst and David Atkinson designed the study and wrote the paper. CRH collected the data and performed the statistical analyses.
- **Chapter 6:** Curtis R. Horne, Andrew G. Hirst, David Atkinson, Rodrigo Almeda and Thomas Kiørboe designed the study and wrote the paper. CRH carried out the experimental work, collected the data and performed the statistical analyses.
- **Chapter 7:** Curtis. R. Horne wrote the chapter.

Although not included as an official chapter, data collected as part of Chapter 2 was also used to examine the temperature dependence of sexual size dimorphism in arthropods. This work was predominantly led by Andrew G. Hirst, the findings from which were subsequently published. Given my contribution both as a co-author, and in collecting the data for this analysis, I also include a copy of this publication at the end of the thesis for reference (see Appendix 7.4).

Finally, thank you to my PhD examiners, David Morritt and Christopher Hassall, for their helpful comments and feedback on the thesis.

Patterns only emerge by ignoring the details.

Lawton (1999)

Major Patterns of Body Size Variation within Arthropod Species: Exploring the Impact of Habitat, Temperature, Latitude, Seasonality and Altitude

Curtis R. Horne

ABSTRACT

Body size affects rates of most biological and ecological processes, from individual performance to ecosystem function. Within species, emergent body size patterns have been formalised into prominent biogeographical and biological rules, including James' Rule (larger individuals are found at higher, colder latitudes), and the Temperature-Size Rule (individuals reared in warmer conditions grow to a smaller adult size). Body size also varies seasonally and with altitude. Yet, the patterns and drivers of these size gradients, and the degree to which they co-vary and share explanatory mechanisms, have never been systematically evaluated. We undertake the most comprehensive meta-analyses to date of temperature- and biogeographical-size clines within arthropod species. Aquatic species show greater reductions in body size with warming and decreasing latitude compared to terrestrial species, likely an adaptive response to deal with increased metabolic demand in the warm and the greater difficulty to uptake oxygen in water than in air. Voltinism explains variation in laboratory temperature- and latitudinal-size clines in terrestrial species. While size decreases with warming and with decreasing latitude in multivoltine terrestrial arthropods, size increases on average in univoltine species, consistent with predictions from size vs. season-length trade-offs. In the globally distributed sub-class Copepoda, seasonal temperature-size (T-S) gradients differ between current-feeding calanoids and ambush-feeding cyclopoids, suggesting that differences in the size- and temperature-dependence of alternative feeding strategies may influence the T-S response. Finally, through experimentation, we explore the progression of the T-S response of Copepoda during ontogeny. The T-S response is more strongly generated in particular life stages, and even reduced in some periods, providing evidence that the temperature-dependence of growth and developmental rates is modified during ontogeny. Ultimately, close similarities between T-S responses measured in controlled laboratory conditions, and seasonal and biogeographical size clines in the field across different arthropod taxa, suggests that these patterns share similar selective pressures.

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PUBLISHED PAPERS

APPENDIX 7.1. Horne C.R., Hirst A.G. & Atkinson D. (2015) Temperature-size responses match latitudinal-size clines in arthropods, revealing critical differences between aquatic and terrestrial species. *Ecology Letters*, **18**, 327-335 **(from Chapter 2 of this thesis)**.

APPENDIX 7.2. Horne C.R., Hirst A.G. & Atkinson D. (2017) Seasonal body size reductions with warming co-vary with major body size gradients in arthropod species. *Proc. Soc. B.* **284**, 20170238 **(from Chapter 3 of this thesis)**.

APPENDIX 7.3. Horne, C.R., Hirst, A.G., Atkinson, D., Neves, A. & Kiørboe, T. (2016) A global synthesis of seasonal temperature-size responses in copepods. *Global Ecology and Biogeography*. **25**, 988-999 **(from Chapter 4 of this thesis)**. *Featured on the journal front cover.*

APPENDIX 7.4. Hirst, A. G., Horne, C. R. & Atkinson, D. (2015) Equal temperature-size responses of the sexes are widespread in arthropod species. *Proc. Soc. B*, **282**, 20152475. *Featured on the journal front cover.* (The data used in this paper was collected by CRH and is also that utilised in Chapter 2).

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CHAPTER 1

General Introduction

In 1999, John Lawton questioned whether general laws in ecology exist, arguing that no ecological ‘laws’ are universal. Instead, he emphasized that ecological patterns are contingent, each emerging only when a particular set of circumstances are satisfied. In essence, biological systems are diverse and complex, and the generality of ecological patterns will vary depending on the environment and species in question. Despite this complexity, emergent ecological patterns have been formalised into prominent biogeographical and biological rules, including those that describe systematic variation in the body size of species with latitude and temperature. These include Bergmann’s rule (inter-specific latitudinal clines: larger bodied species at higher, colder latitudes) (Bergmann, 1847), James’ rule (intra-specific latitudinal clines: larger individuals at higher, colder latitudes) (James, 1970), and the temperature-size rule (TSR) (increased size at maturity when grown through ontogeny at decreased temperature) (Atkinson, 1994). In nature, seasonal temperature variation can also cause substantial changes in adult body size, as subsequent generations experience different developmental conditions over ontogeny. These general biogeographical and biological rules have been the subject of much debate, not least because inconsistencies in the direction of body size gradients between species and taxa have led many researchers to question their generality, particularly in ectotherms (e.g. Mousseau, 1997; Meiri & Dayan, 2003; Stillwell *et al.*, 2008; Shelomi, 2012).

Changes in body size can correlate with temperature across different biological scales, including shifts in the size structure of populations and communities (Daufresne *et al.*, 2009). These effects are also observed at the intra-specific level, as size at maturity in ectothermic species commonly declines in response to warmer temperatures (Atkinson, 1994; Forster *et al.*, 2012). This near-universal phenomenon, formalised as the temperature-size rule (TSR; Atkinson 1994), has been observed in over 80% of ectothermic species, from bacteria and protists to fish and invertebrates, and in over 90% of aquatic species (Atkinson, 1995). This phenotypically plastic response can be achieved within a single generation (Forster & Hirst, 2012; Forster *et al.*, 2013), and its proximate cause in many metazoans is attributed to differences in the temperature dependence of growth and development during ontogeny (van der Have & de Jong, 1996; Forster *et al.*, 2011b; Forster *et al.*, 2011a). Variation in both the strength and direction of temperature-body size (T-S) responses between different species is not well understood, and the ultimate cause is likely a combination of several factors.

In his paper, Lawton (1999) also pointed out that in order to make useful ecological generalizations, we have to look at the correct scale. For those of us attempting to understand, interpret and predict general patterns in nature, scale is incredibly important. We could conduct a detailed assessment of every aspect of a species' life history, population dynamics and trophic interactions, but to quote Lawton (1999) directly: "*put like that, the task is clearly hopeless!"* In the case of body size variation, alternatively and much more appropriately, we can begin to make better sense of the patterns (or lack thereof) by more broadly categorizing species based on a few key life history characteristics, habitat or functional groups. Ultimately, this method can help us to better identify the likely underlying selective pressures driving clines in body size, and provides a more comprehensive hypothesis-driven approach to explaining the biological and biogeographical patterns we observe.

The approach described above is based on the assumption that body size responses to temperature and across geographic clines are adaptive, though this has rarely been tested. Variation in body size could be the product of environmental stress or genetic drift, both of which can result in maladaptive phenotypic changes (Merilä & Hendry, 2014). Body size is often referred to as a ‘master trait’, affecting all vital rates, from individual performance including growth, survival, and reproduction, to ecosystem function, such as food web dynamics, productivity, and predator-prey interactions (Kleiber, 1947; Peters, 1983; Hildrew *et al.*, 2007; Hirst *et al.*, 2014). For this reason, adaptive explanations are typically considered for these body size gradients because important fitness costs and benefits are associated with them (Angilletta *et al.*, 2004; Kingsolver & Huey, 2008). Furthermore, despite profound differences in the proximate mechanisms driving them, the commonality of T-S responses in unicellular and multicellular organisms (Forster *et al.*, 2011b), and between different species, for example in insects (Ghosh *et al.*, 2013), suggests they are most likely adaptive responses. Given the intrinsic link between body size and fitness, as well as the likely cascading ecological impacts caused by potential shifts in body size with climate change, identifying and understanding variation in body size with temperature presents a significant ecological challenge.

Combining body size data from multiple species and studies provides an excellent opportunity to identify systematic trends in body size with temperature in the laboratory and across seasons, as well as clines in body size across latitude and altitude. Though studies of single species may lack power individually, and may not always find significant patterns, pooling data allows us to detect effects over and above what can be obtained from individual studies, uncovering broader patterns that may have otherwise gone undetected due to an increased risk of a Type 2 error. This is particularly true for ecological field studies, where the size of any effects can be relatively small and sample sizes are often limited for logistical reasons. On the other hand, excluding these relevant but often non-significant body size gradients from an analysis could lead to inflated effect size estimates and increase the risk of a Type 1 error (Koricheva *et al.*, 2013). Greater statistical power can also be gained by quantifying gradients in body size, instead of simply counting studies that show a negative, positive or no body size gradient; such an approach can lead to a biased synthesis of the data because a greater number of studies would be treated as having no gradient, and any effect across studies would be underestimated. Therefore, a primary objective of this thesis is to examine variation not just in the direction but also the magnitude of body size gradients. In doing so, we aim to identify the extent to which variation in the T-S response relates to key life history traits, as well as resolve recent contradictions in the literature - two opposing claims have been made of the extent to which T-S responses differ between aquatic and terrestrial species, and are also mass-dependent, such that the magnitude of the T-S varies with species' body size (Forster *et al.*, 2012; Klok & Harrison, 2013). We introduce and discuss these issues in more detail in Chapter 2, and more importantly provide strong evidence to resolve these contradictions throughout the thesis.

T-S responses are commonly measured in the laboratory under saturating food conditions. Thus, this thesis also aims to address the question: how accurately do laboratory T-S responses reflect body size patterns observed in nature, and thus to what extent does temperature correlate with potential drivers of size responses in the field, where any number of confounding factors might also influence body size across geographic and temporal scales? These confounding factors include, but are not limited to, resource availability, season length, mortality risk, competition, and genetic differences between populations. Determining the extent to which laboratory T-S responses co-vary with size patterns observed in the field, not only in direction but also in relative magnitude, can provide critical insight in whether these body size phenomena share common drivers. Yet to our knowledge, these size gradients have not been compared quantitatively before. This objective lies at the heart of this thesis (Chapters 2 to 5), encompassing quantitative comparisons of laboratory T-S responses, seasonal T-S gradients, and latitude- and altitude-size clines. This is a critical step in increasing our understanding of body size patterns in nature. We choose here to focus on the Arthropoda, the most taxonomically diverse and numerous phylum on earth, including marine, freshwater and terrestrial species (May, 1988). This well-studied group also shares a common ancestry and a related body plan, and has huge ecological and economic importance, including pollinator species and agricultural pests, as well as zooplankton, the most abundant animals in our ocean and important prey species for higher trophic levels including fish (Hill, 1997; Klein *et al.*, 2007).

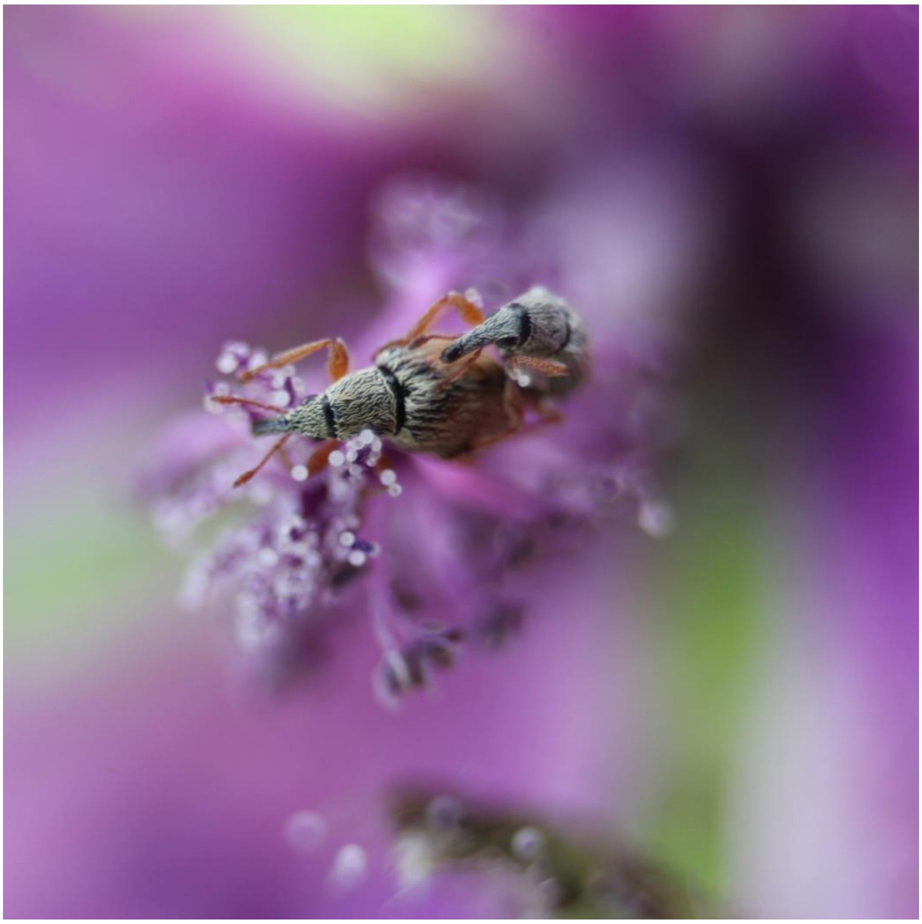
Sexual size dimorphism (SSD) can also vary with environmental conditions. For example, the effects of juvenile density, as well as food quantity and quality commonly result in greater size plasticity in females, suggesting these variables have different adaptive effects on SSD (Stillwell *et al.*, 2010). However, the effect of temperature on SSD in ectotherms is still relatively unexplored. Consequently, the data we present in Chapter 2 was also used to compare plastic responses of size-at-maturity to temperature between males and females in arthropod species. This work was led by Andrew Hirst and is presented in Appendix 7.4. On average, we found that the sexes show similar relative (proportional) temperature-body size responses. The high degree of similarity occurs despite an analysis which includes a wide range in animal body sizes, variation in degree of SSD, and differences in the sign of the T-S response. Consequently, we find no support for Rensch's rule (Rensch, 1960), which predicts greater variation in male size, or indeed the reverse, greater female size variation, suggesting that the same proportional T-S response may generally have equivalent fitness costs and benefits in both sexes.

As already mentioned, the proximate cause of the T-S response is attributed to differences in the thermal sensitivity of growth and development rates; as temperature increases, development rate increases disproportionately more than growth rate, leading to earlier maturation at a smaller size (van der Have & de Jong, 1996; Forster *et al.*, 2011b; Forster *et al.*, 2011a). Understanding how the T-S response is generated during ontogeny is also important in understanding the proximate and ultimate causes of this widespread phenomenon, yet only a handful of studies have examined the progression of T-S responses at high temporal resolution over the course of development (e.g. Gulbrandsen & Johnsen, 1990; Leandro *et al.*, 2006; Forster *et al.*, 2011b). These studies often report non-linear, discontinuous patterns in the progression of the T-S response over ontogeny, suggesting the extent to which growth and development are decoupled varies over the course of development. As such, in Chapter 6 we present an experimental and conceptual examination of the progression of the T-S response over ontogeny. Specifically, using pelagic marine copepods as model organisms, we test the prediction that the temperature dependence of growth and/or development rate varies between life stages.

Reductions in the body size of species has been described as the third universal response to climate warming, alongside shifts in phenology and the spatial redistribution of species (Gardner *et al.*, 2011). Shifts in the size of organisms and biological communities as a result of climate change are likely to have worldwide ecological and economic impacts (Sheridan & Bickford, 2011), and therefore it is in our interest to identify, understand and predict variation in body size. Indeed, biologists have intensively studied body size variation for more than a century (e.g. Darwin, 1859; Schmidt-Nielsen, 1984; Walters & Hassall, 2006; Karl & Fischer, 2008). Consequently, the work presented in this thesis should interest a wide range of researchers studying marine, freshwater and terrestrial systems, including but not limited to ecologists and macro-ecologists, fundamental biologists and physiologists, and evolutionary biologists, including those interested in the different selective pressures driving body size plasticity.

CHAPTER 2

Temperature-size responses match latitudinal-size clines in arthropods, revealing critical differences between aquatic and terrestrial species



Introduction

As emphasized in Chapter 1, body size is a master trait that strongly relates to individual fitness, and has profound physiological and ecological consequences (Hildrew *et al.*, 2007). Adult size in ectotherms commonly co-varies with rearing temperature under controlled laboratory conditions (Atkinson, 1994; Forster *et al.*, 2011b), can vary on a seasonal basis in multivoltine species (Hirst *et al.*, 1999; Kari & Huey, 2000; Sun *et al.*, 2013), and is modified along latitudinal and altitudinal gradients (Blanckenhorn & Demont, 2004; Chown & Gaston, 2010). Yet the correlation between these size responses and clines has never been systematically quantified. It is important that we quantify the degree to which body size responses to experimental temperature match geographical size clines, to determine how these responses differ between environments and identify the likely causative factor(s). This will aid in predicting how size will respond to environmental change (Daufresne *et al.*, 2009), which is critical given global warming trends and latitude- and altitude-dependent shifts in temperature and season length (IPCC, 2014).

The Temperature-Size Rule (TSR) describes the phenotypically plastic response in which size-at-maturity is inversely related to temperature experienced during ontogeny (Atkinson, 1994). Commonly, ectotherms reared at cooler temperatures accumulate mass at a slower rate, but develop to adulthood at a much slower rate than those reared in the warm, thereby achieving a larger adult size (Atkinson, 1994; van der Have & de Jong, 1996; Forster & Hirst, 2012). Proximally, this effect can arise from differences in the sensitivity of growth and development rates to temperature (Walters & Hassall, 2006; Forster & Hirst, 2012). The TSR has been observed across a diverse range of ectotherms, including single-celled and multicellular species, invertebrates and vertebrates, such as fish and amphibians (Atkinson, 1994; Atkinson & Sibly, 1997; Atkinson *et al.*, 2003; Forster *et al.*, 2012; Forster *et al.*, 2013). Temperature-Size (T-S) responses of organisms are typically examined under controlled laboratory conditions, with food supplied *ad libitum*, as nourishment also impacts size at maturity (Diamond & Kingsolver, 2010).

In the field a relationship between latitude and body size has also been described, known as Bergmann's Rule (Bergmann, 1847). Though originally proposed as an inter-specific phenomenon in which larger species of endotherm tend to be found at higher, colder, latitudes (Meiri & Dayan, 2003), the terms "Bergmann's cline" (an increase in size with latitude) and "converse Bergmann's cline" (a decrease in size with latitude) are typically used to describe both inter- and intra-specific latitudinal-size clines in endotherms and ectotherms. Here, we focus specifically on intra-specific latitudinal adult size (L-S) clines in ectotherms, to enable appropriate comparisons with T-S responses.

T-S responses and L-S clines may co-vary despite the fact that adult size can be influenced not just by environmental temperature, but also by season length, productivity, and mortality (Blanckenhorn & Demont, 2004; Chown & Gaston, 2010), and may involve genetic differences, somatic plasticity or a combination of factors. Our objective is to measure the strength of association between T-S and L-S gradients and their sign (positive or negative association) across species and groups at higher taxonomic ranks; this is more powerful than just indicating whether they occur in the same direction for particular species (Kingsolver & Huey, 2008). Crucially, we aim to use this correlative approach to test the predictions of two major mechanistic explanations for temperature- and latitudinal-size patterns (outlined below); the oxygen hypothesis and the optimal resource allocation model. We limit our study to arthropods to allow an examination of size patterns and their drivers within a single extensively studied phylum with a related bauplan, and which has huge ecological and economic importance (Klein *et al.*, 2007; Richardson, 2008).

Both the magnitude and direction of T-S responses relates strongly to whether organisms develop in air or in water, and to species body size, supporting the “oxygen hypothesis” (Woods, 1999; Atkinson *et al.*, 2006) - the idea that more costly uptake of oxygen in water and the pressures that large bodies face to maintain aerobic scope in the warm plays a dominant role in determining mature size (Forster *et al.*, 2012). In comparison, major patterns in L-S clines of ectotherms have been related to season length, voltinism and temperature. Despite the relatively large number of studies that attempt to explain L-S clines, few predict differences in the strength and sign of this gradient between terrestrial and aquatic environments (Chown & Gaston, 2010). One model postulated that simple metabolic constraints in water would result in a stronger Bergmann cline in aquatic than terrestrial species (Makarieva *et al.*, 2005), but so far there has been no empirical test of this difference. We address this issue empirically here, testing the prediction that oxygen availability in water is a major mechanistic determinant of both T-S and L-S gradients.

The optimal resource allocation model of Kozlowski *et al.* (2004) suggests that changes in season length across latitudinal gradients, and variation in the optimal trade-off between growth and reproductive investment among univoltine and multivoltine species, can explain why we observe both Bergmann and converse Bergmann clines.

One prediction is that univoltine species could take advantage of a longer growing season at lower latitudes by developing to a larger adult size, and would therefore exhibit a converse Bergmann cline. Thus, we also aim to investigate whether differences in L-S gradients reflect differences in voltinism, and to what extent these patterns are also seen in laboratory T-S responses. If a close match between T-S and L-S gradients is observed, it would provide further evidence to suggest that both are driven by the same selective pressures.

Two opposing claims have been made of the extent to which T-S responses differ between environments, and depend on species body size. A meta-analysis of 110 metazoan species, including fish, amphibians and a range of invertebrates, showed the T-S response of aquatic organisms to be significantly greater than in terrestrial organisms, and that the slope of the response became more negative with increased species body mass in aquatic organisms, but less negative in terrestrial organisms (Forster *et al.*, 2012). This suggests a major difference in T-S responses between terrestrial and aquatic ectotherms, with a proposed explanation based on the oxygen hypothesis (Woods, 1999; Atkinson *et al.*, 2006), rather than alternative hypotheses also examined by Forster *et al.* (2012). In contrast, a meta-analysis focussing exclusively on arthropods found that habitat type had no significant effect on the magnitude of the T-S response (Klok & Harrison, 2013). Furthermore, this later study showed that smaller individuals, regardless of habitat type, exhibited a more negative T-S response than larger individuals in both terrestrial and aquatic species. Klok and Harrison (2013) proposed that differences between their findings and those of Forster *et al* (2012) may be due to the latter pooling different taxonomic groups, including large aquatic vertebrates. As the two datasets were largely independent, a more comprehensive, appropriately screened, dataset can be constructed, which draws on both sources plus additional data, in order to resolve this issue.

The objectives of this chapter are therefore to: (i) establish whether there is a difference between aquatic and terrestrial arthropods in the magnitude of T-S responses and L-S clines; (ii) determine the degree to which the T-S and L-S gradients co-vary in sign and relative magnitude; and (iii) examine the degree to which both of these size relationships can be explained by major environmental differences, voltinism and species body size.

Methods

Temperature-Size Responses

Published data compilations of Forster *et al.* (2012) and Klok and Harrison (2013) were revisited to obtain a single comprehensive arthropod dataset. Rather than rely upon either set of T-S responses or body sizes, we obtained the original data ourselves in this new compilation, adding more data by searching the Institute for Scientific Information (ISI) Web of Knowledge and from references cited in other publications. Studies were systematically screened to include only laboratory studies where individuals were reared at a range of constant temperatures, with food concentrations at or above saturation, in order to remove the confounding impact of food limitation. Only adult size measurements were used for analysis, except in a small minority of cases where pupal size was considered to be a reliable correlate of size at maturity. The minimum period of acclimation for the inclusion of adult size data was set so that only individuals that were raised from egg or first larval stage were included. Adult data were collected as lengths, volumes, and dry, wet, or carbon mass. These measurements were subsequently converted to dry mass (mg) using intraspecific regressions. Where these were unobtainable, regressions for closely related species, and very occasionally more general inter-specific regressions, were used. Where wet mass was reported, we chose to use a dry mass to wet mass ratio of 0.4, based on average values reported in the literature (Sage, 1982). Data and conversions are provided in Appendix 2.1.

We used Akaike's information criterion (AIC) to compare linear vs. quadratic and also allometric vs. exponential equation forms, to determine which model best explained the response of adult body mass to rearing temperature/latitude. An exponential form was favoured as it offered the advantage of both the highest mean Akaike weights (w_i) and percentage best fit when compared with allometric for both L-S and T-S gradients. Furthermore, the exponential form allowed a clear best model choice for both response types; something neither linear nor quadratic achieved (see Appendix 2.2). The species-specific slopes of the natural log (ln) of the dry mass vs. temperature were then transformed into percentage change in dry mass per degree Celsius, using the formula $(\exp^{(slope)} - 1) * 100$ = % change in mass per °C (Forster *et al.*, 2012). A negative percentage indicates a decrease in size with increasing temperature (following the Temperature-Size Rule) and a positive percentage an increase in size (converse-TSR). This value represents the species-specific T-S response and was used as the dependent variable. Size responses from multiple studies of a single species were combined into a simple mean to generate single species-specific values. The effect of environment type (marine, freshwater, terrestrial), species body mass (adult body mass at 20°C calculated using species-specific slopes) and median rearing temperature were incorporated into a generalized linear mixed model (GLMM), in which four levels of taxonomic classification (subphylum, class, order, family) were incorporated as nested (hierarchical) random effects to control for phylogeny. Because of the phylogenetic relatedness and ecological similarity of species in our sample of the sub-class Copepoda, we combine the orders in our figures and consider the entire sub-class. We allowed for the interaction of all three parameters (i.e. environment type, species body mass, median rearing temperature), and best fit models were derived using AIC.

Latitudinal-Size Clines

To describe species-specific L-S clines we obtained published field measurements of individual adult size from a range of latitudes. Laboratory studies in which individuals from different latitudes were reared at different temperatures were excluded. We followed the conventions applied to our T-S data set; specifically, converting lengths or masses to dry mass (mg), and transforming species-specific slopes of ln dry mass vs. latitude into percentage change in body mass per degree of latitude. A positive percentage indicates an increase in adult size (Bergmann's cline) and a negative percentage a decrease in size (converse-Bergmann's cline) with increasing latitude.

Environment type and species body mass (adult body mass at 50° latitude calculated using species-specific slopes) were incorporated into a GLMM, in which both parameters were allowed to interact and four levels of taxonomic classification were incorporated as nested (hierarchical) random effects. Best fit models were determined using AIC. Mean L-S and T-S gradients were calculated for the five aquatic-developing (Amphipoda, Copepoda, Isopoda, Odonata and Mysida) and seven terrestrial groups (Blattodea, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera and

Orthoptera). Taxon- and species-specific L-S values were compared against their respective T-S values using Reduced Major Axis regressions (RMA) and tested for a significant correlation.

The terrestrial arthropods were categorized by voltinism (uni-, bi- or multivoltine). Multivoltine defined here as >2, bivoltine as 2, and univoltine as 1 generation yr^{-1}. In aquatic arthropods voltinism did not relate significantly to any of the observed variation in L-S clines ($F_{3,8}$=0.18, p=0.91) or T-S responses ($F_{2,39}$=1.71, p=0.19), and so was excluded from further analysis for these species. Voltinism of each species was determined from the literature that provided the size-gradient data or from other pertinent literature sources. For L-S clines, 7 species described in the original literature as switching voltinism or altering generation number with latitude were excluded, as this can obscure within-generation clines. In univoltine and bivoltine species we only considered those species for which we found no evidence that they switch generation number (e.g. if they are regarded as obligatorily univoltine). We note the potential for biased recording within the literature; a switch in voltinism is more likely to be reported for univoltine and bivoltine species for which the change is clearly defined, whilst for multiple generational species any change may go unreported. However, this bias should not substantially affect our analysis, as a change in voltinism is more diluted in multivoltine species, so is less likely to impose major differences in season-length constraints. Voltinism and body mass were incorporated into a GLMM following the same conventions previously outlined, and AIC was used to determine parameter importance. A t-test was used to test for a significant difference between univoltine and multivoltine terrestrial species, and RMA regression analysis was used to test for a significant correlation between voltinism and body mass.

Results

Our meta-analysis includes T-S responses [% change in dry mass (DM) $°C^{-1}$] for 114 arthropod species (aquatic, n=45; terrestrial, n=69); a 36% increase on Klok and Harrison's (2013) sample size (including a 60% increase in the number of marine species considered), and a 25% increase in the number of arthropod species sampled in Forster *et al.* (2012). There was no significant difference in the T-S responses of marine and freshwater species (*t test*; t_{39}=1.30, p>0.05). Species-specific T-S responses had negative slopes in 93% of aquatic arthropods, and 70% in terrestrial. There were highly significant differences in the strength of the T-S response among taxonomic orders ($F_{17,98}$=4.70, p<0.001) (Figure 2.1a).

We found that environment type (aquatic and terrestrial) had a significant effect on species-specific % change in mass $°C^{-1}$ with warming ($F_{1,114}$=29.72, p<0.001) (Figure 2.1a). The mean aquatic T-S response was -2.95% body mass $^{o}C^{-1}$ (±0.76; 95%CI), whilst for terrestrial species it was -0.35% body mass $^{o}C^{-1}$ (±0.59; 95%CI), representing a ~8½-fold difference in the mean response between environments. The significant difference was similarly observed within the Diptera, which contained aquatic- and terrestrial-developing species (*t test*; t_{21}=-2.46, p=0.02). Aquatic-developing Diptera had a mean T-S response of -2.54% (±1.27; 95%CI), whilst those in air had a mean response of -0.95% (±0.53 95%CI).

We obtained L-S clines for 44 arthropod species (aquatic, n=15; terrestrial, n=29). As with the T-S response, there is a significant difference in the strength of the L-S clines

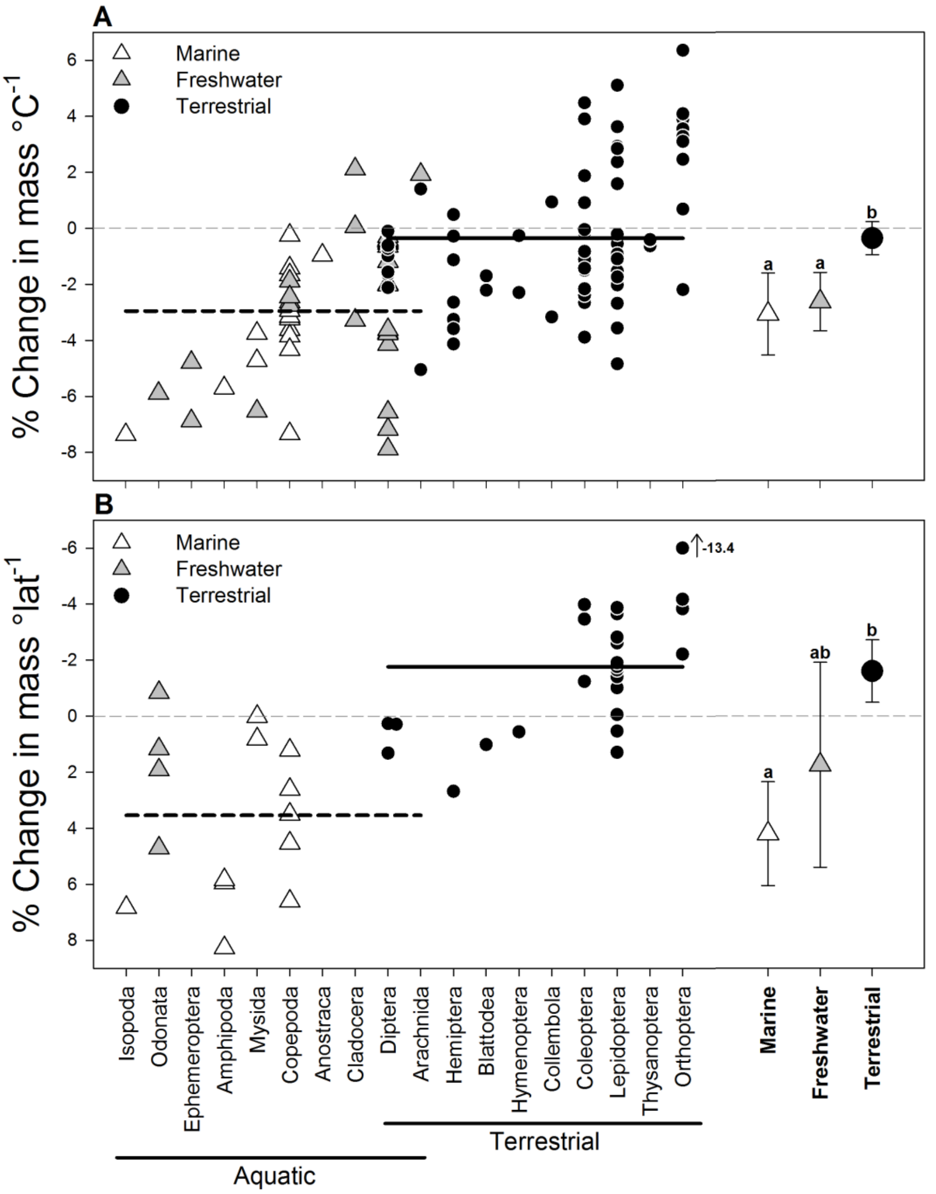


Figure 2.1. (**A**) Comparison of the % change in body mass per °C in aquatic (n=45, mean -2.95% °C^{-1}; dashed line) and terrestrial (n=71, mean -0.35% °C^{-1}; solid line) arthropod species, categorized by taxonomic order. (**B**) Comparison of the % change in body mass per °latitude in aquatic (n=15, mean 3.54% °lat^{-1}; dashed line) and terrestrial (n=29, mean -1.61% °lat^{-1}; solid line) arthropod species, categorized by order. In both panels mean gradient ±95% CI are shown for marine, freshwater and terrestrial arthropod species. Different letters above data points indicate significant differences, whilst shared letters indicate no significant difference. Note the reverse scale on the y-axis in B.

between aquatic and terrestrial species ($F_{1,40}$=34.05, p<0.001) (Figure 2.1b), with the former showing a much greater increase in size with latitude (and hence with average temperature decline, as experienced by the shallow-water animals included here). While the mean Bergmann cline in aquatic species is 3.54% body mass °lat^{-1} (±1.55; 95%CI), for terrestrial species a converse-Bergmann cline was observed, with a mean of -1.61% body mass °lat^{-1} (±1.11; 95%CI).

As reported in Forster *et al.* (2012), and in contrast to Klok and Harrison (2013), we find overwhelming support for the interactive effect of environment type and mass (mean adult or pupal DM at 20°C) on the strength of the T-S response, with this interaction firmly favoured by our AIC model competition framework (w_i = 0.90. see Appendix 2.3). Specifically, the responses of aquatic and terrestrial arthropods diverged with increasing species size; terrestrial arthropods exhibited a significant positive regression ($F_{1,66}$=9.28, p=0.003, r^2=0.11), contrasting with a significant negative regression in aquatic species ($F_{1,43}$=5.40, p=0.02, r^2=0.09) (Figure 2.2a). L-S clines show a close similarity: as mean species body size increases, terrestrial species have a significantly stronger negative (converse Bergmann) cline ($F_{1,27}$=4.56, p=0.04, r^2=0.11) (Figure 2.2b), while the L-S clines of terrestrial and aquatic arthropods diverge with increasing species size. Thus, the effect of species body mass on the strength of the cline is significantly dependent on environment type ($F_{1,40}$=5.16, p=0.03).

In contrast with the effects of voltinism on aquatic species, voltinism significantly affects the T-S response in terrestrial arthropods ($F_{3,61}$=5.08, p=0.003; Figure 2.3a). Indeed, there is a significant difference in the gradient between univoltine and multivoltine terrestrial species for both T-S responses (*t-test*; t_{31}=3.18, p=0.003; Figure

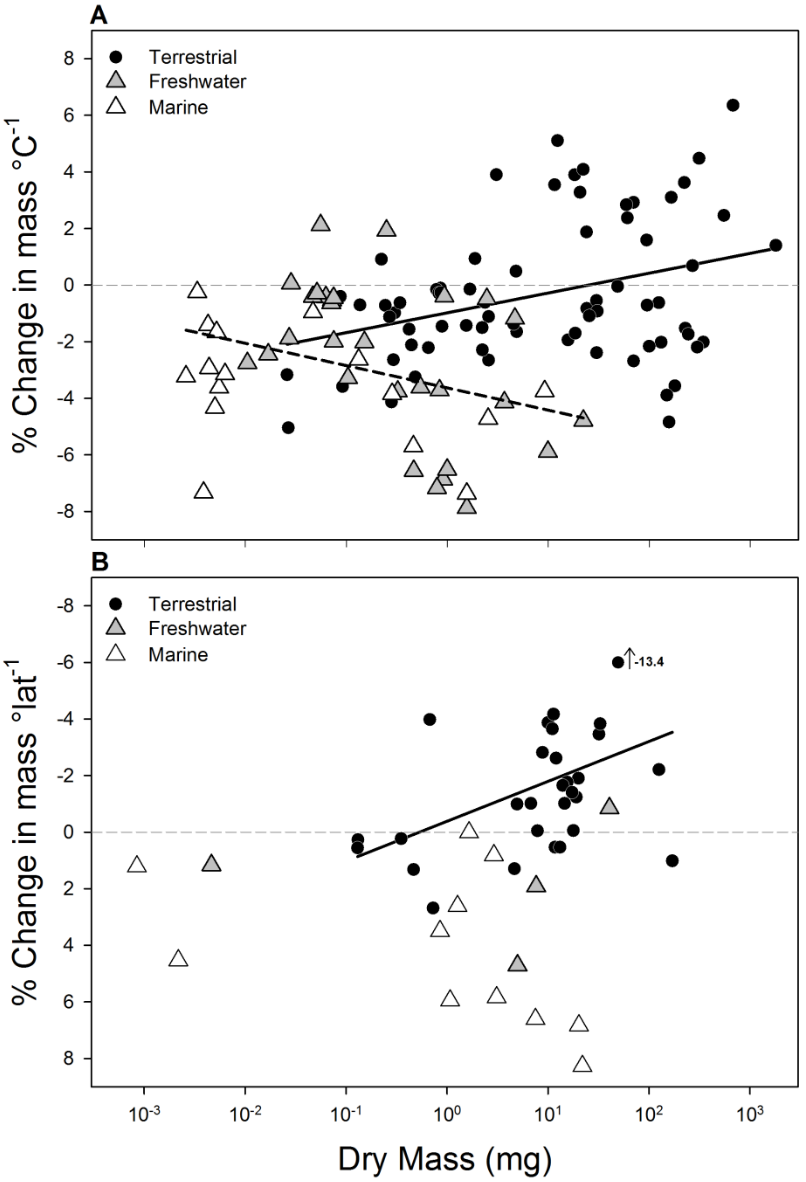

A
Terrestrial
Freshwater
Marine
% Change in mass °C^{-1}
8
6
4
2
0
-2
-4
-6
-8
B
Terrestrial
Freshwater
Marine
-13.4
% Change in mass °lat^{-1}
-8
-6
-4
-2
0
2
4
6
8
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
Dry Mass (mg)

Figure 2.2. (**A**) Species-specific temperature-size responses (% change in body mass per °C) expressed as a function of organism size (dry mass) at 20°C in aquatic and terrestrial arthropods. The effect of body size on the temperature-size response of aquatic and terrestrial arthropods is dependent on environment. Aquatic arthropods exhibit a significant negative regression (dashed line; r^2=0.09), and terrestrial arthropods a significant positive regression (solid line; r^2=0.11). (**B**) Species-specific latitudinal-size clines (% change in body mass per °lat) expressed as a function of organism size (dry mass) at 50°lat in aquatic and terrestrial arthropods. The effect of body size on the temperature-size response of aquatic and terrestrial arthropods is significantly dependent on environment. Aquatic arthropods exhibit a non-significant regression, terrestrial arthropods exhibit a significant positive regression (solid line; r^2=0.11). Note the reverse scale on the y-axis in B.

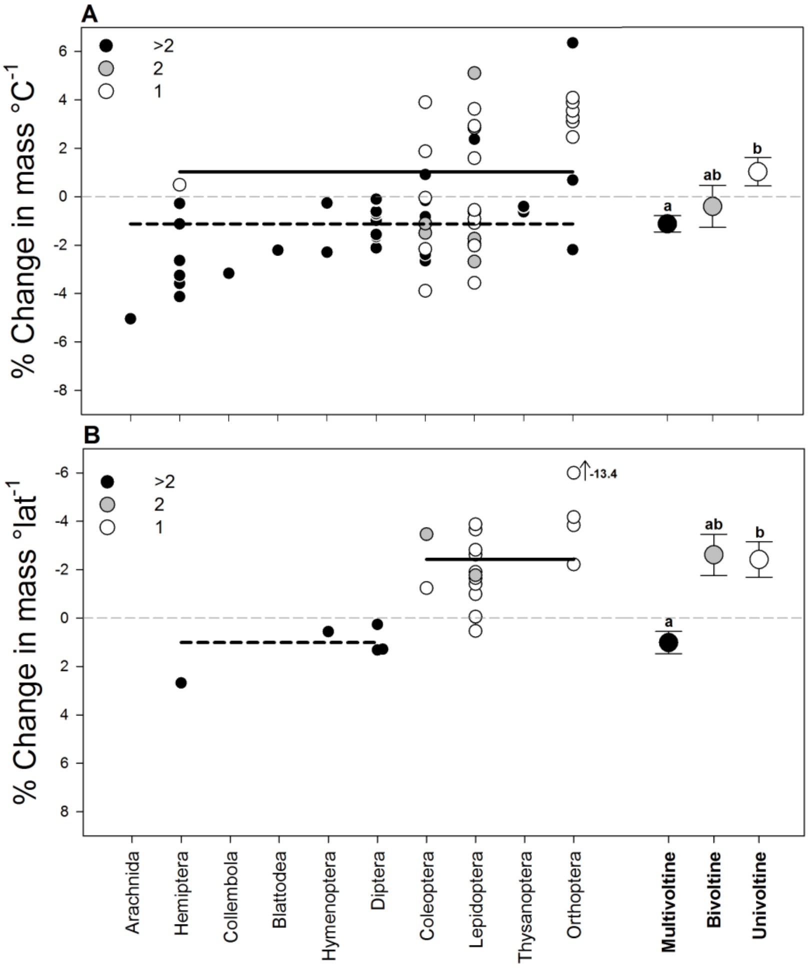


Figure 2.3. (**A**) Comparison of the % change in body mass per °C in multivoltine (mean, -1.12% °C^{-1}; dashed line), bivoltine (mean, -0.41% °C^{-1}) and univoltine (mean, 1.03% °C^{-1}; solid line) terrestrial species, categorized by taxonomic order. There is a significant difference between univoltine and multivoltine species in the size and sign of T-S responses. (**B**) Comparison of the % change in body mass per °lat in multivoltine (mean, 1.01% °lat^{-1}; dashed line), bivoltine (mean, -2.62% °lat^{-1}) and univoltine (mean, -2.42% °lat^{-1}; solid line) terrestrial species. There is a significant difference between univoltine and multivoltine species in the size and sign of L-S responses. In both panels mean ±S.E are given for multivoltine (black circle), bivoltine (grey circle) and univoltine (open circle) arthropod species. Different letters above data points indicate significant differences, whilst shared letters indicate no significant difference. Note the reverse y-axis scale in panel B.

2.3a) and L-S clines (t_{20}=-3.96, $p<0.001$; Figure 2.3b), with different degrees of voltinism producing opposing gradient directions. Univoltine species had a mean T-S and L-S gradient of 1.03% body mass °C^{-1} (±1.23; 95%CI) and -2.42% body mass °lat^{-1} (±1.64; 95%CI) respectively, whilst multivoltine species had a mean T-S and L-S gradient of -1.12% body mass °C^{-1} (±0.68; 95%CI) and 1.01% body mass °lat^{-1} (±0.42; 95%CI) respectively. Voltinism is significantly correlated with species adult mass in both the T-S (RMA regression; $p<0.001$, r^2=0.33; Figure 2.4a) and L-S (RMA regression; $p<0.001$, r^2=0.72; Figure 2.4b) datasets. Voltinism was a more powerful predictor of response size (T-S w_i=1.00; L-S w_i=0.63) than was species adult mass in terrestrial species (T-S w_i=0.59; L-S w_i=0.20), as inferred from the relative Akaike weights of each parameter. Though species mass and voltinism are correlated, this finding suggests that voltinism may be more important than mass *per se* in terrestrial size gradients. Generally, larger terrestrial species are univoltine and exhibit a positive T-S response and a converse Bergmann cline, whilst smaller species are multivoltine and follow the TSR (a negative T-S response) and a typical Bergmann cline.

If L-S and T-S gradients are driven by similar factors then we would expect a negative relationship between the two, given the general decline in temperature away from the equator. Indeed, there is a significant negative correlation between T-S and L-S gradients across both orders and species (r^2=0.72, n=12, $p<0.001$; and r^2=0.73, n=6, p=0.015 respectively) despite the small number of species-specific data (see Appendix 2.4). Across 10 of the 12 taxonomic orders included here, those which on average exhibit a negative T-S response show a positive L-S cline, and *vice versa* (Figure 2.5), the exceptions being Lepidoptera and Coleoptera, in which both T-S and L-S gradients

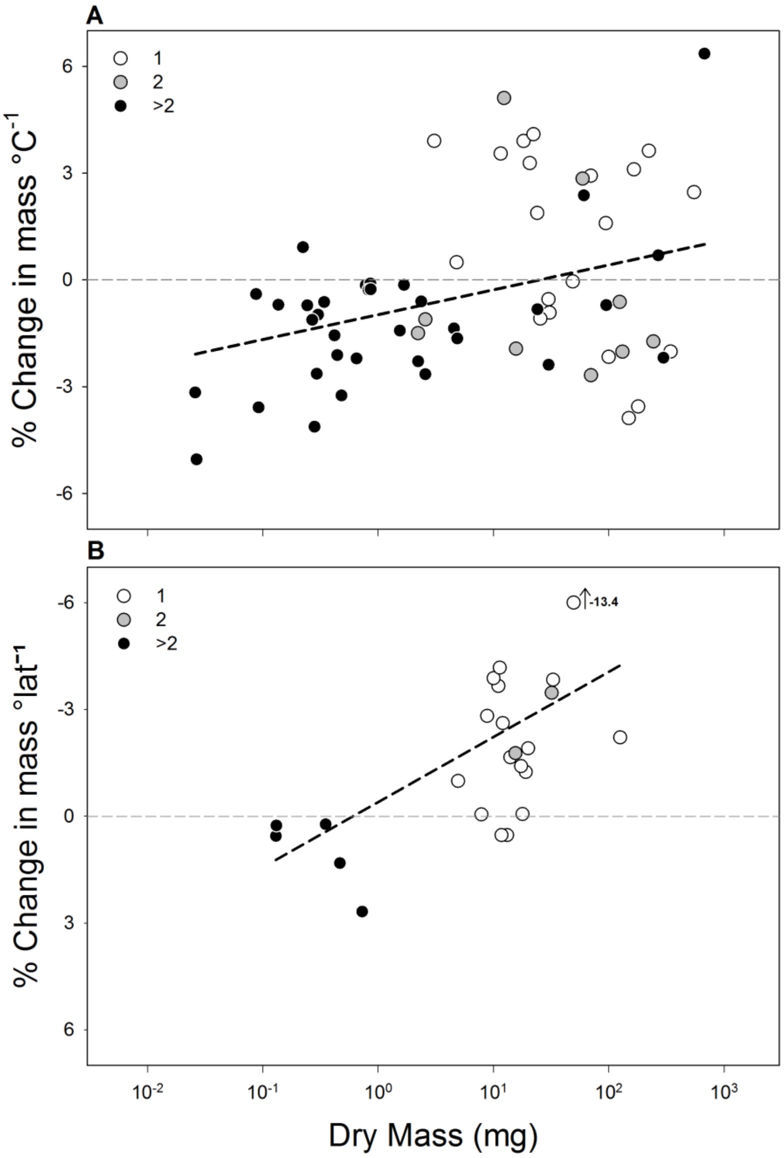


Figure 2.4. (**A**) Species-specific temperature-size responses (% change in body mass per °C) expressed as a function of organism size (dry mass) at 20°C, and (**B**) species-specific latitudinal-size clines (% change in body mass per °lat) expressed as a function of organism size (dry mass) at 50°lat, categorized by voltinism. Voltinism is significantly correlated with mass in both the T-S (RMA regression; $p<0.001$, $r^2=0.33$) and L-S (RMA regression; $p<0.001$, $r^2=0.72$) datasets, and hence may explain the body mass dependence of both T-S and L-S gradients in terrestrial arthropods. When considered together, voltinism has a greater relative Akaike weight than mass, suggesting it is a more powerful response predictor. Note the reverse scale on the y-axis in B.

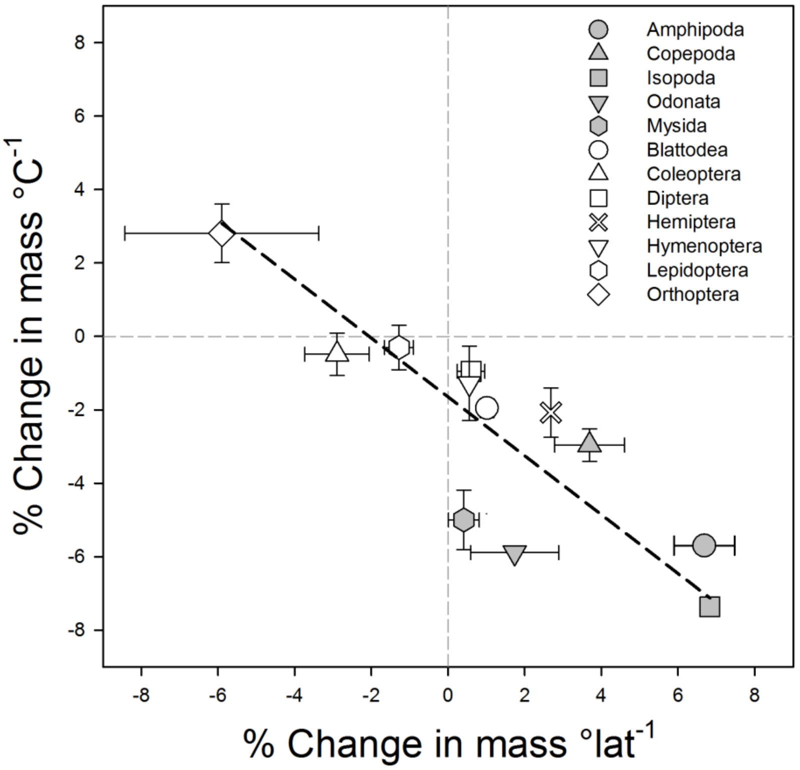


Figure 2.5. Temperature-Size responses (% change in body mass per °C ± S.E) vs. Latitudinal-Size clines (% Change in body mass °lat^{-1} ± S.E) for specific taxa. There is a significant negative correlation between T-S and L-S gradients (RMA regression; $p<0.001$, $r^2=0.72$). On average, those taxa that exhibit the strongest reduction in body size with increasing temperature show the greatest decrease in size with decreasing latitude, and *vice versa*.

are slightly negative. The RMA regression passes close to the zero-zero intercept of the two axes, further indicating a similarity in these gradients. When analysed independently, terrestrial arthropods still show a significant negative correlation between order-specific T-S and L-S gradients ($p=0.002$, $r^2=0.88$, $n=7$). Therefore, the significance of the regression overall is not just driven by the stark difference in size responses between aquatic and terrestrial orders.

Though we made every effort to ensure that length-mass regressions were accurate and species-specific, we often had to rely on broader family- and order-specific conversions for an array of different body measurements. This can increase the likelihood of inaccuracy when determining T-S responses and L-S clines, particularly as any small deviation in the equation's power term can result in substantial over- or under-estimation of dry mass, and thus percentage change in body size. Given these concerns, in order to test the robustness of our findings, we repeated our analysis using length (mm) in place of dry mass (mg) to generate a second set of T-S responses (% change in length $°C^{-1}$) and L-S clines (% change in length $°lat^{-1}$). To do this we simply relied on original recorded length measurements and calculated the cube-root of original mass measurements. We followed the same conventions as previously outlined in our methods, examining the sensitivity of both gradients to environment type (aquatic vs. terrestrial), voltinism, and their mass dependence. Similarly, we re-examined the extent to which T-S responses and L-S clines co-vary among 12 arthropoda taxa.

The results strongly support those presented in our primary analysis, and thus we are confident in our original conclusions. Specifically, we find that environment type (aquatic vs. terrestrial) has a significant effect on species-specific % change in length

°C^{-1} with warming ($F_{1, 110}$=29.53, $p<0.001$). Similarly, there is a significant difference in the strength of the L-S clines between aquatic and terrestrial species ($F_{1,40}$=55.09, $p<0.001$). Importantly, we still find clear support for the interactive effect of environment type and mass on the strength of the T-S response ($F_{1,110}$=17.32, $p<0.001$) and L-S cline ($F_{1,40}$=17.97, $p<0.001$), with the responses of aquatic and terrestrial arthropods diverging with increasing species body size. We still find a significant effect of voltinism on the strength and direction of T-S responses ($F_{2,59}$=7.41, $p=0.001$) and L-S clines ($F_{2,21}$=11.00, $p<0.001$) in terrestrial species, with univoltine species exhibiting both a converse TSR and converse-Bergmann cline, and multivoltine species conforming to the TSR and Bergmann's rule, on average. There remains a clear correlation between voltinism and mean species body mass, suggesting that voltinism drives the body mass dependence of T-S and L-S gradients in terrestrial arthropods. Lastly and reassuringly, we still observe a significant negative correlation between T-S responses and L-S clines, finding a close match between both gradients across taxa (r^2=0.77, n=12, $p<0.001$). Taxa that decrease their size with warming also increase in size with increasing latitude, away from the equator (see Appendix 2.4).

Discussion

We find significant differences between T-S responses of aquatic and terrestrial arthropods (Figure 2.1a), hence supporting the environment-dependence observed by Forster *et al.* (2012) in ectotherms generally. Aquatic arthropods show a significantly stronger negative T-S response with warming than do terrestrial, and followed the TSR in over 90% of cases. These environmental differences are further supported within the order Diptera, in which species with aquatic larval and juvenile stages had a significantly stronger negative T-S response than terrestrial-developing species.

We present compelling evidence for a similarity between T-S responses and L-S clines, observing a significant difference in the strength and direction of T-S and L-S gradients between environments. Individual body size typically declines with increasing latitude in many terrestrial species, but increases with increasing latitude in most aquatic species considered here, matching the general trends in T-S responses (Figure 2.1b). All the aquatic orders show on average both a negative T-S response and a positive L-S cline. Similar covariation between magnitudes of T-S and L-S associations are found in the terrestrial orders, with Orthoptera showing the most extreme positive T-S responses and negative L-S clines (Figure 2.5). The overall negative relationship between these gradients suggests a general ability to predict one from the other within arthropods, and that the driving forces that dictate much of the phenotypically plastic size responses to temperature in the laboratory may also shape the magnitude and sign of latitudinal size changes observed in the field.

This covariation between T-S and L-S gradients is remarkably robust, given the range of confounding variables that can influence L-S clines (Shelomi, 2012), including altitudinal variation, habitat variability (local climate, food availability, natural enemies), the variable match between mean temperature and latitude or season length, and the geographical extent of data for each species. Previously the proportion of the total range has been shown to influence the apparent shape of the L-S cline (Hassall, 2013). However, having tested a small subset of our data (n=8), when we compare the best-fit response (linear vs. quadratic) with the proportion of range sampled, we find no apparent pattern. Certainly genetic variation can determine body size differences between populations. Evidence for genetic influence on L-S clines includes laboratory studies of species collected along a latitudinal gradient and reared under constant temperature and food conditions, which still demonstrate clinal variation in body size (James *et al.*, 1995; Land *et al.*, 1999). Nonetheless, even though body size clines in the field may be influenced partly by genetic differences as well as phenotypic plasticity, the difference between terrestrial and aquatic environments in both T-S and L-S gradients suggests that there may be consistent differences in temperature-related selection pressures on body size between aquatic and terrestrial environments.

The effect of species body mass on both T-S and L-S gradients is significantly dependent on environment type (Figure 2.2). In contrast to Klok and Harrison (2013), but in concordance with Forster *et al.* (2012), T-S responses became significantly more negative with increasing species body mass in aquatic species, while terrestrial arthropods, which are dominated numerically by the insects – both globally (Zhang, 2013) and in our dataset - exhibited a significant positive regression between T-S response and species body mass. The divergence culminated in a ~16-fold difference in the strength of the T-S response between aquatic and terrestrial species of 10mg dry body mass. We propose that the differences in our findings from Klok and Harrison (2013) in T-S patterns may be attributed to their inclusion of data from studies that did not confirm saturating food or controlled temperature conditions. To reduce confounding effects of uncontrolled conditions, and of food limitation, which can reverse the direction of the T-S response (Diamond & Kingsolver, 2010), we excluded studies in which conditions were not controlled, including those with no evidence that food supply was saturating. For example, unlike Klok and Harrison (2013) we excluded the study of Babin-Fenske *et al.* (2008) within the T-S data set, as the size of field-collected museum specimens were related to their field temperatures. Similarly, we excluded the study of Sweeney and Vannote (1978) on species of Ephemeroptera: this study had a large influence on Klok and Harrison's (2013) conclusion, but gave no indication of whether food was provided to saturation or *ad libitum*. Including Sweeney and Vannote's (1978) data in our set has a significant outcome on the species body mass dependence of the T-S response in aquatic species, resulting in the negative regression becoming non-significant, though the inclusion of these data do not affect the overall significant difference in T-S responses between environments. Finally, while we converted all sizes to mass, Klok and Harrison (2013) used various metrics of size, which were then normalised assuming isomorphism (i.e. mass was proportional to lengths^3); this may be problematic as not all arthropods grow isomorphically (Benke *et al.*, 1999; Hirst, 2012).

Of the models proposed to explain intra-specific geographical trends in body size, some can be applied more broadly than others (Chown & Gaston, 2010). For example, the biophysical model of van der Have and de Jong (1996) , often considered a proximate physiological explanation for the TSR, and the starvation resistance model (Cushman *et al.*, 1993), which proposes that species at higher latitudes grow to a larger size to withstand extended periods of food deprivation, cannot account for converse Bergmann clines, of which there are numerous examples in terrestrial arthropods (Blanckenhorn & Demont, 2004; Chown & Gaston, 2010) (see Figures 2.1 and 2.2). Furthermore, we demonstrate effects of environment type (aquatic, terrestrial) and voltinism on L-S gradients, which are not predicted by either of these models. We therefore explore alternative explanations which can account for the variation we observe in the strength and direction of L-S clines among taxa and environments.

We find that differences in voltinism can provide a mechanistic explanation for the dependence of T-S and L-S gradients on species body mass in terrestrial (but not aquatic) arthropods. Voltinism is significantly correlated with mass in terrestrial species (Figure 2.4), with larger species often having a single generation and smaller species producing multiple generations annually. As predicted, on average, larger univoltine terrestrial species have a positive T-S response and exhibit a converse Bergmann cline, whilst smaller multivoltine species tend to conform to the TSR and Bergmann's Rule (Figure 2.3). The variation in body size of terrestrial arthropods, both at different temperatures under controlled laboratory conditions, and along latitudinal clines in the field, may thus reflect an evolutionary adaptation to changing season length. More specifically, at lower latitudes where season length is longest, a large obligatorily univoltine species could take advantage of a longer growing season by maturing to a bigger adult size. Conversely, in the same environment, a smaller shorter-lived multivoltine species may maximize fitness by maturing earlier and at a smaller size, thus decreasing generation time and increasing the number of generations per year. As shown by Roff (1980) , there is a point at which the fitness advantage of large size is outweighed by the advantage of adding an additional generation. These differences in voltinism describe well both the species body mass dependence and order-specific variation we observe in terrestrial T-S responses and L-S clines. Interestingly, Odonata, which develop in water but emerge into air and are commonly univoltine or even semivoltine, show a weaker positive L-S response than most other aquatic orders considered here (Figure 2.5). Their semi-aquatic life history would make for an intriguing case study to determine how the forces dictating latitude and temperature body size gradients in aquatic and terrestrial environments interact. Indeed, Hassall *et al.* (2014) suggested that the typical Bergmann clines observed in Odonata may arise because structural growth occurs during the aquatic larval stage, supporting oxygen limitation as the overriding explanation for body size determination in this order. Unlike most univoltine terrestrial species, one univoltine Odonata species, which showed no evidence of a switch in voltinism with latitude, exhibited a significant Bergmann cline and a typical negative T-S response (Hassall, 2013). Unfortunately, available data are too sparse to evaluate whether voltinism plays a significant role in determining variation in the T-S and L-S gradients between aquatic insects with a terrestrial adult phase. More data on semi-aquatic insects would help determine whether major effects of voltinism on T-S and L-S gradients extend generally to these species too.

The strongly negative T-S responses and positive Bergmann-type clines in aquatic arthropods, especially in larger species, follow the prediction of the oxygen hypothesis (Woods, 1999; Atkinson *et al.*, 2006; Forster *et al.*, 2012). Decreasing latitude relates strongly to increasing mean temperature (Sunday *et al.*, 2011), and increased temperature increases metabolic demand, but results in a relatively much lower rate of increase in oxygen availability in water (Verberk *et al.*, 2011). Makarieva *et al.* (2005) used similar reasoning to explain across-species patterns in maximum body size, highlighting that the largest aquatic poikilotherms are often found at higher latitudes towards the poles. Indeed, experimental studies have shown that oxygen limitation can impede growth in arthropods, such as amphipods (Rudolf & Or, 2005), as well as other phyla including many fish species (Pauly, 2010). Such limitation also predicts the species body mass dependence of both the T-S responses (Forster *et al.*, 2012) and L-S clines, since larger species struggle most to meet their oxygen requirements, whilst no discernible effect can be found for voltinism, as larger univoltine aquatic species do not reduce or reverse their body size responses in comparison to multi-generational species. Our findings therefore support the mechanistic explanation that oxygen demand-supply constraints drive both strong negative T-S and strong positive L-S gradients within aquatic species (Woods, 1999; Atkinson *et al.*, 2006).

In the largest database of its kind to date on a single large phylum, the Arthropoda, we present compelling evidence of a correlation between phenotypically plastic responses to temperature, and body size clines in the field, therefore providing a conceptual unification of the TSR and Bergmann's Rule in ectotherms. Though our findings are correlative rather than the outcome of manipulative experiments, we observe clear differences in the strength and direction of T-S and L-S gradients between aquatic and terrestrial arthropods that match the predictions of adaptive models, supporting the importance of the oxygen hypothesis in aquatic ectotherms, and the effects of seasonal constraints and other possible advantages of large size in warm environments in terrestrial arthropods. The parallel patterns between T-S and L-S gradients suggest that the major selective pressures that produce L-S clines, by either genetic or phenotypically plastic variation, may also be the ones that produce T-S responses. Above all, we demonstrate the value of combining physiological and ecological perspectives in explaining major environmental patterns. This suggests that multi-disciplinary studies, which combine large-scale spatial and temporal trends and lower-level physiological variation, can better reveal macrophysiological patterns and their underlying mechanisms (Gaston *et al.*, 2009).

CHAPTER 3

Seasonal body size reductions with warming co-vary with major body size gradients in arthropod species

Introduction

The causes of intraspecific body size clines across latitudes can differ from those of size responses to ontogenetic temperature treatments in the laboratory. The former can be influenced not just by phenotypic plasticity, but also by genetic variation among geographic populations (Stillwell, 2010), as well as many biotic and abiotic factors that could confound the effects of temperature, such as voltinism, season length, food supply, and natural enemies (Roff, 1980; Mousseau, 1997; Chown & Gaston, 2010; Kivelä *et al.*, 2011; Zeuss *et al.*, 2016). Despite these confounding factors, in Chapter 2 we showed that temperature-size (T-S) responses measured under controlled laboratory conditions and latitudinal-size (L-S) clines measured in the field significantly co-vary across taxonomic orders within the Arthropoda. Specifically, taxonomic orders whose species demonstrate particularly strong negative T-S responses also show strong intra-specific declines in adult size at lower latitudes, whereas those with less negative T-S responses tend to show reduced or reversed latitudinal-size clines (Horne *et al.*, 2015). This co-variation suggests that similar forces may be driving these important patterns.

As we discussed briefly in Chapter 1, it has been debated whether size responses are adaptive, or a maladaptive outcome of environmental stress or genetic drift (Merilä & Hendry, 2014), or simply a consequence of how constraints imposed by the architecture of the maturation mechanism may affect phenotypic outcomes of selection on body size, growth and development rate (Davidowitz *et al.*, 2012). However, given the important influence of body size on vital rates and ecological processes, systematic size responses to temperature are often considered adaptive (Angilletta *et al.*, 2004; Kingsolver & Huey, 2008). For example, variation in the direction of T-S responses and L-S clines can be partly attributed to differences in voltinism in terrestrial arthropods, likely an evolutionary adaptation to changing season length (Roff, 1980; Kozłowski *et al.*, 2004; Horne *et al.*, 2015). Striking differences in the T-S response also occur between environments; aquatic-developing species show greater reductions in adult size per °C of warming, and stronger reductions in size with decreasing latitude towards the equator, than do air-breathing species (Forster *et al.*, 2012; Horne *et al.*, 2015). Oxygen availability, which includes both its concentration and diffusivity, is approximately 3×10^5 times lower in water than in air (Verberk *et al.*, 2011), and body size reduction with warming is thought to be an important mechanism by which aquatic species maintain aerobic scope when faced with increased metabolic rate at elevated temperatures (Atkinson *et al.*, 2006; Forster *et al.*, 2012; Horne *et al.*, 2015). Indeed, hypoxic conditions also commonly lead to reductions in size within species, both under natural conditions (Gibson & Atkinson, 2003) and in laboratory manipulations, especially at warmer temperatures and/or larger body sizes (Hoefnagel & Verberk, 2014).

Multivoltine ectotherms, which have more than one generation per year, can experience considerable differences in temperature, resources and suitable habitat between seasons, hence between generations. The effects of seasonal changes in temperature on optimum body sizes may therefore be easily confounded by other seasonally varying effects such as food, water, oxygen availability and mortality risks (Chown & Gaston, 2010). Nonetheless, seasonal body size variation commonly correlates strongly with changes in environmental temperature in a wide range of uni- and multicellular organisms, including bacteria (Chrzanowski *et al.*, 1988), rotifers (Diéguez *et al.*, 1998), copepods (Hirst *et al.*, 1999; Horne *et al.*, 2016), cladocerans (Miyashita *et al.*, 2011) and insects (Kari & Huey, 2000), examples of which are presented in Figure 3.1. Yet, despite the huge implications of environmental seasonality for global ecology, no broad exploration of seasonal size gradients has been performed to date. Such intra-annual shifts in size have important physiological, ecological and fitness consequences (Chown & Gaston, 2010), and the magnitude and variation of such seasonal change across diverse taxa, and between environments, needs to be investigated (cf. with latitudinal and altitudinal descriptions (Ashton *et al.*, 2003; Blanck & Lamouroux, 2007)). Moreover, the question of whether the differences in body size gradients observed between environments and taxonomic orders, both in the laboratory and across latitudes, are also observed across seasons still remains unanswered. Improved understanding of size gradients in organisms across season will not only help to determine the ultimate causes of body size variation, but will also aid ecologists, including macro-ecologists, in understanding and predicting individual and community level responses to climate change (IPCC, 2014). This is critical given the link between decadal-scale changes in the body sizes of ectotherms and shifts in climate (Daufresne *et al.*, 2009; Sheridan & Bickford, 2011; Rice *et al.*, 2015).

In this chapter we present, to our knowledge, the largest synthesis of seasonal T-S gradients in multivoltine arthropods to date, including those of marine, freshwater and terrestrial species. Given that aquatic-developing species show greater reductions in body size with warming than terrestrial species under controlled laboratory conditions, and we observe similar differences in latitudinal-size clines between these two groups (Forster *et al.*, 2012; Horne *et al.*, 2015), we predict that across seasons, species developing in water will also demonstrate a greater reduction in size per ^{o}C of warming than will species developing in air. We also assess the extent to which the seasonal T-S gradient depends on mean

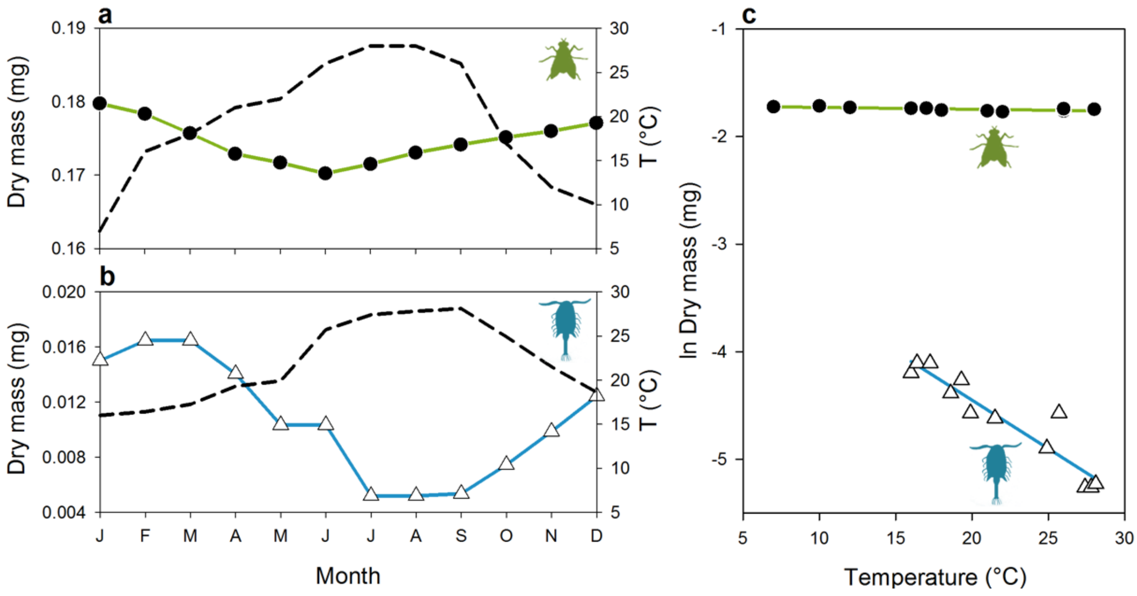


Figure 3.1. Seasonal variation in adult dry mass (mg) (solid line) and environmental temperature (°C) (dashed line) for (**A**) the terrestrial dipteran *Musca domestica*, adapted from Bryant (1977) and (**B**) the aquatic calanoid copepod *Paracartia latisetosa*, adapted from El-Maghraby (1965) . In both cases data are for females. (**C**) Natural log (ln) of dry mass vs. environmental temperature for each of the species described in panels A and B. Body size decreases with increasing temperature in both species, though proportional change in body mass per °C is much greater in the aquatic copepod (open triangles) than in the terrestrial dipteran (filled circles).

annual temperature, latitude and species body mass. Finally, we quantitatively compare seasonal T-S gradients with both T-S responses measured under controlled laboratory conditions and with latitudinal-size (L-S) clines, to establish whether differences observed between environments and among taxa are consistent in these three major size gradients.

Methods

We searched the literature extensively using both the Web of Science database (http://apps.webofknowledge.com/) and Google Scholar for studies in which the adult body size of multivoltine arthropod species (≥2 generations per year) was assessed in nature on multiple occasions during an annual cycle. We used records for which we could model a change in body size that occurred over at least a 3 month period; in the most basic scenario, if a study reported body size and temperature at only 2 time points, it would be included only if these time points were a minimum of 3 months apart. This criterion for data inclusion ensured that body size was measured at different seasonal periods, thereby increasing the likelihood of capturing variation in body size in different cohorts or generations. The primary search term combinations used were: (“seasonal” OR “temporal”) AND “body size” AND (“arthropod” OR “<insert taxonomic order>”) AND “temperature”. We also identified related studies from reference lists in the papers we found, and sought further direction to key literature from relevant experts. Adult size data were collected as lengths, or dry, wet or carbon masses and subsequently standardised to dry mass (mg) using published intra-specific regressions and conversion factors (data and conversions are given in Appendix 3.1). If regressions for the species were not available, regressions for closely related species, or more general inter-specific regressions were used (in ~26% of cases). Taxonomic order and family were confirmed for each species using the World Registry of Marine Species (WoRMS Editorial Board, 2016) or the National Center for Biotechnology Information (NCBI) Taxonomy Database for freshwater and terrestrial species. In the case of planktonic species, to reduce potential sampling bias in the sizes of animals collected, only those studies in which the adults were sampled across the entire depth of the water column, or across most of the depth range of the species, were included. Maximum water sampling depth across all aquatic studies in our data set was 125m.

For each study included in our data set, we derived species-specific slopes of ordinary least-squares regressions between ln-transformed dry mass (mg) and environmental temperature at time of collection, using individuals of species as data points. We derived slopes for males and females separately wherever possible. This exponential function is overwhelmingly favoured for modelling seasonal T-S gradients, rather than linear, quadratic and allometric relationships, giving an Akaike weight of 1 (see Appendix 3.2). The exponential function was also the best for fitting body size-temperature relationships under controlled laboratory conditions and for latitudinal-size clines, again judged using Akaike weights (Hirst *et al.*, 2015; Horne *et al.*, 2015). This common use of an exponential function allows us to easily compare all three of these size gradients. These ‘seasonal T-S slopes’ were also transformed into percentage change in dry mass per degree Celsius (hereby referred to as ‘seasonal T-S gradients’), using the formula $(\exp^{(slope)} -1)*100$ = % change in mass per °C (Forster *et al.*, 2012). A negative gradient shows a reduction in body size with increasing temperature, and hence follows the same trend as the temperature-size rule (Atkinson, 1994).

Where temperature measurements in a study were not reported for the entire year (n=19 of 79), we used high resolution global climate data to estimate mean annual temperature and annual temperature range (ATR) at each sampling location (from NOAA/OAR/ESRL PSD, Boulder, Colorado, USA), available online at http://www.esrl.noaa.gov/psd/. Specifically, we used weekly mean sea surface temperatures (SST) from 1989/12/31 to 2015/10/25 [year/month/day] (NOAA Optimum Interpolation Sea Surface Temperature dataset; 1.0 degree latitude x 1.0 degree longitude global grid) and long term monthly mean air temperature data from 1981 to 2010 (University of Delaware Air Temperature and Precipitation dataset; 0.5 degree latitude x 0.5 degree longitude global grid) to calculate global mean annual ranges in SST for marine environments and surface air temperature ranges for freshwater and terrestrial environments. Surface air temperature has been shown to correlate linearly with water temperature, particularly on a monthly time scale, and thus is a reasonably good indicator of temperature variation in freshwater systems (Pilgrim *et al.*, 1998). In cases where the estimated ATR was less than that of the range derived from the original study, we used the latter given that it represents a direct measurement. Sampling locations were mapped using package ggplot2 in the statistical software package R (R Core Team, 2014) and are presented in Figure 3.2.

Statistical analyses were conducted in R (R Core Team, 2014). We compared several candidate models to best predict seasonal T-S gradients based on the Akaike's information criterion (AIC). Using seasonal T-S gradient as the dependent variable, developmental environment (aquatic-developing vs. terrestrial-developing), log10-transformed species body mass (at 15°C calculated using species-specific T-S slopes) and mean annual temperature were incorporated as fixed variables in a global linear

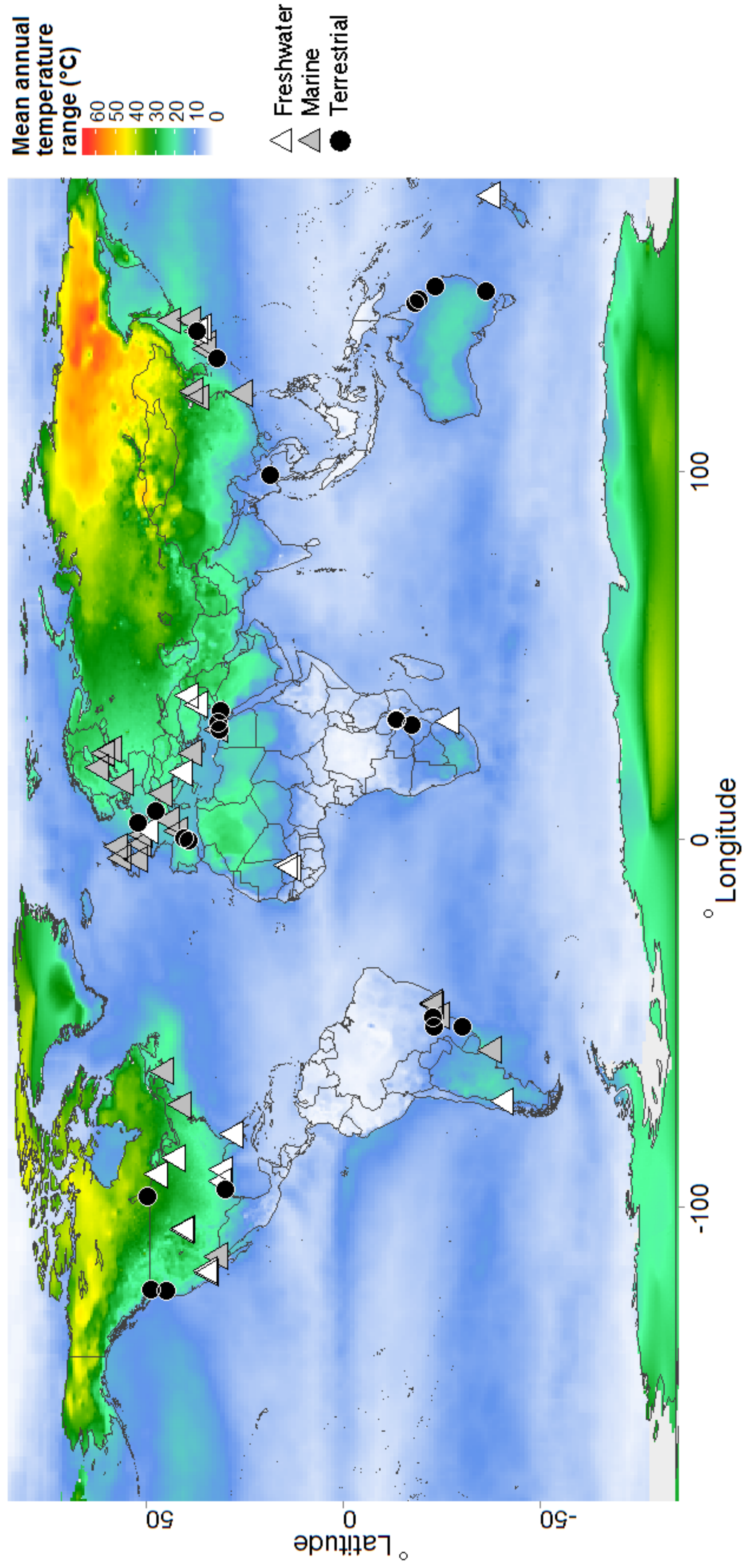

Mean annual temperature range (°C)
60
50
40
30
20
10
0
Freshwater
Marine
Terrestrial
Latitude °
50
0
-50
Longitude °
-100
0
100

Figure 3.2. World map (equirectangular projection) indicating the location of studies (n=71) from which seasonal temperature-size gradients were recorded, categorized by environment type. Colour gradient indicates mean annual temperature ranges. Sea surface temperature data was used for marine environments. Air surface temperature data was used for terrestrial and freshwater environments. Data sources are given in the Methods.

mixed effects model (using package lme4). Log10-transformed species body mass was included to determine if the seasonal T-S gradient was mass dependent, (i.e. to determine if larger species adjusted their body size more strongly with intra-annual warming), following the results of Forster *et al.* (2012) and Horne *et al.* (2015) (Chapter 2) . Given the strong association between latitude and mean annual temperature, we modelled the effect of latitude on the seasonal T-S gradient separately. Gradients of size across seasonal temperatures from multiple studies of the same species were included in our analyses. Given that species have shared evolutionary histories and are not completely statistically independent, we included levels of taxonomic classification (subphylum, class, order, family, and species) as nested (hierarchical) random effects on the intercept in all models to help control for phylogeny (Felsenstein, 1985). We also included habitat (marine, freshwater, terrestrial) as a random effect on the intercept, to control for the fact that we had aquatic-developing species from both marine and freshwater habitats. Including sex as a random effect did not improve the fit of the model, and so this was excluded. Finally, as the dependent variable in our models (the seasonal T-S gradient) is derived from data that vary between studies and species in their goodness of fit, we accounted for variation in information quality by weighting each seasonal T-S gradient by the inverse of the variance of its T-S slope estimate (using the 'weights' function in R) (Koricheva *et al.*, 2013). All possible combinations of the global model terms were compared using the dredge function in the MuMIn package. The best model was identified as that with the lowest small-samples corrected AIC (AICc). Akaike weights (w_i) were used to determine which candidate model was the best fit model. Where the difference between a model's AICc and the lowest AICc (i.e. ΔAICc) was <2, a set of best fit models, rather than a single best model, was assumed. Model averaging was then used to identify the best predictor variables across the top candidate models, and determine their relative importance (computed for each variable as the sum of the Akaike weights from all models in which they appear). In addition to AIC, a series of *F* tests were used to verify the significance ($p<0.05$) of each parameter's effect on the strength of the seasonal T-S gradient. *Post hoc* comparisons were made using a Tukey HSD test.

To estimate the total change in body mass that a species could achieve over a season, we multiplied the seasonal T-S slope by the ATR of the sampling location for each species. This value was transformed into total percentage change in body size using the formula $(\exp^{(\text{slope} \times \text{ATR})} - 1) * 100$ = total % change in mass. We compared total percentage change in body size between aquatic-developing and terrestrial species using a two-sample *t*-test. For both groups, an ordinary least squares (OLS) regression of total % change in mass against ATR was used to determine whether species from more or less thermally variable environments exhibited a greater total percentage change in body size over a season. Given that the slope of this regression did not differ significantly from zero, and thus total percentage change in body size appeared relatively invariant with ATR, we also estimated the maximum total percentage change in body size with warming for aquatic-developing and terrestrial species. To do this, we used package quantreg in R to fit the lowest possible quantile regression that complied with the sample size of each data set, following recommendations by Rogers (1992) , such that $n > 5/q$ (where n is the sample size and q is the quantile of interest). This gives the most reliable estimate of the edge of the data set appropriate to the sample size. In each case, this quantile regression had a slope that did not differ significantly from zero; thus, we simply used the intercept to estimate the limit to total percentage change in body size over the season. Similarly, we also estimated the minimum total percentage change in body size with warming by fitting the highest possible quantile regression through the data that complied with the sample size of each data set, where $n > 5(1 - q)$ (Rogers, 1992).

To compare seasonal T-S gradients with laboratory controlled T-S responses and L-S clines, we used the data compilations from Chapter 2. For each of these data sets, we first combined size gradients from multiple studies of the same species into a simple mean to generate single species-specific values. Order-specific gradients were then calculated by averaging species-specific gradients for each taxonomic order, and reduced major axis (RMA) regression analysis was used to compare order-specific seasonal T-S gradients with laboratory T-S responses and L-S clines.

As in Chapter 2, we note that using interspecific length-mass conversions can increase the likelihood of inaccuracy when determining body size gradients, particularly as any small deviation in the equation's power term can result in substantial over- or under-estimation of the percentage change in body size. Wherever possible we ensured that length-mass regressions were species-specific, but given that family- and order-specific conversions have been used, and that authors have employed a variety of equation forms, we repeated our analysis using length in place of dry mass to generate a second set of seasonal temperature-length (T-L) gradients (% change in length °C^{-1}). To do this we used either the original length measurements reported, or calculated the cube-root of mass when this was given. This length-based analysis confirms the difference in responses between environment types (aquatic, terrestrial), and the major findings from this approach are also summarised in our Results.

Results

Our analysis included data from 71 sites in both temperate and tropical habitats between -38.1° and 61.5° latitude, from Argentina to Scandinavia, although 52% of all study locations are found either in Europe or North America and hence dominated by northern hemisphere temperate areas (Figure 3.2). We obtained 3,725 seasonal body mass measurements in nature, representing 30 freshwater, 47 marine and 25 terrestrial arthropod species from 9 taxonomic orders, resulting in 225 seasonal T-S slopes (see Supplementary Information for species list and individual plots of ln- transformed body mass vs. temperature). Most species (~86%) conformed to the temperature-size rule; that is 88 of the 102 species exhibited a seasonal decrease in adult body size with increased temperature in the field.

Aquatic vs. Terrestrial Species

The model with the lowest AICc score, and hence the better supported model for explaining variation in seasonal T-S gradients, contained only developmental environment (aquatic vs. terrestrial) as a fixed variable. Three other models, including an 'intercept only' model, had a ΔAICc <2. Therefore, we calculated combined parameter Akaike weights across all four candidate models to determine the relative importance of each variable (see Appendix 3.2). The developmental environment was the most important variable in explaining variation in the magnitude of the seasonal T-S gradient, such that aquatic-developing species showed a ~2½-fold stronger reduction in body size with seasonal warming (-3.1% body mass $°C^{-1}$ ±0.8; 95% CI) than terrestrial species (-1.4% body mass $°C^{-1}$ ±0.9; 95% CI; $F_{1,211}$=16.90, p<0.001; see Figure 3.3). Similarly, within the order Diptera, which contains species that develop in water and on land, aquatic-developing species reduced their body size significantly more per °C of

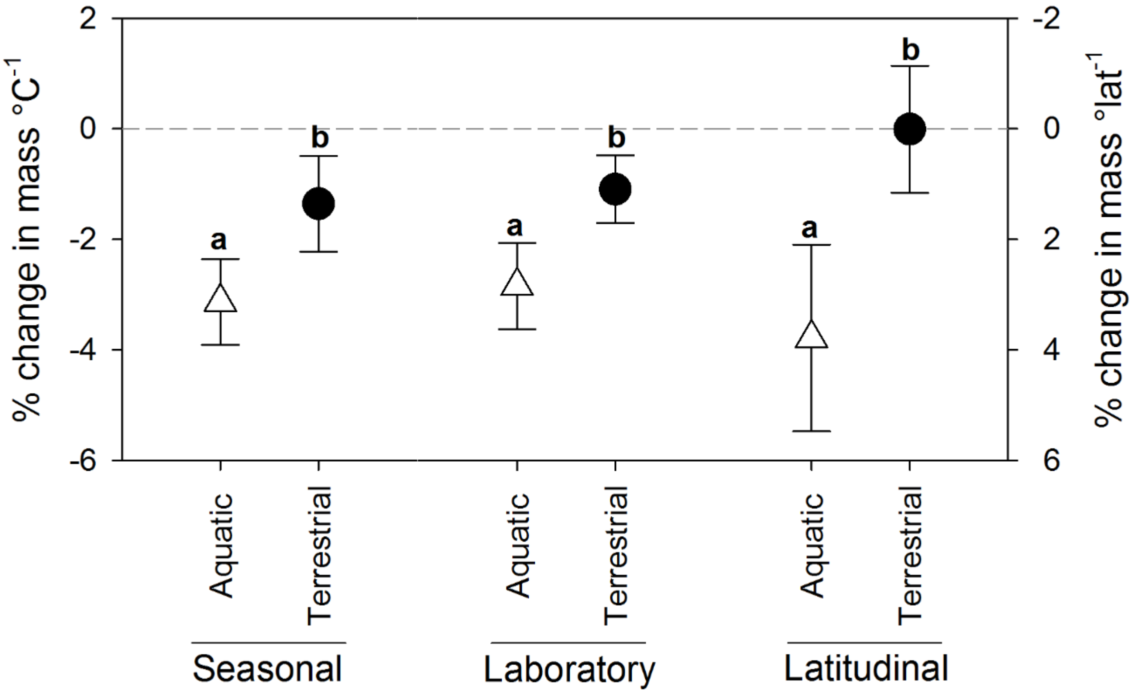


Figure 3.3. Comparison of mean aquatic-developing and terrestrial-developing seasonal temperature-body size gradients (% change in mass per °C ±95% CI, left-hand y-axis) in arthropods with laboratory temperature-size responses (% change in mass per °C ±95% CI, left-hand y-axis) and latitudinal-size clines (% change in mass per °latitude ±95% CI, right-hand y-axis) for multivoltine species, using data from this study and Chapter 2. Different letters above data points indicate significant differences. Dashed grey line indicates no change in body size with warming or increasing latitude. Note the reversal of the right-hand y-axis (for the latitudinal-size cline) for ease of comparison (a reduction in body size with increasing temperature is then comparable with an increase in body size with increasing latitude).

seasonal warming than did terrestrial-developing species ($F_{1,34}$=10.17, p<0.01). These differences between aquatic and terrestrial environments were also important in influencing both laboratory T-S responses and L-S clines in the field (Horne *et al.*, 2015) (Figure 3.3).

Developmental environment (aquatic vs. terrestrial) alone, accounted for ~30% of the total variance in seasonal T-S gradients, whereas mean annual temperature ($\bar{T}$) and $\log_{10}$ species body mass were less important parameters (Appendix 3.2). $\bar{T}$ had no significant effect on the seasonal T-S gradient in either aquatic-developing ($F_{1,172}$=0.42, p=0.32) or terrestrial arthropods ($F_{1,35}$=2.80, p=0.10). The seasonal T-S gradient across aquatic-developing species became more strongly negative with increasing body mass ($F_{1,172}$=6.60, p=0.01), but the goodness of fit was extremely low (R^2=0.02). Thus body mass explained relatively little of the variation in aquatic seasonal T-S gradients in our dataset. There was no significant mass-dependence in terrestrial species ($F_{1,35}$=0.06, p=0.80). There were significant differences in the strength of the seasonal T-S gradient between taxonomic orders, specifically within the sub-class Copepoda ($F_{9,215}$=5.89, p<0.001). The order Calanoida (-3.66±0.70% body mass $^{o}C^{-1}$; 95%CI) had a significantly stronger negative seasonal T-S gradient than both Cyclopoida (-0.91±0.59% body mass $^{o}C^{-1}$; 95%CI) and Poecilostomatoida (1.36±3.06% body mass $^{o}C^{-1}$; 95%CI) (see Chapter 4 for a detailed discussion of these findings). Latitude of the sampling location had no significant effect on the strength of the seasonal T-S gradient ($F_{1,122}$=1.13, p=0.29).

Total percentage change in body size over the annual cycle differed significantly between aquatic-developing and terrestrial species (t_{223}=-3.52, p<0.001), but did not

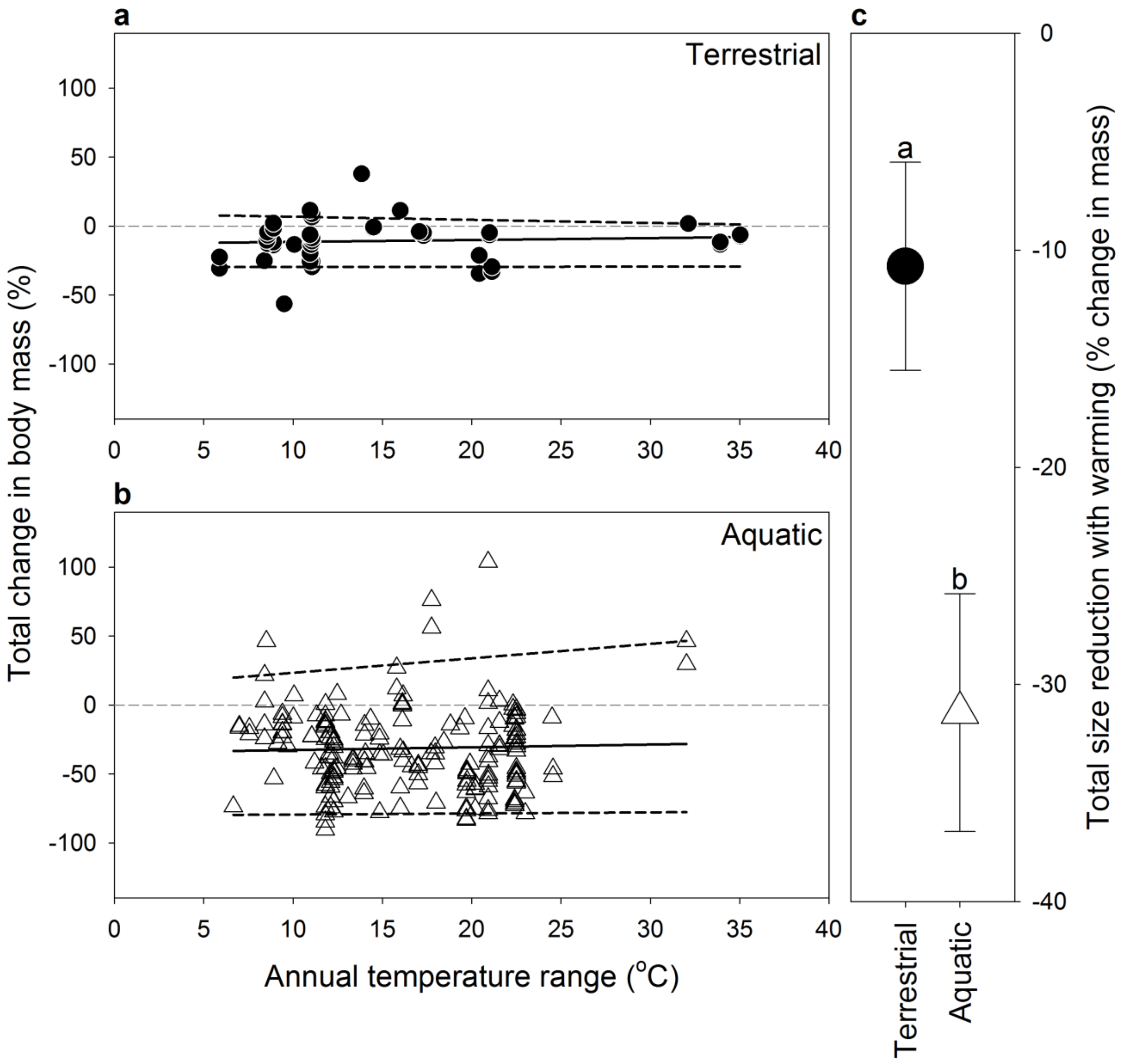


Figure 3.4 Total change in body mass (%) vs. annual temperature range (°C) for (**A**) terrestrial and (**B**) aquatic arthropods. Solid black line represents the OLS regression, the slope of which does not differ significantly from zero in either environment, such that total percentage change in mass appears invariant with annual temperature range. Dashed black lines show the lowest and highest possible quantile regressions through the data and represent the upper and lower limits to total body size reduction with warming respectively (**C**) Mean total size reduction with warming (expressed as a % change in body mass (±95% CI)) for terrestrial and aquatic-developing species. Different letters above data points indicate significant differences.

vary with mean annual temperature range in either group, such that, on average, total size change appeared relatively constant (t_{180}=0.37, p=0.71 and t_{41}=0.47, p=0.64 respectively; see Figure 3.4). Mean overall size reduction in terrestrial species was –10.7±4.8% (95% CI), whereas overall size reduction in aquatic-developing species was almost 3-fold greater at -31.3±5.5% (95% CI) (Figure 3.4c). Additionally, the lowest and highest possible quantile regressions through these data (see Methods) had slopes that did not differ significantly from zero, and thus in each case we used the value of the intercept to also estimate the near-maximum and near-minimum limits to total body size reduction with warming. We estimated a limit for total percentage change in body mass in aquatic-developing species of -80.2±22.6% (95% CI), which is more than 2½-fold greater than in terrestrial-developing species at -29.7±24.9% (95% CI). The lower limit to total percentage change in size with warming did not differ significantly from 0% in either aquatic (t_{180}=0.17, p=0.87) or terrestrial species (t_{41}=0.55, p=0.58).

Coherence among seasonal, laboratory and latitudinal body size patterns

If temperature is a major driver of seasonal body size variation in the field, seasonal and controlled laboratory T-S responses should be significantly correlated. Indeed, across taxonomic orders these two gradients showed a positive correlation, which did not differ significantly from a 1:1 relationship (R^2=0.59; Figure 3.5a). This 1:1 match was supported by the RMA slope differing significantly from zero but not from 1 (0.73±0.38; 95% CI), whilst the intercept did not differ significantly from zero (-0.39±1.16; 95%CI) (inferred from 95% confidence intervals; see inset panel in Figure 3.5a). Given the relatively strong T-S gradients (seasonal and laboratory) of aquatic Isopoda compared with those of the other taxonomic orders, we also tested whether the RMA regression, and hence co-variation between seasonal and laboratory T-S

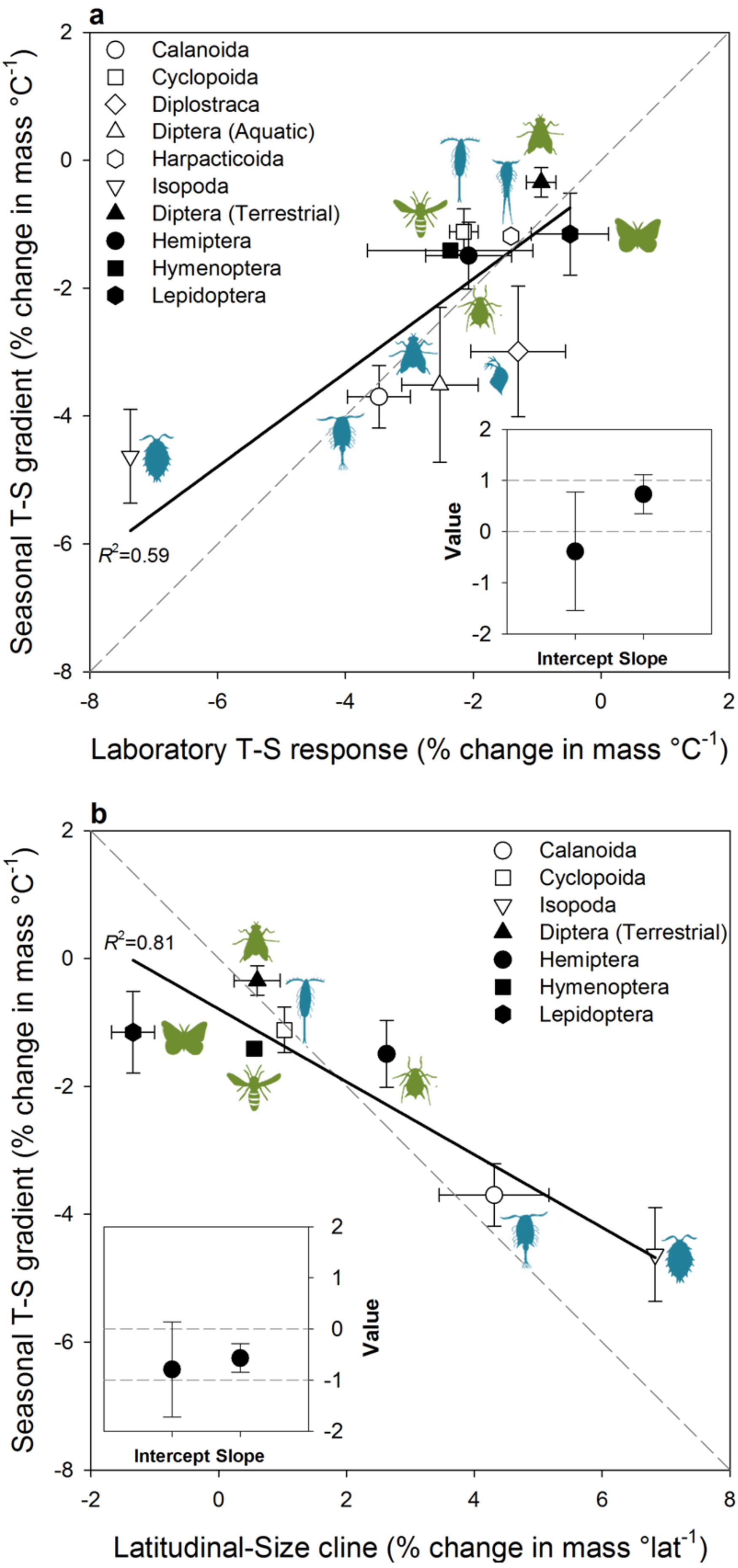

a
Seasonal T-S gradient (% change in mass °C-1)
Laboratory T-S response (% change in mass °C-1)
2
0
-2
-4
-6
-8
-8
-6
-4
-2
0
2
○ Calanoida
□ Cyclopoida
◇ Diplostraca
△ Diptera (Aquatic)
⬡ Harpacticoida
▽ Isopoda
▲ Diptera (Terrestrial)
● Hemiptera
■ Hymenoptera
⬢ Lepidoptera
R2=0.59
Value
2
1
0
-1
-2
Intercept Slope
b
Seasonal T-S gradient (% change in mass °C-1)
Latitudinal-Size cline (% change in mass °lat-1)
2
0
-2
-4
-6
-8
-2
0
2
4
6
8
○ Calanoida
□ Cyclopoida
▽ Isopoda
▲ Diptera (Terrestrial)
● Hemiptera
■ Hymenoptera
⬢ Lepidoptera
R2=0.81
Value
2
1
0
-1
-2
Intercept Slope

Figure 3.5. Reduced major axis (RMA) regression comparing seasonal temperature-size gradients (% change in body mass per °C ±SE) in arthropods with (**A**) laboratory temperature-size responses (% change in body mass per °C ±SE), and (**B**) latitudinal-size clines (% change in body mass per °lat ±SE), categorized by taxonomic order and developmental environment (aquatic=open symbols; terrestrial=filled symbols). Dashed lines indicate a 1:1 relationship. Inset graphs show the intercept and slope values for each regression (±95% CI).

gradients, was dependent on this taxonomic order. The RMA regression did not differ significantly from a 1:1 relationship when the aquatic Isopoda were excluded (slope=1.31±0.90; intercept=0.56±1.85; R^2=0.41). The seasonal and laboratory datasets largely contained different species, yet, even for the small number of species for which we had both sets of data (n=22), there was positive correlation between the two. Once again the RMA slope differed significantly from zero but not 1 (1.51±0.61; 95%CI), whereas the intercept did not differ significantly from zero (1.80±2.28; 95%CI).

Seasonal T-S gradients negatively correlated with L-S clines at the level of taxonomic order (R^2=0.81; Figure 3.5b): those orders (e.g. Isopoda) whose members grew to a smaller adult size in warmer seasons also showed a decrease in size towards lower, warmer latitudes. Although we would not expect a 1:1 relationship between these size gradients (1° increase in latitude does not equal 1°C change in temperature), the gradient of the RMA slope did differ significantly from zero (-0.57±0.28; 95% CI), confirming a significant correlation, whilst the intercept did not differ significantly from zero (-0.79±0.93; 95% CI; see inset panel in Figure 3.5b). As before, there remained a significant correlation even when the aquatic Isopoda were excluded (slope=-0.58±0.52; intercept=-0.78±1.14; R^2=0.59).

As outlined in our Methods, we also we repeated our analysis using length (mm) in place of dry mass (mg). We find that developmental environment (aquatic-developing vs. terrestrial-developing) has a significant effect on % change in length °C^{-1} ($F_{1,223}$=4.13, p=0.04). On average, aquatic-developing species have a seasonal T-S gradient of -1.16% (±0.19; 95% CI) and terrestrial-developing species have a gradient of -0.72% (±0.31; 95% CI). We find no significant effect of mean annual temperature on the seasonal T-S gradient in freshwater ($F_{1,50}$=0.62, p=0.44), marine ($F_{1,128}$=0.55, p=0.46) or terrestrial species ($F_{1,41}$=1.53, p=0.22). As with body mass, we find significant length-dependence in the strength of the seasonal T-S gradient in aquatic species ($F_{1,180}$=6.06, p=0.01), such that larger species tend to show the strongest reduction in length with warming. We find no significant length-dependence in terrestrial species ($F_{1,41}$=0.26, p=0.61). Similarly, we find significant differences in the strength of the seasonal T-S gradient between taxonomic orders when using length in place of body mass, once again in the sub-class Copepoda ($F_{9,215}$=3.22, p<0.01), in which the order Calanoida (-1.37±0.23% length $^{o}C^{-1}$; 95%CI) has a significantly stronger negative seasonal T-S gradient than both Cyclopoida (-0.43±0.28% length $^{o}C^{-1}$; 95%CI) and Poecilostomatoida (0.48±1.07% length $^{o}C^{-1}$; 95%CI).

Discussion

Our analysis of seasonal T-S gradients leads us to present four major conclusions: (i) multivoltine arthropod species inhabiting thermally varying seasonal habitats commonly demonstrate a negative seasonal T-S gradient, (ii) aquatic-developing species exhibit a stronger decline in adult body size with seasonal warming than those developing in air, (iii) total size reduction with warming appears relatively invariant despite variation in the annual temperature range experienced, and (iv) seasonal T-S gradients correlate significantly with both laboratory T-S responses and latitudinal-size clines.

The aquatic-terrestrial differences in seasonal T-S gradients per °C parallel those observed in laboratory T-S responses and latitudinal-size clines (Forster *et al.*, 2012; Horne *et al.*, 2015) (Figure 3.3). Further, mean overall size reduction through the year is almost 3-fold greater in aquatic (31.3%) than terrestrial (10.7%) arthropods (Figure 3.4c), whilst the lower quantile estimate of the near-maximum limit to total body size reduction with warming also varies considerably between these two groups. The greatest overall reduction in body mass with temperature for an aquatic species in our dataset is 90.4%, estimated for the calanoid copepod *Temora longicornis*, whereas in terrestrial species it is 56.4%, estimated for the isopod *Porcellionides pruinosus*. These consistent differences in seasonal T-S gradients between environments suggest that the drivers of body size reduction with warming are much stronger in aquatic than terrestrial arthropods.

The difference in seasonal body size change between environments is consistent with the hypothesis that greater constraints on oxygen availability in water than in air have either selected for greater plasticity in adult body size of aquatic species in response to temperature (both per °C and overall), or imposed constraints directly on their growth, compared with terrestrial species (Atkinson *et al.*, 2006). Specifically, metabolic demand increases much faster with increased size and temperature than does oxygen availability in water (Forster *et al.*, 2012); consequently, aquatic-developing species may have adapted to meet these increased metabolic demands with warming by reducing body size, and/or oxygen limitation may also have limited growth directly. An alternative explanation based on thermoregulatory ability also requires consideration. In the field, behavioural thermoregulation may allow arthropod species to maintain a narrower body temperature range over a season relative to the ambient temperature range, be this through seeking shade or basking in terrestrial species, or vertical / horizontal migration in aquatic species. For this reason, the seasonal T-S gradient in thermoregulating species may seem weaker. Due to the higher heat capacity of water than air, thermoregulation is much more difficult for aquatic than terrestrial species. However, we discount the explanation that thermoregulation may explain the differences seen between environments, because this ability is unlikely to account for a 2½-fold difference in body size reduction with warming between these two groups – such an explanation would imply that, where aquatic species experience an annual temperature range of 30°C, terrestrial species experience a range in body temperature of only 12°C, i.e. are able to reduce their body temperature range by 18°C. At least in some environments, this major reduction in body temperature range is highly improbable (Kearney *et al.*, 2009). Furthermore, larger aquatic species often exhibit the greatest reduction in body size with warming (Forster *et al.*, 2012; Horne *et al.*, 2015), yet we see no reason why behavioural thermoregulation would be reduced in larger compared to smaller aquatic species. Instead, this pattern supports the prediction that due to their lower surface area to volume ratio, larger species would struggle most to meet their oxygen requirements in the warm, leading to a stronger T-S gradient. Therefore, behavioural buffering does not seem capable of explaining the observed mass-dependence of the T-S gradient in aquatic species, which instead is consistent with the oxygen hypothesis (Forster *et al.*, 2012).

Although body size reduction with warming is thought to provide fitness benefits by balancing resource demand and supply at elevated temperatures, this likely comes at a cost, given the link between body size and other vital rates and physiological processes. For example, body size is often strongly positively correlated with fecundity, including in insects (Honěk, 1993) and zooplankton (Bunker & Hirst, 2004), whilst smaller body size may also reduce survival during periods of low food availability, or increase vulnerability to predation (Chown & Gaston, 2010). Thus, there will eventually come a point at which the fitness benefits of reducing body size no longer outweigh the costs. These widespread fitness trade-offs may dictate overall limits to total proportional size change in arthropods; an optimal point at which the selective pressures in a given environment over the annual cycle no longer favour more extreme size reductions with warming. The relative consistency in total proportional size change in relation to ATR, despite variation in ATR of up to 30°C between sampling locations, may be an indication of such limits (Figure 3.4). Although these optima vary between species and environments, as is observed in the ~3-fold difference in mean total body size reduction between aquatic and terrestrial species, and in the scatter in total proportional change, the lack of a relationship with ATR suggests that, on average, arthropods from similar environments may share and frequently realize these limits, regardless of the degree of thermal variability across the year. Consequently, species inhabiting environments with a greater thermal range on average reduce their body size less per ^{o}C of warming than those from less thermally varying environments.

Unexplained variation in the magnitude of T-S gradients between species and higher taxonomic groupings is likely to be attributed to differences in life history, physiology and behaviour. Indeed, in Chapter 4 we explore these issues in more detail within the sub-class Copepoda, in which the ~4-fold difference between the seasonal T-S gradients of calanoid and cyclopoid copepods may relate to differences in the temperature-dependence of energy supply and expenditure in current-feeding calanoids vs. ambush-feeding cyclopoids (Horne *et al.*, 2016). Differences in the strength of seasonal variation in resource availability (e.g., food and water in terrestrial species, and food and oxygen in aquatic species) or seasonal mortality risk (e.g. associated with predation, desiccation or both) are also likely to modify the T-S gradient. Although the arthropods in our data set all have more than one generation per year, some groups, such as some of the Lepidoptera species included here, have just two generations in a year, whereas others, including the smaller terrestrial Diptera and aquatic Copepoda, have many overlapping generations. Voltinism is highly temperature-dependent and can constrain body size (Roff, 1980; Kozłowski *et al.*, 2004), and differences in perceived seasonality (including temperature and resource availability) between species with these different generation times, might lead to differences in the strength of the seasonal T-S gradient. Specifically, smaller species with short generation times are likely to perceive reduced seasonality within each generation (Kozłowski *et al.*, 2004). Hence, we might predict that the adaptive advantage of tuning body size to prevailing conditions during development will be strong, leading to a greater reduction in body size and a greater seasonal T-S gradient. Our data largely applies to species with many overlapping generations in a year, making it difficult to assign body size measurements to specific generations or cohorts. A synthesis of changes in mature body size in univoltine terrestrial species, measured over multiple years, would be an informative next step, not least because these species often exhibit a reverse T-S response in the laboratory (i.e. increase in size with warming) and an increase in size towards the equator. In accordance with these patterns, a recent study of a univoltine butterfly species showed that adult male forewing length was positively correlated with temperature during development across multiple years (Fenberg *et al.*, 2016). Whether such an inter-annual size trend extends more generally to other univoltine terrestrial arthropods remains to be tested.

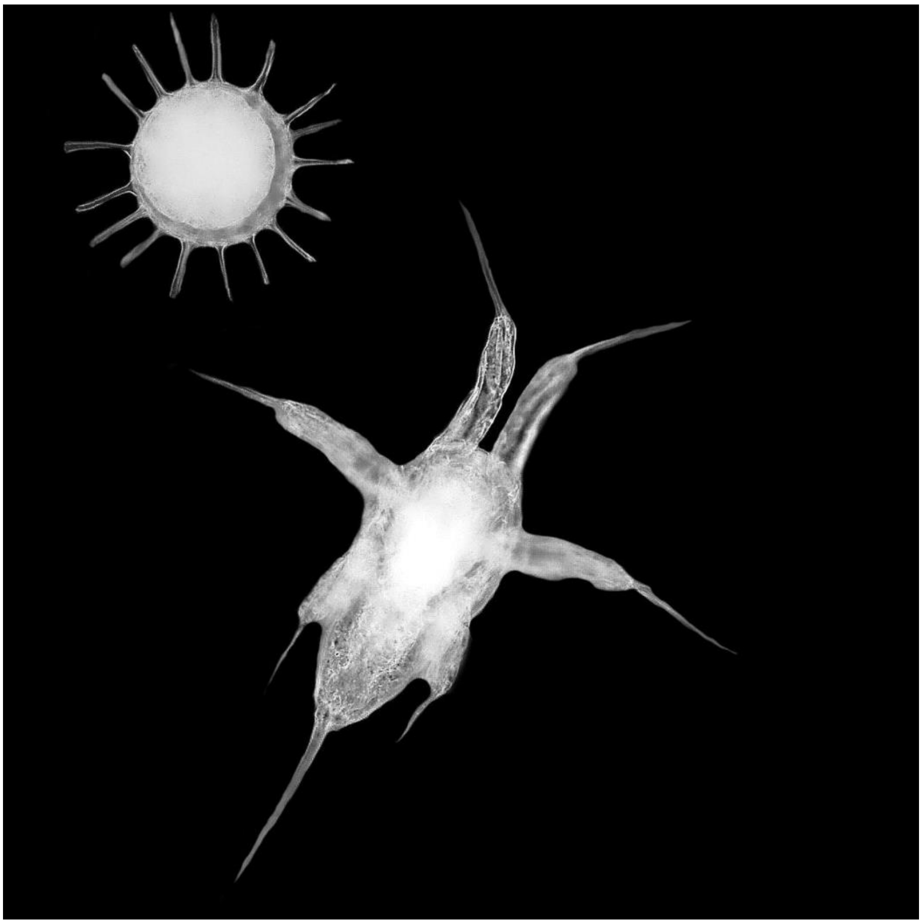
Unlike laboratory T-S responses which are measured at constant temperatures, we note the potential for a mismatch between temperature at the time of collection of adults in the field and the temperature the animals experienced during ontogenetic development. This is particularly true for larger species with longer development times and/or in those species from strongly seasonal environments. However, as discussed above, many of the multivoltine species considered here generally have short generation times, often of just a few weeks; thus, in most cases any temperature fluctuations experienced within a generation should be fairly conserved in these shorter-lived species, and temperature at time of collection of adults should be a reasonable proxy for developmental temperature. Similar issues could arise in species that either undergo extended periods of reproductive diapause, or live a long time as adults, particularly the larger Lepidoptera species in our data set (6 of 10 lepidopteran species considered), during which time juvenile recruitment to the population is ceased. In this case, adults collected during periods of diapause, or towards the end of long adult lives, may actually develop much earlier in the season, when environmental conditions were very different. This is further complicated because larger individuals generally have a greater chance of surviving periods of dormancy, and this could be an important factor influencing body size variation in diapausing generations, obscuring any effects of temperature and/or resource availability (Teder *et al.*, 2010). Yet, when we further explored this issue, by excluding body size measurements recorded during suspected periods of reproductive diapause, we observed no significant shift in the T-S slope in any of the 6 species of Lepidoptera that exhibited this behaviour. These species represent the few extreme cases in our data set where juvenile recruitment is ceased for relatively long periods, giving us confidence in the overall patterns we present.

Despite the potential pitfalls in our data and the many confounding factors that can influence body size variation in the field, we find a statistically significant match between body size responses measured in the laboratory and in nature, which suggests that they share common drivers. The consistency in both the strength and direction of all three of these body size gradients observed both at the levels of taxonomic order (Figure 3.5) and of species, as well as between environments, and together with the weighting of data by data quality (as the inverse of the variance of the slope), gives us confidence that these patterns are unlikely to arise simply from differences in sample size between groups or potential sampling error in the individual T-S slopes. Ultimately, the close match between laboratory and seasonal T-S gradients (see Figures 3.3 and 3.5a) suggests that temperature is an important driver of variation in mature body size in arthropods in the diverse seasonal systems we have explored, despite changes in other abiotic and biotic factors that can directly influence body size variation, such as food quantity and quality (Diamond & Kingsolver, 2010).

Once again we demonstrate a simple yet powerful correlative approach to understand major patterns in body size. Although our data set represents only a tiny fraction of all arthropod species globally, by simultaneously comparing large-scale temporal, spatial and laboratory-derived temperature-body size gradients, each of which are largely composed of different species, we identify important patterns in body size that are common to all. Evidently, changes in the body sizes of ectotherms associated with climate change can be both substantial (Daufresne *et al.*, 2009; Sheridan & Bickford, 2011; Rice *et al.*, 2015) and widespread (Gardner *et al.*, 2011). Advancing our understanding of what drives temperature-body size gradients in the field is essential if we are to accurately predict how body size will change with projected increases in temperature and with more extreme seasonality (IPCC, 2014).

CHAPTER 4

A global synthesis of seasonal temperature-size gradients in copepods



Introduction

As demonstrated in Chapter 3, ectothermic species that have short life-cycles and inhabit seasonal environments are typically subjected to varying environmental conditions across subsequent generations within a year. This is commonly linked to marked temporal shifts in adult body size over an annual cycle, as sequentially recruited adults are affected by different abiotic and biotic conditions during their ontogeny. Intra-specific variation in size related to seasonal variation in temperature has been found across a wide range of uni- and multicellular organisms, including bacteria (Chrzanowski *et al.*, 1988), rotifers (Diéguez *et al.*, 1998), copepods (Liang & Uye, 1997; Hirst *et al.*, 1999; Riccardi & Mariotto, 2000; Dutz *et al.*, 2012), cladocerans (Miyashita *et al.*, 2011) and insects (Kari & Huey, 2000), yet broad-scale syntheses of temporal changes in adult body size are rare.

Uncertainty still remains in the degree to which body size responses to temperature measured in the laboratory accurately reflect size variation with temperature in natural field conditions, where multiple variables can act simultaneously to influence body size. For instance, the relative contribution of food and temperature in determining seasonal shifts in adult size still needs to be resolved. Food availability impacts size at maturity, but while slower growth at lower temperature is frequently coupled with an increase in adult size, slower growth with reduced food availability is typically associated with smaller size at maturity (Berrigan & Charnov, 1994). Further, food quality can dramatically alter the T-S response, even to the extent that the sign of the T-S response can be reversed under poor food quality (Diamond & Kingsolver 2010). Identifying and understanding seasonal variation in body size will not only help to determine the ultimate causes of such variation, but will also aid in predicting future shifts in body size associated with changes in climate (IPCC, 2014) and phenology (Visser & Both, 2005).

Our meta-analysis of terrestrial and aquatic arthropods in Chapter 2 identified an impressive match between T-S responses measured under controlled laboratory conditions and intra-specific body size clines observed in the field across latitudes (Horne *et al.*, 2015). The magnitude and direction of these gradients revealed consistent differences in the strength and sign of the response between aquatic and terrestrial species. These results suggest that laboratory T-S responses and latitudinal body size clines may be driven by similar selective pressures within arthropods, specifically, by voltinism and season length trade-offs in terrestrial species (Kozłowski *et al.*, 2004; Walters & Hassall, 2006), and the need to balance oxygen demand and supply in larger aquatic species (Woods, 1999; Atkinson *et al.*, 2006; Forster *et al.*, 2012). However, in many small organisms, in which oxygen diffusion under normoxic conditions is likely to adequately meet metabolic demand, size reductions with warming are still very common; they are for example observed in bacteria, protists and small metazoans, such as copepods (Atkinson *et al.*, 2003; Forster *et al.*, 2012). Copepods are possibly the most numerous metazoans on the planet, are ecologically important, and play a pivotal role in marine and freshwater biogeochemistry and trophodynamics (Banse, 1995). Reduction in size with increasing temperature, consistent with the temperature-size rule (Atkinson, 1994), has been shown in many copepod species, both in controlled laboratory experiments (Horne *et al.*, 2015), and across seasons in the field (Seasonal T-S gradients) (Uye *et al.*, 1983; Hirst *et al.*, 1999; Riccardi & Mariotto, 2000; Drif *et al.*, 2010). Furthermore, the strength of the laboratory temperature-size response varies widely between species, to the extent that we observed an approximate 30-fold difference between the strongest and weakest copepod T-S responses in Chapter 2. It would appear, therefore, that another factor (or other factors) may be responsible for size reductions with warming observed in these smaller taxa, and identifying the likely causes is an important next step. Planktonic copepods are excellent model organisms in which to investigate seasonal size gradients. Temporal changes in adult body size have commonly been examined in this taxon (Figure 4.1), especially in mid-latitude environments which demonstrate strong shifts in temperature and food, while most species have multiple generations within a year and short generation times of >10 to <100 days (Hirst & Kiørboe, 2002). Thus, in this chapter we present and test a number of alternative hypotheses that may help to explain the considerable variation observed in body size sensitivity to warming in planktonic copepods.

Mature adult size is dependent in part upon obtaining sufficient food to meet maintenance and growth requirements, and size at maturity is controlled by different body size scaling of catabolism and anabolism (von Bertalanffy, 1957; Perrin, 1995):

$$\frac{dM}{dt} = c_s \cdot M^s - c_l \cdot M^l$$

where $s > 0$ and $l > 0$ are exponents for energy supply and loss respectively, and $c_s > 0$ and $c_l > 0$ represent the temperature dependence of the intercept terms in log-log scale. The point at which metabolic supply and demand intersect defines an organism's asymptotic mass ($\left.\frac{dM}{dt}\right|_{M_A} = 0$). In mathematical terms, the asymptotic mass, M_A, is given by

$$c_s \cdot (M_A)^s = c_l \cdot (M_A)^l \Rightarrow \log(M_A) = \frac{\log\left({}^{c_s}/_{c_l}\right)}{l - s}$$

Temperature changes will affect both energy supply and expenditure, forcing the organism into a new asymptotic mass. Hence, we can predict the induced relative change in asymptotic mass per degree Celsius, noting that f'(x) / f(x) = f'[log(x)]:

$$\frac{1}{M_A}\frac{dM_A}{dT} = \frac{d}{dT}[\log(M_A)] = \frac{1}{l-s} \cdot \frac{d}{dT}\left[\log\left(\frac{c_s}{c_l}\right)\right]$$

Thus, the temperature dependence scales inversely with the difference in the mass scaling of supply and demand (*l*-*s*), and is also influenced by the temperature dependence of the intercepts. Moreover, within this framework, the strength of the T-S response should be independent of body mass.

Despite overwhelming evidence in favour of the temperature-size rule (TSR) in a diverse range of ectotherms, there remains considerable unexplained variation in the strength of the response between species and taxonomic groups, which are most likely attributed to key differences in life history traits and their associated metabolic constraints. In copepods and many other small zooplankton, food acquisition is governed by prey availability and uptake. Species within the order Calanoida largely utilise feeding currents to entrain and capture prey (Kiørboe, 2011), with a few exceptions; by contrast, species within the non-Calanoida orders, i.e., the Harpacticoida, Cyclopoida and Poecilostomatoida, lack an ability to produce a feeding current and are either ambush feeders (Cyclopoida; Paffenhöfer 1993), or they feed on surfaces, which in the planktonic environment is provided by marine snow aggregates (Harpacticoida; Koski *et al.* 2005), or they are parasitic (e.g. many Poecilostomatoida; Huys & Boxshall 1991). There is evidence that feeding mode is an important correlate of metabolic rates (respiration), clearance, growth and ingestion rates (Kiørboe & Hirst, 2014). Ignoring parasitic copepods, the body mass dependence of clearance rate differs between feeding current feeders and more passive ambush and surface feeders (Kiørboe, 2011), suggesting a possible difference in the temperature-body size sensitivity between different feeding behaviours. We cannot yet predict the magnitude and direction of the T-S response since we do not know how metabolic rates change with mass during ontogeny, and we also do not know how the intercept terms vary with temperature. However, these considerations lead us to suggest that some of the variability in T-S responses may be due to differences in feeding behaviour.

Another potential influence on the T-S response is reproductive strategy. In copepods, reproductive strategy can be divided into broadcast spawning and sac spawning. Sac spawners carry eggs in external sacs, and have much lower fecundity rates than broadcast spawners (Hirst & Kiørboe, 2002). Sac spawners commonly do not lay the next batch of eggs until the previous batch has hatched from the attached sac(s) (Ward & Hirst, 2007), hence egg production is limited by the egg hatch time (Hirst & Bunker, 2003). By contrast, broadcasters have much higher fecundity rates, and are less likely to be limited by clutch size or egg hatch rates in the same way. The potentially different thermal sensitivities of egg development versus egg production rates may produce different solutions for size at maturity (and in turn its temperature dependence) between these two reproductive strategies. However, even in the absence of clear evidence of such a difference in thermal sensitivity of egg production and hatching, optimum size may change to different degrees if the cost of carrying *versus* not carrying egg sacs is temperature dependent. For example, feeding rates of ectotherms, including predators such as fish, typically increase with warming (Barneche *et al.*, 2008), and such increased risk of mortality to prey organisms may amplify any small differences in size- and fecundity- related trade-offs observed between broadcast and sac spawners at cooler temperatures. In principle, therefore, differences in the optimum body size response to temperature between the two spawning strategies can be hypothesized.

This chapter therefore aims to: 1. Quantify and synthesise seasonal temperature-size gradients of a wide range of planktonic copepod species, and to compare these with responses under controlled laboratory conditions. 2. Examine the temperature dependence of size at maturity in copepods, based around major differences between taxonomic orders, species body sizes, modes of feeding (feeding current vs. active ambush feeding), and reproductive strategy. 3. Assess the relative importance of food concentration and temperature in driving seasonal size change.

Methods

We searched the literature for studies in which the adult body size of planktonic copepods was assessed on multiple occasions during an annual cycle. In addition to temperature we also recorded the concentration of phytoplankton pigment chlorophyll-*a* (Chl-*a*) when this was reported. Chl-*a* concentration is commonly used as a proxy for phytoplankton biomass and food availability; indeed, adult fecundity and juvenile growth in many copepods correlates to this term (Hirst & Bunker, 2003; Bunker & Hirst, 2004). To reduce potential sampling bias in the sizes of animals collected, only those studies in which the adults were sampled across the entire depth of the water column, or across most of the depth range of the species, were included. Adult size data were collected as either lengths or dry, wet, or carbon mass. These measurements were subsequently converted to dry mass (mg) using published intra-specific regressions. If these were not available, regressions for closely related species, or more general interspecific regressions, were used. All raw data and conversions are detailed in Appendix 4.1. Taxonomic order and family were confirmed for each species using the World Registry of Marine Species (WoRMS Editorial Board, 2016).

In Chapter 3 we showed that the exponential equation form was overwhelmingly favoured for modelling seasonal T-S gradients, as judged by Akaike weights. We therefore used an exponential equation form to model the seasonal temperature-size gradient for each species from each study in our dataset, separating gradients by sex. Species-specific slopes of the natural log (ln) of dry mass vs. temperature were transformed into percentage change in dry mass per degree Celsius, using the formula $(\exp^{(slope)} -1)*100$ = % change in mass per °C (Forster *et al.*, 2012). This value represents the seasonal temperature-size gradient, with a negative value showing a reduction in body mass with increasing temperature, and hence following the same trend as the temperature-size rule. Size gradients from multiple studies of the same species were then combined into a simple mean to generate a single species-specific seasonal T-S gradient, separated by sex.

To quantify relationships between body mass and Chl-*a*, the species-specific slopes of the natural log (ln) of dry mass vs. Chl-*a* concentration (µg per litre) were determined for all individuals and transformed into percentage change in dry mass per $\mu g.L^{-1}$, again using the formula $(\exp^{(slope)} -1)*100$ = % change in mass per $\mu g.L^{-1}$, to generate a chlorophyll-size (C-S) gradient. For all datasets in which we had both a measure of temperature and Chl-*a* concentration (n=80), we compared the coefficient of determination (R^2) of both parameters (i.e. by comparing the R^2 of each seasonal T-S gradient with its corresponding C-S gradient), to determine whether one consistently explained significantly more of the variation in seasonal body size gradients than the other. Given that temperature is a mechanistic driver of variation in primary productivity, we also utilised an alternate approach to examine these relationships; first we regressed body mass against temperature and then subsequently regressed the residuals from this on Chl-*a* concentration, to determine how much of the seasonal variation in body size could be attributed to Chl-*a* after accounting for temperature.

All statistical analyses were conducted using R (R Core Team, 2014). We derived several candidate models to determine the best predictors of seasonal T-S gradients based on the Akaike's information criterion (AIC). In order to determine whether species body size impacts the seasonal T-S gradient, we included log10 species mass at a reference temperature (15°C) as a predictor, following the significant allometric relationships shown previously (Forster *et al.*, 2012; Horne *et al.*, 2015). Taxonomic order, log10 body mass (at 15°C calculated using species and sex-specific slopes) and sex were incorporated as fixed variables in a global linear mixed effects model (using package lme4), with species nested within family, and latitude included as random effects on the intercept. When selecting our random effects, we considered the estimates of variance explained by each of our proposed random variables (environment type (marine vs. freshwater), latitude, and species nested within family) and used stepwise elimination of non-significant terms to determine which parameters to include in the final model. All possible combinations of the global model terms were compared using the dredge function in the MuMIn package in *R*. The best model was identified as that with lowest small-samples corrected AIC (AICc), and Akaike weights (w_i) were used to determine the probability (0-1) of each candidate model being the best fit model. Where the difference between a model's AICc and the lowest AICc (i.e. ΔAICc) is <2, a set of best fit models, rather than a single best model, can be assumed, and model averaging may be used to identify the best predictor variables across the top candidate models and determine their relative importance (computed for each variable as the sum of the Akaike weights from all models in which they appear). In addition to AIC, a series of F tests (using the ‘anova’ function in *R*) were used to verify the significance ($p<0.05$) of each parameter’s effect on the strength of the seasonal T-S gradient. *Post hoc* comparisons were made using a Tukey HSD test.

To compare seasonal T-S gradients with laboratory T-S responses, we used the extensive T-S response dataset from Chapter 2.

Results

We derived a total of 140 seasonal T-S gradients from 33 different global locations (Figure 4.1), within the latitudinal range of 25°N/S to 61°N/S, hence largely falling around mid-latitudes (with a dominance of northern hemisphere locations). This in part reflects well-studied temperate environments with strong seasonality, while also being inhabited by copepod species with multiple generations in a year. The data set included 48 planktonic copepod species from 4 taxonomic orders (Calanoida, Cyclopoida, Harpacticoida, Poecilostomatoida). These species-specific seasonal T-S gradients had negative slopes in 87% of cases, with a mean reduction in size of -2.87±0.65% (95%CI) body mass per °C (Figure 4.2), reinforcing the generality of the negative T-S response in copepods. The overall strength or direction of the seasonal T-S gradient did not vary significantly across latitudes ($F_{1,138}=1.20$, $p=0.27$). Of the 80 seasonal body size clines

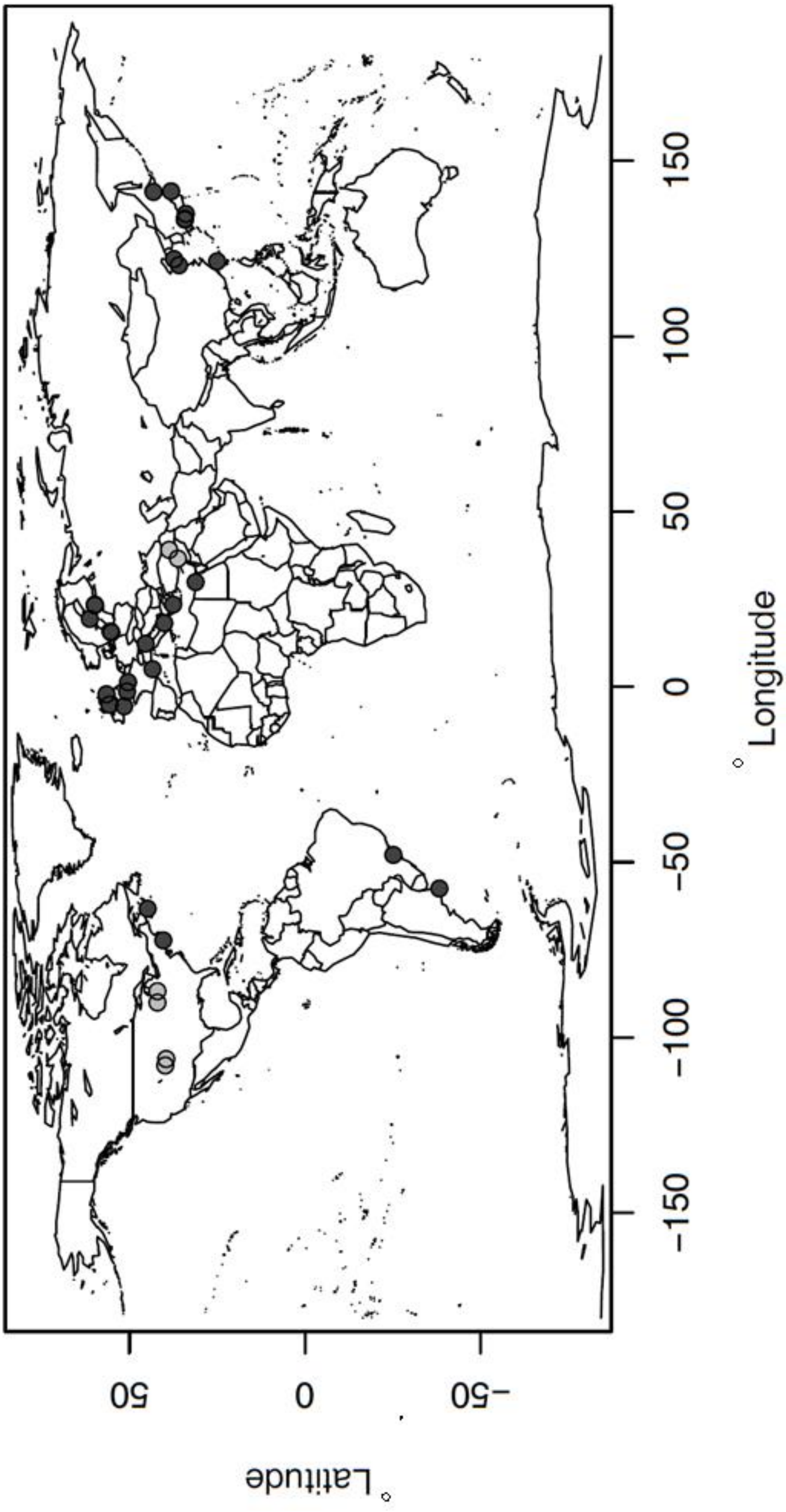

Latitude °
50
0
−50
Longitude °
−150
−100
−50
0
50
100
150

Figure 4.1. World map indicating the location of studies (n=33) from which copepod seasonal size gradients were recorded. Studies from freshwater environments are indicated by the light grey circles whilst marine environments are indicated by the dark grey circles.

for which we had a measure of Chl-*a* concentration (corresponding to 33 species), across all orders we observed a mean body mass response of 0.98±2.01% (95%CI) per µg.L^{-1}, which does not differ significantly from zero (t_{79}=0.97, p=0.34) (Figure 4.2). On average, across all taxonomic orders temperature explained more of the variation in seasonal body mass than Chl-*a* concentration: this is inferred from the mean R^2 values of each parameter when both were modelled separately (0.44±0.07 vs. 0.22±0.05 respectively (95%CI)), and also when comparing body mass-temperature regressions with regressions of the resulting residuals against Chl-*a* concentration (0.44±0.07 vs. 0.07±0.03 respectively (95%CI)). Considering each of the four orders separately, temperature always explained more of the variation in adult body mass than did Chl-a concentration.

In explaining variation in the strength of the seasonal T-S gradient among planktonic copepods, the model with the lowest AICc includes only taxonomic order as a fixed variable, whilst all other candidate models have a ΔAICc>2 (Table 4.1). Thus, given the data available, we may reject the other candidate models in favour of a single best fit model in which taxonomic order has a significant independent effect on the strength of the seasonal T-S gradient ($F_{3,82}$=9.43, p<0.001). As briefly described in Chapter3, *post hoc* comparisons (Tukey HSD) show that Calanoida (*n*=66, mean=-3.66±0.70% body mass $^{o}C^{-1}$; 95%CI) have a significantly stronger negative seasonal T-S gradient than both Cyclopoida (*n*=12, mean=-0.91±0.59% body mass $^{o}C^{-1}$; 95%CI) and Poecilostomatoida (*n*=6, mean=1.36±3.06% body mass $^{o}C^{-1}$; 95%CI), but not Harpacticoida (*n*=2, mean=-1.19±3.60% body mass $^{o}C^{-1}$; 95%CI), though our seasonal data for this order are sparse, including only male and female *Euterpina acutifrons*. We

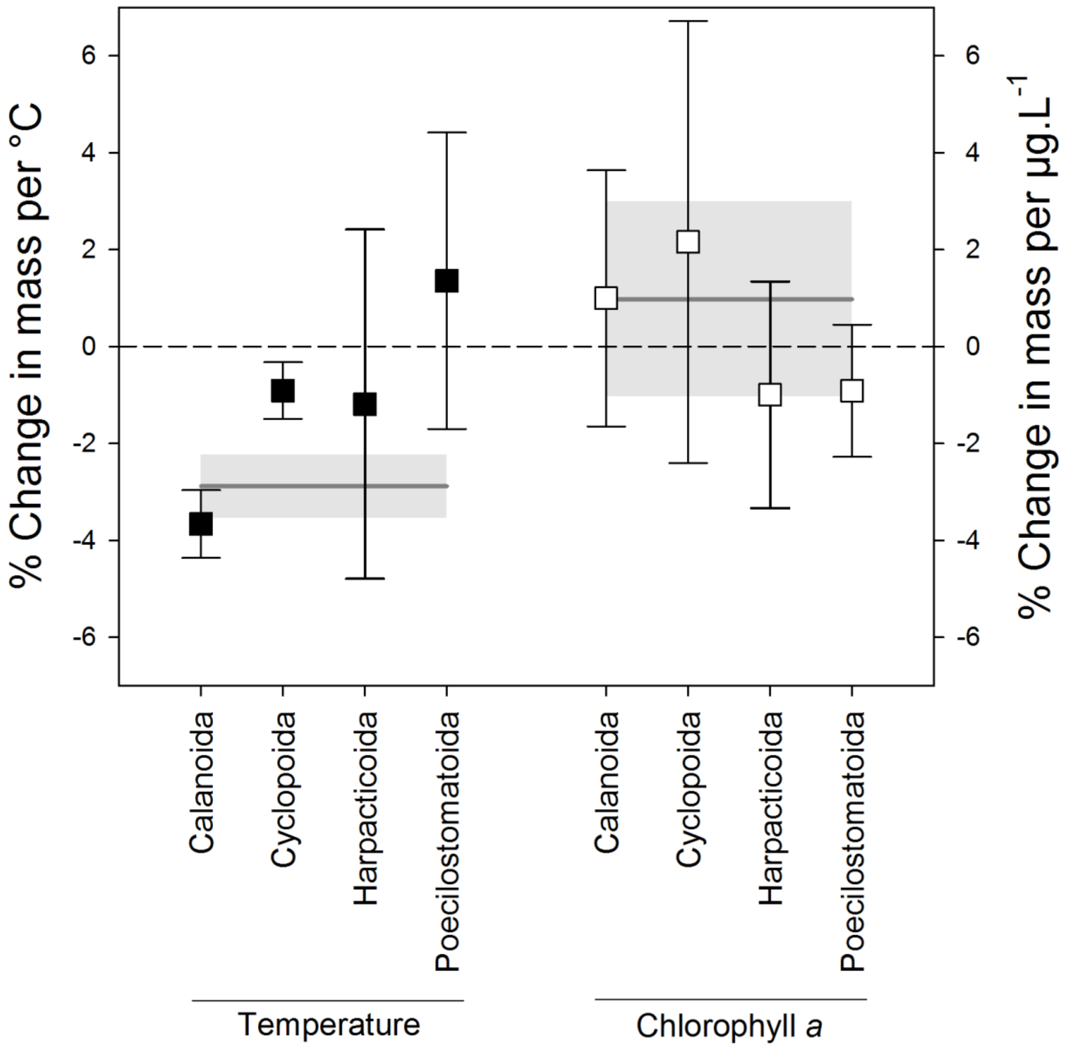


Figure 4.2. Species-specific % change in body mass (±95% CI) for seasonal T-S (per °C) and C-S (per µg.L^{-1}) gradients, averaged by order. Solid grey lines shows the mean seasonal T-S and C-S gradient across all orders with 95%CI indicated by the shaded area.

Table 4.1. AIC output comparing the relative strength of candidate models in explaining variation in seasonal T-S gradients. The best model, shown in bold, is identified as that with the lowest small-samples corrected AIC (AICc). Given that the difference between the lowest AICc and those of the alternative models (i.e. ΔAICc) is >2, we may favour a single best fit model in which taxonomic order has a significant independent effect on the strength of the seasonal T-S gradient. An 'intercept only' model, shown in italics, is included for comparison. Akaike weight (w_i) denotes the probability of a given model being the best fit model in the candidate set. The number of parameters (K) in each model is shown. Mass is the species adult body mass at 15°C.

Model	K	Log-likelihood	AICc	ΔAICc	w_i
Intercept	*5*	*-328.40*	*667.25*	*5.08*	*0.04*
Order	**8**	**-322.53**	**662.16**	**0.00**	**0.52**
Order+Sex	9	-322.51	664.40	2.24	0.17
Log_{10}Mass+Order	9	-322.53	664.44	2.27	0.17
Log_{10}Mass+Order+Sex	10	-322.51	666.72	4.55	0.05
Log_{10}Mass	6	-327.89	668.41	6.25	0.02
Sex	6	-328.38	669.40	7.24	0.01
Log_{10}Mass+Sex	7	-327.77	670.38	8.22	0.01

note specifically the different temperature gradient between the calanoids, which use feeding-currents, and ambush feeding cyclopoid copepods, with a 4-fold difference in the strength of the seasonal T-S gradient observed between these two groups (Figure 4.3). We find no significant change in the strength of the gradient with mean species body mass in either the Calanoida ($F_{1,101}$=0.11, p=0.75) or non-Calanoida orders ($F_{1,35}$=2.75, p=0.11), supporting our prediction that any change in mature body size is independent of mean species body mass in these smaller taxa (Figure 4.4).

Reproductive strategy also varies within and between orders; calanoid species can be either broadcast or sac spawners, but are more commonly the former (n=44 vs. n=22 for broadcast and sac spawners respectively in our dataset), whilst all species in the three remaining orders considered here are sac spawners. Given that taxonomic order and reproductive strategy correlate exactly in 3 of the 4 orders in our dataset, while in calanoids both reproductive strategies occur, we tested for differences in the seasonal T-S gradient between broadcasters and sac spawners exclusively in calanoids, finding no significant effect ($F_{1,64}$=0.71, p=0.40). Equally, we tested for order-level differences in the seasonal T-S gradient exclusively in sac spawners (i.e. by excluding any broadcast spawning calanoid species), and find significant differences in the strength of the seasonal T-S gradient between taxonomic orders, still observing a 4-fold significant difference between calanoids and cyclopoids (*t*-test; *t*=-4.51, df=31, p<0.0001). This leads us to suggest that reproductive strategy is not responsible for driving the observed differences in seasonal T-S gradients between taxonomic orders and, hence, explains why we chose to exclude the latter from our global linear mixed effects model.

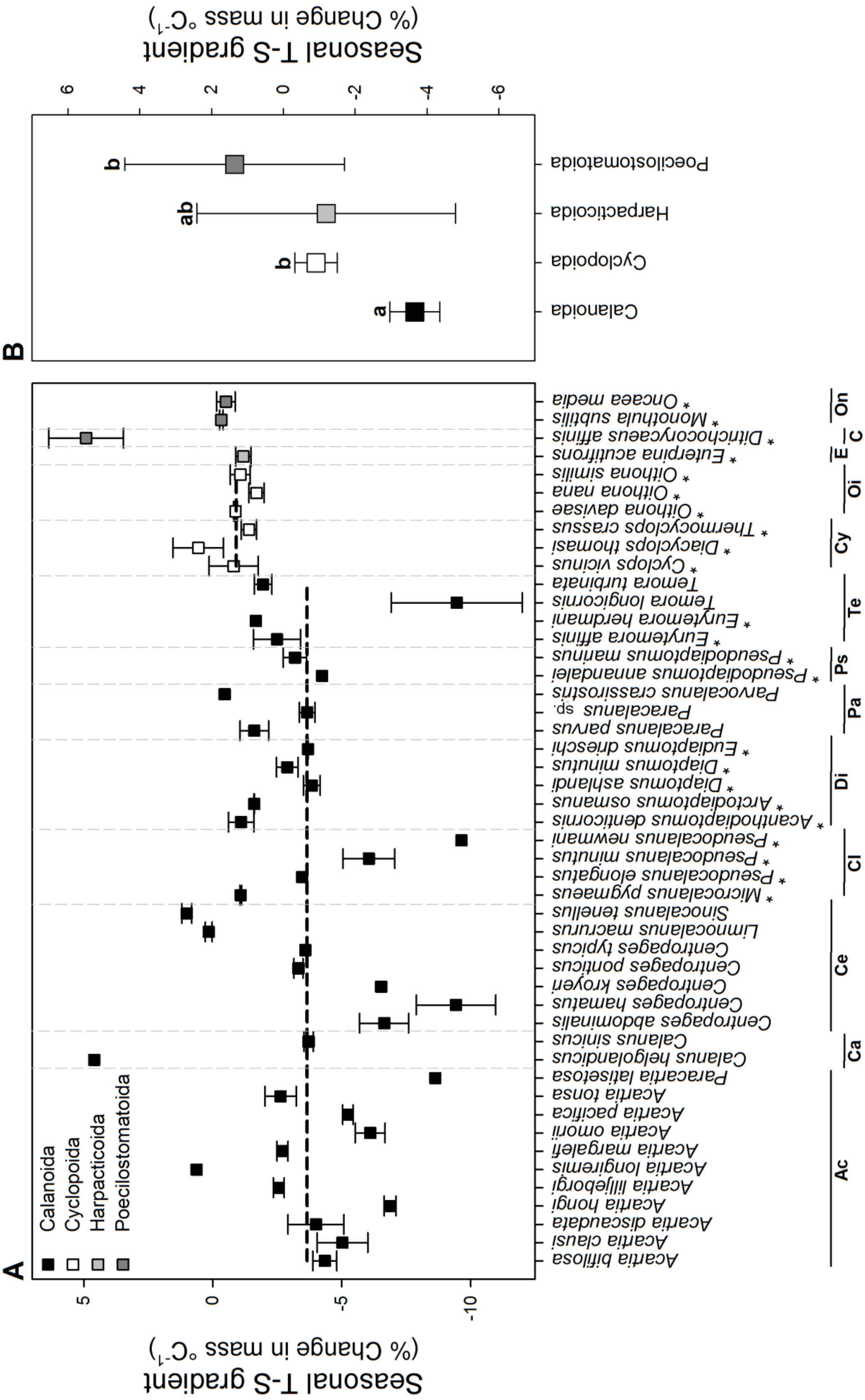

A
B
Seasonal T-S gradient
(% Change in mass °C-1)
Calanoida
Cyclopoida
Harpacticoida
Poecilostomatoida
Acartia bifilosa
Acartia clausi
Acartia discaudata
Acartia hongi
Acartia lilljeborgi
Acartia longiremis
Acartia margalefi
Acartia omorii
Acartia pacifica
Acartia tonsa
Paracartia latisetosa
Calanus helgolandicus
Calanus sinicus
Centropages abdominalis
Centropages hamatus
Centropages kroyeri
Centropages ponticus
Centropages typicus
Limnocalanus macrurus
Sinocalanus tenellus
* Microcalanus pygmaeus
* Pseudocalanus elongatus
* Pseudocalanus minutus
* Pseudocalanus newmani
* Acanthodiaptomus denticornis
* Arctodiaptomus osmanus
* Diaptomus ashlandi
* Diaptomus minutus
* Eudiaptomus driescii
Paracalanus parvus
Paracalanus sp.
Parvocalanus crassirostris
* Pseudodiaptomus annandalei
* Pseudodiaptomus marinus
* Eurytemora affinis
* Eurytemora herdmani
Temora longicornis
Temora turbinata
* Cyclops vicinus
* Diacyclops thomasi
* Thermocyclops crassus
* Oithona davisae
* Oithona nana
* Oithona similis
* Euterpina acutifrons
* Ditrichocorycaeus affinis
* Monothula subtilis
* Oncaea media
Ac
Ca
Ce
Cl
Di
Pa
Ps
Te
Cy
Oi
E
C
On
5
0
-5
-10
Calanoida
Cyclopoida
Harpacticoida
Poecilostomatoida
a
b
ab
b
6
4
2
0
-2
-4
-6

Figure 4.3. (**A**) Seasonal temperature-size gradients of adult copepods for individual species, including both males and females, categorized by order (Calanoida, Cyclopoida, Harpacticoida, Poecilostomatoida) and family (Acartidae (Ac), Calaniidae (Ca), Centropagidae (Ce), Clausocalanidae (Cl), Diaptomidae (Di), Paracalanidae (Pa), Pseudodiaptomidae (Ps), Temoridae (Te), Cyclopidae (Cy), Oithonidae (Oi), Euterpinidae (E), Corycaeidae (C), Oncaeidae (On)). Where more than one study has been undertaken on a species, the mean (and ±SE) are plotted. Dashed horizontal lines indicate the mean seasonal T-S gradient for the Calanoida and Cyclopoida orders. Dashed vertical lines divide taxonomic families. Species names preceded by an asterisk are sac spawners, whilst all other species are broadcast spawners. (**B**) Species-specific seasonal T-S gradients (±95% CI), averaged by order. Different letters above data points indicate significant differences, whilst shared letters indicate no significant difference. Note the significant difference between feeding-current feeding Calanoida and ambush feeding Cyclopoida.

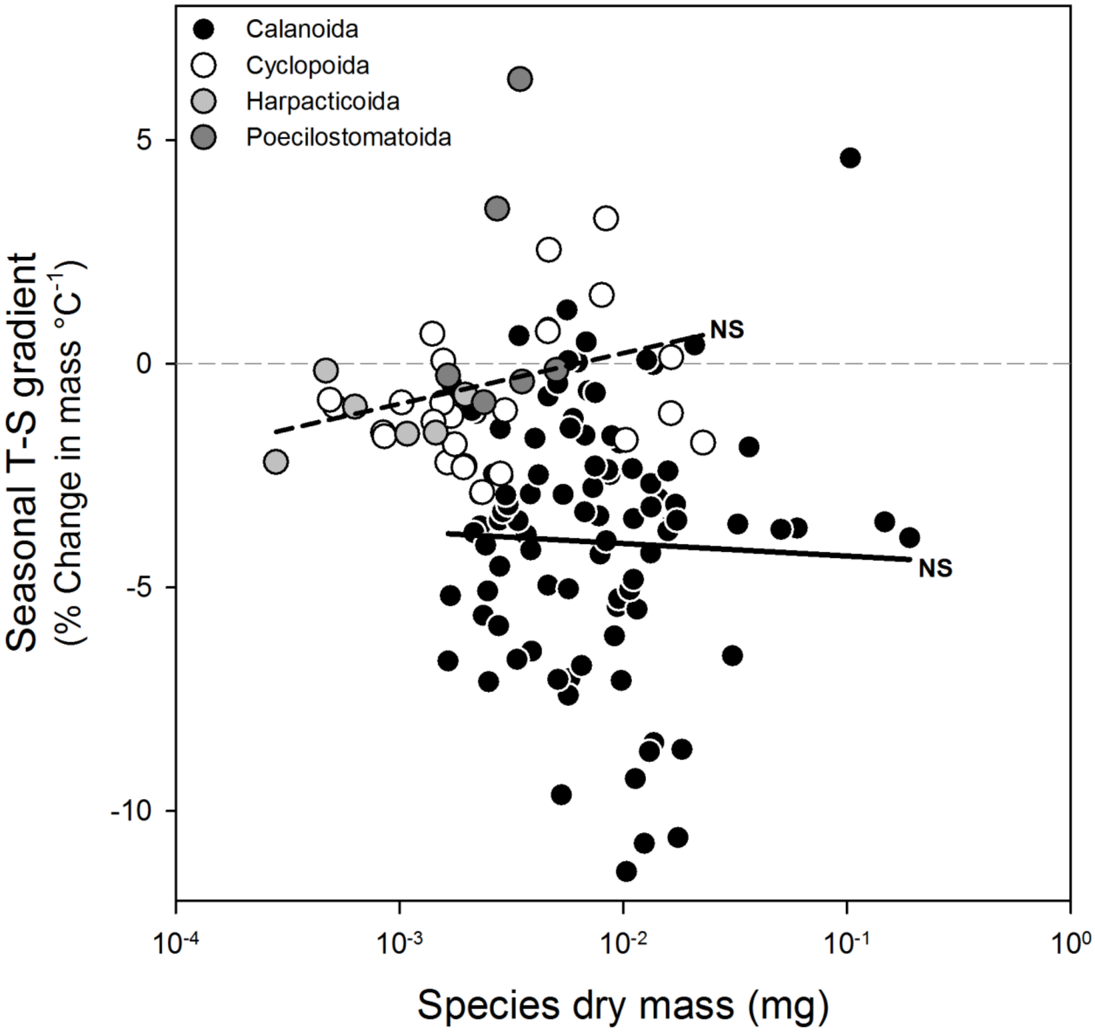


Figure 4.4. Seasonal temperature-size gradients (% change in body mass °C^{-1}) versus species log10 adult dry mass (mg), categorized by taxonomic order. We find no significant relationship between the strength of seasonal T-S gradient and species body mass across either Calanoida ($F_{1,101}$=0.11, p=0.75; solid line) or non-Calanoida species ($F_{1,35}$=2.75, p=0.11; dashed line). Data for both females and males are included where possible. "NS"=not significant.

Despite the numerous other variables that may act to obscure the correlation between body mass and seasonal temperature, we find a strong match between the mean Calanoida seasonal T-S gradient (-3.66±0.70%) and mean T-S response (-3.20±0.49%) measured in the laboratory under conditions of excess food (*t*-test; *t*=-1.09, df=79, p=0.28). However, we note that the two datasets comprise of different species. Indeed, when we regress species-specific seasonal T-S gradients against laboratory T-S responses for the small number of species for which we have both sets of data (n=12), separating responses by sex, we observe much greater variation in seasonal T-S gradients than those measured under controlled laboratory conditions (Figure 4.5). This suggests that other environmental variables are impacting the T-S response in the field. There appears to be no systematic difference in the strength of laboratory and seasonal T-S gradients between the sexes, such that sex has no significant effect on the strength of the seasonal T-S gradient, either across species ($F_{1,84}$=0.03, p=0.86) or intra-specifically (paired *t*-test; *t*=1.35, df=37, p=0.19). Unfortunately we are unable to make further meaningful comparisons between field and laboratory gradients. For example, we could not compare the broad differences between taxonomic orders we observe in the seasonal T-S data with laboratory data, as very few laboratory studies on species other than calanoids have been conducted; our dataset contains male and female laboratory T-S responses for just 2 planktonic cyclopoid species and a single harpacticoid species.

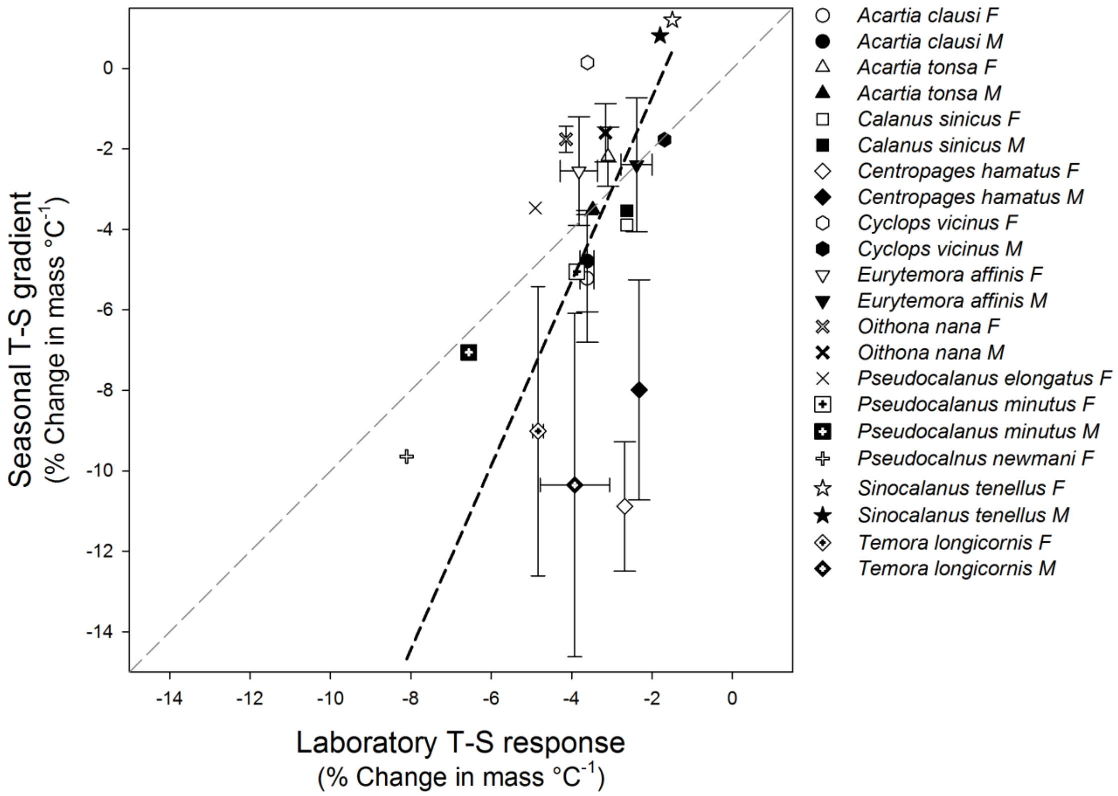


Figure 4.5. Male (M) and female (F) species-specific laboratory temperature-size (T-S) responses versus seasonal T-S gradients in planktonic copepods. Seasonal T-S gradients are much more variable than laboratory T-S responses and there is a significant positive correlation (RMA regression; R^2=0.25; black dashed line) between the two. Dashed grey line indicates y=x for comparison. There appears to be no significant difference in the strength of seasonal and laboratory T-S gradients between the sexes.

Discussion

Our work combines field data from numerous studies worldwide (Figure 4.1), going beyond controlled laboratory-based T-S studies to demonstrate broad patterns in the thermal size responses of marine and freshwater planktonic copepods. Despite numerous other variables that may act to complicate the T-S signal in the field, we show that almost 90% of copepod species in our dataset follow the Temperature-Size Rule (TSR) in seasonal environments, maturing at a smaller adult body mass in warmer conditions. Yet, as we may expect, seasonal T-S gradients appear much more variable compared to those measured under controlled conditions in the laboratory (Figure 4.5), suggesting that other environmental factors, in addition to temperature, may play a role in driving seasonal body size variation in the field. As discussed in Chapter 3, we should also consider that the temperature at which adults are collected is unlikely to correspond exactly to temperatures experienced during ontogeny, and this may be further complicated by the existence of a winter diapause, during which many copepods will cease recruitment over late winter to early spring. Throughout this period their prosome length will change little, and yet temperatures may vary considerably.

Food availability has also been shown to have a direct influence on body size (Berrigan & Charnov, 1994; Diamond & Kingsolver, 2010), though we find that Chl-*a* concentration explains very little of the seasonal variation in body mass, when both modelled independently and after accounting for the effects of temperature. This suggests that temperature is much more significant in driving body size gradients in these natural populations. Higher food quantity typically leads to larger size at maturity in ectotherms, and we observe a positive but non-significant percentage change in adult body mass with increasing Chl-*a* concentration on average (Figure 4.2). Chl-*a* concentration commonly correlates significantly with juvenile growth and adult fecundity rates in many natural populations of planktonic copepods (Hirst & Bunker, 2003; Bunker & Hirst, 2004), and hence is generally considered a reasonable proxy of food availability. However, many copepods have an omnivorous diet that does not exclusively include prey containing this pigment (e.g. including heterotrophic ciliates and flagellates (Calbet & Saiz, 2005)), and the proxy also fails to account for variation in prey quality (Pond *et al.*, 1996), which has been shown to alter the temperature-size response, even reversing its sign at times (Diamond & Kingsolver, 2010). Here we find little evidence for sign reversal when comparing laboratory and field animals. Time lags might also obscure the correlation between Chl-*a* concentration and body size. As food availability commonly varies over a much shorter timescale than generation time, whilst temperature varies over a relatively longer timescale, correlations with the latter are likely to be much more reliable. Although greater chlorophyll concentration is often associated with increased growth (Hirst & Bunker, 2003; Bunker & Hirst, 2004), consumer abundance is also predicted to increase with primary productivity (O'Connor *et al.*, 2009). Our analysis does not account for the abundance of the copepods, and hence we are unable to assess the role of food availability on a *per capita* basis. Assuming metabolic rate has a Q_{10} of 2.5 and scales with body mass$^{0.75}$ (Zuo *et al.*, 2012), a simple calculation suggests that an organism would have to decrease its body mass by approximately 11.5% per °C of warming to offset the increase in metabolic rate associated with this temperature increase. Given that calanoid copepods on average reduce their body mass by only 3.66% $°C^{-1}$, this compensates for approximately a third of the increase in metabolic rate per °C of warming. If resources were limiting and kept constant then population abundance would have to fall substantially with warming to accommodate the extra metabolic demand, even with reduced body size of individuals.

Beyond variation in temperature and food availability, we might expect predation by ectotherms to increase with warming in the field (Kordas *et al.*, 2011). This in turn may lead to increased copepod mortality, selecting for earlier maturation and resulting in a reduced adult body size. Copepods can also detect and perceive chemical signals released by predators, such as fish kairomones, the presence of which has been shown to trigger faster development and earlier maturation at a smaller body size in calanoids (Gutiérrez *et al.*, 2010). Thus, increased predation risk in the warm and associated increases in mortality and the presence of chemical cues may amplify the temperature-size gradient in the field.

The relative strength of the seasonal T-S gradient does not vary significantly between the sexes in this study, evidence of which can also be observed in Figure 4.5. These findings agree with the broader analysis across Arthropoda, for which T-S responses were found to not significantly differ between the sexes (Hirst *et al.*, 2015) (also see Appendix 7.4). Rensch's rule suggests that male body size varies more than female body size, irrespective of which is the larger sex (Rensch, 1960). Applied within species, the rule would predict an increase in sexual size dimorphism (SSD) with increasing body size in species where males are the larger sex, and a decrease in SSD with body size in species where females are larger. Thus males should consistently have the greater size variation, yet we find no evidence to support this pattern at the intra-specific level. Our finding at the intra-specific level here concurs with there being isometry between male and female size seen across copepod species (Hirst & Kiørboe 2014), and suggests that the selection pressures on the seasonal T-S gradient have been equally as strong for both males and females.

Though we have begun to identify broad trends in both the magnitude and direction of the T-S response, for example between terrestrial and aquatic species (Forster *et al.*, 2012; Horne *et al.*, 2015), there remains a large amount of variation in the strength of the response that is yet to be explained. This is especially true for planktonic species which are only a few millimetres in size or less, where oxygen availability in most conditions appears unlikely to be a driver. Indeed, our most compelling finding is the significant difference in the strength of seasonal T-S gradients between species of calanoid and cyclopoid (Figure 4.3), which typically employ different feeding modes. We find that calanoids exhibit much greater size plasticity upon temperature changes than non-calanoids. This is consistent with the hypothesis that feeding mode may influence the T-S response, since all calanoids can produce a feeding current to harvest prey, while none of the other orders do so. The extent to which the T-S response differs between these two feeding modes depends on the differences in both size-scaling and thermal response of feeding in relation to metabolism. Thus, to thoroughly test this hypothesis, one would need estimates of within-species mass scaling and temperature dependence of feeding and metabolism. While some estimates of between-species body mass scaling of respiration and feeding of the two groups exist (e.g., Kiørboe & Hirst 2014), the body mass-dependent changes in vital rates during ontogeny are typically different (Hirst *et al.*, 2014; Glazier *et al.*, 2015), and thus needed for these groups. A further complication arises from the fact that feeding mode may change during ontogeny: while all cyclopoids are ambush feeders throughout their development, many calanoids are ambush feeders during the nauplii stage, and feeding current feeders during the copepodite stages; or they may switch between feeding modes in the copepodite stages (Kiørboe, 2011).

We note the association between taxonomic order and feeding mode in our dataset, and appreciate the potential difficulty in disentangling effects of feeding strategy from other order-specific differences in physiology and behaviour. For example, all cyclopoids in our dataset are sac spawners, whilst calanoid species can be either broadcast or sac spawners, but are more commonly the former. However, we find no substantial effect of reproductive strategy on the sensitivity of mature body mass to temperature. Whilst broadcast and sac spawning planktonic copepods have markedly different rates of adult fecundity (Bunker & Hirst, 2004), egg mass production rates (Hirst & Bunker, 2003) and egg mortality (Hirst & Kiørboe, 2002), they appear to have somewhat similar rates of juvenile growth, development and mortality (Hirst & Kiørboe, 2002; Hirst & Bunker, 2003). The T-S responses of species with determinate growth are largely generated during the juvenile phase of ontogeny (Forster & Hirst, 2012). Similarity of important life history rates during the juvenile phase may therefore explain the lack of difference in the seasonal T-S gradient within the calanoids based upon reproductive strategy. Expanding analyses in future to consider ambush feeding calanoid copepods, such as in the genera *Tortanus* and *Pareuchaeta*, will help to more definitively separate effects of feeding strategy from order-level differences. Unfortunately at present, suitable data are not available on these taxa. We recommend that future experimental studies comparing species-specific size variation in response to temperature, both within and between taxonomic orders, should focus on those taxonomic groups that are currently data deficient.

Given that body size is an important predictor of fitness, and warming is a prominent feature of climate change, there is an urgent need to accurately predict changes in body size with temperature. This is particularly the case in zooplankton which globally represent a primary resource for invertebrates and vertebrates, including fish (Ware & Thomson, 2005). Changes in body size will not only affect individual and population fitness, but may impact feeding rates and alter food web connectivity given the size dependency of trophic processes (Hansen *et al.*, 1994; Rice *et al.*, 2015), as planktonic food webs are especially highly size-structured (Webb, 2012). Measuring and accounting for abundance in the field would also help to define the relationship between food availability *per capita* and adult body size under natural conditions. This may be particularly informative in light of the fact that the temperature-size response in the majority of ectotherms appears to compensate for only a small proportion of the predicted increase in metabolic rate with temperature, whilst metabolic rate in autotrophs (and thus primary productivity) increases substantially less with warming than metabolic rate in heterotrophs (Allen *et al.*, 2005).

CHAPTER 5

Altitudinal body size trends in arthropods are much more variable than laboratory, latitudinal and seasonal temperature-size gradients

Introduction

In the previous chapters we identified close parallels between phenotypically plastic size responses to temperature measured in the laboratory, and intraspecific changes in body size observed in the field, both across latitudes and seasonally over an annual cycle - consistent differences in both the magnitude and direction of body size gradients were found among taxonomic orders, between environments (aquatic vs. terrestrial, suggesting an important role for oxygen availability), and between univoltine and multivoltine terrestrial species (likely an evolutionary adaptation to changing season length) (Horne *et al.*, 2015; Horne *et al.*, 2017). This co-variation suggests that these widespread body size phenomena may be driven by similar selective pressures, and that temperature is an important correlate of size responses in the field, despite a number of confounding factors that can also influence body size (e.g. resource availability, mortality risk and competition) (Chown & Gaston, 2010).

As with increasing latitude, temperature commonly declines with increasing altitude, and therefore we might expect those species that grow to a larger adult size in the cold and with increasing latitude will also exhibit a positive cline in body size with increasing altitude. On average, temperature falls by 5.5 to 6.5°C per 1000m increase in elevation (Anslow & Shawn, 2002), though this, of course, varies considerably based on other climatic and topographic factors (Hodkinson, 2005). In addition to temperature, other important environmental parameters that can vary with altitude include season length, which generally declines at higher altitude and encompasses seasonal variation not just in temperature but also in resource availability (Hodkinson, 2005), and a decline in the partial pressure of atmospheric gases, including oxygen, which decreases near linearly with altitude (Peacock, 1998). These factors have the potential to influence clines in body size and potentially confound the effects of temperature on size at maturity (see Figure 5.1 for an illustration of the predicted effects of these key parameters on body size). Additional environmental parameters also generally increase with altitude, including wind speed, precipitation, and UV radiation (Hodkinson, 2005).

Variation in the magnitude and direction of temperature-size and latitudinal-size gradients in terrestrial arthropods has been attributed partly to differences in voltinism; multivoltine species are predicted to grow to a smaller adult size in the warm, in accordance with the temperature-size rule (Atkinson, 1994), whereas body size in univoltine species is much more dependent on season length (often positively correlated with temperature) and thus time available for growth (Roff, 1980; Kozłowski *et al.*, 2004; Shelomi, 2012). To this end, multivoltine species are predicted to increase their body size with increasing altitude (i.e. with decreasing temperature), whereas univoltine species are predicted to decrease their body size with increasing altitude (i.e. with decreasing season length). Indeed, variation in the prevalence and direction of altitude-size (A-S) clines among species has been attributed to seasonality and differences in reproductive strategy (univoltine vs. multivoltine) (Chown & Klok, 2003; Shelomi, 2012). However, there are few quantitative syntheses of intraspecific A-S clines, with most choosing to focus on whether body size gradients were negative, positive or non-significant (e.g. Shelomi, 2012). Quantifying A-S clines allows us to compare not just the direction of these clines, but also their relative magnitude across species and higher taxonomic groupings. This in turn can help us to more closely relate variation in the magnitude of body size change with particular traits or life history characteristics. Most importantly, quantitative data capturing the magnitude of A-S clines will allow for a more direct comparison with laboratory temperature-size (T-S) responses.

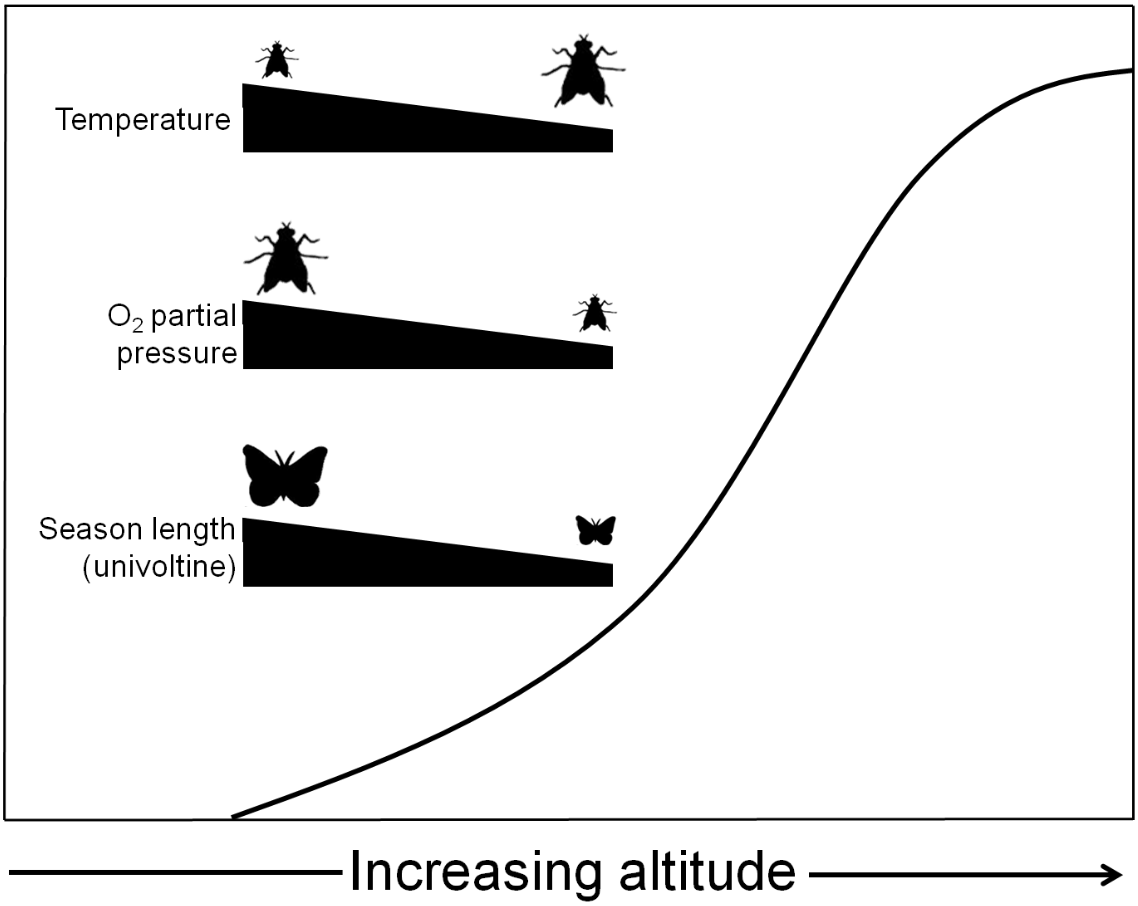


Figure 5.1. The predicted effects of i) decreasing temperature, ii) decreasing oxygen partial pressure and iii) decreasing season length with increasing altitude on size at maturity in arthropods. Body size is predicted to increase with decreasing temperature, following the temperature-size rule, particularly in multivoltine species. However, a decrease in the partial pressure of oxygen at higher altitudes may reduce the available energy for growth, acting in the opposite direction to constrain body size. Similarly, shorter season length is predicted to decrease body size at higher altitude in univoltine species, as resource availability and time available for growth is reduced.

To our knowledge, A-S clines and laboratory T-S responses have not previously been compared quantitatively. Such a comparison provides an opportunity to explore the extent to which changes in other environmental parameters might be confounding the effects of temperature across altitude in the field. This includes the decline in oxygen partial pressure with increasing elevation (Peacock, 1998). Although lower temperatures at higher altitude may favour larger body size at maturity in multivoltine species (i.e. following the temperature-size rule (TSR) (Atkinson, 1994)), a concurrent reduction in oxygen partial pressure (i.e. a reduction in oxygen availability per unit volume of air) could act in the opposite direction, particularly if a species is unable to increase its rate of air intake, thereby reducing available energy for growth (Hodkinson, 2005). Thus, this might act to weaken any potential increase in body size at higher colder altitudes, or even exacerbate body size reduction in those species that already grow to a smaller size in the cold (i.e. many univoltine species). Dispersal or migratory ability may also obscure A-S clines; unlike L-S clines, which are typically measured over much greater distances and can span thousands of kilometres, whole continents and entire species' ranges (e.g. Hassall, 2013), there is more likelihood of interconnectivity and thus movement of individuals between populations along elevation gradients (for example by flight and/or active transport by wind), which are often studied along localized transects typically covering tens of kilometres (e.g. Smith *et al.,* 2000). Consequently, adults collected at one altitude might have developed at another, where environmental conditions were very different and/or more favourable. This might be particularly true for more mobile species with greater dispersal ability, such as winged species capable of flight, which may move more freely between sample sites or, upon reaching maturity, may colonize new areas prior to reproduction (Roff & Fairbairn, 2007). As a result, A-S clines in more mobile species may be obscured or appear weaker. Seasonality, and the extent to which season length decreases with increasing altitude, is also likely to influence the strength of A-S clines, particularly in univoltine species for which time available for growth is an important limiting factor. Consequently, we might expect A-S clines to be stronger in more thermally seasonal environments, such as at higher latitudes, where the decline in thermal season along altitudinal gradients is much more pronounced than in the tropics (Körner, 2000; Chown & Klok, 2003). Of course, voltinism itself can vary with environmental conditions, and any switch in reproductive strategy could also affect the magnitude of any apparent size change (Roff, 1980; Mousseau & Roff, 1989; Zeuss *et al.*, 2016).

In this chapter we quantify and examine variation in intraspecific A-S clines in arthropod species. We compare the magnitude and direction of A-S clines based on taxonomy, voltinism, flight capability and latitude. We then compare these clines to T-S responses measured under controlled laboratory conditions, to determine whether these major size gradients co-vary, and thus to better understand to what extent changes in other environmental parameters might be confounding the effects of temperature on body size across altitude in the field.

Methods

In order to quantitatively describe species-specific altitudinal-size clines, we searched the literature using the Web of Science database (http://apps.webofknowledge.com/) and Google Scholar for published field records of adult body size measured at different altitudes, covering at least 100m in altitudinal range and spanning no more than 5° of latitude (note that 75% of studies spanned 1° of latitude or less). The primary search term combinations used were: (“altitude” OR “elevation”) AND “body size” AND ("insect" OR "[<insert taxonomic order>]"). We also used reference lists from the papers we found to identify additional key literature. Adult size data were collected as lengths, or dry, wet or carbon masses and subsequently standardised to dry mass (mg) using published intra-specific regressions and conversion factors (see Appendix 5.1). In cases where species-specific regressions were unavailable, regressions for related species, or more general inter-specific regressions were used. All altitudinal measurements were standardized to metres above sea level.

We derived species-specific slopes of ordinary least-squares (OLS) regressions between ln-transformed dry mass (mg) and altitude (m). Slopes were calculated for each sex separately where these data were available. We used this exponential function of body mass having consistently found this to be the best for modelling body size gradients with both temperature (in the laboratory and seasonally) and across latitude (Forster *et al.*, 2012; Horne *et al.*, 2015; Horne *et al.*, 2017). Further, this allowed us to easily compare these different size gradients with one another. Altitudinal-size clines were then transformed into percentage change in dry mass per meter, using the formula $(\exp^{(slope)} -1)*100$ = % change in mass per m (Forster *et al.*, 2012). Thus, a positive cline denotes an increase in body size with increasing altitude, whilst a negative cline denotes a decrease in body size with increasing altitude. In general, temperature declines with increasing altitude at a rate of between 5.5°C and 6.5°C per 1000m (Anslow & Shawn, 2002). Thus, we also calculated the percentage change in dry mass per 150m, using the formula $(\exp^{(slope\ x\ 150)} -1)*100$ = % change in mass per 150m. This estimate of proportional body size change across altitude corresponded to a size change over an approximate 1°C decrease in temperature, and allowed us to more appropriately compare the strength of A-S clines measured in the field with T-S responses measured in the laboratory.

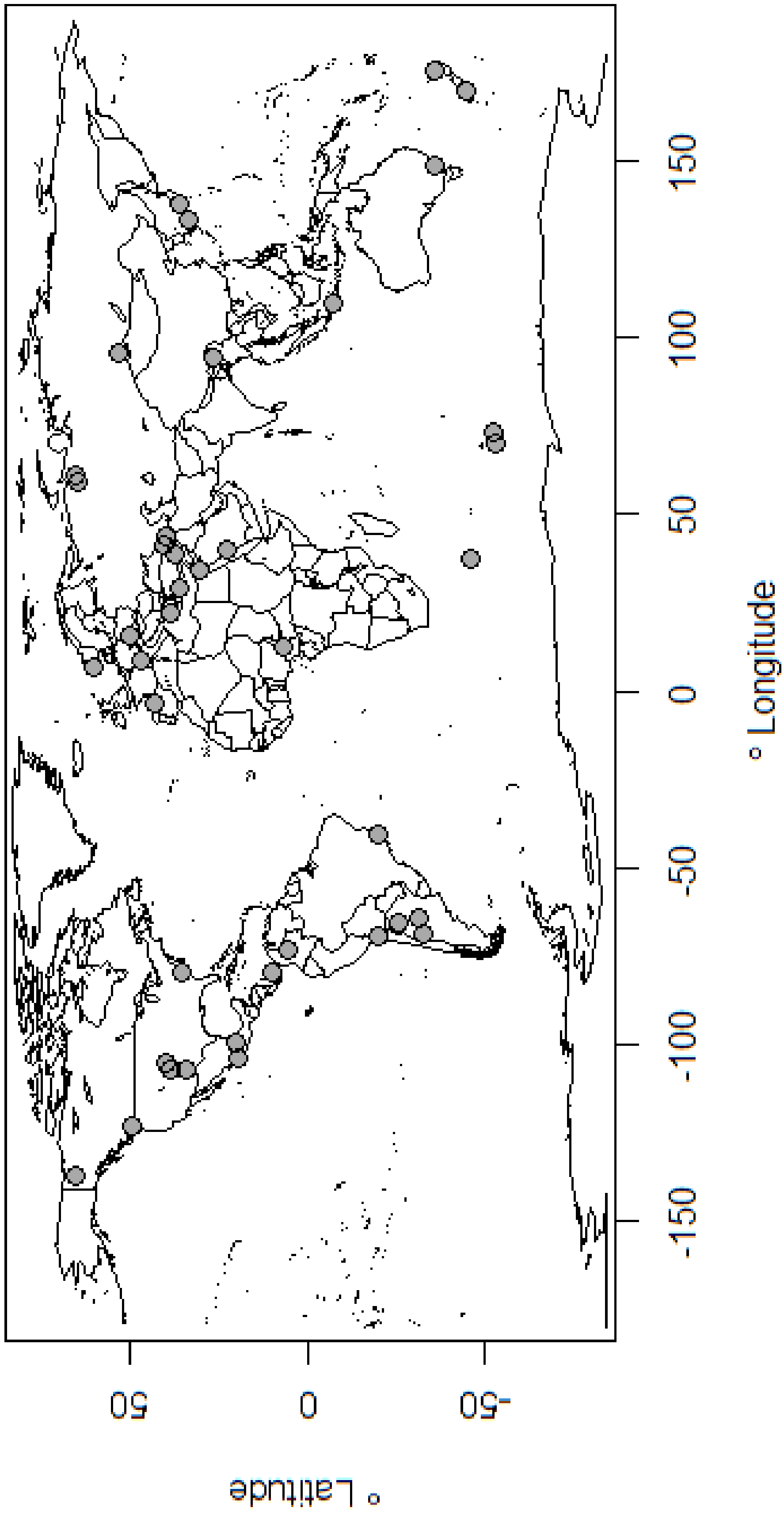
All statistical analyses were conducted in R (R Core Team, 2014). Using A-S clines as the dependent variable, we compared several candidate models to best predict variation in the magnitude and direction of A-S clines based on the Akaike's information criterion (AIC). Voltinism, categorised here as species that are generally regarded as having ≤1 generation per year or >1 generation per year (multivoltine), flight capability (flying vs. flightless), $\log_{10}$-transformed species body mass (at 1500m calculated using species-specific A-S clines), and mean latitude of the study location (degrees from the equator) were incorporated as fixed variables in a global linear mixed effects model (using package lme4). Log_{10}-transformed species body mass was included to determine if A-S clines were mass dependent. A-S clines from multiple studies of the same species were included in our analyses. We included levels of taxonomic classification (order, family, and species) as nested (hierarchical) random effects on the intercept in all models to help control for phylogeny, given that species have shared evolutionary histories, and so are not completely independent. Given that A-S clines did not differ significantly between males and females, and that sex was not reported in all studies, we chose to exclude it as a random effect in the models. We accounted for variation in information quality by weighting each A-S cline by the inverse of the variance of its slope estimate (using the 'weights' function in R) (Koricheva *et al.*, 2013). This helped to account for the fact that A-S clines were derived from data that varied in their goodness of fit between studies and species. We compared all possible combinations of the global model terms using the dredge function in the MuMIn package. The best model was identified as that with the lowest small-samples corrected AIC (AICc).

Where the difference between a model's AICc and the lowest AICc (i.e. ΔAICc) was <2, a set of best fit models, rather than a single best model, was assumed. Model averaging was then used to identify the best predictor variables across the top candidate models, and determine their relative importance (computed for each variable as the sum of the Akaike weights from all models in which they appear). In addition to AIC, a series of F tests were used to verify the significance ($p<0.05$) of each parameter's effect on the strength of the seasonal T-S gradient.

To compare A-S clines with laboratory controlled T-S responses, we used the data compilation from Chapter 2. For each data set, we first generated single species-specific body size gradients by combining size gradients from multiple studies of the same species into a simple mean. We then averaged these species-specific gradients for each taxonomic order, separating species by voltinism, and plotted the resulting order-specific A-S clines against order-specific laboratory T-S responses. We then assessed the extent to which both of these body size gradients co-varied (i.e. whether a 1% increase in body size per 150m altitude = 1% decrease in body size per °C).

Results

We derived a total of 135 altitude-size clines representing 72 arthropod species from 10 taxonomic orders. These clines were recorded at 40 different global locations ranging from 53° South to 66° North, with the majority of locations falling around mid-latitude regions (Figure 5.2). The data set contained a near even distribution of negative and positive A-S clines, with 51% of clines showing a decrease in adult body size with increasing altitude. The magnitude of A-S clines varied considerably between species,



° Latitude
50
0
-50
° Longitude
-150
-100
-50
0
50
100
150

Figure 5.2. World map indicating the location of studies (n=40) from which altitudinal-size clines were recorded.

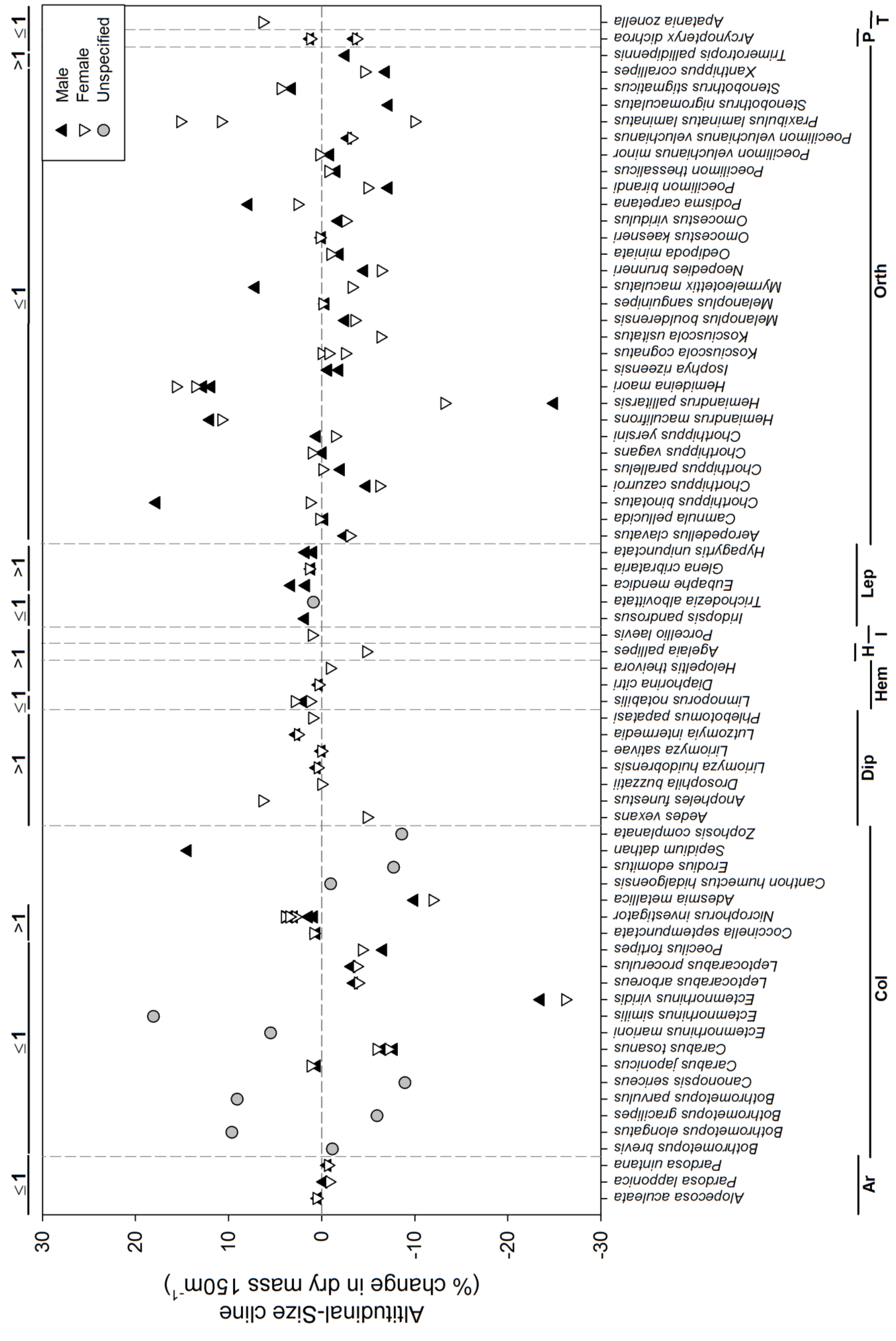

Altitudinal-Size cline
(% change in dry mass 150m-1)
30
20
10
0
-10
-20
-30
Male
Female
Unspecified
≤1
≤1
>1
>1
≤1
>1
≤1
>1
≤1
≤1
>1
Alopecosa aculeata
Pardosa lapponica
Pardosa uintana
Bothrometopus brevis
Bothrometopus elongatus
Bothrometopus gracilipes
Bothrometopus parvulus
Canonopsis sericeus
Carabus japonicus
Carabus tosanus
Ectemnorhinus marioni
Ectemnorhinus similis
Ectemnorhinus viridis
Leptocarabus arboreus
Leptocarabus procerulus
Poecilus fortipes
Coccinella septempunctata
Nicrophorus investigator
Adesmia metallica
Canthon humectus hidalgoensis
Erodius edomitus
Sepidium dathan
Zophosis complanata
Aedes vexans
Anopheles funestus
Drosophila buzzatii
Liriomyza huidobrensis
Liriomyza sativae
Lutzomyia intermedia
Phlebotomus papatasi
Limnoporus notabilis
Diaphorina citri
Helopeltis theivora
Agelaia pallipes
Porcellio laevis
Iridopsis pandrosus
Trichodezia albovittata
Eubaphe mendica
Glena cribrataria
Hypagyrtis unipunctata
Aeropedellus clavatus
Camnula pellucida
Chorthippus binotatus
Chorthippus cazurroi
Chorthippus parallelus
Chorthippus vagans
Chorthippus yersini
Hemiandrus maculifrons
Hemiandrus pallitarsis
Hemideina maori
Isophya rizeensis
Kosciuscola cognatus
Kosciuscola usitatus
Melanoplus bouldrensis
Melanoplus sanguinipes
Myrmeleotettix maculatus
Neopedies brunneri
Oedipoda miniata
Omocestus kaesneri
Omocestus viridulus
Podisma carpetana
Poecilimon birandi
Poecilimon thessalicus
Poecilimon veluchianus minor
Poecilimon veluchianus veluchianus
Praxibulus laminatus laminatus
Stenobothrus nigromaculatus
Stenobothrus stigmaticus
Xanthippus corallipes
Trimerotropis pallidipennis
Arcynopteryx dichroa
Apatania zonella
Ar
Col
Dip
Hem
H
I
Lep
Orth
P
T

Figure 5.3. Altitudinal-size clines (% change in body mass per 150m) of individual arthropod species, including both males and females, categorized by taxonomic order (Araneae (Ar), Coleoptera (Col), Diptera (Dip), Hemiptera (Hem), Hymenoptera (H), Isopoda (I), Lepidoptera (Lep), Orthoptera (Orth), Plecoptera (P) and Trichoptera (T)). Species within each order are also categorized by voltinism (one generation or less per year (≤1), multiple generations per year (>1)). Dashed horizontal line indicates no change in body size with altitude. Dashed vertical lines divide taxonomic orders.

with some of the strongest clines observed in the Coleoptera and Orthoptera, both of which also had the largest number of species in our data set (see Figure 5.3). After controlling for species as a random effect on the intercept, we found that neither of the sexes within species showed a consistently stronger cline in body size with altitude than the other ($F_{1,62}$=0.06, p=0.81; also see Figure 5.3).

The best supported model for explaining variation in A-S clines was an intercept-only model, suggesting that none of the fixed variables included in our global model could significantly explain variation in A-S clines (see Appendix 5.2). Coleoptera (-1.4% body mass $150m^{-1}$ ±4.6 95% CI) and Orthoptera (-0.8% body mass $150m^{-1}$ ±2.3 95% CI) were among those taxonomic orders to show on average a decrease in body size with increasing altitude, whilst orders such as Diptera (0.8% body mass $150m^{-1}$ ±3.1 95% CI), Hemiptera (0.5% body mass $150m^{-1}$ ±3.6 95% CI) and Lepidoptera (1.5% body mass $150m^{-1}$ ±0.8 95% CI) tended to show the opposite pattern (Figure 5.4). Yet, these patterns were not strong enough to detect a significant effect of taxonomic order on the magnitude of the A-S cline ($F_{9,62}$=0.29, p=0.97). Both Coleoptera and Orthoptera in our data set consisted mainly of species with one or fewer generations per year, whilst Diptera, Hemiptera and Lepidoptera were predominantly composed of multivoltine species. Indeed, when we categorized species into those which commonly have one generation or fewer per year, or species that have multiple generations per year, the former reduced their body size with increasing altitude on average (-0.7% body mass $150m^{-1}$ ±1.5 95% CI), whilst the latter showed a mean increase in body size at higher altitudes (1.2% body mass $150m^{-1}$ ±0.8 95% CI), following predictions based on size vs. season length trade-offs (Figure 5.5a). However, these patterns were not strong enough to detect a significant effect of voltinism on the magnitude of the A-S

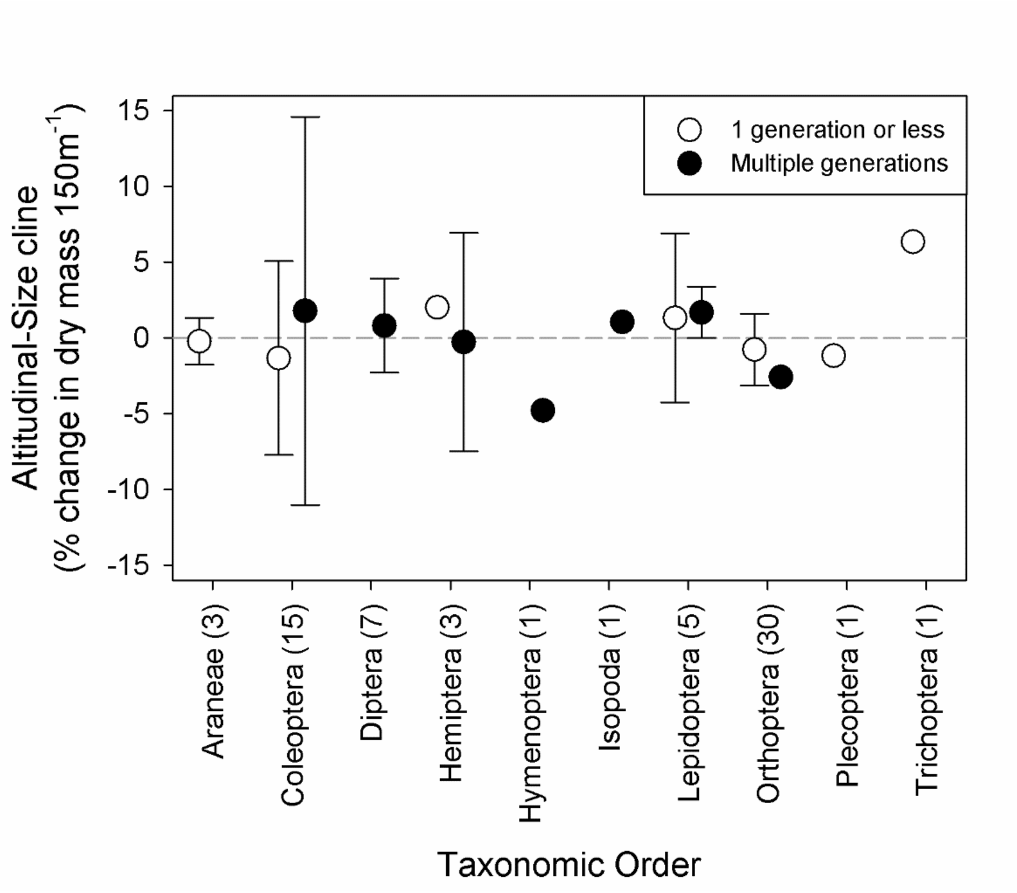


Figure 5.4. A-S clines (±95% CI), averaged by taxonomic order and by voltinism (one generation or less per year (≤1), multiple generations per year (>1)). Number of species is given in brackets after each order. Dashed horizontal line indicates no change in body size with altitude There is no significant effect of taxonomic order on the strength of the A-S cline.

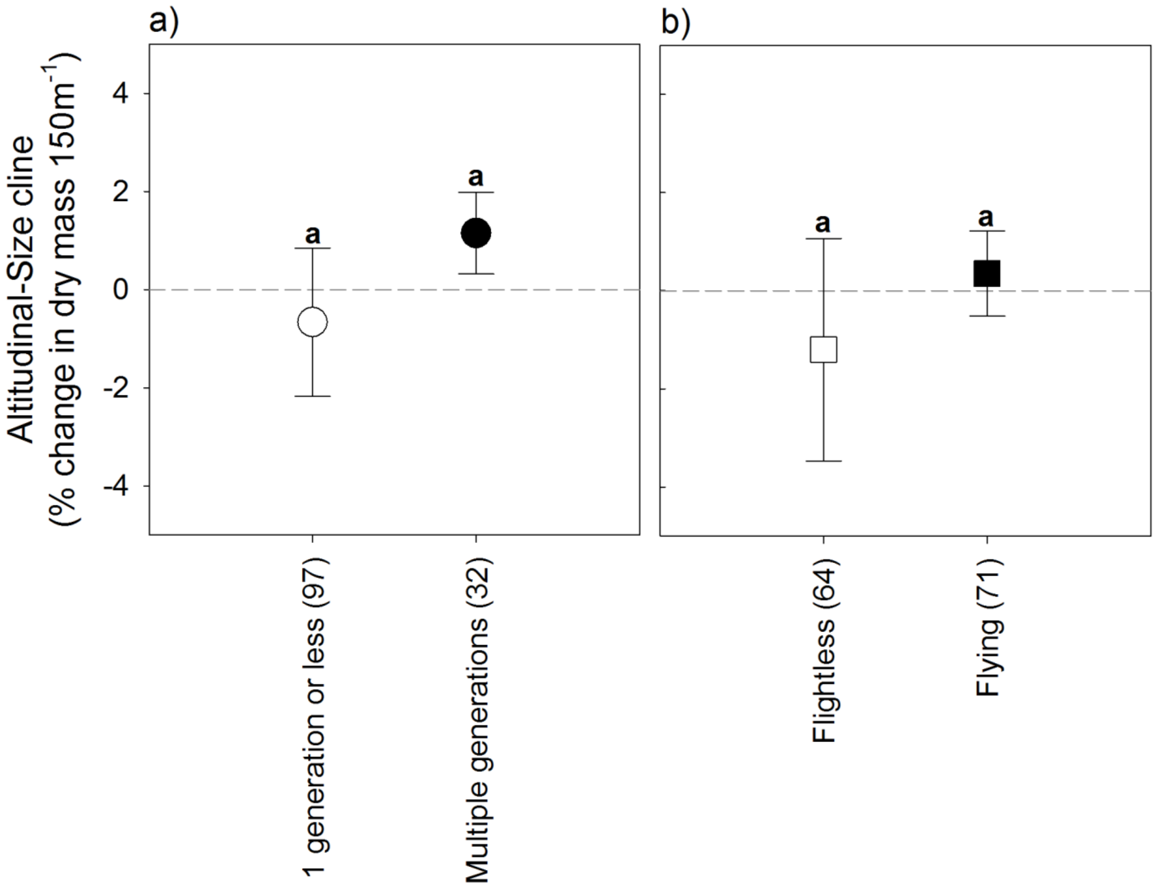


Figure 5.5. Mean A-S clines (±95% CI) in (**A**) arthropods with one generation or less per year vs. those with multiple generations per year, and (**B**) flightless vs. flying species. Sample sizes are given in brackets. Dashed horizontal line indicates no change in body size with altitude. Shared letters above data points indicate no significant difference.

cline ($F_{1,46}$=0.44, p=0.51). Similarly, we found no significant effect of flight capability on A-S clines ($F_{1,62}$=0.02, p=0.90), though on average species that could fly (0.3% body mass $150m^{-1}$ ±0.9 95% CI) showed a weaker A-S cline than flightless species (-1.2% body mass $150m^{-1}$ ±2.3 95% CI), consistent with our predictions (Figure 5.5b). Mean latitude of the study location had no significant effect on the A-S cline ($F_{1,62}$=0.002, p=0.97). We generally observed greater variability in the magnitude of A-S clines measured over relatively small altitudinal ranges. Consequently, we also re-ran our analyses adopting stricter screening criteria, only including A-S clines measured over altitudinal ranges ≥800m (determined by the point at which variation in the magnitude of A-S clines appeared to stabilize). However, the best supported model for explaining variation in A-S clines was still an intercept-only model.

We next examined whether A-S clines were similar in direction and magnitude to temperature-size responses measured under controlled laboratory conditions. The plot of order-specific A-S clines (% change in dry mass per 150m) against laboratory T-S responses (% change in dry mass per °C), taken from the comprehensive data set of Horne *et al.* (2015) , is shown in Figure 5.6. Unlike observations between laboratory T-S responses and latitudinal-size clines when species-specific values have been averaged by taxonomic order (Horne *et al.*, 2015), and also between seasonal T-S gradients and laboratory T-S responses (Horne *et al.*, 2017), the correlation coefficient (r=-0.12) between A-S clines measured in the field and T-S responses measured in the laboratory did not differ significantly from zero (t_8=-0.35, p=0.74), suggesting no linear association between these gradients. Thus, we chose not to fit an RMA regression through the data (Smith, 2009). In most cases however, those orders that exhibited an increase in size with decreasing temperature in the laboratory also showed an increase

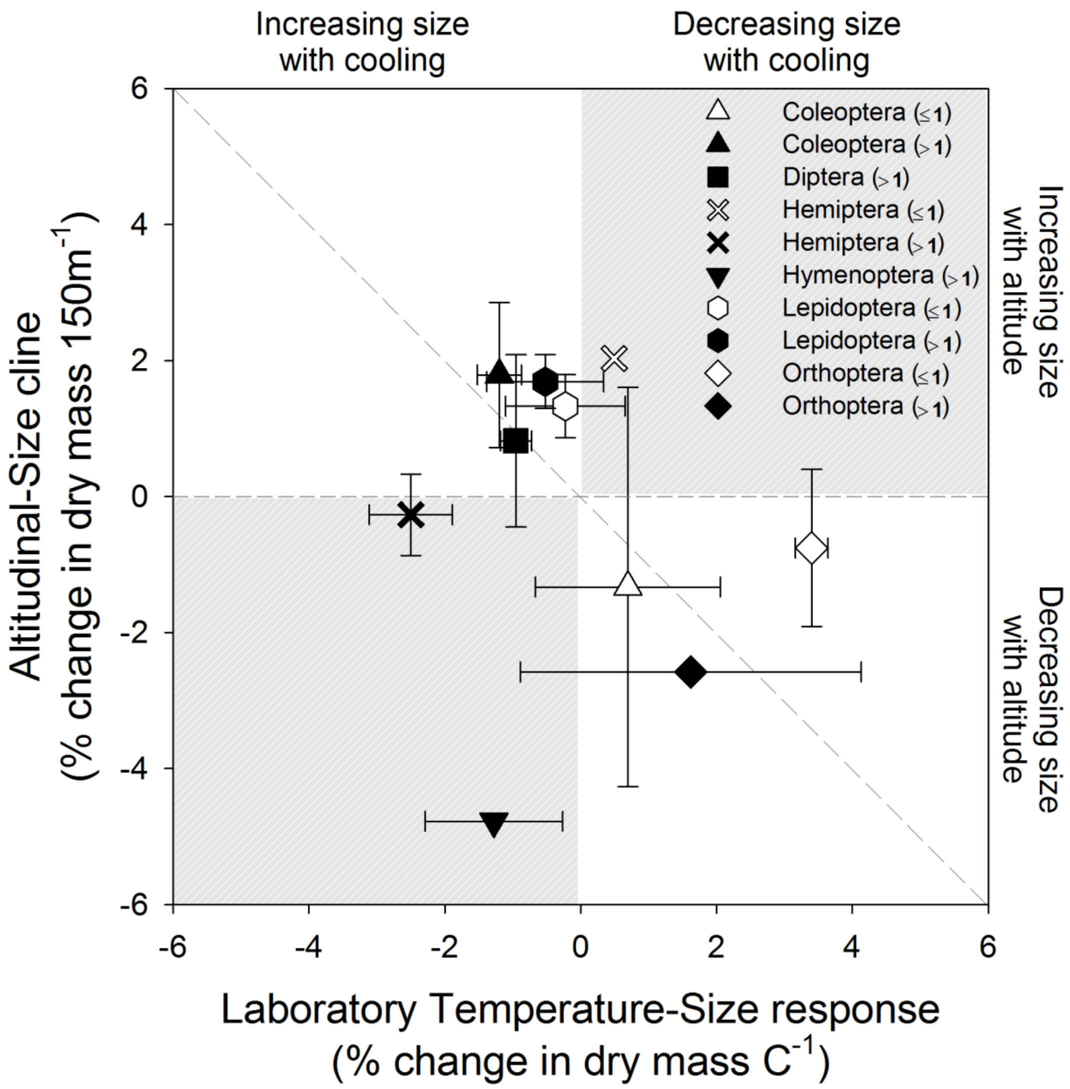


Figure 5.6. A comparison of order-specific altitudinal-size clines (% change in body mass per 150m ±SE) with laboratory temperature-size responses (% change in body mass per °C ±SE) for arthropod species with one generation or less per year (≤1; open symbols) and multiple generations per year (>1; black symbols). Dashed line indicates a 1:1 relationship. Taxonomic orders which fall within the shaded quadrants exhibit a mismatch in their mean A-S cline and T-S gradient.

in size with increasing altitude, suggesting co-variation in the direction of these gradients. We might ascertain from Figure 5.6 that temperature is a more important driver of body size change in some taxa compared to others; in particular, there is a very close match in the strength and direction of A-S clines and T-S responses in the multivoltine Diptera, suggesting altitudinal body size patterns in these species may be more strongly correlated with changes in temperature (or some other parameter that also correlates with temperature), as opposed to other environmental variables, such as season length or resource availability. Similarly, there is a reasonably close match in the magnitude of these gradients in the Coleoptera and Lepidoptera, whereas these gradients appear to deviate more strongly from a 1:1 relationship in the Orthoptera and Hemiptera (Figure 5.6).

Discussion

Our synthesis of A-S clines in arthropods reveals widespread variation not just in the direction of these body size gradients, but also in their magnitude. We predicted that species with one or fewer generations per year would exhibit a negative A-S cline, whereas multivoltine species would exhibit a positive A-S cline. We also hypothesised that species capable of flight would exhibit a weaker cline in body size due to their greater dispersal ability. Although on average the patterns we observe follow these predictions, they lack statistical significance and there is a lot of unexplained variation in the magnitude of A-S clines. This suggests that other environmental factors or life history traits not captured in our models are influencing altitudinal clines in body size.

Although not significant, in concordance with previous studies that identified differences in the direction of A-S clines based on voltinism (Chown & Klok, 2003; Shelomi, 2012), in our quantitative synthesis we also find that on average species with one or fewer generations per year show a decrease in body size with increasing altitude (Figure 5.5a). Season length generally declines at higher elevations, and unlike species with multiple generations a year, these species are much more perceptive of changes in seasonality - as season length decreases, so does time available for growth and reproduction, which imposes limits on size at maturity; thus, the maximisation of fitness may come from utilising as much of the amenable season length as possible. In multivoltine species, however, which reproduce multiple times per year, generation time is relatively short and consequently each generation is less perceptive of changes in season length (Kozłowski *et al.*, 2004). Instead, size at maturity in multivoltine species is predicted to be more dependent on changes in temperature. Development rate is generally considered to have a stronger temperature dependence than growth rate in many species, especially multivoltine species (van der Have & de Jong, 1996; Forster & Hirst, 2012), resulting in larger size at maturity in the cold. Indeed, in the multivoltine species considered here, we observe a mean increase in body size with altitude, corresponding with a decrease in temperature.

In species with one or fewer generations per year, we also expected A-S clines to be greater in magnitude in habitats with stronger altitudinal gradients in season length, such as at higher latitudes, where decreasing seasonality with increasing elevation is often more pronounced (Körner, 2000). Indeed, a study by Chown and Klok (2003) provides support for this hypothesis, in which the authors observed opposing A-S clines in weevil species sampled from two regions that differed in their seasonality. Yet here we find no effect of latitude on the strength of the A-S cline. Of course, latitude is only a proxy for seasonality, corresponding with relatively broad changes in environmental conditions. High resolution climate data, including changes in season length and resource availability with altitude, would provide a much more robust test of this hypothesis. This is particularly true for mountainous regions, as fine-scale variation in environmental conditions due to local topography can result in microclimates that differ from surrounding regions (Suggitt *et al.*, 2011).

Differences in the direction of A-S clines observed between taxa with different reproductive strategies corresponds with intraspecific body size patterns observed across latitudes, and also with T-S responses measured in the laboratory (Horne *et al.*, 2015). This reiterates the importance of voltinism in dictating body-size gradients in the field. A-S clines observed at the level of taxonomic order were generally similar in direction to temperature-body size responses measured under controlled laboratory conditions; those orders that commonly grow to a larger adult size when reared at colder temperatures in the laboratory also show an increase in size at higher colder altitudes. However, these size gradients are not significantly correlated in magnitude (Figure 5.6). This is in contrast to the strong correlations observed between L-S clines and T-S responses in Chapter 2, and also between laboratory and seasonal T-S responses in Chapter 3, which did not differ significantly from a 1:1 relationship on average. Unlike these other comparisons, the correlation coefficient was extremely low and did not differ significantly from zero, which would suggest that other environmental parameters are confounding the effects of temperature on the strength of the A-S cline. We note that the A-S and T-S data sets largely contain different species that will vary in their life history. We also re-emphasize that the extent to which these two body size gradients co-vary is based upon the assumption that temperature declines by 1°C per 150m increase in altitude. This is a general estimation, and the extent to which temperature varies with elevation will differ between study locations depending on local environmental conditions and topography, including aspect, wind speed and cloud cover amongst others (Hodkinson, 2005). Nevertheless, given this assumption, what confounding factors might be causing deviations in the magnitude of A-S clines away from those predicted by controlled laboratory T-S responses?

Here we assume that temperature is a more important driver of A-S clines in those orders that exhibit close to a 1:1 relationship with laboratory T-S responses (dashed line in Figure 5.6), whereas other confounding factors are acting more strongly in those orders that deviate from a 1:1 relationship. We find that A-S clines and T-S responses are more closely matched in some taxonomic orders more than others (Figure 5.6). For example, we observe a very close match between A-S clines and T-S responses in the Diptera; these consist largely of multivoltine species with short generation times, and we would predict that these species are likely to tune their body size more to temperature than to other variables. There is also a relatively close match both in multivoltine Coleoptera species and those with one or fewer generations per year. The Orthoptera in our data set consist predominantly of species that have one generation or less per year, and thus these species should be particularly responsive to declining season length at higher altitudes. However, we observe a relatively weak A-S cline in this order compared with their T-S response. Voltinism itself can vary with environmental conditions (Zeuss *et al.*, 2016), and this can obscure body size gradients in the field. It is plausible that some of the species included in our data set could extend their life cycle beyond a year at higher altitudes, allowing more time for growth despite a decrease in season length, thus weakening the negative cline in body size. Such a switch in voltinism can lead to a ‘saw-tooth’ body size cline (Roff, 1980), and consequently the slope of size change across the entire altitudinal gradient would appear shallower. Many Orthoptera are also highly mobile and strong fliers (Daly *et al.*, 1978), increasing the likelihood of dispersal and/or migration between sampling locations, and this could potentially obscure their A-S cline. Indeed, Alexander (1964) reported collecting many species of montane grasshopper as much as several thousand feet above their normal breeding range. We did observe a weaker mean A-S response in flying vs. flightless species, though again this was not significant (Figure 5b). We also note that, unlike many of the other taxonomic orders in our data set, which have a relatively sedentary larval stage and undergo metamorphosis (e.g. Diptera), Orthoptera are hemimetabolous, reproducing as nymphs that resemble the adult phase (Daly *et al.*, 1978). The latter may show greater mobility during juvenile growth and development; for example, a study of the movement and dispersal patterns in the bush cricket *Pholidoptera griseoaptera*, found that juveniles and imagos exhibited equally good dispersal ability (Diekötter *et al.*, 2005). Consequently, altitude at time of collection may not necessarily resemble altitude during ontogeny in these more mobile species.

A reduction in oxygen partial pressure with altitude might also limit energy available for growth if species cannot increase their air intake, confounding the effects of temperature on body size (Peacock, 1998). Indeed, lower proportional oxygen concentrations have been shown to reduce body size experimentally (Frazier *et al.*, 2001; Peck & Maddrell, 2005; Atkinson *et al.*, 2006; Walczyńska *et al.*, 2015). Of those taxonomic orders that showed an increase in size with altitude and decreasing temperature, only multivoltine Hemiptera appeared to exhibit a much weaker A-S cline relative to their T-S response, but we as yet have no clear explanation as to why this order would be more sensitive to changes in oxygen partial pressure than any other. We also acknowledge that the Hemiptera A-S clines in our data set represent very few species (n=3) (Figure 5.3), and so these patterns should be viewed with caution. Like Orthoptera, species in the order Hemiptera are also hemimetabolous (Daly *et al.*, 1978). In an assessment of flight ability of the Asiatic citrus psyllid (*Diaphorina citri*), a species included in our data set, Arakawa & Miyamoto (2007) found that flight duration in this species was similar at all ages. Thus, the increased mobility of nymphs during ontogeny might similarly obscure A-S clines observed in this taxon.

A reduction in the partial pressure of respiratory gases with altitude not only reduces oxygen availability, but also serves to reduce overall air density (Hodkinson, 2005). This can be particularly problematic for active fliers, such as Lepidoptera, and selection in these species may favour larger wings with greater surface area to maintain flight performance at higher altitudes (Hodkinson, 2005). Lepidoptera do show a stronger A-S cline relative to their T-S response, and body size measurements in these species were in fact derived from forewing length. Not only does this provide a potential adaptive explanation for the stronger A-S clines seen in Lepidoptera, it also highlights the importance of which body part or organ is measured when assessing size clines. Approximately 58% of the A-S clines in our data set are based on measurements of body length, width or direct measurements of mass, whilst the remaining clines were derived from limb measurements, including femur length and wing length, as well as head width. Variation in the size of one body part may not always be proportional to a change in another, and this can potentially obscure the magnitude and direction of altitudinal size clines. To this end, Shelomi (2012) in his assessment of A-S clines reported a higher prevalence of non-significant clines among body-part studies relative to whole-body studies. Yet, whether A-S clines were derived from whole- or part-body measurements had no significant effect on the magnitude of A-S clines in our data set ($F_{1,49}$=0.40, p=0.53).

Here we quantify variation in the magnitude and direction of A-S clines in terrestrial arthropods. By comparing A-S clines with laboratory T-S responses, in which many confounding variables are controlled, we begin to postulate which environmental factors and/or life history traits are important in driving variation in A-S clines between different taxonomic groups. Unlike latitudinal-size clines and seasonal T-S gradients, for which systematic trends in the magnitude of body size gradients have been identified, and both of which correlate body size with relatively broad and predictable changes in climate, patterns in A-S clines are much more obscure. Given that the environmental changes associated with variation in altitude can differ substantially between study locations, as yet we can make only broad predictions about the direction of A-S clines. For more accurate predictions on the magnitude of intraspecific clines, a detailed understanding of the life history of the species in question, in particular reproductive strategy and behaviour, coupled with fine scale information on local environmental conditions and topography, should be assessed on a case by case basis.

CHAPTER 6

Temperature-size responses signal rapid developmental shifts in the thermal-dependence of life history rates

Introduction

In the previous chapters we identified systematic variation in the magnitude and direction of adult temperature-size (T-S) responses between taxa and environments, suggesting that T-S responses are adaptive, and also that the selective pressures driving body size change with temperature differ between groups with different life histories (Forster *et al.*, 2012; Horne *et al.*, 2015; Horne *et al.*, 2016). This is further supported by the fact that the actual mechanisms by which size changes are achieved differ fundamentally between single celled organisms and metazoans (Forster *et al.*, 2011b; Forster *et al.*, 2011a; Forster *et al.*, 2013). However, few studies have examined T-S responses over ontogeny at high temporal resolution (e.g. Gulbrandsen & Johnsen, 1990; Leandro *et al.*, 2006; Forster *et al.*, 2011b), and most report non-linear or discontinuous patterns in the progression of the T-S response over the course of development. Such studies are important because the T-S response provides an important insight into the processes underlying ontogenetic growth and development; size-at-stage is ultimately dependent upon both of these rates, as well as the size of progeny. Indeed, the proximate mechanism underlying the T-S response can be attributed to differences in the temperature dependence of growth and development rate (van der Have & de Jong, 1996; Forster *et al.*, 2011a). For metazoans, one proposed mechanism lies in the greater sensitivity of DNA replication (associated with differentiation) than protein synthesis (associated with growth) to temperature; specifically, diffusion is less rate-limiting in DNA replication than in protein synthesis, and thus the temperature coefficient of differentiation is higher than the temperature coefficient of growth (van der Have & de Jong, 1996). This results in the decoupling of growth and development rates. As temperature increases, development rate increases disproportionately more than growth rate, hence causing individuals to develop faster but mature smaller (Forster *et al.*, 2011b; Forster *et al.*, 2011a).

Many arthropod species are assumed to have developmental rate isomorphy (DRI), or equi-proportional development (Hart, 1990; Jarosik *et al.*, 2004). The former is the term used in the insect literature to describe equivalent temperature dependence of development rates across distinct ontogenetic stages (Jarosik *et al.*, 2004), the latter is the terminology to describe the same phenomenon in zooplankton, especially copepods (Hart 1990). A similar concept might also be hypothesized for growth rate, i.e., an assumption that growth rate of any particular juvenile stage has the same temperature dependency as all other juvenile stages. However, recent advances have questioned such an assumption (Forster *et al.*, 2011b). In cases where the T-S response has been examined over ontogeny, progeny often show comparatively little or no variation in size with rearing temperature in comparison to adults (Forster *et al.*, 2011b), but the T-S response appears to be generated discontinuously during development, such that the strength of the T-S response both increases and decreases sporadically between life stages. For example, Forster & Hirst (2012) in their study of the brine shrimp *Artemia franciscana,* observed a rapid increase in the T-S response during some periods of ontogeny, but much less so in other periods. Furthermore, the authors found that growth rate and its temperature dependence varied significantly between stages, whilst development rate had the same temperature dependence throughout ontogeny. However, whether the discontinuous nature of the T-S response over ontogeny commonly results from variation in the temperature dependence of growth rate (as in *A. franciscana*), as opposed to development rate, or both, is unknown.

Examining the T-S response at high temporal resolution provides an excellent opportunity to test the degree of thermal equivalence of growth and development rates across ontogeny, and is an important step to understanding why the T-S response has evolved. Here we examine the generation of the T-S response over the course of a single generation in several pelagic copepod species. Copepods globally represent a primary food resource for invertebrate and vertebrate predators, including fish, and are one of the most abundant metazoans on the planet (Ware & Thomson, 2005). In general, the postembryonic development of planktonic copepods is characterized by 6 naupliar stages (N1-N6) and 5 copepodite stages (C1-C5) that can be distinguished based on morphological features. We construct a model to produce preliminary predictions of the impact of different growth and developmental rates and their temperature sensitivities on the ontogenetic progression of the T-S response, following an initial assumption that growth and development have different temperature dependencies that are constant during ontogeny. In comparing these model estimates with our own measurements of ontogenetic variation in the T-S response, we show how the generation of the T-S response over ontogeny deviates from the model predictions, indicative of variation in the temperature dependency of growth and/or development rate between life stages. We then empirically test this assumption by calculating stage-specific growth and development rates, using our own data for the cyclopoid copepod *Oithona nana* as an example, to determine whether the discontinuous progression of the T-S response results from variation in the temperature dependence of growth rate, development rate, or both.

Methods

Experimentation and Data Collection

Three calanoid copepod species (*Acartia tonsa, Centropages hamatus, and Temora longicornis*) and one cyclopoid species (*Oithona nana*), were reared from egg or early nauplii to maturity under three constant temperature treatments (10, 15, 20°C) using two replicates per temperature, resulting in 24 experimental cultures. Copepods were obtained from continuous laboratory cultures at DTU Aqua. Details about the conditions used in the stock cultures and the geographic origin of the copepod species can be found in Almeda *et al.* (2017) . Eggs of *C. hamatus* (starting density of ~700 eggs L^{-1}) and *T. longicornis* (starting density ~150 eggs L^{-1}), and stage 1 nauplii (N1) of *O. nana* (starting density ~1875 individuals L^{-1}) were harvested directly from stock cultures maintained at 16°C and immediately transferred to each treatment. *O. nana* cultures were established using nauplii instead of eggs due to the fact that females carry the eggs until hatching. *A. tonsa* cultures were seeded with eggs held for long periods at 4°C, at which temperature they do not hatch (starting density ~5300 eggs L^{-1}, assuming 25% hatch success (Drillet *et al.*, 2011)).

All experimental cultures were reared in open-top 2L Duran bottles containing filtered seawater (salinity 32). Cultures were incubated at each constant temperature treatment by placing the bottles into 3 high-density polyethylene (HDPE) containers filled with freshwater, each connected via a water pump to a closed loop temperature control system equipped with a digital thermostat (TECO *TK2000* aquarium chiller; ±0.5°C). Insulating foam was placed around the connecting tubing to reduce heat transfer. Cultures were permanently aerated by bubbling a constant low flow of atmospheric air directly into each bottle. All species were fed the dinoflagellate *Oxyrrhis marina* obtained from stock cultures kept at 16°C, with food levels maintained at ≥3000 cells ml^{-1} (≥1500 cells ml^{-1} for *O. nana*) to ensure saturated food conditions (Leandro *et al.*, 2006; Saage *et al.*, 2009; Almeda *et al.*, 2010; Gonçalves *et al.*, 2014). Food concentrations were measured either daily, or every 48h at 10°C, using a Beckman Coulter Multisizer^{TM} 3 Coulter Counter® and adjusted accordingly to maintain saturation and avoid possible confounding effects of food limitation.

To obtain a measure of body size at different developmental stages throughout ontogeny, an 80ml sample was collected from each culture every 24h and filtered through a 40μm mesh. Bottle contents were mixed thoroughly prior to sampling, to ensure a homogenous distribution of individuals, and cultures were immediately replenished with filtered seawater. Individuals were preserved in 0.5% Lugol's solution for staging and sizing. Due to the much lower stocking density of *T. longicornis* in our experimental cultures, samples of this species were collected and preserved from only 3 time points (nauplii stage 6, copepodite stage 1 and copepodite stage 6), determined by regularly staging and then returning a sub-sample of individuals from each culture.

All preserved individuals were staged by eye with an inverted microscope using taxonomic guides (Conway, 2006; Banse & Hirst, In press). To determine body size, digital pictures of ~30 random individuals from each temperature treatment and developmental stage (separated by sex in later copepodites stages) were taken with a camera attached to an inverted microscope. Total body length without spines (BL, μm) for nauplii, and prosome length (PL, μm) for copepodites were measured using image analysis software (Volocity® v.5.3.1, PerkinElmer) and subsequently converted to dry mass using published nauplii- and copepodite-specific length-weight regressions for each species (see Appendix 6.1). Body width of all stages was also measured (also see Appendix 6.1). For each species the slopes of ln-transformed mass vs. temperature were then determined for each developmental stage. These stage-specific slopes were transformed into percentage change in mass per degree Celsius, using the formula $(\exp^{(slope)} -1)*100$ = % change in mass per °C (Forster *et al.*, 2012). A negative percentage change indicates a decrease in body size with increasing temperature, following the TSR.

To obtain quantitative data on stage-specific development and growth rates, and to determine their temperature dependence, in addition to body size measurements, we also recorded the daily frequency distribution (based on the first 20 individuals per sample) of developmental stages in our *O. nana* cultures. Development time was estimated by plotting the cumulative proportion of each stage against time using a 3rd order polynomial function, and determining the time at which 50% of individuals had reached each stage**.** Stage-specific development times (days) were then calculated as the median development times, i.e., from the point at which 50% of individuals reached stage *i* to the point at which 50% of individuals reached stage *i* + 1 (Peterson & Painting, 1990). The reciprocal of development time was used to determine stage-specific development rate (day^{-1}). In addition, stage durations at 15°C for *A. tonsa, C. hamatus and T. longicornis* were obtained from relevant literature sources (Breteler *et al.*, 1982; Leandro *et al.*, 2006) in order to describe the progression of their T-S response over ontogeny.

Mass-specific growth rates from one stage to the next ($g_{i \rightarrow i+1}$) for *Oithona nana* for each of the temperature treatments were calculated combining data on arithmetic mean weights of each stage, and development times across consecutive stages, using appropriate methodologies as laid out by Hirst *et al.* (2005) (their equation 22). All statistical analyses were conducted in R (R Core Team, 2014). The effect of temperature on *O. nana* growth and development rates was modelled using an exponential equation form, so as to be consistent with the temperature coefficient (Q10) adopted for growth rate in our conceptual model. We applied multiple variations of this model in which developmental stage was incorporated as a random effect, either on: i) the intercept only, ii) the slope only, or iii) both the intercept and the slope. These models were compared using a log-likelihood ratio test (ANOVA function in R) to identify which model best described the data. This allowed us to determine whether growth and development rate varied by intercept (a), and thus between different life stages, and/or varied by the slope (b), i.e. had different temperature dependences across different life stages. Similarly, we compared models with and without 'replicate' incorporated as a random factor, to determine whether the effect of temperature on growth and development rate was consistent across our replicate cultures.

Modelling the Progression of the T-S Response

The progression of the T-S response through ontogeny was examined using parameter values based on measurements of pelagic copepod growth, although it can be easily modified for other taxa and under alternative assumptions. In this case, ontogeny begins as eggs (E), which were assumed here to have no T-S response. Development time between stages was initially assumed to be isochronal (i.e. that each juvenile stage occupies the same period of time), and to have a constant degree of temperature dependence, that does not change over ontogeny, as is an implicit assumption of the equiproportional development concept (Hart, 1990). Development time was estimated using a Bělehrádek function, defined as:

$$D_t = 675\ (T + 2.7)^{-2.05} \tag{1}$$

where D_t is the stage-specific median development time (days) and T is the temperature (°C). We chose to fix the shape of the response, such that b = 2.05, which is assumed to be relatively conserved among different species within a taxon (McLaren, 1995). The remaining parameter values were chosen based on iteration, using copepod development times typically reported in the literature (e.g. Leandro *et al.*, 2006; Almeda *et al.*, 2010). Mass was assumed to increase exponentially over time, i.e., have a constant mass-specific growth rate, throughout ontogeny, with a rate initially set at 0.2d^{-1} at 15^{o}C, and having a Q10 of 2.5. Egg mass was assigned an arbitrary value that did not vary with temperature, and we used our estimates of growth rate and development time to model variation in body mass with temperature at each life stage. The slopes of ln-transformed mass at stage vs. temperature were determined for each developmental stage and transformed into a percentage change in mass per degree Celsius, as above. These stage-specific T-S responses were then converted to a proportion of the final adult T-S response, allowing us to model the progression of the T-S response across life stages and through time (for comparative purposes we express this as a proportion of total development time), assuming isochronal development.

Many copepods have near exponential growth over much of ontogeny under non-limiting food conditions (e.g. *Acartia*, *Oithona* and many other genera) (Miller *et al.*, 1977; Almeda *et al.*, 2010), while other copepods grow somewhat slower in the later development stages (Hirst & Bunker, 2003). Some copepods also commonly have longer development times in these later copepodite stages too (Landry, 1983). To allow for this, we also used the model to explore how variation in growth and development rates over ontogeny might impact the generation of the T-S response. Specifically, mass-specific growth rate was set either to decline or increase through ontogeny (growth rate declining or increasing progressively by 10% per stage), and/or development time was set to increase in successive stages (assuming a 25% increase in stage duration per stage). We also varied the initial growth rate set at 15°C, ranging between 0.1 to 0.4 day^{-1} (based on values reported in Kiørboe & Sabatini 1995), as well as its temperature dependence, ranging from a Q10 of 1.5 to 4 (based on values reported in Hirst & Bunker 2003). In all cases, growth and development rate were assumed to have different but constant temperature dependence throughout ontogeny, the latter being assumed to always have the greater temperature dependence (van der Have & de Jong, 1996; Forster *et al.*, 2011a). We ran the combinations of these model parameters and compared the predictions with ontogenetic T-S responses measured in copepod species under experimental conditions.

Results

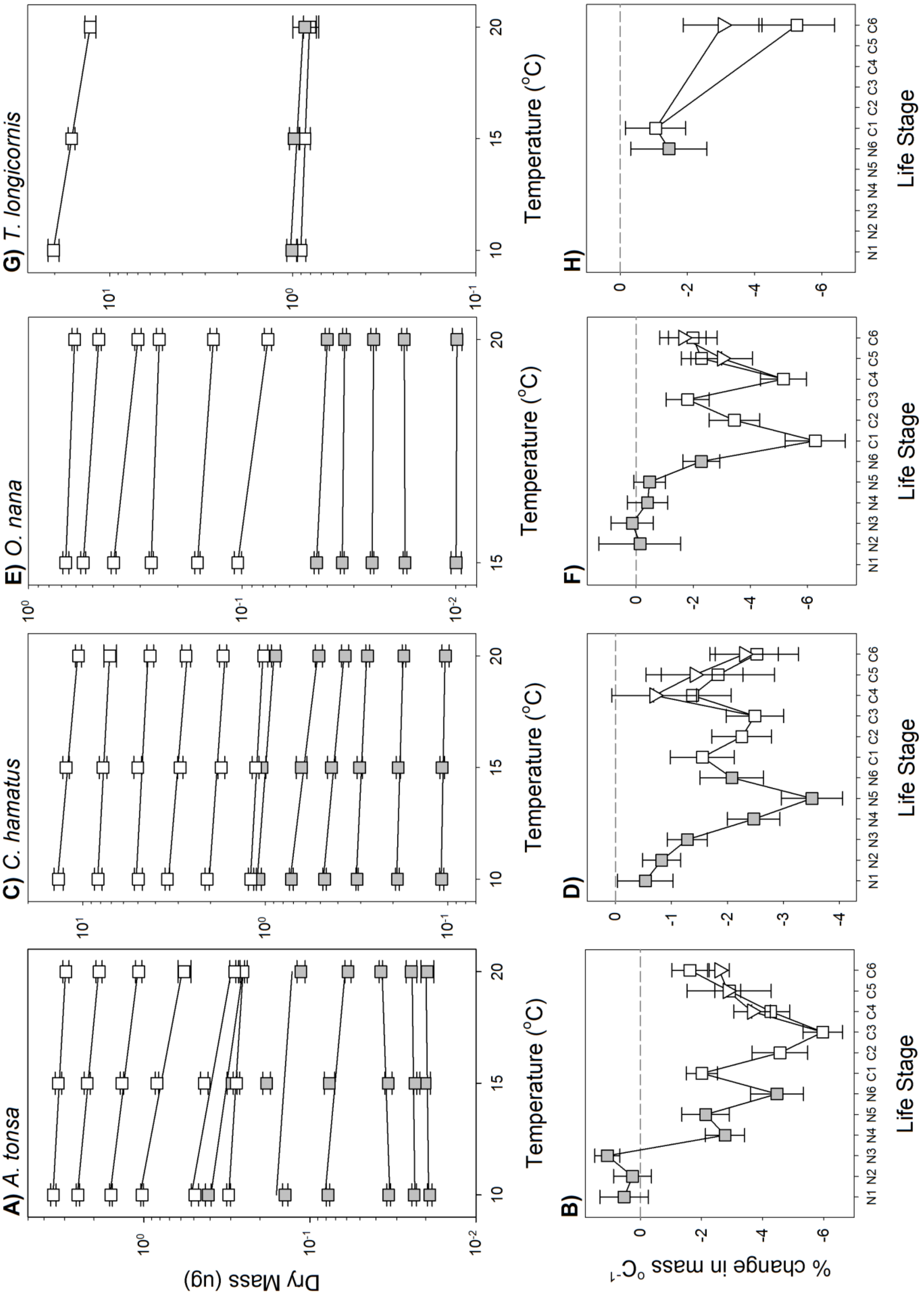
Body size measurements of 5,620 individuals were recorded across all four copepod species, developmental stages and treatments (see Appendix 6.1). All species were successfully reared to maturity at each temperature treatment, with the exception of *O. nana*, which did not mature at 10°C within our experimental period. Thus, *O. nana* individuals reared at 10°C were excluded from our analyses. Arithmetic mean body mass for each species at each life stage and rearing temperature are presented in Table 6.1. All species adhered to the TSR; that is, they exhibited a decrease in adult body size with increasing temperature. *T. longicornis,* the largest of the four species, exhibited the greatest adult T-S response (-4.16% $°C^{-1}$), followed by *C. hamatus* (-2.41% $°C^{-1}$) and *A. tonsa* (-2.10% $°C^{-1}$), whilst *O. nana*, the smallest of the species, exhibited the weakest T-S response (-1.82% $°C^{-1}$).

Stage-specific T-S responses in each of the 4 species are shown in Figure 6.1. Early nauplii stages generally showed a weak or inverse T-S response, particularly in *A. tonsa* and *O. nana,* whilst later nauplii stages exhibited stronger reductions in body size with temperature. All species except *O. nana* exhibited a reduction in the strength of the T-S response in the transition from nauplii (N6) to copepodite (C1), which corresponds with a radical shift in body form. In 3 of the 4 species, the strongest T-S response did not occur in the adult stage, but rather in the late nauplii and early copepodite stages, the subsequent stages then tended to show a reduction in the strength of the T-S response

Table 6.1. Stage-specific arithmetic mean body mass. Values have been converted from total body length for nauplii and prosome length for copepodites using published length-mass regressions (see Appendix 6.1).

	Dry Mass (µg)										
	A. tonsa			***C. hamatus***			***O. nana***		***T. longicornis***		
	10°C	**15°C**	**20°C**	**10°C**	**15°C**	**20°C**	**15°C**	**20°C**	**10°C**	**15°C**	**20°C**
N1	0.019 (60)	0.020 (14)	0.020 (12)	0.108 (60)	0.108 (46)	0.102 (47)	-	-	-	-	-
N2	0.024 (28)	0.023 (70)	0.024 (29)	0.189 (49)	0.187 (60)	0.174 (58)	0.010 (60)	0.010 (60)	-	-	-
N3	0.034 (60)	0.035 (40)	0.037 (60)	0.313 (39)	0.304 (33)	0.275 (26)	0.017 (60)	0.017 (46)	-	-	-
N4	0.078 (60)	0.076 (60)	0.059 (55)	0.474 (42)	0.430 (19)	0.366 (17)	0.025 (60)	0.024 (60)	-	-	-
N5	0.141 (60)	0.182 (23)	0.114 (50)	0.719 (32)	0.635 (15)	0.510 (46)	0.034 (60)	0.033 (60)	-	-	-
N6	0.409 (50)	0.295 (51)	0.263 (50)	1.086 (55)	1.037 (40)	0.872 (35)	0.045 (60)	0.040 (60)	1.006 (19)	0.985 (10)	0.890 (7)
C1	0.309 (60)	0.277 (30)	0.254 (31)	1.200 (60)	1.131 (42)	1.022 (32)	0.105 (60)	0.076 (60)	0.902 (24)	0.869 (12)	0.839 (13)
C2	0.493 (110)	0.434 (62)	0.290 (30)	2.083 (60)	1.772 (29)	1.701 (18)	0.162 (60)	0.136 (60)	-	-	-
C3	1.026 (60)	0.834 (54)	0.569 (47)	3.447 (54)	2.928 (42)	2.713 (24)	0.267 (60)	0.244 (60)	-	-	-
C4	1.579 (117)	1.356 (66)	1.095 (104)	4.938 (46)	5.023 (46)	4.269 (37)	0.400 (60)	0.307 (61)	-	-	-
C5	2.506 (41)	2.203 (69)	1.857 (48)	8.246 (45)	7.768 (48)	7.114 (18)	0.556 (77)	0.468 (113)	-	-	-
C6	3.506 (95)	3.277 (120)	2.947 (127)	13.309 (71)	12.377 (120)	10.583 (120)	0.669 (120)	0.608 (120)	20.303 (87)	16.149 (120)	12.830 (97)

Note: *n* values are given in brackets



A) A. tonsa
C) C. hamatus
E) O. nana
G) T. longicornis
B)
D)
F)
H)
Dry Mass (ug)
% change in mass °C-1
Temperature (°C)
Life Stage
10
15
20
10^0
10^-1
10^-2
10^1
0
-2
-4
-6
-1
-3
N1 N2 N3 N4 N5 N6 C1 C2 C3 C4 C5 C6

Figure 6.1. Stage-specific regressions of dry body mass (μg) (log10 scale) vs. temperature for nauplii (N1-N6; filled symbols) and copepodites (C1-C6; open symbols), and associated temperature-size responses (percentage change in mass per ^{o}C) for the species *Acartia tonsa* (panels A and B), *Centropages hamatus* (panels C and D), *Oithona nana* (panels E and F; nauplii begin at stage N2) and *Temora longicornis* (panels G and G; stages N6, C1 and C6 only). Where body size measurements were separated by sex (i.e. later copepodite stages), temperature-size responses are depicted for males (triangles) and females separately (lower panels only). We find no difference in the strength of the temperature-size responses between the sexes.

into adulthood. Across all four species, we found no significant interaction between temperature and sex acting on body size, suggesting that the strength of the T-S response does not differ between males and females ($F_{1,2065}$=0.18, p=0.67; also see Figure 6.1).

Modelling the Progression of the T-S Response

Figures 6.2a and 6.2b show the predicted generation of the T-S response through ontogeny when development is isochronal and growth is exponential (growth rate at 15°C is 0.2 day^{-1}; and the Q10 is 2.5). Two alternative models are also depicted, in which, i) development is isochronal and growth rate declines through ontogeny (decreasing by 10% for consecutive stages; growth rate at 15°C=0.4 day^{-1}; Q10=1.5), and, ii) development time of each stage increases through ontogeny (+25% stage duration per stage) and growth is exponential (growth rate at 15°C is 0.1 day^{-1}; and the Q10 is 4). These alternative models were chosen because they contained the combination of parameters that resulted in the greatest deviation in the predicted generation of the T-S response away from the predictions of the initial model. When compared to the model predictions, both in terms of proportion of the development time and adult body mass at 15°C, our own empirical data suggests that the T-S response is not generated following a similar pattern, i.e. evenly through ontogeny (as occurs when the temperature dependence of growth and development rate are constant, or when the model is modified to account for a decrease in growth rate or an increase in stage duration over ontogeny). The strongest reductions in body size with warming seem to occur mid-way through development (Figure 6.2a), on average corresponding with the point at which individuals reach ~20% of their adult mass (Figure 6.2b). Thus, at high resolution the generation of the T-S response appears irregular, with some stage changes showing an actual reversal in the strength of the T-S

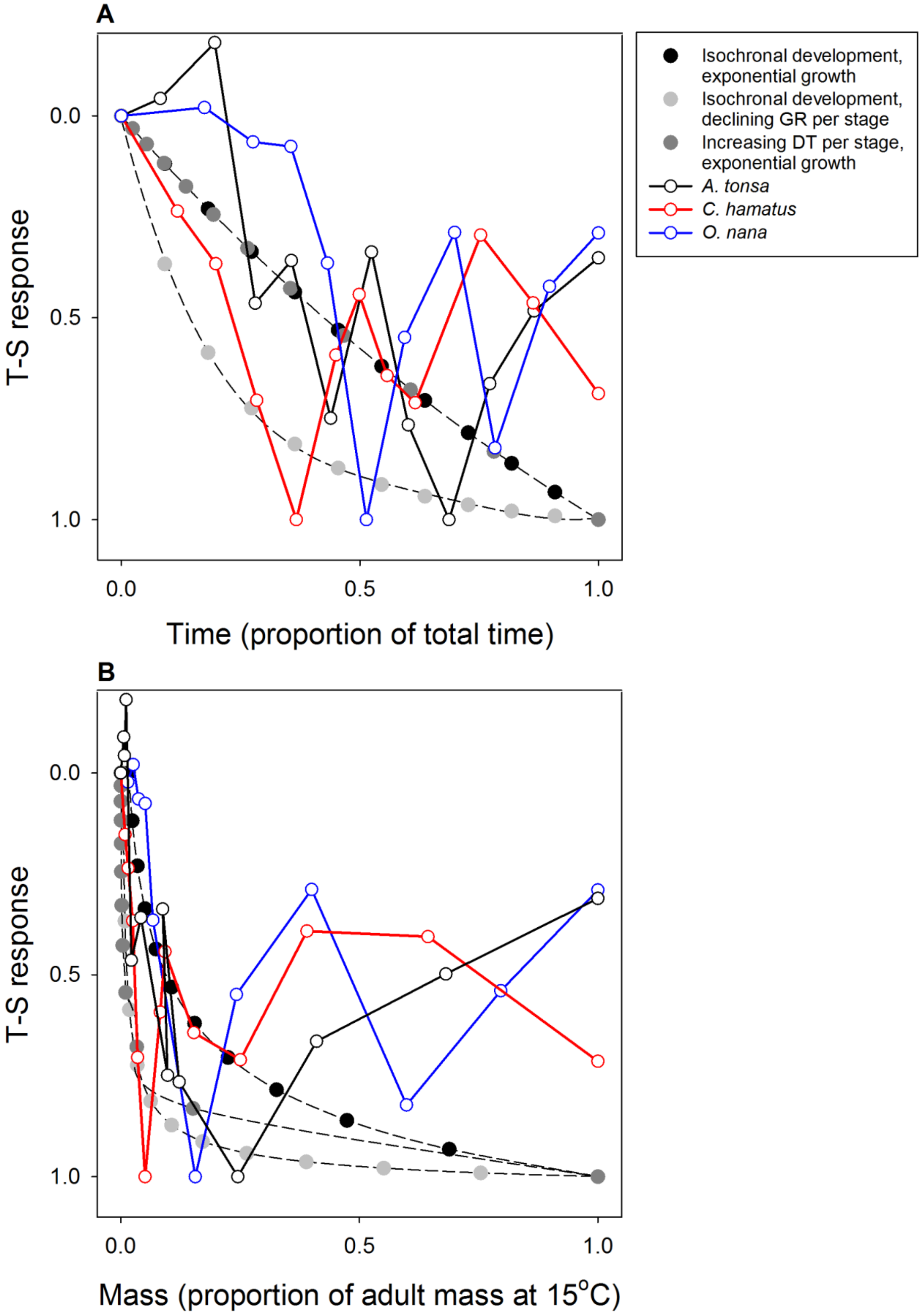

A
B
T-S response
0.0
0.5
1.0
Time (proportion of total time)
Mass (proportion of adult mass at 15°C)
● Isochronal development, exponential growth
● Isochronal development, declining GR per stage
● Increasing DT per stage, exponential growth
A. tonsa
C. hamatus
O. nana

Figure 6.2. T-S response in copepods (normalised by strongest response) vs. (**A**) time (represented as a proportion of total development time) and (**B**) mass (represented as a proportion of adult mass at 15°C), based on model predictions and experimental data for copepods. Data points represent each life stage. The initial model output assumed isochronal development and exponential growth though ontogeny (black circles; GR=0.2 day^{-1} at 15°C; Q10=2.5). Alternative outputs containing the combination of parameters that resulted in the greatest deviation from the initial model output are also shown, assuming either i) isochronal development with growth rate declining progressively by 10% per stage in comparison to that in the first stage (light grey circles; GR=0.4 day^{-1} at 15°C; Q10=1.5), or ii) an increase in stage duration by 25% per stage from that in the first stage and exponential growth (dark grey circles; GR=0.1 day^{-1} at 15°C; Q10=4). In all cases development rate was assumed to have greater temperature dependence than growth rate, with neither having temperature dependence that varied over ontogeny in the model. Experimental data for stage-specific T-S responses of *A. tonsa* (black line), *C. hamatus* (red line) and *O. nana* (blue line) are included for comparison. Note the discontinuous progression of the T-S response in the empirical data compared to the model predictions.

response. We conclude that this is only possible if growth and/or development rates differ in their thermal sensitivity across life stages.

Growth and Development Rates

We tested for ontogenetic variation in the temperature dependence of growth and development rates using the experimental data from *O. nana*. When modelling the effect of temperature on growth rate, the best fit model contained developmental stage as a random effect on the intercept (suggesting that different stages have significantly different growth rates), and also on the slope (suggesting that the temperature dependence of growth rate varies between stages) (Figure 6.3). Conversely, neither incorporating developmental stage as a random effect on the intercept, or on the slope, significantly improved the fit of the model for development rate, suggesting its temperature dependence remains conserved over ontogeny in this species (Figure 6.3). In both instances, including replicate as a random effect did not improve the fit of the model, and so data from both replicates at each temperature were combined. The discontinuous progression of the T-S response through ontogeny in *O. nana* appears to be caused by variation in the temperature dependence of the growth rate, as oppose to development rate.

Discussion

All four of our experimental species exhibited a decrease in adult body size with increasing temperature. These findings support the broader patterns in T-S responses observed in nature, in which nearly 90% of copepod species were found to adhere to the TSR when body size was correlated with seasonal variation in temperature, as subsequent generations experienced different developmental conditions during ontogeny (Horne *et al.*, 2016). Furthermore, the weakest adult T-S response in our

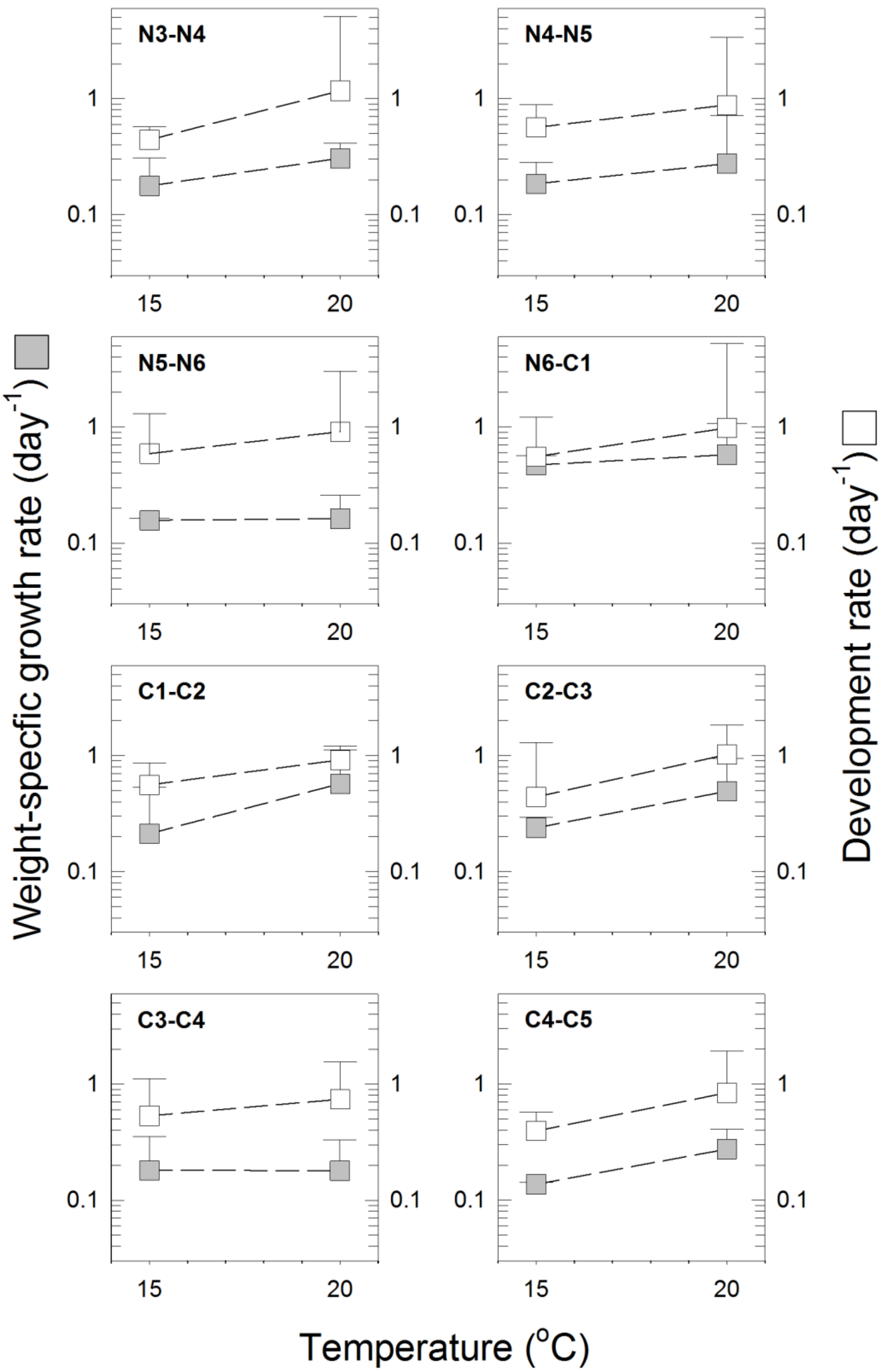

N3-N4
N4-N5
N5-N6
N6-C1
C1-C2
C2-C3
C3-C4
C4-C5
1
0.1
15
20
Weight-specfic growth rate (day^{-1}) ▨
Development rate (day^{-1}) ☐
Temperature (°C)

Figure 6.3. *O. nana* weight-specific growth (shaded symbols) and development rates (open symbols) vs. temperature at different developmental stages (left and right hand axes respectively). Regressions are fitted through data for both replicates combined. The best fit model for the effect of temperature on growth rate contains developmental stage as a random effect on the intercept and slope, suggesting that growth rate and its temperature dependence varies significantly between stages. Incorporating developmental stage as a random effect on either the intercept or slope did not improve the fit of the model for the effect of temperature on development rate, suggesting that development rate and its temperature dependence are constrained across different life stages. Error bars denote 95% CIs. Note the logged Y axes.

experimental species was observed in the cyclopoid *O. nana*, which corresponds with the findings of Horne *et al.* (2016) , in which ambush-feeding cyclopoids were shown to exhibit relatively weaker seasonal T-S responses than current-feeding calanoids. Similarly, *T. longicornis* showed the strongest adult T-S response in our study, and this species also exhibits one of the strongest reductions in body size with temperature in the field (Horne *et al.*, 2016). We also find support for there being similar temperature-size responses in male and female size within single species, in agreement with a broader analysis across Arthropoda, in which T-S responses were found to not significantly differ between the sexes (Hirst *et al.*, 2015).

Beyond observing variation in the strength of the adult T-S response between species, our work highlights that important and systematic shifts in temperature dependence occur over very short time periods and across consecutive life stages, providing evidence that the degree to which the temperature dependence of growth and development rates differ is not consistent over ontogeny. Indeed, a switch to greater temperature dependence in growth than development rates is not only confirmed by the weakening of the T-S response from stage to stage in all four of the copepod species we studied, but is also apparent in *O. nana*, in which the slope of weight-specific growth rate vs. temperature is at times stronger than that of development rate (e.g. C1-C2 in Figure 6.3). This also corresponds with a reduction in the strength of the T-S response across these life stages (C1-C2 in Figure 6.1d). Similarly, where the slope of weight-specific growth rate vs. temperature is relatively shallower than development rate (e.g. N5-N6 in Figure 6.3), this corresponds with a sharp increase in the strength of the T-S response (N5-N6 in Figure 6.1d). These findings lead us to suggest that the variable progression in the strength of the T-S response observed in our other experimental species also arises due to variation in the temperature dependence of at least one of these rates over ontogeny.

Our findings support those previously reported by Forster & Hirst (2012) , in which growth rate and its thermal sensitivity were found to vary over different developmental phases in the brine shrimp *Artemia franciscana*. Like Forster & Hirst (2012), we find that the thermal sensitivity of development rate is conserved over ontogeny while the sensitivity of growth to temperature varies. In addition to their own experimental data, Forster & Hirst (2012) also gave examples from the literature of T-S responses through ontogeny in a variety of crustacean species, most of which demonstrated discontinuous patterns in the generation of their T-S response over the course of development. Having revisited these data and their original sources, following the same methodology described herein, we were also able to test for changes in the temperature dependence of development rate across ontogeny in six of the ten species presented. Of these species, four showed no significant variation in the temperature dependence of development rate between life stages, suggesting instead that variation in the temperature dependence of growth rate is accountable for the discontinuous generation of the T-S response over ontogeny. Correspondingly, in their assessment of growth and development rates in 15 marine copepod species, Forster *et al.* (2011b) observed that the thermal response of development rate was consistent through the life cycle, whilst the thermal response of growth rate was more variable, differing between nauplii and copepodites, albeit not significantly. Thus, the pattern emerging is that variation in the TSR during ontogeny is driven by variation in the temperature dependence of growth rather than of development rate. These findings contradict the model presented in Figure 6.2, which assumes that growth rate is to a consistent degree less temperature dependent than development rate.

Such an assumption would lead to a rather monotonous pattern with a strengthening of the T-S response over time and across stages.

The discontinuous progression of the T-S response over ontogeny in part contradicts the prediction of van der Have and de Jong (1996), who suggested that the mechanistic basis of the TSR lies in the greater sensitivity of DNA replication (associated with differentiation) than protein synthesis (associated with growth) to temperature. Why the thermal sensitivity of these rates, and in particular growth rate, varies over ontogeny, and determining whether this variation is systematic and therefore predictable, is challenging. Furthermore, although among our experimental species we see similarities in the progression of the T-S response, the pattern itself is somewhat irregular. One potential explanation is that measurements of whole organism growth rate reflect different processes at the cellular level, encompassing not just individual cell growth but also cell differentiation. Although copepods are thought to be eutelic (i.e., have a determinate number of somatic cells at maturity) (McLaren & Marcogliese, 1983; Escribano *et al.*, 1992), the extent to which growth occurs by cell division (likely in earlier life stages), or by individual cell growth (likely in later life stages) may vary from one life stage to the next. Given that the biological rates underlying these processes have different temperature dependence, variation in the prevalence of these two processes over ontogeny would be reflected in the thermal sensitivity of growth rate at the whole organism level. Indeed, in our experimental species, the majority of the T-S response appears to be generated in earlier life stages approaching ‘metamorphosis’ (the transition from nauplii to copepodites), when individuals on average reached just 20% of their adult mass (Figure 6.2b). Similarly, in *Acartia tonsa* and *Calanus finmarchicus,* Forster *et al.* (2011a) found that the majority of the T-S response was established at a proportion of 0.2 of the adult weight. Up to this point, cell differentiation (assumed to be more sensitive to temperature than individual cell growth via protein synthesis) is likely to be the primary method accounting for whole organism growth.

One alternative, though not mutually exclusive, explanation, is that the discontinuous progression of the T-S response arises from a mismatch between ontogenetic demands on energy (and thus scope for growth) in the laboratory versus in nature. Should an organism find itself growing bigger or smaller than would be 'expected' given its evolutionary history in the field, for example because it is investing less in locomotion or 'defence', or because food quality and quantity do not match those encountered in typical field conditions, then feeding rates and size at a particular moult may be adjusted in subsequent larval stages. Mismatches at each life stage might generate irregular patterns in the thermal sensitivity of growth, and thus in the progression of the T-S response over ontogeny. At present, these seem the most plausible explanations for the broader patterns in our data.

We show both conceptually and empirically that the discontinuous progression of the T-S response over ontogeny signals rapid developmental shifts in the thermal-dependence of life history rates, in particular growth rate. Just as we observe variation in the magnitude of T-S responses between organisms with different life histories (e.g. aquatic vs. terrestrial, univoltine vs. multivoltine) (Forster *et al.*, 2012; Horne *et al.*, 2015), we should also consider that the selective pressures acting on body size may differ within species over ontogeny. Limiting factors other than temperature, such as resource availability, may be constraining growth more strongly at certain life stages than others, thereby confounding the effects of temperature on growth rate. A greater awareness of how these trade-offs manifest themselves over ontogeny, and how they are likely to affect allocation of energy to growth and development vs. maintenance, could help us to better understand the progression of the T-S response at high resolution, including how and why it may have evolved.

CHAPTER 7

General Conclusions

Major biological and biogeographical rules have linked body size variation with latitude, altitude and environmental temperature, but these rules are often studied in isolation. Consequently, the degree to which these body size gradients co-vary and share explanatory mechanisms has not been systematically evaluated. To our knowledge, this thesis provides the largest quantitative comparison of these size gradients to date in arthropods, including marine, freshwater and terrestrial species. By comparing both large spatial scale and temporal body size data within diverse arthropod species with laboratory-derived size data from controlled temperature manipulations, we develop a powerful approach for explaining body size patterns. In doing so, our comprehensive analysis leads us to present the following major conclusions:

i. Body size in aquatic species generally reduces with warming, both in the laboratory and across seasons in the field, and with decreasing latitude, whereas terrestrial species have much reduced and even opposite gradients. These patterns support the prediction that greater oxygen limitation in water than in air forces aquatic arthropods to exhibit greater plasticity in body size with temperature than terrestrial arthropods.

ii. Voltinism explains much of the variation in laboratory temperature-size responses and latitudinal-size clines in terrestrial but not aquatic species. While body size generally decreases with warming and with decreasing latitude in multivoltine terrestrial arthropods, size increases on average in univoltine species, consistent with predictions from size vs. season-length trade-offs.

iii. The majority of multivoltine arthropod species reduce their body size with seasonal warming, as subsequent generations experience different developmental conditions. However, total percentage change in size with warming over the annual cycle appears relatively constant with annual temperature range, but still varies between aquatic and terrestrial species. This suggests there may be an optimal point at which the selective pressures in a given environment over the annual cycle no longer favour more extreme size reductions with warming.

iv. We describe how seasonal size variation relates to temperature, food concentration (chlorophyll-*a*) and life history characteristics in planktonic copepods. We find that temperature, rather than food, is the dominant explanatory variable of adult body size variation across seasons. Furthermore, our findings lead us to suggest that variation in the temperature dependence of energy supply and expenditure between different feeding strategies may play a significant role in dictating the magnitude of seasonal temperature-size gradients in copepods, with potential implications for other ectotherms with diverse feeding methods.

v. Crucially, although our data sets represent only a tiny fraction of all arthropod species globally, we show that strong correlations exist between laboratory temperature-size responses, seasonal temperature-size gradients and latitudinal-size clines, suggesting that these patterns share common drivers. In contrast, despite similarities in the general direction of temperature-size responses and altitude-size clines, deviation in the magnitude of these gradients suggests that other environmental parameters are confounding the effects of temperature on body size across altitude in the field, in some taxa more than others.

Understanding how the temperature-size response is generated during ontogeny is also important to determine the proximate and ultimate causes of this widespread rule, yet only a handful of studies have examined the progression of the T-S response at high temporal resolution over the course of development. In Chapter 6, we examined the progression of the T-S response over the ontogeny of four species of marine planktonic copepods. Contrary to model predictions based on the assumption that growth and development have different temperature dependencies that do not change over ontogeny, the temperature-size response is not generated in a monotonous way; rather, the difference in temperature dependency between growth and development rates varies rapidly from stage to stage. Furthermore, the strongest temperature-size response appears around the time of metamorphosis. Using the copepod *Oithona nana,* we show that weight-specific growth rate and its temperature dependence differ significantly among life stages, whilst development rate remains rather conserved. Thus, this thesis also provides direct evidence that the extent to which growth and development rate are thermally decoupled changes rapidly among stages in planktonic copepods.

This thesis adds to a growing literature that aims to understand and predict intra-specific variation in body size. Conservative estimates predict a rise in average global temperatures of more than 2°C by the end of this century (IPCC, 2014), and physiological plastic responses to temperature are almost universal, particularly in ectotherms whose internal body temperature is dictated by the environmental temperature. Although the degree of change will vary from species to species, generally the rate of most biochemical reactions will increase with increasing temperature. This means that the majority of species are likely to experience an increase in their metabolic costs, demands and other biochemical rates with warming. In the majority of ectothermic species, changes in these rates are accompanied by a decline in body size with increasing temperature, as observed in the laboratory and across seasons and latitude in the natural environment.

The consequences of smaller body size could impact individuals, populations and communities. Although growing to a smaller size at higher temperatures might provide a coping mechanism to overcome the physiological challenges associated with warming, any reduction in adult size is likely traded-off against other life history traits that scale with body size. For example, in many ectotherms, larger body size often infers a reproductive advantage, and thus a reduction in size with warming is likely to be accompanied by a decrease in fecundity (Honěk, 1993; Arendt, 2011). Smaller individuals may also face a greater risk of mortality, including but not limited to reduced probability of survival during periods of low resource availability (e.g. Sogard, 1997), and/or a greater susceptibility to desiccation from evaporative heat loss (e.g. Heatwole *et al.*, 1969). Changes in the body size of species could also impact the ecosystem services we depend upon. For example, body size has been shown to positively correlate with foraging distance in a number of taxa, including bees, and in turn can determine the spatial scale at which they can provide pollination services to crops (Kremen, 2005; Greenleaf *et al.*, 2007). Should body size reduction with warming be accompanied by a reduction in foraging distance, this could influence the sexual reproduction of flowering plants, altering the genetic structure of plant populations, and ultimately affect agricultural production (Greenleaf *et al.*, 2007). In aquatic systems, the size-distribution of zooplankton can dictate the level of grazing pressure on phytoplankton, such that larger species have a wider range of food-size spectra, and can more effectively control phytoplankton standing crop (Vanni, 1987; Gliwicz, 1990).

Consequently, a reduction in body size with warming could constrain the food-size spectra of zooplankton, leading to less effective control of algal blooms, some of which can be toxic and can dramatically alter water quality.

Predicting the impact of changing body size at the community and ecosystem level becomes increasingly complex, particularly because, as we show here, not all taxa adjust their body size equally with temperature. Mismatches in the sensitivity of species body size to temperature have the potential to alter trophic dynamics, including interactions between consumers and their resource, especially if body size changes at the individual level lead to changes in total biomass, and thus population productivity. At the time of writing this conclusion, a study by Osmond *et al.* (2017) was published online, in which the effects of warming on body size were incorporated into models predicting consumer-resource dynamics. Based on body size declines that are consistent with empirical observations, including those presented in Chapter 2 of this thesis, the authors predicted consumer-resource biomass ratios would remain stable or increase under warming, whilst particularly strong body size responses can even facilitate an increase in system stability with warming. These outcomes arise specifically from changes in both the consumer conversion efficiency and the intrinsic growth rate of the resource; each of these rates increases with declining body mass, supporting a relatively larger consumer biomass at higher temperatures, whilst greater stability at higher temperatures arises from the increase in the resource's intrinsic growth rate, combined with a reduction in attack rate at smaller consumer body sizes (Osmond *et al.*, 2017). These predictions contrast with those when body sizes are assumed to be constant across temperatures, emphasizing the importance of accounting for body size plasticity. Clearly then, the quantitative data presented in this thesis could prove to be invaluable for the parameterization of these models, leading to more accurate predictions. Understanding how changes in body size with warming impact food web dynamics is a challenge, but a necessary advance in this field if we are to truly understand the wider ecosystem level impacts of body size change.

Perhaps most notably, changes in the body size of species could have a direct impact on human food consumption, particularly in aquatic species, which we would expect to exhibit the greatest reduction in body size with warming. The total output of marine molluscs from coastal waters in 2008 was 12.8 million tonnes and valued at US$12.8 billion (Bostock *et al.*, 2010), whilst fish, in particular smaller-sized pelagic species, globally represent one of the most important sources of high quality protein and essential nutrients for human consumption and as feed in aquaculture (Tacon & Metian, 2013). Quantifying the effects of temperature on the body size of these and other aquatic taxa with ecological and economic value is urgently needed. This becomes even more poignant when we consider that warming and eutrophication have each led to an increase in the severity and extent of deoxygenation in regions of the world's oceans and freshwaters, and this is predicted to worsen significantly over the coming decades (Ficke *et al.*, 2007; Long *et al.*, 2016). Reductions in absolute oxygen availability (as opposed to reductions in oxygen supply relative to metabolic demand) can also result in smaller adult body size, as has been observed in many ectothermic species (Gibson & Atkinson, 2003; Hoefnagel & Verberk, 2014), creating a double jeopardy for aquatic taxa. Thus, having a deeper understanding of how underlying biological rates and processes change with size reduction, and how this may impact the partitioning of biomass across trophic levels, could provide valuable insights into how total productivity and efficiency of transfer from food to flesh might be affected in warmer conditions. Whether the T-S response fully compensates for increased metabolic demand at higher temperatures, and thus the same total biomass of a population can be supported in warmer conditions (composed simply of many small rather than fewer large individuals) is debatable. As highlighted in Chapter 4, given an initial general assumption that metabolic rate increases 2.5-fold with every 10°C increase in temperature, and that metabolic rate scales with body mass to the power of 0.75 (Zuo *et al*. 2012), an organism would have to decrease its body mass by ~11.5% per °C of warming to fully compensate for the increase in metabolic demand associated with warming (i.e. to keep metabolic rate constant with temperature). Yet, the majority of species examined to date reduce their body size by less than this amount (Forster *et al.*, 2012; Horne *et al.*, 2015; Horne *et al.*, 2016; Horne *et al.*, 2017). The extent to which T-S responses compensate for increased metabolic demand with warming has not been explored in any detail. For aquaculture management, such information may be extremely useful when predicting future harvest yields, particularly in natural systems (e.g. coastal or offshore pens), where cultures are likely to be effected by climate warming. Quantitative data exploring the relationship between gross growth efficiency integrated over ontogeny and body size plasticity with warming and reduced oxygen are needed to better explore how population productivity may be impacted by changing conditions.

It is important to acknowledge that any predicted reductions in the body size of species with climate warming are, of course, based on the assumption that these species will maintain the same geographical and/or temporal distribution. However, an increasing number of studies have reported shifts in the latitudinal and/or altitudinal distribution of species, as well as shifts in phenology (timing of life cycle events), with climate warming (Chen *et al.*, 2011). These shifts may enable species to track more favourable thermal environments, thereby negating any potential effects of warming on body size. Some species will be better equipped to shift their range distribution than others; physical limitations (e.g. poor dispersal ability), lack of suitable habitat, and physical barriers to migration can all limit the ability of species to track favourable temperatures. Similarly, phenological shifts with climate warming may be less pronounced in those species that rely primarily on environmental cues other than temperature, such a photoperiod or light intensity. Perhaps then, efforts to quantify and predict T-S responses should be focussed on those species that are less able to shift their geographical and/or temporal distribution? As reported in Chapter 5, although we observe a relatively close match in the magnitude of altitude-size clines and laboratory T-S responses in some taxa, suggesting a particularly important role for temperature, deviations away from a 1:1 relationship in other taxa may indicate that alternative environmental variables are confounding the effects of temperature on body size along elevation gradients in the field. Whilst high-altitude environments may become more similar in temperature to those at lower altitudes with climate warming, and thus species may shift their altitudinal distribution to track favourable temperatures, altitudinal variation in other environmental parameters is likely to remain unchanged (e.g. oxygen partial pressure, photoperiod). Therefore, in those species for which temperature appears a less important correlate of size at maturity, such altitudinal range shifts may still be accompanied by significant changes in the size of species.

While advances have been made in understanding phenology and spatial redistribution of species over recent decades in relation to climate change, little progress has been made in determining how and to what extent body size is changing in the field over longer temporal timescales (several years to decades). Only a small number of studies have isolated the importance of temperature in causing major shifts in size in natural communities over time, including fish species in the North Pacific and North Sea (Cheung *et al.,* 2013; Baudron *et al.*, 2014). This is surprising given that these long-term responses have the greatest ecological relevance to predicting future variation in body size with climate change. As well as plasticity, long-term body size change is also likely to be driven by adaptive evolution; clearly, we cannot rely solely on laboratory estimates to predict future shifts in size. Therefore, describing body size changes over decades in natural populations is also a critical next step in understanding natural responses, and importantly, will increase predictive power. For example, continuous environmental monitoring, particularly in oceanic ecosystems, has produced a wealth of time series data and preserved biological samples. Whilst these are often used to investigate changes in community abundance and species composition, little focus has been given to quantifying body size changes of species. Revisiting these historic samples and data collections provides an opportunity to investigate long-term changes in the body size of aquatic species, such as zooplankton, and relate these to records of environmental conditions.

In this thesis we have identified parallels between phenotypically plastic size responses to temperature measured in the laboratory and changes observed in the field across latitudes and seasonally over an annual cycle. This suggests that these widespread body size phenomena may be driven by similar selective pressures, and that temperature plays a pivotal role in influencing size responses in the field, despite a number of confounding factors that can also affect body size. We have also identified important traits associated with variation in the strength and direction of these responses. At a time when the World Meteorological Organization declared 2016 as the year that made history, with record global temperatures, unabated ocean heat and extreme weather conditions set to continue, there has never been a more urgent need to quantify, understand, predict and develop strategies to deal with warming-induced changes in body size.

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LETTER

Temperature-size responses match latitudinal-size clines in arthropods, revealing critical differences between aquatic and terrestrial species

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Abstract

Two major intraspecific patterns of adult size variation are plastic temperature-size (T-S) responses and latitude-size (L-S) clines. Yet, the degree to which these co-vary and share explanatory mechanisms has not been systematically evaluated. We present the largest quantitative comparison of these gradients to date, and find that their direction and magnitude co-vary among 12 arthropod orders ($r^2 = 0.72$). Body size in aquatic species generally reduces with both warming and decreasing latitude, whereas terrestrial species have much reduced and even opposite gradients. These patterns support the prediction that oxygen limitation is a major controlling factor in water, but not in air. Furthermore, voltinism explains much of the variation in T-S and L-S patterns in terrestrial but not aquatic species. While body size decreases with warming and with decreasing latitude in multivoltine terrestrial arthropods, size increases on average in univoltine species, consistent with predictions from size vs. season-length trade-offs.

Keywords

Body size, oxygen availability, phenotypic plasticity, voltinism.

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INTRODUCTION

Body size is a master trait that strongly relates to individual fitness, and has profound physiological and ecological consequences (Hildrew *et al.* 2007). Adult size in ectotherms commonly co-varies with rearing temperature under controlled laboratory conditions (Atkinson 1994; Forster *et al.* 2011), can vary on a seasonal basis in multivoltine species (Hirst *et al.* 1999; Kari & Huey 2000; Sun *et al.* 2013), and is modified along latitudinal and altitudinal gradients (Blanckenhorn & Demont 2004; Chown & Gaston 2010). Yet, the correlation between these size responses and clines has never been systematically quantified. It is important that we quantify the degree to which body size responses to experimental temperature match geographical size clines, to determine how these responses differ between environments and identify the likely causative factor(s). This will aid in predicting how size will respond to environmental change (Daufresne *et al.* 2009), which is critical given global warming trends and latitude- and altitude-dependent shifts in temperature and season length (IPCC 2014).

The temperature-size rule (TSR) describes the phenotypically plastic response in which size at maturity is inversely related to temperature experienced during ontogeny (Atkinson 1994). Commonly, ectotherms reared at cooler temperatures accumulate mass at a slower rate, but develop to adulthood at a much slower rate than those reared in the warm, thereby achieving a larger adult size (Atkinson 1994; van der Have & de Jong 1996; Forster & Hirst 2012). Proximally, this effect can arise from a difference in sensitivities of growth and development rates to temperature (Walters & Hassall 2006; Forster & Hirst 2012). The TSR has been observed across a diverse range of ectotherms, including single-celled and multicellular species, invertebrates and vertebrates (Atkinson 1994; Atkinson & Sibly 1997; Atkinson *et al.* 2003; Forster *et al.* 2012, 2013). Temperature-size (T-S) responses of organisms are typically examined under controlled laboratory conditions, with food supplied *ad libitum*, as nourishment also impacts size at maturity (Diamond & Kingsolver 2010).

In the field a relationship between latitude and body size has also been described, known as Bergmann's Rule (Bergmann 1847). Though originally proposed as an interspecific phenomenon in which larger species of endotherm tend to be found at higher, colder latitudes (Meiri & Dayan 2003), the terms 'Bergmann cline' (an increase in size with latitude) and 'converse Bergmann cline' (a decrease in size with latitude) are typically used to describe both inter- and intraspecific latitudinal-size clines in endotherms and ectotherms. Here, we focus specifically on intraspecific latitudinal adult size (L-S) clines in ectotherms, to enable appropriate comparisons with T-S responses.

T-S responses and L-S clines may co-vary despite the fact that adult size can be influenced not just by environmental temperature, but also by season length, productivity, and mortality (Blanckenhorn & Demont 2004; Chown & Gaston 2010), and may involve genetic differences, somatic plasticity

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or a combination of factors. Our objective is to measure the strength of association between T-S and L-S gradients and their sign (positive or negative association) across species and groups at higher taxonomic ranks; this is more powerful than just indicating whether they occur in the same direction for particular species (Kingsolver & Huey 2008). Crucially, we aim to use this correlative approach to test the predictions of two major mechanistic explanations for T-S and L-S patterns; the oxygen hypothesis and the optimal resource allocation model (outlined below). We limit our study to arthropods to allow an examination of size patterns and their drivers within a single extensively studied phylum with a related bauplan, and which has huge ecological and economic importance (Klein *et al.* 2007; Richardson 2008).

The strength and sign of T-S responses relate strongly to whether organisms breathe air or water and to species body size, supporting the 'oxygen hypothesis' (Woods 1999; Atkinson *et al.* 2006) – the idea that more costly uptake of oxygen in water and the pressures that large bodies face to maintain aerobic scope in the warm plays a dominant role in determining mature size (Forster *et al.* 2012). In comparison, major patterns in L-S clines of ectotherms have been related to season length, voltinism and temperature. Despite the relatively large number of studies that attempt to explain L-S clines, few predict differences in the strength and sign of this gradient between terrestrial and aquatic environments (Chown & Gaston 2010). One model postulated that simple metabolic constraints in water would result in a stronger Bergmann cline in aquatic than terrestrial species (Makarieva *et al.* 2005), but so far there has been no empirical test of this difference. We address this issue empirically here, testing the prediction that oxygen availability in water is a major mechanistic determinant of both T-S and L-S gradients.

The optimal resource allocation model of Kozlowski *et al.* (2004) suggests that changes in season length across latitudinal gradients, and variation in the optimal trade-off between growth and reproductive investment among univoltine and multivoltine species, can explain why we observe both Bergmann and converse-Bergmann clines. One prediction is that univoltine species could take advantage of a longer growing season at lower latitudes by developing to a larger adult size, and would therefore exhibit a converse-Bergmann cline. Thus, we also aim to investigate whether differences in L-S gradients reflect differences in voltinism, and to what extent these patterns are also seen in laboratory T-S responses. If a close match between T-S and L-S gradients is observed, it would provide further evidence to suggest that both are driven by the same selective pressures.

Two opposing claims have been made of the extent to which T-S responses differ between environments, and depend on species body size. A meta-analysis of 110 metazoan species, including fish, amphibians and a range of invertebrates, showed the T-S response of aquatic organisms to be significantly greater than in terrestrial organisms, and that the slope of the response became more negative with increased species body mass in aquatic organisms, but less negative in terrestrial organisms (Forster *et al.* 2012). This suggests a major difference in T-S responses between terrestrial and aquatic ectotherms, with a proposed explanation based on the oxygen hypothesis (Woods 1999; Atkinson *et al.* 2006), rather than alternative hypotheses also examined by Forster *et al.* (2012). In contrast, a meta-analysis focussing exclusively on arthropods found that habitat type had no significant effect on the magnitude of the T-S response (Klok & Harrison 2013). Furthermore, this later study showed that smaller individuals, regardless of habitat type, exhibited a more negative T-S response than larger individuals in both terrestrial and aquatic species. Klok & Harrison (2013) proposed that differences between their findings and those of Forster *et al.* (2012) may be due to the latter pooling different taxonomic groups, including large aquatic vertebrates. As the two data sets were largely independent, a more comprehensive, appropriately screened, data set can be constructed, which draws on both sources plus additional data, in order to resolve this issue.

The objectives of this study are therefore to: (1) establish whether there is a difference between aquatic and terrestrial arthropods in the magnitude of T-S responses and L-S clines, (2) determine the degree to which the T-S and L-S gradients co-vary in sign and relative magnitude, and (3) examine the degree to which both of these size relationships can be explained by major environmental differences, voltinism and species body size.

METHODS

Temperature-size response

Published data compilations of Forster *et al.* (2012) and Klok & Harrison (2013) were revisited to obtain a single comprehensive arthropod data set. Rather than rely upon either set of T-S responses or body sizes, we obtained the original data ourselves in this new compilation, adding more data by searching the Institute for Scientific Information (ISI) Web of Knowledge and from references cited in other publications.

Studies were systematically screened to include only laboratory studies where individuals were reared at a range of constant temperatures, with food concentrations at or above saturation, to remove the confounding impact of food limitation. Only adult size measurements were used for analysis, except in a small minority of cases where pupal size was considered to be a reliable correlate of size at maturity. The minimum period of acclimation for the inclusion of adult size data was set so that only individuals that were raised from egg or first larval stage were included. Adult data were collected as lengths, volumes, and dry, wet or carbon mass. These measurements were subsequently converted to dry mass (mg) using intraspecific regressions. Where these were unobtainable, regressions for closely related species, and very occasionally more general interspecific regressions, were used. All data and conversions are detailed in our Data S1.

We used Akaike's information criterion (AIC) to compare linear vs. quadratic and also allometric vs. exponential equation forms, to determine which model best explained the response of adult body mass to rearing temperature/latitude. An exponential form was favoured as it offered the advantage of both the highest mean Akaike weights (w_i) and percentage best fit when compared with allometric for both L-S and T-S gradients. Furthermore, the exponential form allowed a clear best model choice for both response types; something neither linear nor quadratic achieved (see Appendix S1). The species-specific slopes of the natural log (ln) of the dry mass vs. temperature were then transformed into percentage change in dry mass per degree Celsius, using the formula $(\exp^{(\mathrm{slope})} - 1)*100 = \%$ change in mass per °C (Forster *et al.* 2012). A negative percentage indicates a decrease in size with increasing temperature (following the TSR) and a positive percentage an increase in size (converse-TSR). This value represents the species-specific T-S response and was used as the dependent variable. Size responses from multiple studies of a single species were combined into a simple mean to generate single species-specific values. The effect of environment type (marine, freshwater, terrestrial), species body mass (adult body mass at 20 °C calculated using species-specific slopes) and median rearing temperature were incorporated into a generalised linear mixed model (GLMM), in which four levels of taxonomic classification (subphylum, class, order, family) were incorporated as nested (hierarchical) random effects to control for phylogeny. In the graphs we combine species from different orders within the sub-class Copepoda, to reflect their phylogenetic relatedness and ecological similarity (note that our statistical approaches still consider the orders separately). We allowed for the interaction of all three parameters (i.e. environment type, species body mass, median rearing temperature), and best-fit models were derived using AIC (see Appendix S2).

Latitudinal-size clines

To describe species-specific L-S clines we obtained published field measurements of individual adult size from a range of latitudes. Laboratory studies in which individuals from different latitudes were reared at different temperatures were excluded. We followed the conventions applied to our T-S data set; specifically, converting lengths or masses to dry mass (mg), and transforming species-specific slopes of ln dry mass vs. latitude into percentage change in body mass per degree of latitude. A positive percentage indicates an increase in adult size (Bergmann's cline) and a negative percentage a decrease in size (converse-Bergmann's cline) with increasing latitude.

Environment type and species body mass (adult body mass at 50° latitude calculated using species-specific slopes) were incorporated into a GLMM, in which both parameters were allowed to interact and four levels of taxonomic classification were incorporated as nested (hierarchical) random effects. Best-fit models were determined using AIC. Mean L-S and T-S gradients were calculated for the five aquatic (Amphipoda, Copepoda, Isopoda, Odonata and Mysida) and seven terrestrial groups (Blattodea, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera and Orthoptera). Taxon- and species-specific L-S values were compared against their respective T-S values using Reduced Major Axis regressions (RMA) and tested for a significant correlation.

The terrestrial arthropods were categorised by voltinism (uni-, bi- or multivoltine). Multivoltine defined here as > 2, bivoltine as 2 and univoltine as 1 generation year^{-1}. In aquatic arthropods voltinism did not relate significantly to any of the observed variation in L-S clines ($F_{3,8} = 0.18$, $P = 0.91$) or T-S responses ($F_{2,39} = 1.71$, $P = 0.19$), and so was excluded from further analysis for these species. Voltinism of each species was determined from the literature that provided the size-gradient data or from other pertinent literature sources. For L-S clines, seven species described in the original literature as switching voltinism or altering generation number with latitude were excluded, as this can obscure within-generation clines. In univoltine and bivoltine species we only considered those species for which we found no evidence that they switch generation number (e.g. if they are regarded as obligatorily univoltine). We note the potential for biased recording within the literature; a switch in voltinism is more likely to be reported for univoltine and bivoltine species for which the change is clearly defined, whilst for multiple generational species any change may go unreported. However, this bias should not substantially affect our analysis, as a change in voltinism is more diluted in multivoltine species, so is less likely to impose major differences in season-length constraints. Voltinism and body mass were incorporated into a GLMM following the same conventions previously outlined, and AIC was used to determine parameter importance. A *t*-test was used to test for a significant difference between univoltine and multivoltine terrestrial species, and RMA regression analysis was used to test for a significant correlation between voltinism and body mass.

RESULTS

Our meta-analysis includes T-S responses [% change in dry mass (DM) °C^{-1}] for 114 arthropod species (aquatic, $n = 45$; terrestrial, $n = 69$); a 36% increase on Klok & Harrison's (2013) sample size (including a 60% increase in the number of marine species considered), and a 25% increase in the number of arthropod species sampled in Forster *et al.* (2012). There was no significant difference in the T-S responses of marine and freshwater species (*t*-test; $t_{39} = 1.30$, $P > 0.05$). Species-specific T-S responses had negative slopes in 93% of aquatic arthropods, and 70% in terrestrial. There were highly significant differences in the strength of the T-S response among taxonomic orders ($F_{17,98} = 4.70$, $P < 0.001$) (Fig 1a).

We found that environment type (aquatic and terrestrial) had a significant effect on species-specific % change in mass °C^{-1} with warming ($F_{1,114} = 29.72$, $P < 0.001$) (Fig. 1a). The mean aquatic T-S response was −2.95% body mass °C^{-1} (± 0.76; 95% CI), whilst for terrestrial species it was −0.35% body mass °C^{-1} (± 0.59; 95% CI), representing a ~ 8½-fold difference in the mean response between environments. The significant difference was similarly observed within the Diptera, which contained aquatic- and terrestrial-developing species (*t*-test; $t_{21} = -2.46$, $P = 0.02$). Aquatic-developing Diptera had a mean T-S response of −2.54% (± 1.27; 95% CI), whilst those in air had a mean response of −0.95% (± 0.53 95% CI).

We obtained L-S clines for 44 arthropod species (aquatic, $n = 15$; terrestrial, $n = 29$). As with the T-S response, there is a significant difference in the strength of the L-S clines between aquatic and terrestrial species ($F_{1,40} = 34.05$, $P < 0.001$) (Fig. 1b), with the former showing a much greater increase in size with latitude (and hence with average temperature decline, as experienced by the shallow-water animals

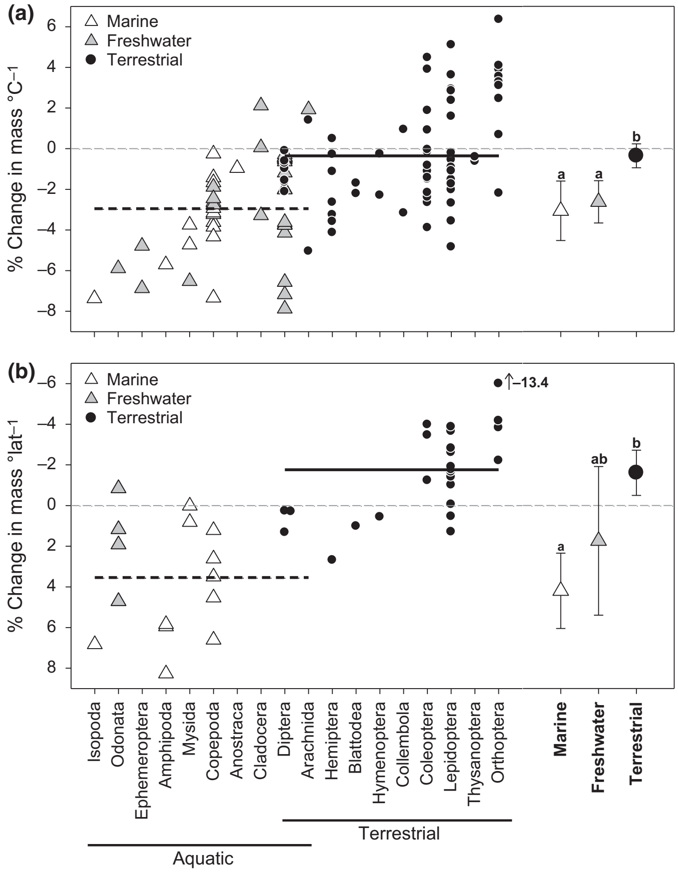

Figure 1 (a) Comparison of the % change in body mass per °C in aquatic (n = 45, mean −2.95% °C^{-1}; dashed line) and terrestrial (n = 71, mean −0.35% °C^{-1}; solid line) arthropod species, categorised by order. Environment type (aquatic and terrestrial) has a significant effect on % change in mass per °C ($F_{1,114}$ = 29.72, $P < 0.001$). (b) Comparison of the % change in body mass per °latitude in aquatic (n = 15, mean 3.54% °lat^{-1}; dashed line) and terrestrial (n = 29, mean −1.61% °lat^{-1}; solid line) arthropod species, categorised by order. Environment type (aquatic and terrestrial) has a significant effect on % change in mass per °latitude ($F_{1,40}$ = 34.05, $P < 0.001$). In both panels mean gradient ±95% CI are shown for marine, freshwater and terrestrial arthropod species. Different letters above data points indicate significant differences, whilst shared letters indicate no significant difference. Note the reverse scale on the y-axis in (b).

included here). While the mean Bergmann cline in aquatic species is 3.54% body mass °lat^{-1} (± 1.55; 95% CI), for terrestrial species a converse-Bergmann cline was observed, with a mean of −1.61% body mass °lat^{-1} (± 1.11; 95% CI).

As reported in Forster *et al.* (2012), and in contrast to Klok & Harrison (2013), we find overwhelming support for the interactive effect of environment type and mass (mean adult or pupal DM at 20 °C) on the strength of the T-S response, with this interaction firmly favoured by our AIC model competition framework (w_i = 0.90. see Appendix S2). Specifically, the responses of aquatic and terrestrial arthropods diverged with increasing species size; terrestrial arthropods exhibited a significant positive regression ($F_{1,66}$ = 9.28, P = 0.003, r^2 = 0.11), contrasting with a significant negative regression in aquatic species ($F_{1,43}$ = 5.40, P = 0.02, r^2 = 0.09) (Fig. 2a). L-S clines show a close similarity: as mean species body size increases, terrestrial species have a significantly stronger negative (converse Bergmann) cline ($F_{1,27}$ = 4.56, P = 0.04, r^2 = 0.11), while the L-S clines of terrestrial and aquatic arthropods diverge with increasing species size. Thus, the effect of species body mass on the strength of the cline is significantly dependent on environment type ($F_{1,40}$ = 5.16, P = 0.03).

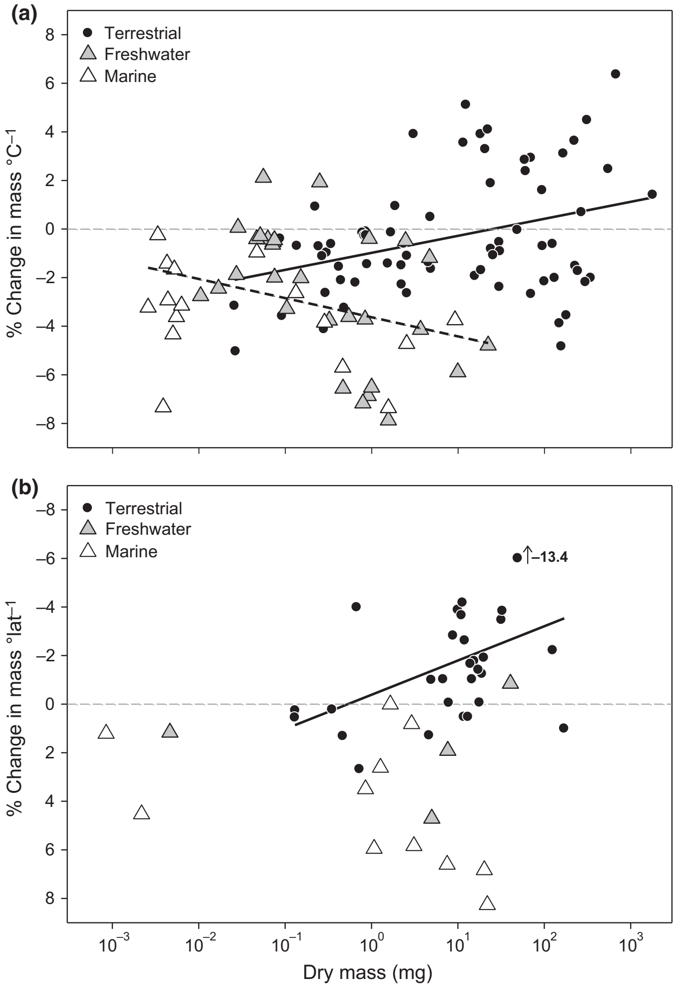

Figure 2 (a) Species-specific temperature-size (T-S) responses (% change in body mass per °C) expressed as a function of organism size (dry mass) at 20 °C in aquatic and terrestrial arthropods. The effect of body size on the T-S response is dependent on environment ($F_{1,112}$ = 13.41, $P < 0.001$). Aquatic arthropods exhibit a significant negative (dashed line, $F_{1,43}$ = 5.40, P = 0.02, r^2 = 0.09), and terrestrial arthropods a significant positive regression (solid line $F_{1,69}$ = 9.28, P = 0.003, r^2 = 0.11). (b) Species-specific latitudinal-size (L-S) clines (% change in body mass per °lat) expressed as a function of organism size (dry mass) at 50°lat in aquatic and terrestrial arthropods. The effect of body size on the L-S response of aquatic and terrestrial arthropods is significantly dependent on environment ($F_{1,40}$ = 5.16, P = 0.03). Aquatic arthropods exhibit a non-significant regression ($F_{1,13}$ = 0.90, P = 0.36, r^2 = 0.06); terrestrial arthropods exhibit a significant positive regression (solid line, $F_{1,27}$ = 4.56, P = 0.04, r^2 = 0.11). Note the reverse scale on the y-axis in (b).

In contrast with the effects of voltinism on aquatic species, voltinism significantly affects the T-S response in terrestrial arthropods ($F_{3,61}$ = 5.08, P = 0.003; Fig 3a). Indeed, there is a

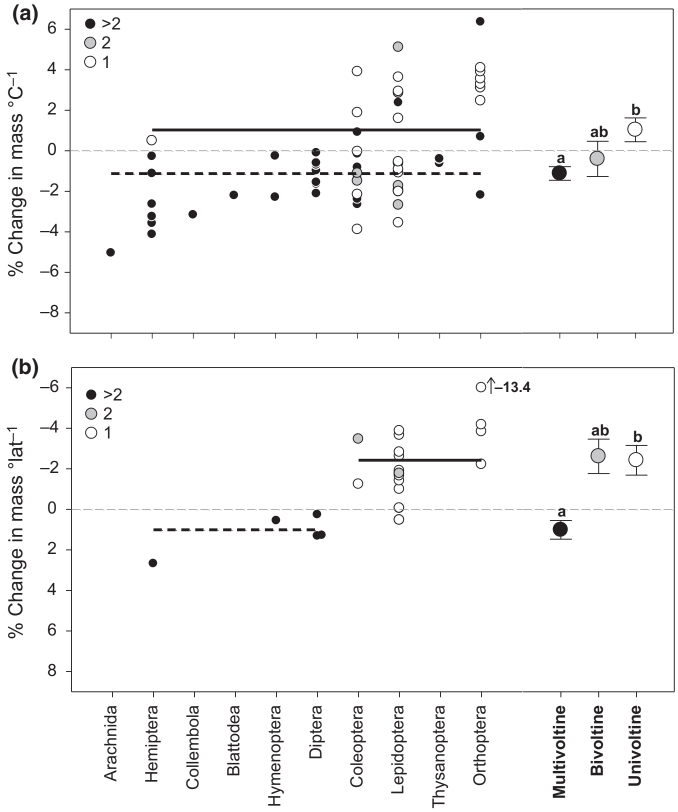

Figure 3 (a) Comparison of the % change in body mass per °C in multivoltine (mean, −1.12% °C^{-1}; dashed line), bivoltine (mean, −0.41% °C^{-1}) and univoltine (mean, 1.03% °C^{-1}; solid line) terrestrial species, categorised by taxonomic order. There is a significant difference between univoltine and multivoltine species in the size and sign of T-S responses (*t*-test; $t_{31} = 3.18$, $P = 0.003$). (b) Comparison of the % change in body mass per °lat in multivoltine (mean, 1.01% °lat^{-1}; dashed line), bivoltine (mean, −2.62% °lat^{-1}) and univoltine (mean, −2.42% °lat^{-1}; solid line) terrestrial species. There is a significant difference between univoltine and multivoltine species in the size and sign of latitude-size responses ($t_{20} = -3.96$, $P < 0.001$). In both panels mean ± SE are given for multivoltine (black circle), bivoltine (grey circle) and univoltine (open circle) arthropod species. Different letters above data points indicate significant differences, whilst shared letters indicate no significant difference. Note the reverse *y*-axis scale in panel (b).

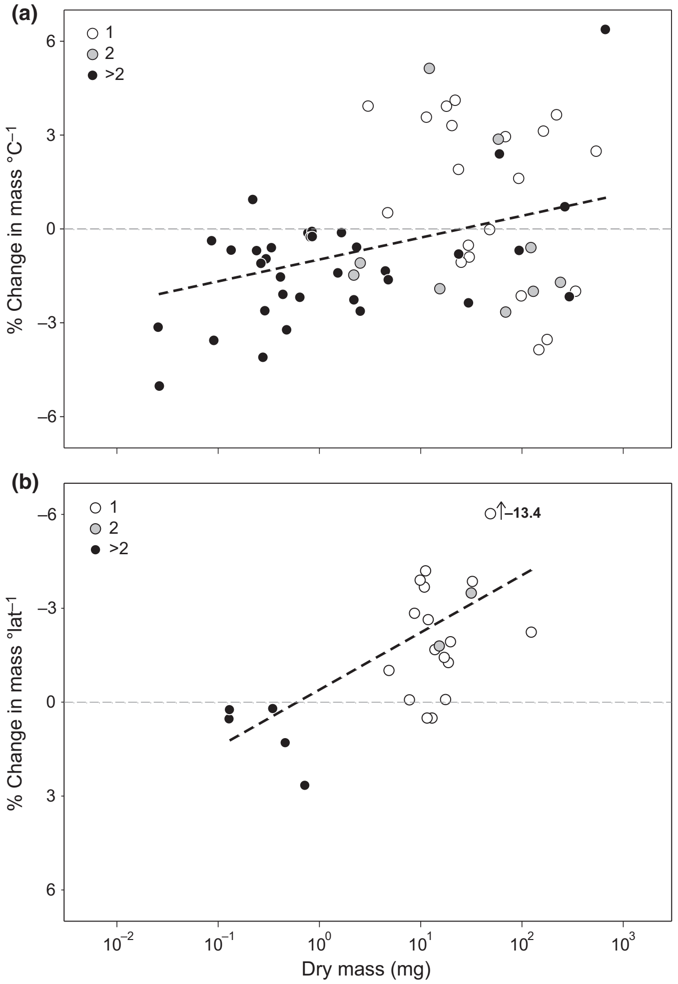

Figure 4 (a) Species-specific temperature-size (T-S) responses (% change in body mass per °C) expressed as a function of organism size (dry mass) at 20 °C, and (b) species-specific latitudinal-size (L-S) clines (% change in body mass per °lat) expressed as a function of organism size (dry mass) at 50°lat, categorized by voltinism. Voltinism is significantly correlated with mass in both the T-S (Reduced Major Axis regressions, RMA; $P < 0.001$, $r^2 = 0.33$) and L-S (RMA regression; $P < 0.001$, $r^2 = 0.72$) data sets, and hence may explain the body mass dependence of both T-S and L-S gradients in terrestrial arthropods. When considered together, voltinism has a greater relative Akaike weight than mass, suggesting it is a more powerful response predictor. Note the reverse scale on the *y*-axis in (b).

significant difference in the gradient between univoltine and multivoltine terrestrial species for both T-S responses (*t*-test; $t_{31} = 3.18$, $P = 0.003$; Fig. 3a) and L-S clines ($t_{20} = -3.96$, $P < 0.001$; Fig. 3b), with different degrees of voltinism producing opposing gradient directions. Univoltine species had a mean T-S and L-S gradient of 1.03% body mass °C^{-1} (± 1.23; 95% CI) and −2.42% body mass °lat^{-1} (± 1.64; 95% CI) respectively, whilst multivoltine species had a mean T-S and L-S gradient of −1.12% body mass °C^{-1} (± 0.68; 95% CI) and 1.01% body mass °lat^{-1} (± 0.42; 95% CI) respectively. Voltinism is significantly correlated with species adult mass in both the T-S (RMA regression; $P < 0.001$, $r^2 = 0.33$; Fig. 4a) and L-S (RMA regression; $P < 0.001$, $r^2 = 0.72$; Fig. 4b) data sets. Voltinism was a more powerful predictor of response size (T-S $w_i = 1.00$; L-S $w_i = 0.63$) than was species adult mass in terrestrial species (T-S $w_i = 0.59$; L-S $w_i = 0.20$), as inferred from the relative Akaike weights of each parameter. Though species mass and voltinism are correlated, this finding suggests that voltinism may be more important than mass *per se* in terrestrial size gradients. Generally, larger terrestrial species are univoltine and exhibit a positive T-S response and a converse-Bergmann cline, whilst smaller species are multivoltine and follow the TSR (a negative T-S response) and a typical Bergmann cline.

If L-S and T-S gradients are driven by similar factors then we would expect a negative relationship between the two, given the general decline in temperature away from the equator. Indeed, there is a significant negative correlation between T-S and L-S gradients across both orders and species ($r^2 = 0.72$, $n = 12$, $P < 0.001$; and $r^2 = 0.73$, $n = 6$, $P = 0.015$ respectively) despite the small number of species-specific data

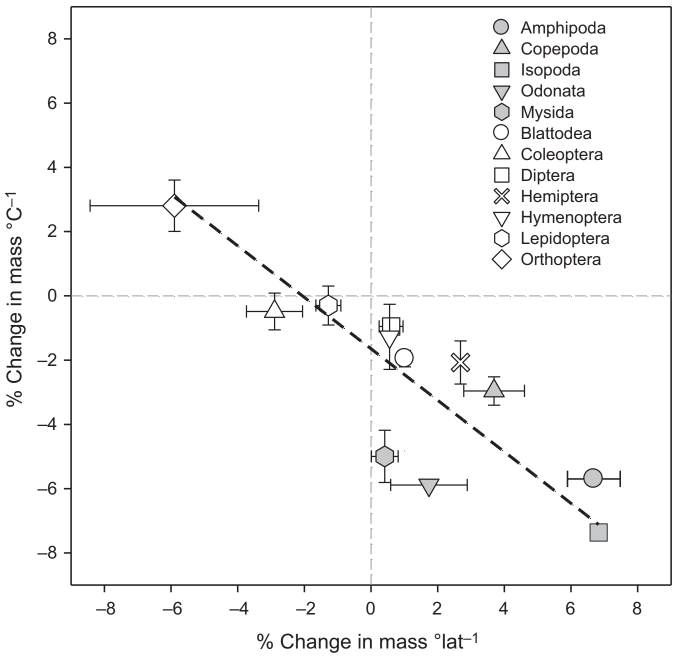

Figure 5 Temperature-size (T-S) responses (% change in body mass per °C ± SE) vs. latitudinal-size (L-S) clines (% change in body mass °lat^{-1} ± SE) for specific taxa. There is a significant negative correlation between T-S and L-S gradients (Reduced Major Axis regressions, RMA; $P < 0.001$, $r^2 = 0.72$; $y = -1.65–0.80x$). On average, those taxa that exhibit the strongest reduction in body size with increasing temperature show the greatest decrease in size with decreasing latitude, and *vice versa.*

(see Appendix S3). Across 10 of the 12 orders included here, those which on average exhibit a negative T-S response show a positive L-S cline, and *vice versa* (Fig. 5), the exceptions being Lepidoptera and Coleoptera, in which both T-S and L-S are slightly negative. The RMA regression passes close to the zero-zero intercept of the two axes, further indicating a similarity in these gradients. When analysed independently, terrestrial arthropods still show a significant negative correlation between order-specific T-S and L-S gradients ($P = 0.002$, $r^2 = 0.88$, $n = 7$). Therefore, the significance of the regression overall is not just driven by the stark difference in size responses between aquatic and terrestrial orders.

DISCUSSION

We find significant differences between T-S responses of aquatic and terrestrial arthropods (Fig. 1a), hence supporting the environment dependence observed by Forster *et al.* (2012) in ectotherms generally. Aquatic arthropods show a significantly stronger negative T-S response with warming than do terrestrial, and follow the TSR in over 90% of cases. These environmental differences are further supported within the order Diptera, in which species with aquatic larval and juvenile stages had a significantly stronger negative T-S response than terrestrial-developing species.

We present compelling evidence for a similarity between T-S responses and L-S clines, observing a significant difference in the strength and direction of T-S and L-S gradients between environments. Individual body size typically declines with increasing latitude in many terrestrial species, but increases with increasing latitude in most aquatic species considered here, matching the general trends in T-S responses (Fig. 1b). All the aquatic orders show on average both a negative T-S response and a positive L-S cline. Similar covariation between magnitudes of T-S and L-S associations are found in the terrestrial orders, with Orthoptera showing the most extreme positive T-S responses and negative L-S clines (Fig. 5). The overall negative relationship between these gradients suggests a general ability to predict one from the other within arthropods, and that the driving forces that dictate much of the phenotypically plastic size responses to temperature in the laboratory may also shape the magnitude and sign of latitudinal-size changes observed in the field.

This covariation between T-S and L-S gradients is remarkably robust, given the range of confounding variables that can influence L-S clines (Shelomi 2012), including altitudinal variation, habitat variability (local climate, food availability, natural enemies), the variable match between mean temperature and latitude or season length, and the geographical extent of data for each species. Previously, the proportion of the total range has been shown to influence the apparent shape of the L-S cline (Hassall 2013). However, having tested a small subset of our data ($n = 8$), when we compare the best-fit response (linear vs. quadratic) with the proportion of range sampled (data not presented), we find no apparent pattern. Certainly genetic variation can determine body size differences between populations. Evidence for genetic influence on L-S clines includes laboratory studies of species collected along a latitudinal gradient and reared under constant temperature and food conditions, which still demonstrate clinal variation in body size (James *et al.* 1995; Land *et al.* 1999). Nonetheless, even though body size clines in the field may be influenced partly by genetic differences as well as phenotypic plasticity, the difference between terrestrial and aquatic environments in both T-S and L-S gradients suggests that there may be consistent differences in temperature-related selection pressures on body size between aquatic and terrestrial environments.

The effect of species body mass on both T-S and L-S gradients is significantly dependent on environment type (Fig. 2). In contrast to Klok & Harrison (2013), but in concordance with Forster *et al.* (2012), T-S responses became significantly more negative with increasing species body mass in aquatic species, while terrestrial arthropods, which are dominated numerically by the insects – both globally (Zhang 2013) and in our data set – exhibited a significant positive regression between T-S response and species body mass. The divergence culminated in a ~ 16-fold difference in the strength of the T-S response between aquatic and terrestrial species of 10 mg dry body mass. We propose that the differences in our findings from Klok & Harrison (2013) in T-S patterns may be attributed to their inclusion of data from studies that did not confirm saturating food or controlled temperature conditions. To reduce confounding effects of uncontrolled conditions, and of food limitation, which can reverse the direction of the T-S response (Diamond & Kingsolver 2010), we excluded studies in which conditions were not controlled, including those with no evidence that food supply was saturating. For example, unlike Klok & Harrison (2013) we excluded the study of

Babin-Fenske *et al.* (2008) within the T-S data set, as the size of field-collected museum specimens were related to their field temperatures. Similarly, we excluded the study of Sweeney & Vannote (1978) on species of Ephemeroptera: this study had a large influence on Klok & Harrison's (2013) conclusion, but gave no indication of whether food was provided to saturation or *ad libitum*. Including Sweeney & Vannote's (1978) data in our set has a significant outcome on the species body mass dependence of the T-S response in aquatic species, resulting in the negative regression becoming non-significant, though the inclusion of these data do not affect the overall significant difference in T-S responses between environments. Finally, while we converted all sizes to mass, Klok & Harrison (2013) used various metrics of size, which were then normalised assuming isomorphism (i.e. mass was proportional to lengths^3); this may be problematic as not all arthropods grow isomorphically (Benke *et al.* 1999; Hirst 2012).

Although often assumed, it is challenging to establish whether body size variation with latitude and temperature is adaptive, and indeed this has rarely been tested. Variation in body size could be the product of environmental stress or genetic drift, both of which can result in maladaptive phenotypic changes (Merilä & Hendry 2014). Given these caveats, adaptive explanations are typically considered for T-S and L-S gradients because important fitness costs and benefits are associated with them (Angilletta *et al.* 2004; Kingsolver & Huey 2008). Furthermore, despite profound differences in the proximate mechanisms driving them, the commonality of T-S responses in unicellular and multicellular organisms (Forster *et al.* 2011), and similarity between different insect species (Ghosh *et al.* 2013), suggests they are most likely adaptive responses.

Of the models proposed to explain intraspecific geographical trends in body size, some can be applied more broadly than others (Chown & Gaston 2010). For example, the biophysical model of van der Have & de Jong (1996), often considered a proximate physiological explanation for the TSR, and the starvation resistance model (Cushman *et al.* 1993), which proposes that species at higher latitudes grow to a larger size to withstand extended periods of food deprivation, cannot account for converse-Bergmann clines, of which there are numerous examples in terrestrial arthropods (Blanckenhorn & Demont 2004; Chown & Gaston 2010) (see Figs 1 and 2). Furthermore, we demonstrate effects of environment type (aquatic, terrestrial) and voltinism on L-S gradients, which are not predicted by either of these models. We therefore explore alternative explanations which can account for the variation we observe in the strength and direction of L-S clines among taxa and environments.

We find that differences in voltinism can provide a mechanistic explanation for the dependence of T-S and L-S gradients on species body mass in terrestrial (but not aquatic) arthropods. Voltinism is significantly correlated with mass in terrestrial species (Fig. 4), with larger species often having a single generation and smaller species producing multiple generations annually. As predicted, on average, larger univoltine terrestrial species have a positive T-S response and exhibit a converse-Bergmann cline, whilst smaller multivoltine species tend to conform to the TSR and Bergmann's Rule (Fig. 3). The variation in body size of terrestrial arthropods, both at different temperatures under controlled laboratory conditions, and along latitudinal clines in the field, may thus reflect an evolutionary adaptation to changing season length. More specifically, at lower latitudes where season length is longest, a large obligatorily univoltine species could take advantage of a longer growing season by maturing to a bigger adult size. Conversely, in the same environment, a smaller shorter-lived multivoltine species may maximise fitness by maturing earlier and at a smaller size, thus decreasing generation time and increasing the number of generations per year. As shown by Roff (1980), there is a point at which the fitness advantage of large size is outweighed by the advantage of adding an additional generation. These differences in voltinism describe well both the species body mass dependence and order-specific variation we observe in terrestrial T-S responses and L-S clines. Interestingly, Odonata, which develop in water but emerge into air and are commonly univoltine or even semivoltine, show a weaker positive L-S response than most other aquatic orders considered here (Fig. 5). Their semi-aquatic life history would make for an intriguing case study to determine how the forces dictating latitude and temperature body size gradients in aquatic and terrestrial environments interact. Indeed, Hassall *et al.* (2014) suggested that the typical Bergmann clines observed in Odonata may arise because structural growth occurs during the aquatic larval stage, supporting oxygen limitation as the overriding explanation for body size determination in this order. Unlike most univoltine terrestrial species, one univoltine odonate species, which showed no evidence of a switch in voltinism with latitude, exhibited a significant Bergmann cline and a typical negative T-S response (Hassall 2013). Unfortunately, available data are too sparse to evaluate whether voltinism plays a significant role in determining variation in the T-S and L-S gradients between aquatic insects with a terrestrial adult phase. More data on semi-aquatic insects would help determine whether major effects of voltinism on T-S and L-S gradients extend generally to these species too.

The strongly negative T-S responses and positive Bergmann-type clines in aquatic arthropods, especially in larger species, follow the prediction of the oxygen hypothesis (Woods 1999; Atkinson *et al.* 2006; Forster *et al.* 2012). Increasing latitude relates strongly to mean temperature (Sunday *et al.* 2011), and increased temperature increases metabolic demand, but results in a relatively much lower rate of increase in oxygen availability in water (Verberk *et al.* 2011). Makarieva *et al.* (2005) used similar reasoning to explain across-species patterns in maximum body size, highlighting that the largest aquatic poikilotherms are often found at higher latitudes towards the poles. Indeed, experimental studies have shown that oxygen limitation can impede growth in arthropods, such as amphipods (Rudolf & Or 2005), as well as other phyla including many fish species (Pauly 2010). Such limitation also predicts the species body mass dependence of both the T-S responses (Forster *et al.* 2012) and L-S clines, since larger species struggle most to meet their oxygen requirements, whilst no discernible effect can be found for voltinism, as larger univoltine aquatic species do not reduce or reverse their body size responses in comparison to multi-generational species. Our findings therefore support the mechanistic explanation that oxygen demand-supply constraints drive both strong negative T-S and strong positive L-S gradients within aquatic species (Woods 1999; Atkinson *et al.* 2006).

In the largest database of its kind to date on a single large phylum, the Arthropoda, we present compelling evidence of a correlation between phenotypically plastic responses to temperature, and body size clines in the field, therefore providing a conceptual unification of the TSR and Bergmann's Rule in ectotherms. Though our findings are correlative rather than the outcome of manipulative experiments, we observe clear differences in the strength and direction of T-S and L-S gradients between aquatic and terrestrial arthropods that match the predictions of adaptive models, supporting the importance of the oxygen hypothesis in aquatic ectotherms, and the effects of seasonal constraints and other possible advantages of large size in warm environments in terrestrial arthropods. Future research should aim to explore whether these same size patterns are evident in altitudinal and seasonal clines, and also in other phyla. The parallel patterns between T-S and L-S gradients suggest that the major selective pressures that produce L-S clines, by either genetic or phenotypically plastic variation, may also be the ones that produce T-S responses. Above all, we demonstrate the value of combining physiological and ecological perspectives in explaining major environmental patterns, and suggest that multi-disciplinary studies, which combine large-scale spatial and temporal trends and lower-level physiological variation, can better reveal macrophysiological patterns and their underlying mechanisms (Gaston *et al.* 2009).

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AUTHORSHIP

CH, AGH and DA designed the study and wrote the paper. CH collected the data and performed the meta-analysis.

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Seasonal body size reductions with warming covary with major body size gradients in arthropod species

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Major biological and biogeographical rules link body size variation with latitude or environmental temperature, and these rules are often studied in isolation. Within multivoltine species, seasonal temperature variation can cause substantial changes in adult body size, as subsequent generations experience different developmental conditions. Yet, unlike other size patterns, these common seasonal temperature–size gradients have never been collectively analysed. We undertake the largest analysis to date of seasonal temperature-size gradients in multivoltine arthropods, including 102 aquatic and terrestrial species from 71 global locations. Adult size declines in warmer seasons in 86% of the species examined. Aquatic species show approximately 2.5-fold greater reduction in size per °C of warming than terrestrial species, supporting the hypothesis that greater oxygen limitation in water than in air forces aquatic species to exhibit greater plasticity in body size with temperature. Total percentage change in size over the annual cycle appears relatively constant with annual temperature range but varies between environments, such that the overall size reduction in aquatic-developing species (approx. 31%) is almost threefold greater than in terrestrial species (approx. 11%). For the first time, we show that strong correlations exist between seasonal temperature–size gradients, laboratory responses and latitudinal–size clines, suggesting that these patterns share common drivers.

1. Introduction

Body size is a 'master trait', affecting vital rates (growth, survival, reproduction) and ecological processes ranging from individual performance (e.g. fitness) to ecosystem function (e.g. food web dynamics, productivity) [1–4]. Biologists have intensively studied body size variation for more than a century [5–8], including describing size clines over latitude and altitude in the field [9,10]. Populations grown under controlled laboratory conditions show strong associations between mature body size and temperature [11–13], and food [14]. These emergent body size patterns have been formalized into prominent biogeographical and biological rules, including Bergmann's rule (interspecific latitudinal clines: larger bodied species at higher, colder latitudes) [9], James' rule (intra-specific latitudinal clines: larger individuals at higher, colder latitudes) [15] and the temperature–size rule (TSR) (increased size at maturity when grown through ontogeny at decreased temperature) [11]. Furthermore, body size reduction has been described as the third universal response to climate warming [16].

The causes of intra-specific body size clines across latitudes can differ from those of size responses to ontogenetic temperature treatments in the laboratory. The former can be influenced not just by phenotypic plasticity, but also by genetic variation among geographical populations [17], as well as many biotic and abiotic factors that could confound the effects of temperature, such as voltinism, season length, food supply and natural enemies [18–22]. Despite these confounding

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factors, temperature–size (T-S) responses measured under controlled laboratory conditions and latitudinal–size (L-S) clines measured in the field significantly covary across taxonomic orders within the Arthropoda. Specifically, taxonomic orders whose species demonstrate particularly strong negative T-S responses (i.e. following the TSR) also show strong intraspecific declines in adult size at lower latitudes (i.e. following James' rule), whereas those with less negative T-S responses tend to show reduced or reversed L-S clines [13]. This covariation suggests that similar forces may be driving these important patterns.

It has been debated whether size responses are adaptive, or a maladaptive outcome of environmental stress or genetic drift [23], or simply a consequence of how constraints imposed by the architecture of the maturation mechanism may affect phenotypic outcomes of selection on body size, growth and development rate [24]. However, given the important influence of body size on vital rates and ecological processes, systematic size responses to temperature are often considered adaptive [25,26]. For example, variation in the direction of T-S responses and L-S clines has been attributed to differences in voltinism in terrestrial arthropods, likely an evolutionary adaptation to changing season length [13,19,27]. Striking differences in the T-S response also occur between environments; aquatic-developing species show greater reductions in adult size per °C of warming, and stronger reductions in size with decreasing latitude towards the equator, than do air-breathing species [12,13]. Oxygen availability, which includes both its concentration and diffusivity, is approximately 3×10^5 times lower in water than in air [28], and body size reduction with warming is thought to be an important mechanism by which aquatic species maintain aerobic scope when faced with increased metabolic rate at elevated temperatures [12,13,29]. Indeed, hypoxic conditions also commonly lead to reductions in size within species, both under natural conditions [30] and in laboratory manipulations, especially at warmer temperatures and/or larger body sizes [31].

Multivoltine ectotherms, which have more than one generation per year, can experience considerable differences in temperature, resources and suitable habitat between seasons, hence between generations. The effects of seasonal changes in temperature on optimum body sizes may, therefore, be easily confounded by other seasonally varying effects such as food, water, oxygen availability and mortality risks [18]. Nonetheless, seasonal body size variation commonly correlates strongly with changes in environmental temperature in a wide range of uni- and multicellular organisms, including bacteria [32], rotifers [33], copepods [34,35], cladocerans [36] and insects [37], examples of which are presented in electronic supplementary material, figure S1. Yet, despite the huge implications of environmental seasonality for global ecology, no broad exploration of seasonal size gradients has been performed to date. Such intra-annual shifts in size have important physiological, ecological and fitness consequences [18], and the magnitude and variation of such seasonal change across diverse taxa, and between environments, needs to be investigated (cf. with latitudinal and altitudinal descriptions [38,39]). Moreover, the question of whether the differences in body size gradients observed between environments and taxonomic orders, both in the laboratory and across latitudes, are also observed across seasons still remains unanswered. Improved understanding of size gradients across seasons will not only help to determine the ultimate causes of body size variation, but will also aid ecologists, including macro-ecologists, in understanding and predicting individual and community level responses to climate change [40]. This is critical given the link between decadal-scale changes in the body sizes of ectotherms and shifts in climate [41–43].

Our analysis focuses on the Arthropoda, which is the most taxonomically diverse and numerous phylum on the Earth [44], and which has huge ecological and economic importance [45,46]. This well-studied group also shares a common ancestry and a related body plan. Here we present, to our knowledge, the largest synthesis of seasonal T-S gradients in multivoltine arthropods to date, including those of marine, freshwater and terrestrial species. Following from the stronger observed laboratory T-S responses and L-S clines in aquatic-developing than terrestrial species [12,13], we predict that across seasons, species developing in water will also demonstrate a greater reduction in size per °C of warming than will species developing in air. We also assess the extent to which the seasonal T-S gradient depends on mean annual temperature, latitude and species body mass. Finally, we quantitatively compare seasonal T-S gradients with both T-S responses measured under controlled laboratory conditions and with L-S clines, to establish whether differences observed between environments and among taxa are consistent in these three major size gradients.

2. Material and methods

We searched the literature extensively using both the Web of Science database (http://apps.webofknowledge.com/) and Google Scholar for studies in which the adult body size of multivoltine arthropod species (greater than or equal to two generations per year) was assessed in nature on multiple occasions during an annual cycle. We used records for which we could quantify a change in body size that occurred over at least a three-month period. This criterion for data inclusion increased the likelihood of capturing variation in body size in different cohorts or generations. The primary search term combinations used were ('seasonal' OR 'temporal') AND 'body size' AND ('arthropod' OR '<insert taxonomic order>)' AND 'temperature'. We also identified related studies from reference lists in the papers we found, and sought further direction to key literature from relevant experts. Adult size data were collected as lengths, or dry, wet or carbon masses and subsequently standardized to dry mass (milligrams) using published intra-specific regressions and conversion factors (see electronic supplementary material, dataset S1). If regressions for the species were not available, regressions for closely related species, or more general interspecific regressions were used (in approx. 26% of cases). Taxonomic order and family were confirmed for each species using the World Registry of Marine Species [47] or the National Center for Biotechnology Information (NCBI) Taxonomy Database for freshwater and terrestrial species. In the case of planktonic species, to reduce potential sampling bias in the sizes of animals collected, only those studies in which the adults were sampled across the entire depth of the water column, or across most of the depth range of the species, were included. Maximum water sampling depth across all aquatic studies in our dataset was 125 m.

For each study included in our dataset, we derived species-specific slopes of ordinary least-squares (OLS) regressions between ln-transformed dry mass (milligrams) and environmental temperature at time of collection, using individuals of species as data points. We derived slopes for males and females separately wherever possible. This exponential function is overwhelmingly favoured for modelling seasonal T-S gradients, rather than linear, quadratic and allometric relationships, giving an Akaike

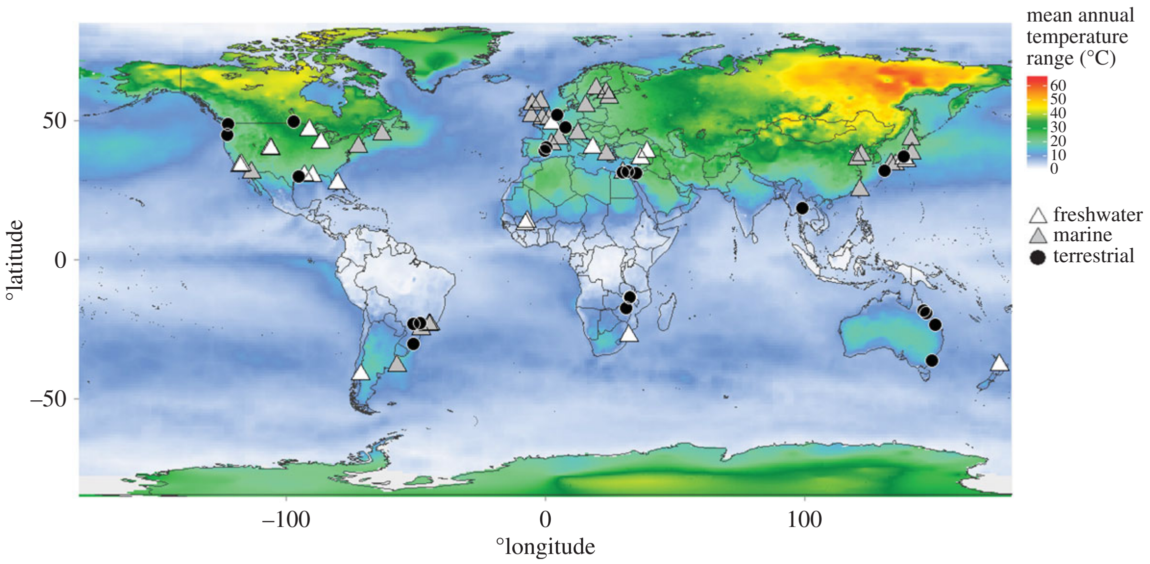

Figure 1. World map (equirectangular projection) indicating the location of studies ($n = 71$) from which seasonal T-S gradients were recorded, categorized by environment type. Colour gradient indicates mean annual temperature ranges. Sea surface temperature data were used for marine environments. Air surface temperature data were used for terrestrial and freshwater environments. Data sources are given in the Material and methods.

weight of 1 (see electronic supplementary material, table S1). The exponential function is also the best for fitting body size–temperature relationships under controlled laboratory conditions and for L-S clines, again judged using Akaike weights [13,48]. This common use of an exponential function allows us to easily compare all three of these size gradients. These 'seasonal T-S slopes' were also transformed into percentage change in dry mass per °C (hereby referred to as 'seasonal T-S gradients'), using the formula $(\exp^{(\mathrm{slope})} - 1) \times 100 = \%$ change in mass per °C [12]. A negative gradient shows a reduction in body size with increasing temperature, and hence follows the same trend as the TSR [11].

Where temperatures in a study were not reported for the entire year ($n = 19$ of 79), we used high-resolution global climate data to estimate mean annual temperature and annual temperature range (ATR) at each sampling location (from NOAA/OAR/ESRL PSD, Boulder, CO, USA), available online at http://www.esrl.noaa.gov/psd/. Specifically, we used weekly mean sea surface temperatures (SST) from 1989/12/31 to 2015/10/25 (year/month/day) (NOAA Optimum Interpolation Sea Surface Temperature dataset; 1.0° latitude × 1.0° longitude global grid) and long-term monthly mean air temperature data from 1981 to 2010 (University of Delaware Air Temperature and Precipitation dataset; 0.5° latitude × 0.5° longitude global grid) to calculate global mean annual ranges in SST for marine environments and surface air temperature ranges for freshwater and terrestrial environments. Surface air temperature has been shown to correlate linearly with water temperature, particularly on a monthly time scale, and thus is a reasonably good indicator of temperature variation in freshwater systems [49]. In cases where the estimated ATR was less than that of the range derived from the original study, we used the latter given that it represents a direct measurement. Sampling locations are presented in figure 1.

Statistical analyses were conducted in R [50]. We compared several candidate models to best predict seasonal T-S gradients based on the Akaike's information criterion (AIC). Using seasonal T-S gradient as the dependent variable, developmental environment (aquatic-developing versus terrestrial-developing), log10-transformed species body mass (at 15°C calculated using species-specific T-S slopes) and mean annual temperature were incorporated as fixed variables in a global linear mixed effects model (using package lme4). Log10-transformed species body mass was included to determine if the seasonal T-S gradient was mass dependent, (i.e. to determine if larger species adjusted their body size more strongly with intra-annual warming), following the results of Forster *et al*. [12] and Horne *et al*. [13]. Given the strong association between latitude and mean annual temperature, we modelled the effect of latitude on the seasonal T-S gradient separately. Gradients from multiple studies of the same species were included in our analyses. Species have shared evolutionary histories and are not completely statistically independent; we therefore included levels of taxonomic classification (subphylum, class, order, family and species) as nested (hierarchical) random effects on the intercept in all models to help control for phylogeny [51]. We also included habitat (marine, freshwater, terrestrial) as a random effect on the intercept, to control for the fact that we had aquatic-developing species from both marine and freshwater habitats. Including sex as a random effect did not improve the fit of the model, and so this was excluded. Finally, as the dependent variable in our models (the seasonal T-S gradient) is derived from data that vary between studies and species in their goodness of fit (see electronic supplementary material for individual plots of ln-transformed body mass versus temperature), we accounted for variation in information quality by weighting each seasonal T-S gradient by the inverse of the variance of its T-S slope estimate (using the 'weights' function in R) [52]. All possible combinations of the global model terms were compared using the dredge function in the MuMIn package. The best model was identified as that with the lowest small-samples corrected AIC (AICc). Where the difference between a model's AICc and the lowest AICc (i.e. ΔAICc) was less than 2, a set of best fit models, rather than a single best model, was assumed. Model averaging was then used to identify the best predictor variables across the top candidate models, and determine their relative importance (computed for each variable as the sum of the Akaike weights from all models in which they appear). In addition to AIC, a series of *F*-tests were used to verify the significance ($p < 0.05$) of each parameter's effect on the strength of the seasonal T-S gradient. *Post hoc* comparisons were made using a Tukey's HSD test.

To estimate the total change in body mass that a species could achieve over a season, we multiplied the seasonal T-S slope by the ATR of the sampling location for each species. This value was transformed into total percentage change in body size using the formula $(\exp^{(\mathrm{slope} \times \mathrm{ATR})} - 1) \times 100 = \mathrm{total}\ \%$ change in mass.

We compared total percentage change in body size between aquatic-developing and terrestrial species using a two-sample *t*-test. For both groups, an OLS regression of total percentage change in mass against ATR was used to determine whether species from more or less thermally variable environments exhibited a greater total percentage change in body size over a season. Given that the slope of this regression did not differ significantly from zero, and thus total percentage change in body size appeared relatively invariant with ATR, we also estimated the maximum total percentage change in body size with warming for aquatic-developing and terrestrial species. To do this, we used the package quantreg in R to fit the lowest possible quantile regression that complied with the sample size of each dataset, following recommendations by Rogers [53], such that $n > 5/q$ (where n is the sample size and q is the quantile of interest). This gives the most reliable estimate of the edge of the dataset appropriate to the sample size. Each quantile regression had a slope that did not differ significantly from zero; thus, we simply used the intercept to estimate the limit to total percentage change in body size over the season. Similarly, we also estimated the minimum total percentage change in body size with warming by fitting the highest possible quantile regression through the data that complied with the sample size of each dataset, where $n > 5(1 - q)$ [53].

To compare seasonal T-S gradients with laboratory controlled T-S responses and L-S clines, we used the data compilations of Horne *et al*. [13]. Where possible, we added data from our own search to these two body size datasets, using identical methods to screen and quantify size changes. For each of these datasets, we first combined size gradients from multiple studies of the same species into a simple mean to generate single species-specific values. Order-specific gradients were then calculated by averaging species-specific gradients for each taxonomic order, and reduced major axis (RMA) regression analysis was used to compare order-specific seasonal T-S gradients with laboratory T-S responses and L-S clines.

We note that using interspecific length-mass conversions can increase the likelihood of inaccuracy when determining body size gradients, particularly as any small deviation in the equation's power term can result in substantial overestimation or underestimation of the percentage change in body size. Given that we sometimes had to use family- and order-specific conversions, and that authors have employed a variety of equation forms, we repeated our analysis using length in place of dry mass to generate a second set of seasonal temperature–length (T-L) gradients (% change in length $^{\circ}C^{-1}$). To do this we used either the original length measurements reported or calculated the cube-root of mass when this was given. This length-based analysis confirms the difference in responses between environment types (aquatic, terrestrial), and the major findings from this approach are summarized in the electronic supplementary material.

3. Results

Our analysis included data from 71 sites in both temperate and tropical habitats between −38.1° and 61.5° latitude, although 52% of all study locations are found either in Europe or North America and hence dominated by Northern Hemisphere temperate areas (figure 1). We obtained 3725 seasonal body mass measurements in nature, representing 30 freshwater, 47 marine and 25 terrestrial arthropod species from nine taxonomic orders, resulting in 225 seasonal T-S slopes (see electronic supplementary material for species list and individual plots of ln-transformed body mass versus temperature). Most species (approx. 86%) conformed to the TSR; that is 88 of the 102 species exhibited a seasonal decrease in adult body size with increased temperature in the field.

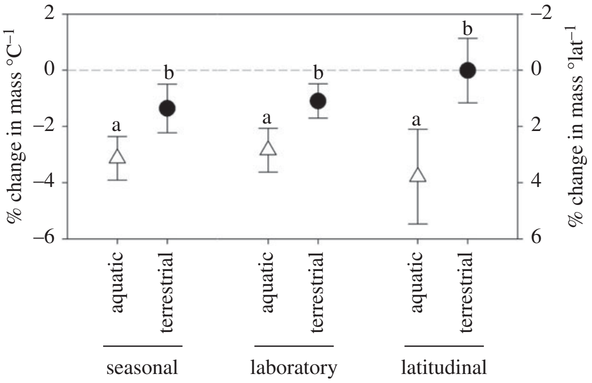

Figure 2. Comparison of mean aquatic- and terrestrial-developing seasonal temperature–body size gradients (% change in mass per °C ± 95% CI, left-hand *y*-axis) in arthropods with laboratory T-S responses (% change in mass per°C ± 95% CI, left-hand *y*-axis) and L-S clines (% change in mass per °latitude ± 95% CI, right-hand *y*-axis) for multivoltine species, using data from this study and Horne *et al.* [13]. Different letters above data points indicate significant differences. Dashed grey line indicates no change in body size with warming or increasing latitude. Note the reversal of the right-hand *y*-axis (for the L-S cline) for ease of comparison (a reduction in body size with increasing temperature is then comparable with an increase in body size with increasing latitude).

(a) Aquatic versus terrestrial species

The best supported model for explaining variation in seasonal T-S gradients contained only developmental environment (aquatic versus terrestrial) as a fixed variable. Three other models, including an 'intercept only' model, had a $\Delta AICc < 2$. Therefore, we calculated combined parameter Akaike weights across all four candidate models to determine the relative importance of each variable (electronic supplementary material, table S2). Developmental environment was the most important variable, accounting for approximately 30% of the total variance in the seasonal T-S gradient. Aquatic-developing species showed an approximately 2.5-fold stronger reduction in body size with seasonal warming (−3.1% body mass $^{\circ}C^{-1} \pm 0.8$; 95% CI) than terrestrial species (−1.4% body mass $^{\circ}C^{-1} \pm 0.9$; 95% CI; $F_{1,211} = 16.90$, $p < 0.001$; figure 2). Similarly, within the order Diptera, which contains species that develop in water and on land, aquatic-developing species reduced their body size significantly more per °C of seasonal warming than did terrestrial-developing species ($F_{1,34} = 10.17$, $p < 0.01$). These differences between aquatic and terrestrial environments were also important in influencing both laboratory T-S responses and L-S clines in the field [13] (figure 2).

$\bar{T}$ had no significant effect on the seasonal T-S gradient in either aquatic-developing ($F_{1,172} = 0.42$, $p = 0.32$) or terrestrial arthropods ($F_{1,35} = 2.80$, $p = 0.10$). The seasonal T-S gradient across aquatic-developing species became more strongly negative with increasing body mass ($F_{1,172} = 6.60$, $p = 0.01$), but the goodness of fit was extremely low ($R^2 = 0.02$). Thus body mass explained relatively little of the variation in aquatic seasonal T-S gradients in our dataset. There was no significant mass-dependence in terrestrial species ($F_{1,35} = 0.06$, $p = 0.80$). There were significant differences in the strength of the seasonal T-S gradient between taxonomic orders within the sub-class Copepoda; the order Calanoida (−3.66 ± 0.70% body mass $^{\circ}C^{-1}$; 95% CI) had a significantly stronger negative seasonal T-S gradient than both Cyclopoida (−0.91 ± 0.59%

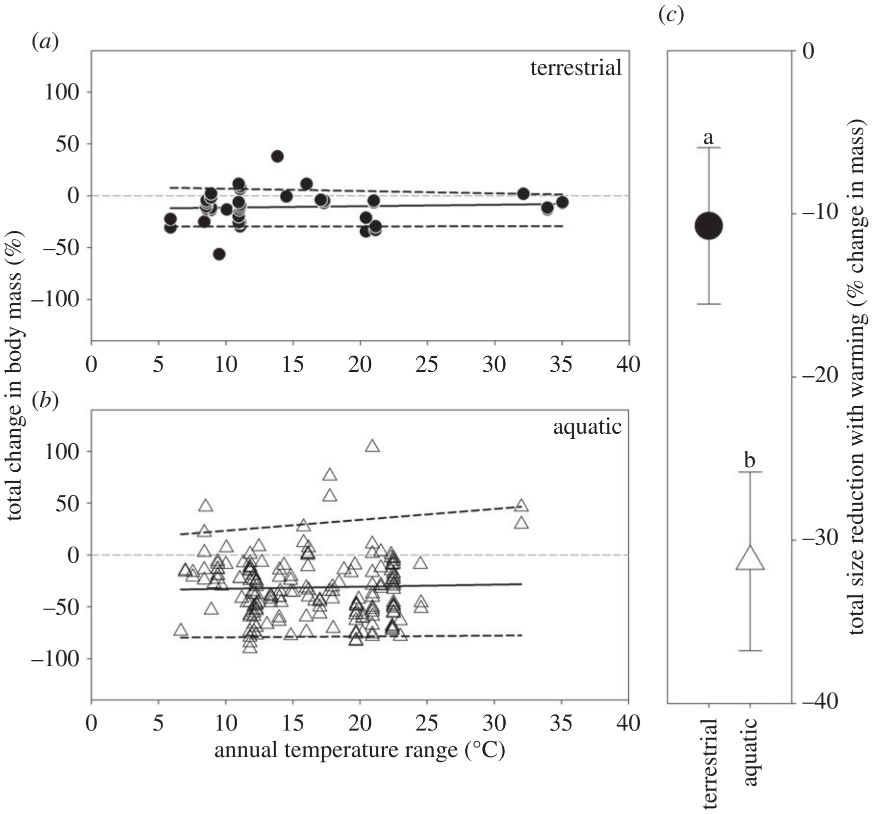

Figure 3. Total change in body mass (%) versus annual temperature range (°C) for (*a*) terrestrial and (*b*) aquatic arthropods. Solid black line represents the OLS regression, the slope of which does not differ significantly from zero in either environment, such that total percentage change in mass appears invariant with annual temperature range. Dashed black lines show the lowest and highest possible quantile regressions through the data and represent the upper and lower limits to total body size reduction with warming, respectively (*c*). Mean total size reduction with warming (expressed as a % change in body mass (±95% CI)) for terrestrial and aquatic-developing species. Different letters above data points indicate significant differences.

body mass $°C^{-1}$; 95% CI) and Poecilostomatoida (1.36 ± 3.06% body mass $°C^{-1}$; 95% CI). Latitude of the sampling location had no significant effect on the strength of the seasonal T-S gradient ($F_{1,122} = 1.13$, $p = 0.29$).

Total percentage change in body size over the annual cycle differed significantly between aquatic-developing and terrestrial species ($t_{223} = -3.52$, $p < 0.001$), but did not vary with mean annual temperature range in either group, such that, on average, total size change appeared relatively constant ($t_{180} = 0.37$, $p = 0.71$ and $t_{41} = 0.47$, $p = 0.64$, respectively; figure 3). Mean overall size reduction in terrestrial species was −10.7 ± 4.8% (95% CI), whereas overall size reduction in aquatic-developing species was almost threefold greater at −31.3 ± 5.5% (95% CI) (figure 3*c*). Additionally, based on the lowest and highest possible quantile regressions through these data (see Material and methods), we estimated a limit for total percentage change in body mass in aquatic-developing species of −80.2 ± 22.6% (95% CI), which is more than 2.5-fold greater than in terrestrial-developing species at −29.7 ± 24.9% (95% CI). The lower limit to total percentage change in size with warming did not differ significantly from 0% in either aquatic ($t_{180} = 0.17$, $p = 0.87$) or terrestrial species ($t_{41} = 0.55$, $p = 0.58$).

(b) Coherence among seasonal, laboratory and latitudinal body size patterns

If temperature is a major driver of seasonal body size variation in the field, seasonal and controlled laboratory T-S responses should be significantly correlated. Indeed, across taxonomic orders these two gradients showed a positive correlation, which did not differ significantly from a 1 : 1 relationship ($R^2 = 0.59$; figure 4*a*). This 1 : 1 match was supported by the RMA slope differing significantly from zero but not from 1 (0.73 ± 0.38; 95% CI), whilst the intercept did not differ significantly from zero (−0.39 ± 1.16; 95% CI) (inferred from 95% CIs; see inset panel in figure 4*a*). Given the relatively strong T-S gradients (seasonal and laboratory) of aquatic Isopoda compared with those of the other taxonomic orders, we also tested whether the RMA regression, and hence covariation between seasonal and laboratory T-S gradients, was dependent on this taxonomic order. The RMA regression did not differ significantly from a 1 : 1 relationship when the aquatic Isopoda were excluded (slope = 1.31 ± 0.90; intercept = 0.56 ± 1.85; $R^2 = 0.41$). The seasonal and laboratory datasets largely contained different species, yet, even for the small number of species for which we had both sets of data ($n = 22$), there was positive correlation between the two. Once again the RMA slope differed significantly from zero but not 1 (1.51 ± 0.61; 95% CI), whereas the intercept did not differ significantly from zero (1.80 ± 2.28; 95% CI).

Seasonal T-S gradients negatively correlated with L-S clines at the level of taxonomic order ($R^2 = 0.81$; figure 4*b*): those orders (e.g. Isopoda) whose members grew to a smaller adult size in warmer seasons also showed a decrease in size towards lower, warmer latitudes. Although we would not expect a 1 : 1 relationship between these size gradients (1° increase in latitude does not equal 1°C change in temperature), the gradient of the RMA slope did differ significantly from zero (−0.57 ± 0.28; 95% CI), confirming a significant correlation, whilst the

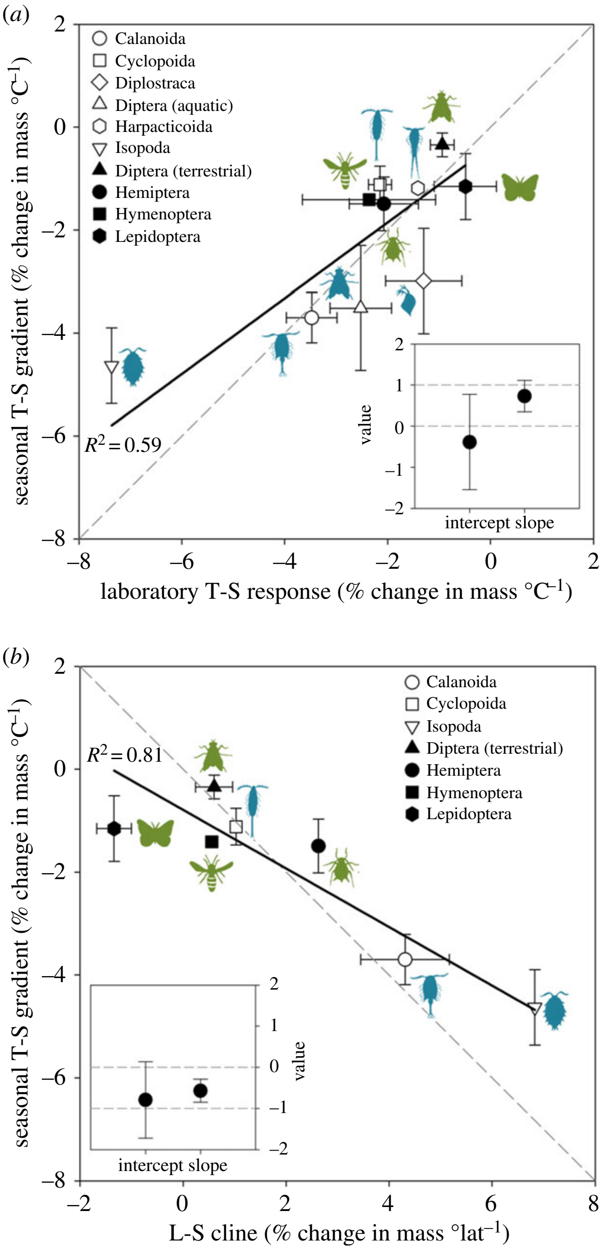

Figure 4. Reduced major axis (RMA) regression comparing seasonal T-S gradients (% change in body mass per °C ± s.e.) in arthropods with (*a*) laboratory T-S responses (% change in body mass per°C ± s.e.) and (*b*) L-S clines (% change in body mass per °lat ± s.e.), categorized by taxonomic order and developmental environment (aquatic, open symbols; terrestrial, filled symbols). Dashed lines indicate a 1 : 1 relationship. Inset graphs show the intercept and slope values for each regression (±95% CI). (Online version in colour.)

intercept did not differ significantly from zero (−0.79 ± 0.93; 95% CI; see inset panel in figure 4*b*). As before, there remained a significant correlation even when the aquatic Isopoda were excluded (slope = −0.58 ± 0.52; intercept = −0.78 ± 1.14; $R^2 = 0.59$).

4. Discussion

Our analysis of seasonal T-S gradients leads us to present four major conclusions: (i) multivoltine arthropod species inhabiting thermally varying seasonal habitats commonly demonstrate a negative seasonal T-S gradient, (ii) aquatic-developing species exhibit a stronger decline in adult body size with seasonal warming than those developing in air, (iii) total size reduction with warming appears relatively invariant despite variation in the annual temperature range experienced and (iv) seasonal T-S gradients correlate significantly with both laboratory T-S responses and latitudinal-size clines.

The aquatic-terrestrial differences in seasonal T-S gradients per °C parallel those observed in laboratory T-S responses and L-S clines [12,13] (figure 2). Further, mean overall size reduction through the year is almost threefold greater in aquatic (31.3%) than terrestrial (10.7%) arthropods (figure 3*c*). The greatest overall reduction in body mass with temperature for an aquatic species in our dataset is 90.4%, estimated for the calanoid copepod *Temora longicornis*, whereas in terrestrial species it is 56.4%, estimated for the isopod *Porcellionides pruinosus*. These consistent differences in seasonal T-S gradients between environments suggest that the drivers of body size reduction with warming are much stronger in aquatic than terrestrial arthropods.

The difference in seasonal body size change between environments is consistent with the hypothesis that greater constraints on oxygen availability in water than in air have either selected for greater plasticity in adult body size of aquatic species in response to temperature (both per °C and overall), or imposed constraints directly on their growth, compared with terrestrial species [29]. Specifically, metabolic demand increases much faster with increased size and temperature than does oxygen availability in water [12]; consequently, aquatic-developing species may have adapted to meet these increased metabolic demands with warming by reducing body size, and/or oxygen limitation may also have limited growth directly. An alternative explanation based on thermoregulatory ability also requires consideration. In the field, behavioural thermoregulation may allow arthropod species to maintain a narrower body temperature range over a season relative to the ambient temperature range, be this through seeking shade or basking in terrestrial species, or vertical/horizontal migration in aquatic species. For this reason, the seasonal T-S gradient in thermoregulating species may seem weaker. Owing to the higher heat capacity of water than air, thermoregulation is much more difficult for aquatic than terrestrial species. However, we discount the explanation that thermoregulation may explain the differences seen between environments, because this ability is unlikely to account for a 2.5-fold difference in body size reduction with warming between these two groups—such an explanation would imply that, where aquatic species experience an annual temperature range of 30°C, terrestrial species experience a range in body temperature of only 12°C, i.e. are able to reduce their body temperature range by 18°C. At least in some environments, this major reduction in body temperature range is highly improbable [54]. Furthermore, larger aquatic species often exhibit the greatest reduction in body size with warming [12,13], yet we see no reason why behavioural thermoregulation would be reduced in larger compared to smaller aquatic species. Instead, this pattern supports the prediction that due to their lower surface area to volume ratio, larger species would struggle most to meet their oxygen requirements in the warm, leading to a stronger T-S gradient. Therefore, behavioural buffering does not seem capable of explaining the observed mass-dependence of the T-S gradient in aquatic species, which instead is consistent with the oxygen hypothesis [12].

Although body size reduction with warming is thought to provide fitness benefits by balancing resource demand and supply at elevated temperatures, this likely comes at a cost, given the link between body size and other vital rates and physiological processes. For example, body size is often strongly positively correlated with fecundity, including in insects [55] and zooplankton [56], while smaller body size may also reduce survival during periods of low food availability, or increase vulnerability to predation [18]. Thus, there will eventually come a point at which the fitness benefits of reducing body size no longer outweigh the costs. These widespread fitness trade-offs may dictate overall limits to total proportional size change in arthropods; an optimal point at which the selective pressures in a given environment over the annual cycle no longer favour more extreme size reductions with warming. The relative consistency in total proportional size change in relation to ATR, despite variation in ATR of up to 30°C between sampling locations, may be an indication of such limits (figure 3). Although these optima vary between species and environments, as is observed in the approximately threefold difference in mean total body size reduction between aquatic and terrestrial species, and in the scatter in total proportional change, the lack of a relationship with ATR suggests that, on average, arthropods from similar environments may share and frequently realize these limits, regardless of the degree of thermal variability across the year. Consequently, species inhabiting environments with a greater thermal range on average reduce their body size less per °C of warming than those from less thermally varying environments.

Unexplained variation in the magnitude of T-S gradients between species and higher taxonomic groupings is likely to be attributed to differences in life history, physiology and behaviour. Indeed, such effects have been explored in the sub-class Copepoda, in which the approximately fourfold difference between the seasonal T-S gradients of calanoid and cyclopoid copepods may relate to differences in the temperature-dependence of energy supply and expenditure in current-feeding calanoids versus ambush-feeding cyclopoids [35]. Differences in the strength of seasonal variation in resource availability (e.g. food and water in terrestrial species, and food and oxygen in aquatic species) or seasonal mortality risk (e.g. associated with predation, desiccation or both) are also likely to modify the T-S gradient. Although the arthropods in our dataset all have more than one generation per year, some groups, such as some of the Lepidoptera species included here, have just two generations in a year, whereas others, including the smaller terrestrial Diptera and aquatic Copepoda, have many overlapping generations. Voltinism is highly temperature-dependent and can constrain body size [19,27], and differences in perceived seasonality (including temperature and resource availability) between species with these different generation times, might lead to differences in the strength of the seasonal T-S gradient. Specifically, smaller species with short generation times are likely to perceive reduced seasonality within each generation [27]. Hence, we might predict that the adaptive advantage of tuning body size to prevailing conditions during development will be strong, leading to a greater reduction in body size and a greater seasonal T-S gradient. Our data largely apply to species with many overlapping generations in a year, making it difficult to assign body size measurements to specific generations or cohorts. A synthesis of changes in mature body size in univoltine terrestrial species, measured over multiple years, would be an informative next step, not least because these species often exhibit a reverse T-S response in the laboratory (i.e. increase in size with warming) and an increase in size towards the equator. In accordance with these patterns, a recent study of a univoltine butterfly species showed that adult male forewing length was positively correlated with temperature during development across multiple years [57]. Whether such an inter-annual size trend extends more generally to other univoltine terrestrial arthropods remains to be tested.

We note the potential for a mismatch between temperature at the time of collection of adults in the field and the temperature the animals experienced during ontogenetic development. This is particularly true for larger species with longer development times and/or in those species from strongly seasonal environments. However, as discussed above, many of the multivoltine species considered here generally have short generation times, often of just a few weeks; thus, in most cases any temperature fluctuations experienced within a generation should be fairly conserved, and temperature at time of collection of adults should be a reasonable proxy for developmental temperature. Similar issues could arise in species that either undergo extended periods of reproductive diapause, or live a long time as adults, particularly the larger Lepidoptera species in our dataset (six of 10 Lepidopteran species considered), during which time juvenile recruitment to the population is ceased. In this case, adults collected during periods of diapause, or towards the end of long adult lives, may actually develop much earlier in the season, when environmental conditions were very different. This is further complicated because larger individuals generally have a greater chance of surviving periods of dormancy, and this could be an important factor influencing body size variation in diapausing generations, obscuring any effects of temperature and/or resource availability [58]. Yet, when we further explored this issue, by excluding body size measurements recorded during suspected periods of reproductive diapause, we observed no significant shift in the T-S slope in any of the six species of Lepidoptera that exhibited this behaviour. These species represent the few extreme cases in our dataset where juvenile recruitment is ceased for relatively long periods, giving us confidence in the overall patterns we present.

Despite the potential pitfalls in our data and the many confounding factors that can influence body size variation in the field, we find a statistically significant match between body size responses measured in the laboratory and in nature, which suggests that they share common drivers. The consistency in both the strength and direction of all three of these body size gradients observed both at the levels of taxonomic order (figure 4) and of species, as well as between environments, and together with the weighting of T-S slopes by the inverse of their variance, gives us confidence that these patterns are unlikely to arise simply from differences in sample size between groups or potential sampling error in the individual T-S slopes. Ultimately, the close match between laboratory and seasonal T-S gradients (figures 2 and 4*a*) suggests that temperature is an important driver of variation in mature body size in arthropods in the diverse seasonal systems we have explored, despite changes in other abiotic and biotic factors that can directly influence body size variation, such as food quantity and quality [59].

Here we use a simple yet powerful correlative approach to understand major patterns in body size. Although our dataset represents only a tiny fraction of all arthropod species globally, we identify important patterns in body size that covary with major body size gradients. Evidently, changes in the body sizes of ectotherms associated with climate change can be both substantial [41–43] and widespread [16]. Advancing our understanding of what drives T-S gradients in the field is essential if we are to accurately predict how body size will change with projected increases in temperature and with more extreme seasonality [40].

Authors' contributions. C.R.H., A.G.H. and D.A. designed the study and wrote the paper. C.R.H. collected the data and performed the statistical analyses.

Competing interests. We declare we have no competing interests.

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RESEARCH PAPER

A global synthesis of seasonal temperature–size responses in copepods

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ABSTRACT

Aim Body size is a master trait with significant ecological importance. Seasonal changes in body size within diverse ectothermic species can result from different environmental conditions experienced during ontogeny in subsequent generations. Whilst intraspecific changes in adult size have been well studied under controlled experimental conditions and across geographical ranges, comprehensive analyses of temporal changes are lacking, and there remains considerable unexplained variation in body size responses within aquatic taxa. Using planktonic copepods as an exemplar taxon, we quantify variation in adult body mass within seasonally varying marine and freshwater environments. We describe how size variation relates to temperature, food concentration (chlorophyll-*a*) and life-history characteristics, including feeding strategy.

Location Global.

Methods Using a meta-analytic approach we extract quantitative data from published literature on seasonal size responses of copepods. We analyse competing models to determine the best predictors of these responses, and compare the relative importance of temperature and chlorophyll-*a* concentration in explaining variation in body size.

Results We quantify 140 seasonal size responses from 33 different global locations, representing 48 planktonic copepod species from four taxonomic orders. We find that temperature ($r^2 = 0.50$), rather than food ($r^2 = 0.22$), is the dominant explanatory variable of changes in adult body size across seasons. A striking outcome is that calanoid copepods, which utilize feeding currents to capture prey, exhibit a four-fold greater reduction in adult body mass per °C (-3.66%) compared with cyclopoid copepods (-0.91%), which are ambush feeders. By contrast, species body size or reproductive strategy did not explain variation in the seasonal temperature–size response.

Main conclusions Our findings lead us to suggest that feeding strategies may play a significant role in dictating the magnitude of seasonal temperature–size responses in copepods, with potential implications for other ectotherms with diverse feeding methods. Seasonal temperature–size responses were typically much more variable than responses in laboratory studies that provided excess food, suggesting that field conditions modify the temperature–size response.

Keywords

Body size, chlorophyll, phenotypic plasticity, temperature, temporal, zooplankton.

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INTRODUCTION

Biologists have long been fascinated by body size variation (Bergmann, 1847; Schmidt-Nielsen, 1984), in part because this 'master trait' affects all vital rates, including feeding (Burns, 1968; Kiørboe, 2011), growth (Poston, 1990; Kiørboe & Hirst, 2014), metabolism (Peters, 1983; Glazier, 2005; Hirst *et al.*, 2014) and reproduction (Honěk, 1993; Arendt, 2011), as well as many other aspects of the biology of an organism (Andersen *et al.*, 2016). Consequently, identifying and understanding what drives variation in body size is of fundamental biological importance. Body size is sensitive to environmental conditions, due to the temperature dependence of physiological processes, as well as other factors such as changes in food availability. Ectothermic species that have short life cycles and inhabit seasonal environments are typically subjected to varying environmental conditions across subsequent generations within a year. This is commonly linked to marked temporal shifts in adult body size over an annual cycle, as sequentially recruited adults are affected by different abiotic and biotic conditions over their ontogeny. Intraspecific variation in size related to seasonal variation in temperature has been found across a wide range of uni- and multicellular organisms, including bacteria (Chrzanowski *et al.*, 1988), rotifers (Diéguez *et al.*, 1998), copepods (Liang & Uye, 1997; Hirst *et al.*, 1999; Riccardi & Mariotto, 2000; Dutz *et al.*, 2012), cladocerans (Miyashita *et al.*, 2011) and insects (Kari & Huey, 2000), yet broad-scale analyses of temporal changes in adult body size are lacking.

By contrast, intraspecific variation in size-at-stage has been well described in the laboratory under different conditions (Atkinson, 1994; Forster *et al.*, 2012), and also spatially over latitude or across regions (Horne *et al.*, 2015). The most frequently observed intraspecific response to warmer temperatures in ectotherms is a reduction in size-at-stage; this has been formalized as the temperature–size (T–S) rule (TSR) (Atkinson, 1994). This phenotypically plastic response can be achieved within a single generation (Forster & Hirst, 2012; Forster *et al.*, 2013), and in many metazoans the proximate cause is attributed to differences in the temperature dependence of growth and development during ontogeny (van der Have & de Jong, 1996; Forster *et al.*, 2011a,b; Zuo *et al.*, 2012). The ultimate cause of this outcome, however, may be a complex of several factors (e.g. see Forster *et al.* 2012; Horne *et al.* 2015). The degree to which these responses are found in natural field conditions, where multiple variables can act simultaneously to influence body size, is still uncertain. For instance, the relative contribution of food and temperature in determining seasonal shifts in adult size still needs to be resolved. Food availability affects size at maturity, but while slower growth at lower temperature is frequently coupled with an increase in adult size, slower growth with reduced food availability is typically associated with smaller size at maturity (Berrigan & Charnov, 1994). Further, food quality can dramatically alter the T–S response, even to the extent that the sign of the T–S response can be reversed under conditions of poor food quality (Diamond & Kingsolver, 2010). Identifying and understanding seasonal variation in body size will not only help to determine the ultimate causes of such variation, but will also aid in predicting future shifts associated with changes in climate (IPCC, 2014) and phenology (Visser & Both, 2005). Our study aims to synthesize and quantify seasonal patterns in adult size of multivoltine species, going beyond previous species- and location-specific studies, so that we might provide a broader understanding of such patterns.

A recent meta-analysis of terrestrial and aquatic arthropods identified an impressive match between T–S responses measured under controlled laboratory conditions and intraspecific body size clines observed in the field across latitudes (Horne *et al.*, 2015). The magnitude and direction of these responses revealed consistent differences in the strength and sign of the response between aquatic and terrestrial species. These results suggest that laboratory T–S responses and latitudinal body size clines may be driven by similar selective pressures within arthropods – specifically, by voltinism and season length trade-offs in terrestrial species (Kozłowski *et al.*, 2004; Walters & Hassall, 2006) and the need to balance oxygen demand and supply in larger aquatic species (Woods, 1999; Atkinson *et al.*, 2006; Forster *et al.*, 2012). However, in many small organisms, in which oxygen diffusion under normoxic conditions is likely to be adequate to meet metabolic demand, size reductions with warming are still very common; they are observed, for example, in bacteria, protists and small metazoans, such as copepods (Atkinson *et al.*, 2003; Forster *et al.*, 2012). Copepods are one of the most numerous metazoans on the planet; they are ecologically important and play a pivotal role in marine and freshwater biogeochemistry and trophodynamics (Banse, 1995). Reduction in size with increasing temperature, consistent with the TSR (Atkinson, 1994), has been shown in many copepod species, both in controlled laboratory experiments (Horne *et al.*, 2015) and across seasons in the field (seasonal T–S responses) (Uye *et al.*, 1982; Hirst *et al.*, 1999; Riccardi & Mariotto, 2000; Drif *et al.*, 2010). Furthermore, the strength of the laboratory T–S response varies widely between species, to the extent that Horne *et al.* (2015) observed an approximate 30-fold difference between the strongest and weakest copepod T–S responses in their dataset on arthropods. It would appear, therefore, that another factor (or other factors) may be responsible for the size reductions with warming observed in these smaller taxa, and identifying the likely causes is an important next step. Planktonic copepods are excellent model organisms in which to investigate seasonal size responses. Temporal changes in adult body size have commonly been examined in this taxon (Fig. 1), especially in mid-latitude environments which demonstrate strong shifts in temperature and food, while most species have multiple generations within a year and short generation times of >10 to <100 days (Hirst & Kiørboe, 2002). Thus, in this paper we present and test a number of alternative hypotheses that may help to explain the considerable variation observed in sensitivity of body size to warming in planktonic copepods.

Mature adult size is dependent in part upon obtaining sufficient food to meet maintenance and growth requirements,

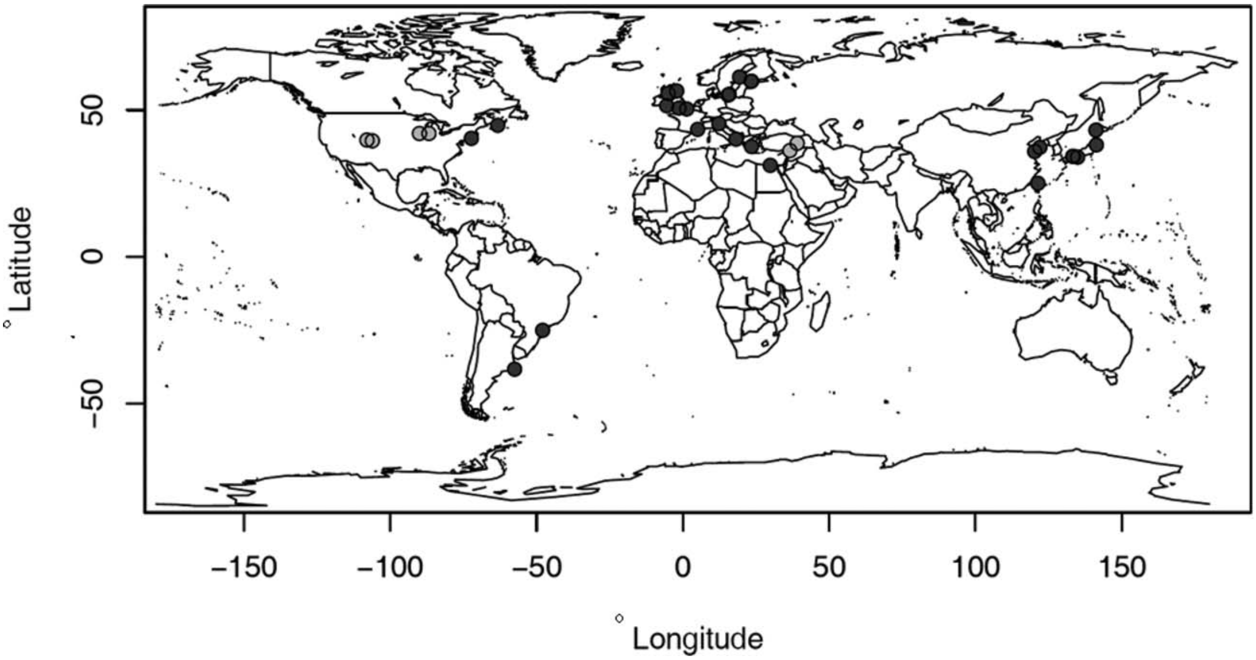

Figure 1 World map indicating the location of studies ($n = 33$) from which copepod seasonal size responses were recorded. Studies from freshwater environments are indicated by the light grey circles whilst marine environments are indicated by the dark grey circles.

and size at maturity is controlled by different body size scaling of catabolism and anabolism (von Bertalanffy, 1957; Perrin, 1995):

$$\frac{\mathrm{d}M}{\mathrm{d}t}=c_s M^s - c_l M^l$$

where $s > 0$ and $l > 0$ are exponents for energy supply and loss respectively, and $c_s > 0$ and $c_l > 0$ represent the temperature dependence of the intercept terms on a log–log scale. The point at which metabolic supply and demand intersect defines an organism's asymptotic mass:

$$\frac{\mathrm{d}M}{\mathrm{d}t}\Big|_{M_\mathrm{A}}=0.$$

In mathematical terms, the asymptotic mass, M_A, is given by

$$c_s(M_\mathrm{A})^s = c_l(M_\mathrm{A})^l \Rightarrow \log(M_\mathrm{A}) = \frac{\log(c_s/c_l)}{l-s}.$$

Temperature changes will affect both energy supply and expenditure, forcing the organism into a new asymptotic mass. Hence, we can predict the induced relative change in asymptotic mass per degree Celsius, noting that $f'(x)/f(x) = f'\,[\log(x)]$:

$$\frac{1}{M_\mathrm{A}}\frac{\mathrm{d}M_\mathrm{A}}{\mathrm{d}T}=\frac{\mathrm{d}}{\mathrm{d}T}[\log(M_\mathrm{A})]=\frac{1}{l-s}\frac{\mathrm{d}}{\mathrm{d}T}\left[\log\left(\frac{c_s}{c_l}\right)\right].$$

Thus, the temperature dependence scales inversely with the difference in the mass scaling of supply and demand ($l - s$), and is also influenced by the temperature dependence of the intercepts. Moreover, within this framework, the strength of the T–S cline should be independent of body mass.

Despite overwhelming evidence in favour of the TSR in a diverse range of ectotherms, there remains considerable unexplained variation in the strength of the response between species and taxonomic groups, which can most likely be attributed to key differences in life-history traits and their associated metabolic constraints. In copepods and many other small zooplankton, food acquisition is governed by prey availability and uptake. With a few exceptions, species within the order Calanoida largely utilize feeding currents to entrain and capture prey (Kiørboe, 2011); by contrast, species within the non-calanoid orders, i.e. the Harpacticoida, Cyclopoida and Poecilostomatoida, lack the ability to produce a feeding current and are either ambush feeders (Cyclopoida; Paffenhöfer, 1993), feed on surfaces, which in the planktonic environment are provided by marine snow aggregates (Harpacticoida; Koski *et al.*, 2005), or are parasitic (e.g. many Poecilostomatoida; Huys & Boxshall, 1991). There is evidence that feeding mode is an important correlate of metabolic rate (respiration), and clearance, growth and ingestion rates (Kiørboe & Hirst, 2014). Ignoring parasitic copepods, the body mass dependence of clearance rate differs between feeding current feeders and more passive ambush and surface feeders (Kiørboe, 2011), suggesting a possible difference in the temperature–body size sensitivity between different feeding behaviours. We cannot yet predict the magnitude and direction of the T–S response since we do not know how metabolic rates change with mass during ontogeny, and we also do not know how the intercept terms vary with temperature. However, these considerations lead us to suggest that some of the variability in T–S responses may be due to differences in feeding behaviour.

Another potential influence on the T–S response is reproductive strategy. In copepods, reproductive strategy can be divided into broadcast spawning and sac spawning. Sac spawners carry eggs in external sacs and have much lower fecundity rates than broadcast spawners (Hirst & Kiørboe, 2002). Sac spawners commonly do not lay the next batch of eggs until the previous batch has hatched from the attached sac(s) (Ward & Hirst, 2007), hence egg production is limited by the egg hatching time (Hirst & Bunker, 2003). By contrast, broadcasters have much higher fecundity rates, and are less likely to be limited by clutch size or egg hatching rates in the same way. The potentially different thermal sensitivities of egg development versus egg production rates may produce different solutions for size at maturity (and in turn its temperature dependence) between these two reproductive strategies. However, even in the absence of clear evidence of such a difference in thermal sensitivity of egg production and hatching, optimum size may change to different degrees if the cost of carrying versus not carrying egg sacs is temperature dependent. For example, feeding rates of ectotherms, including predators such as fish, typically increase with warming (Barneche *et al.*, 2008), and such an increased risk of mortality to prey organisms may amplify any small differences in size- and fecundity-related trade-offs observed between broadcast and sac spawners at cooler temperatures. In principle, therefore, differences in the optimum body size response to temperature between the two spawning strategies can be hypothesized.

Our study therefore aims to: (1) quantify and synthesize for the first time the seasonal temperature–size responses of a wide range of planktonic copepod species, and to compare these with responses under controlled laboratory conditions; (2) examine the temperature dependence of size at maturity in copepods, based around major differences between taxonomic orders, species body sizes, modes of feeding (feeding current versus active ambush feeding), and reproductive strategy; (3) assess the relative importance of food concentration and temperature in driving seasonal size change.

METHODS

We searched the literature for studies in which the adult body size of planktonic copepods was assessed on multiple occasions during an annual cycle. In addition to temperature we also recorded the concentration of the phytoplankton pigment chlorophyll-*a* (Chl-*a*) when this was reported. Chl-*a* concentration is commonly used as a proxy for phytoplankton biomass and food availability; indeed, adult fecundity and juvenile growth in many copepods correlates to this term (Hirst & Bunker, 2003; Bunker & Hirst, 2004). To reduce potential sampling bias in the sizes of animals collected, only those studies in which the adults were sampled across the entire depth of the water column, or across most of the depth range of the species, were included. Adult size data were collected as either lengths or dry, wet or carbon mass. These measurements were subsequently converted to dry mass (mg) using published intraspecific regressions. If these were not available, regressions for closely related species, or more general interspecific regressions, were used. A list of the data sources is given in Appendix 1. All raw data and conversions are detailed in Data S1 in the Supporting Information. Taxonomic order and family were confirmed for each species using the World Registry of Marine Species (WoRMS Editorial Board, 2015).

In order to test which form of equation best describes the relationship between mass and temperature within a species, we used the Akaike information criterion (AIC) to compare linear, quadratic, exponential and allometric fits to the data. We found that the exponential equation form was overwhelmingly favoured for modelling seasonal T–S responses, as judged by Akaike weights (see Table S1 for full details). We therefore used an exponential equation form to model the seasonal T–S response for each species from each study in our dataset, separating responses by sex. Species-specific slopes of the natural log (ln) of dry mass versus temperature were determined and transformed into percentage change in dry mass per degree Celsius, using the formula $[\exp(\text{slope}) - 1] \times 100 = \%$ change in mass per °C (Forster *et al.*, 2012). This value represents the seasonal T–S response, with a negative value showing a reduction in body mass with increasing temperature, hence following the same trend as the TSR. Size responses from multiple studies of the same species were then combined into a simple mean to generate a single species-specific seasonal T–S value, separated by sex.

To quantify relationships between body mass and Chl-*a*, the species-specific slopes of ln(dry mass) versus Chl-*a* concentration ($\mu g\ l^{-1}$) were determined for all individuals and transformed into percentage change in dry mass per $\mu g\ l^{-1}$, again using the formula $[\exp(\text{slope}) - 1) \times 100 = \%$ change in mass per $\mu g\ l^{-1}$, to generate a chlorophyll–size (C–S) response. The mean and 95% confidence intervals of T–S and C–S responses, calculated from the 95% confidence intervals of the individual estimated slopes, are presented for each order in Table S2. For all datasets containing both a measure of temperature and Chl-*a* concentration ($n = 80$), we compared the coefficient of determination (R^2) of both parameters (i.e. by comparing the R^2 of each seasonal T–S response with its corresponding C–S response), to determine whether one consistently explained significantly more of the variation in seasonal body size clines than the other. Given that temperature is a mechanistic driver of variation in primary productivity, we also utilized an alternative approach to examine these relationships; first we regressed body mass against temperature and then subsequently regressed the residuals from this on Chl-*a* concentration, to determine how much of the seasonal variation in body size could be attributed to Chl-*a* after accounting for temperature.

All statistical analyses were conducted using the free statistical software package R (R Core Team, 2014). We derived several candidate models to determine the best predictors of seasonal T–S responses based on the AIC. In order to determine whether species body size affects the T–S response, we included $\log_{10}$ species mass at a reference temperature (15 °C) as a predictor, as such an allometric relationship has previously been shown to be significant (Forster *et al.*, 2012; Horne *et al.*, 2015). Taxonomic order, $\log_{10}$ body mass (at 15 °C, calculated using species- and sex-specific slopes) and sex were incorporated as fixed variables in a global linear mixed effects model (using the package lme4), with species nested within family, and latitude included as a random effect on the intercept. When selecting our random effects, we considered the estimates of variance explained by each of our proposed random variables [environment type (marine versus freshwater), latitude and species nested within family] and used stepwise elimination of non-significant terms to determine which parameters to include in the final model. All possible combinations of the global model terms were compared using the dredge function in the MuMIn package in R. The best model was identified as that with lowest small-sample corrected AIC (AICc), and Akaike weights (w_i) were used to determine the probability (0–1) of each candidate model being the best fit model (i.e. if $w_i = 0.9$, there is a 90% probability that a given model is the best fit model among those considered and given the data available). Where the difference between a model's AICc and the lowest AICc (i.e. ΔAICc) is less than two, a set of best fit models, rather than a single best model, can be assumed, and model averaging may be used to identify the best predictor variables across the top candidate models and determine their relative importance (computed for each variable as the sum of the Akaike weights from all models in which they appear). In addition to AIC, a series of *F* tests (using the 'anova' function in *R*) were used to verify the significance ($P < 0.05$) of each parameter's effect on the strength of the seasonal T–S response. *Post hoc* comparisons were made using the Tukey honest significant difference (HSD) test.

To compare seasonal T–S responses with laboratory T–S responses, we used the extensive T–S response dataset of Horne *et al.* (2015), supplementing this where possible with newly identified data following identical methods for acquiring data.

RESULTS

We derived a total of 140 seasonal T–S responses from 33 different global locations (Fig. 1) within the latitudinal range of 25° to 61°, hence largely falling around mid-latitudes (with a dominance of Northern Hemisphere locations). This range in part reflects well-studied temperate environments with strong seasonality while also being inhabited by copepod species with multiple generations in a year. The data set included 48 planktonic copepod species from four taxonomic orders (Calanoida, Cyclopoida, Harpacticoida, Poecilostomatoida). These species-specific seasonal T–S responses had negative slopes in 87% of cases, with a mean (±95% CI) reduction in size of $-2.87 \pm 0.65\%$ body mass $°\mathrm{C}^{-1}$ (Fig. 2), reinforcing the generality of the negative T–S response in copepods. The overall strength or direction of the seasonal T–S response did not vary significantly across latitudes ($F_{1,138} = 1.20$, $P = 0.27$). Of the 80 seasonal body size clines for which we had a measure of Chl-*a* concentration (corresponding to 33 species), across all orders we observe a mean body mass response of $0.98 \pm 2.01\%$ per μg l^{-1}, which does not differ significantly from zero ($t_{79} = 0.97$, $P = 0.34$) (Fig. 2). On average, across all taxonomic orders temperature explained more of the variation in seasonal body mass than Chl-*a* concentration: this is inferred from the mean R^2 values of each parameter when both were modelled separately (0.44 ± 0.07 vs. 0.22 ± 0.05, respectively), and also when comparing body mass–temperature regressions and the residuals from these against Chl-*a* concentration (0.44 ± 0.07 vs. 0.07 ± 0.03, respectively) (see Fig. S1). Considering each of the four orders separately, temperature always explained more of the variation in adult body mass than did Chl-*a* concentration.

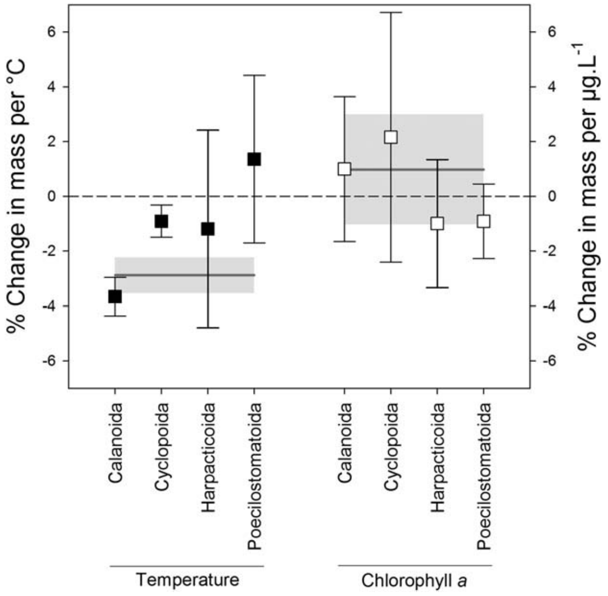

Figure 2 Species-specific percentage change in body mass (±95% CI) for seasonal T–S (per °C) and C–S (per μg l^{-1}) responses, averaged by order. Solid grey lines show the mean seasonal T–S and C–S response across all orders with the 95% CI indicated by the shaded area.

In explaining variation in the strength of the seasonal T–S response among planktonic copepods, the model with the lowest AICc includes only taxonomic order as a fixed variable, whilst all other candidate models have ΔAICc > 2 (Table 1). Thus, given the data available, we may reject the other candidate models in favour of a single best fit model in which taxonomic order has a significant independent effect on the strength of the seasonal T–S response ($F_{3,82} = 9.43$, $P < 0.001$). *Post hoc* comparisons (Tukey HSD) show that Calanoida ($n = 66$, mean $= -3.66 \pm 0.70\%$ body mass $°\mathrm{C}^{-1}$) have a significantly stronger negative seasonal T–S response than both Cyclopoida ($n = 12$, mean $= -0.91 \pm 0.59\%$ body

Table 1 AIC output comparing the relative strength of candidate models in explaining variation in seasonal T–S responses.

Model	K	Log-likelihood	AICc	ΔAICc	w_i
Intercept	*5*	*−328.40*	*667.25*	*5.08*	*0.04*
Order	**8**	**−322.53**	**662.16**	**0.00**	**0.52**
Order + sex	9	−322.51	664.40	2.24	0.17
Log_{10}(mass) + order	9	−322.53	664.44	2.27	0.17
Log_{10}(mass) + order + sex	10	−322.51	666.72	4.55	0.05
Log_{10}(mass)	6	−327.89	668.41	6.25	0.02
Sex	6	−328.38	669.40	7.24	0.01
Log_{10}(mass) + sex	7	−327.77	670.38	8.22	0.01

The best model, shown in bold, is identified as that with the lowest small-sample-corrected AIC (AICc). Given that the difference between the lowest AICc and those of the alternative models (i.e. ΔAICc) is > 2, we may favour a single best fit model in which taxonomic order has a significant independent effect on the strength of the seasonal T–S response. An 'intercept only' model, shown in italics, is included for comparison. Akaike weight (w_i) denotes the probability of a given model being the best fit model in the candidate set. The number of parameters (K) in each model is shown. Mass is the species adult body mass at 15 °C.

mass °C^{-1}) and Poecilostomatoida ($n = 6$, mean $= 1.36 \pm 3.06\%$ body mass °C^{-1}), but not Harpacticoida ($n = 2$, mean $= -1.19 \pm 3.60\%$ body mass °C^{-1}), though our seasonal data for this order are sparse, including only male and female *Euterpina acutifrons*. We note specifically the different temperature response between the calanoids, which use feeding currents, and ambush-feeding cyclopoid copepods, with a four-fold difference in the strength of the seasonal T–S response observed between these two groups (Fig. 3). We find no significant change in the strength of the response with mean species body mass in either the Calanoida ($F_{1,101} = 0.11$, $P = 0.75$) or non-calanoid orders ($F_{1,35} = 2.75$, $P = 0.11$), supporting our prediction that any change in mature body size is independent of mean species body mass in these smaller taxa (Fig. 4).

Reproductive strategy also varies within and between orders; calanoid species can be either broadcast or sac spawners, but are more commonly the former ($n = 44$ vs. $n = 22$ for broadcast and sac spawners, respectively, in our dataset), whilst all species in the three remaining orders considered here are sac spawners. Given that taxonomic order and reproductive strategy correlate exactly in three of the four orders in our dataset, while in calanoids both reproductive strategies occur, we tested for differences in the seasonal T–S response between broadcasters and sac spawners exclusively in calanoids, finding no significant effect ($F_{1,64} = 0.71$, $P = 0.40$). We also tested for order-level differences in the seasonal T–S response exclusively in sac spawners (i.e. by excluding any broadcast spawning calanoid species), and found significant differences in the strength of the seasonal T–S response between taxonomic orders, still observing a four-fold significant difference between calanoids and cyclopoids (t-test; $t = -4.51$, d.f. $= 31$, $P < 0.0001$). This leads us to suggest that reproductive strategy is not responsible for driving the observed differences in seasonal T–S responses between taxonomic orders and, hence, explains why we chose to exclude the latter from our global linear mixed effects model.

Despite the numerous other variables that may act to obscure the correlation between body mass and seasonal temperature, we find a strong match between the mean seasonal T–S response for Calanoida ($-3.66 \pm 0.70\%$) and the mean T–S response ($-3.20 \pm 0.49\%$) measured in the laboratory under conditions of excess food (t-test; $t = -1.09$, d.f. $= 79$, $P = 0.28$). However, we note that the two datasets comprise different species. Indeed, when we regress species-specific seasonal T–S responses against laboratory T–S responses for the small number of species for which we have both sets of data ($n = 12$), separating responses by sex, we observe much greater variation in seasonal T–S responses than those measured under controlled laboratory conditions (Fig. 5). This suggests that food quantity/quality, and potentially other environmental variables, are having an impact on the T–S response in the field. There appears to be no systematic difference in the strength of laboratory and seasonal T–S responses between the sexes, such that sex has no significant effect on the strength of the seasonal T–S response, either across species ($F_{1,84} = 0.03$, $P = 0.86$) or intraspecifically (paired t-test; $t = 1.35$, d.f. $= 37$, $P = 0.19$). Unfortunately, we are unable to make further meaningful comparisons between field and laboratory responses. For example, we could not compare the broad differences between taxonomic orders we observe in the seasonal T–S data with laboratory data, as very few laboratory studies on species other than calanoids have been conducted; our dataset contains male and female laboratory T–S responses for just two planktonic cyclopoid species and a single harpacticoid species.

DISCUSSION

Our work combines field data from numerous studies worldwide (Fig. 1), and goes beyond controlled laboratory-based T–S studies to demonstrate broad patterns in the thermal size responses of marine and freshwater planktonic copepods. Despite numerous other variables that may act to complicate the T–S signal in the field, we show that almost 90% of copepod species in our dataset follow the TSR in seasonal environments, maturing at a smaller adult body mass in warmer conditions. Yet, as we might expect, seasonal T–S responses appear to be much more variable than those measured under controlled conditions in the laboratory (Fig. 5), suggesting that environmental factors in addition to temperature may play a role in driving seasonal body size variation in the field. We should also consider that the temperature at which adults are collected is unlikely to correspond exactly to temperatures experienced during ontogeny, and this may be further complicated by the existence of a winter diapause, during which many copepods will cease recruitment over late winter to early spring. Throughout this period their prosome

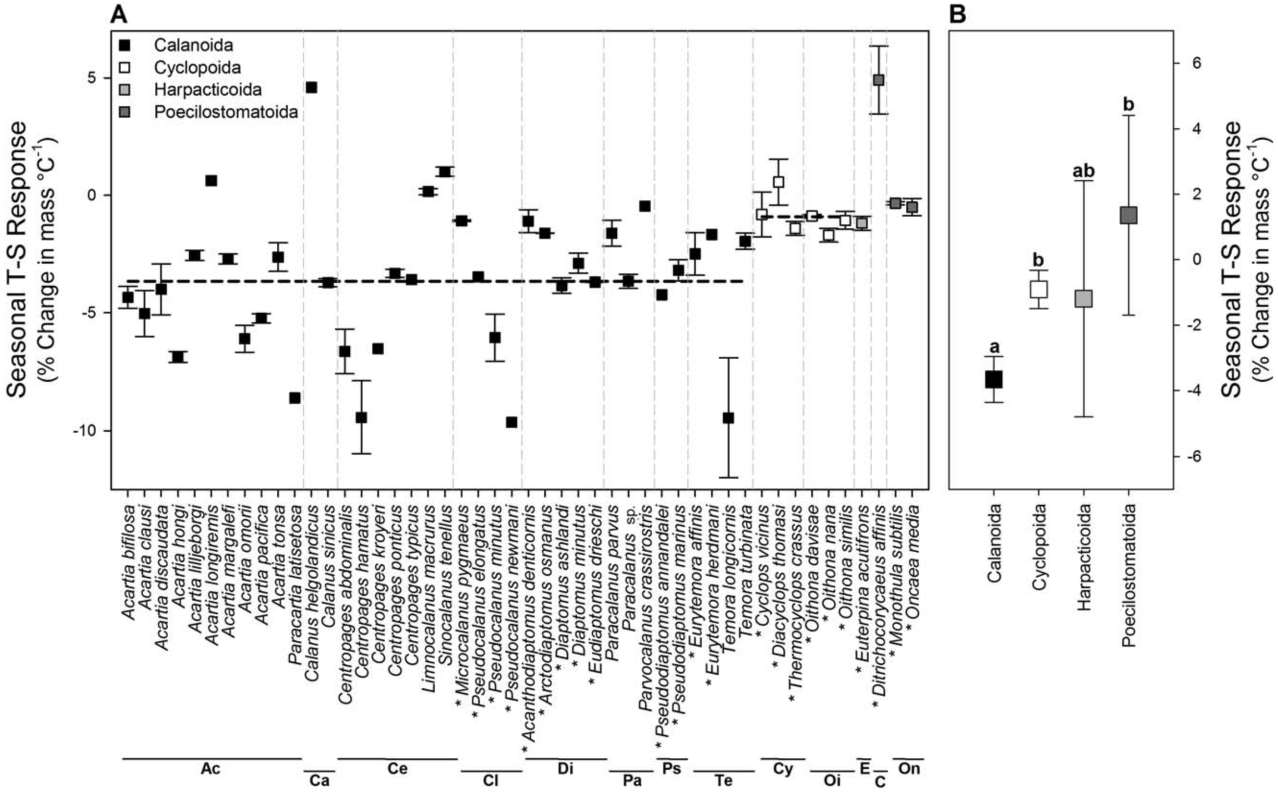

Figure 3 (a) Seasonal temperature–size responses of adult copepods for individual species, including both males and females, categorized by order (Calanoida, Cyclopoida, Harpacticoida, Poecilostomatoida) and family [Acartidae (Ac), Calaniidae (Ca), Centropagidae (Ce), Clausocalanidae (Cl), Diaptomidae (Di), Paracalanidae (Pa), Pseudodiaptomidae (Ps), Temoridae (Te), Cyclopidae (Cy), Oithonidae (Oi), Euterpinidae (E), Corycaeidae (C), Oncaeidae (On)]. Where more than one study has been undertaken on a species, the mean (±SE) are plotted. Dashed horizontal lines indicate the mean seasonal T–S response for the orders Calanoida and Cyclopoida. Dashed vertical lines divide taxonomic families. Species names preceded by an asterisk are sac spawners, whilst all other species are broadcast spawners. (b) Species-specific seasonal T–S responses (±95% CI), averaged by order. Different letters above data points indicate significant differences, whilst shared letters indicate no significant difference. Note the significant difference between current-feeding Calanoida and ambush-feeding Cyclopoida.

length will change little, and yet temperatures may vary considerably.

Food availability has also been shown to have a direct influence on body size (Berrigan & Charnov, 1994; Diamond & Kingsolver, 2010), though we find that Chl-*a* concentration explains very little of the seasonal variation in body mass, both when modelled independently and after accounting for the effects of temperature. This suggests that temperature is much more significant in driving body size responses in these natural populations. Greater quantities of food typically lead to larger size at maturity in ectotherms, and we observe a positive but non-significant percentage change in adult body mass with increasing Chl-*a* concentration on average (Fig. 2). Chl-*a* concentration commonly correlates significantly with juvenile growth and adult fecundity rates in many natural populations of planktonic copepods (Hirst & Bunker, 2003; Bunker & Hirst, 2004), and hence is generally considered a reasonable proxy of food availability. However, many copepods have an omnivorous diet that does not exclusively include prey containing this pigment (e.g. including heterotrophic ciliates and flagellates; Calbet & Saiz, 2005), and the proxy also fails to account for variation in prey quality (Pond *et al.*, 1996), which has been shown to alter the T–S response, even reversing its sign at times (Diamond & Kingsolver, 2010). Here we find little evidence for sign reversal when comparing laboratory and field animals. Time lags might also obscure the correlation between Chl-*a* concentration and body size. As food availability commonly varies over a much shorter time-scale than generation time, whilst temperature varies over a relatively longer time-scale, correlations with the latter are likely to be much more reliable. Although greater chlorophyll concentration is often associated with increased growth (Hirst & Bunker, 2003; Bunker & Hirst, 2004), consumer abundance is also predicted to increase with primary productivity (O'Connor *et al.*, 2009). Our analysis does not account for the abundance of the copepods, and hence we are unable to assess the role of food availability on a per capita basis. Assuming the metabolic rate has a Q_{10} of 2.5 and scales with body mass$^{0.75}$ (Zuo *et al.*, 2012), a simple calculation suggests that an organism would have to decrease its body mass by approximately 11.5% °C^{-1} of warming to offset the increase in metabolic

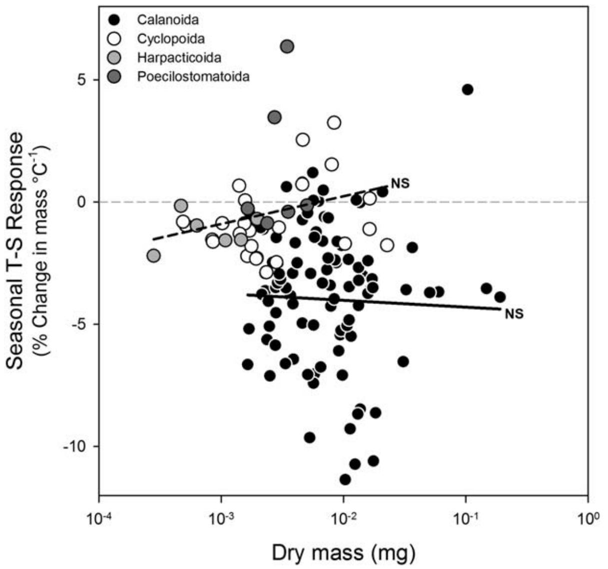

Figure 4 Seasonal temperature–size responses (percentage change in body mass °C^{-1}) versus species adult dry mass (mg) ($\log_{10}$ scale), categorized by taxonomic order. We find no significant relationship between the strength of seasonal T–S responses and species body mass across either Calanoida ($F_{1,101} = 0.11$, $P = 0.75$; solid line) or non-Calanoida species ($F_{1,35} = 2.75$, $P = 0.11$; dashed line). Data for both females and males are included where possible. NS, not significant.

rate associated with this temperature increase. Given that calanoid copepods on average reduce their body mass by only 3.7% °C^{-1}, this compensates for approximately a third of the increase in metabolic rate °C^{-1} of warming. If resources were limiting and kept constant then population abundance would have to fall substantially with warming to accommodate the extra metabolic demand, even with a reduced individual body size.

In the field, beyond variation in temperature and food availability, we might expect predation by ectotherms to increase with warming (Kordas *et al.*, 2011). This in turn may lead to increased copepod mortality, selecting for earlier maturation and resulting in a reduced adult body size. Copepods can also detect and perceive chemical signals released by predators, such as fish kairomones, the presence of which has been shown to trigger faster development and earlier maturation at a smaller body size in calanoids (Gutiérrez *et al.*, 2010). Thus, increased predation risk in the warm and associated increases in mortality and the presence of chemical cues may amplify the T–S response in the field.

The relative strength of the seasonal T–S response does not vary significantly between the sexes in this study, evidence for which can also be observed in Fig. 5. These findings agree with the broader analysis across Arthropoda, for which T–S responses were not found to differ significantly between the sexes (Hirst *et al.*, 2015). Rensch's rule suggests that male body size varies more than female body size, irrespective of which is the larger sex (Rensch, 1960). Applied within species, the rule would predict an increase in sexual size dimorphism (SSD) with increasing body size in species where males are the larger sex, and a decrease in SSD with body size in species where females are larger. Thus males should consistently have the greater size variation, yet we find no evidence to support this pattern at the intraspecific level. Our finding at the intraspecific level here concurs with there being isometry between male and female size seen across copepod species (Hirst & Kiørboe, 2014), suggesting that the selection pressures on the seasonal T–S response have been equally as strong for both males and females.

Though recent studies have begun to identify broad trends in both the magnitude and direction of the T–S response, for example between terrestrial and aquatic species (Forster *et al.*, 2012; Horne *et al.*, 2015), there remains a large amount of variation in the strength of the response that is yet to be explained. This is especially true for planktonic species, which are only a few millimetres or less in size, where in most conditions oxygen availability appears unlikely to be a driver. Indeed, our most compelling finding is the significant difference in the strength of seasonal T–S responses between species of calanoid and cyclopoid (Fig. 3), both of which typically employ different feeding modes. We find that calanoids exhibit much greater size plasticity upon temperature changes than non-calanoids. This is consistent with the hypothesis that feeding mode may influence the T–S response, since all calanoids can produce a feeding current to harvest prey, while none of the other orders do so. The extent to which the T–S response differs between these two feeding modes depends on the differences in both size scaling and the thermal response of feeding in relation to metabolism. Thus, in order to thoroughly test this hypothesis, one would need estimates of within-species mass scaling and temperature dependence of feeding and metabolism. While there are some estimates of between-species body mass scaling of respiration and feeding of the two groups (e.g., Kiørboe & Hirst, 2014), the body mass-dependent changes in vital rates during ontogeny are typically different (Hirst *et al.*, 2014; Glazier *et al.*, 2015), and thus needed for these groups. A further complication arises from the fact that feeding mode may change during ontogeny: while all cyclopoids are ambush feeders throughout their development, many calanoids are ambush feeders during the nauplius stage, and feeding current feeders during the copepodite stages; or they may switch between feeding modes in the copepodite stages (Kiørboe, 2011).

We note the association between taxonomic order and feeding mode in our dataset, and appreciate the potential difficulty in disentangling effects of feeding strategy from other order-specific differences in physiology and behaviour. For example, all cyclopoids in our dataset are sac spawners, whilst calanoid species can be either broadcast or sac spawners, but are more commonly the former. However, we find no substantial effect of reproductive strategy on the sensitivity of mature body mass to temperature. Whilst broadcast and sac spawning planktonic copepods have markedly different rates of adult fecundity (Bunker & Hirst, 2004), egg mass production rates (Hirst & Bunker, 2003) and egg mortality

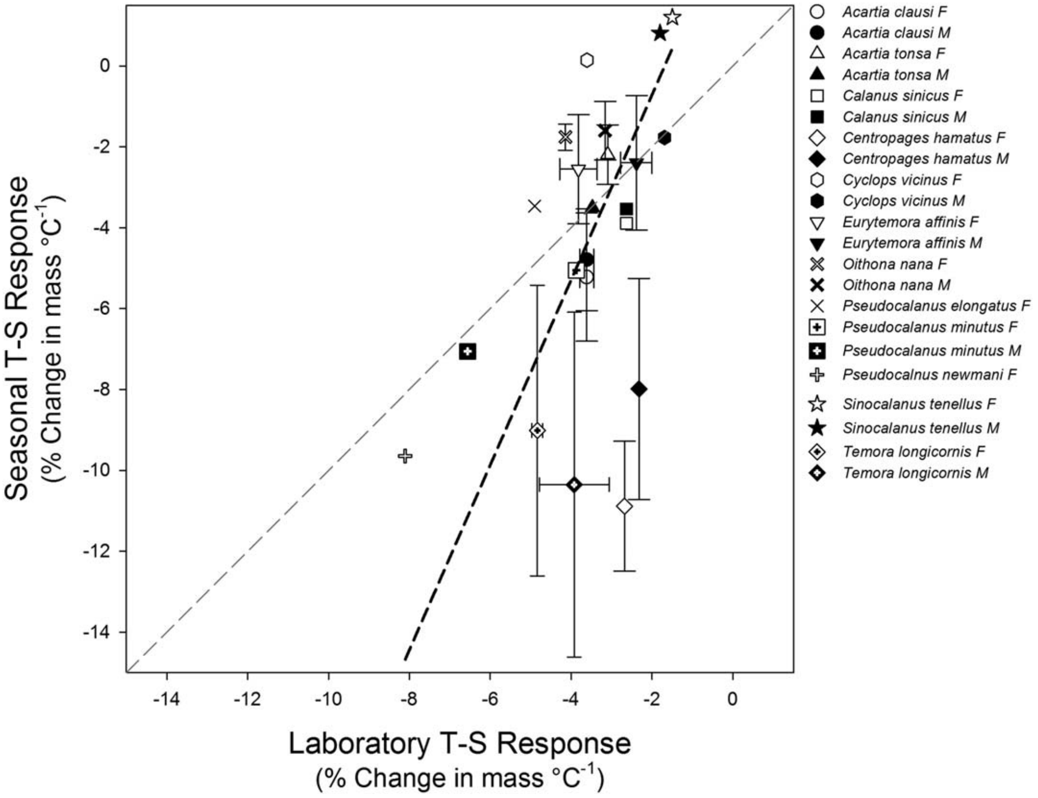

Figure 5 Male (M) and female (F) species-specific laboratory temperature–size (T–S) responses versus seasonal T–S responses in planktonic copepods. Seasonal T–S responses are much more variable than laboratory T–S responses, and there is a significant positive correlation (reduced major axis regression; $R^2 = 0.25$) between the two. The dashed grey line indicates $y = x$ for comparison. There appears to be no significant difference in the strength of seasonal and laboratory T–S responses between the sexes.

(Hirst & Kiørboe, 2002), they appear to have somewhat similar rates of juvenile growth, development and mortality (Hirst & Kiørboe, 2002; Hirst & Bunker, 2003). The T–S responses of species with determinate growth are largely generated during the juvenile phase of ontogeny (Forster & Hirst, 2012). Similarity of important life-history rates during the juvenile phase may therefore explain the lack of difference in the T–S responses within the calanoids based upon reproductive strategy. Expanding our analysis in future to consider ambush-feeding calanoid copepods, such as in the genera *Tortanus* and *Pareuchaeta*, will help to more definitively separate effects of feeding strategy from order-level differences. Unfortunately, at present, suitable data are not available on these taxa. We recommend that future experimental studies comparing species-specific size variation in response to temperature, both within and between taxonomic orders, should focus on those taxonomic groups that are currently data deficient.

Given that body size is an important predictor of fitness, and warming is a prominent feature of climate change, there is an urgent need to accurately predict changes in body size with temperature. This is particularly the case in zooplankton, which globally represent a primary resource for invertebrates and vertebrates, including fish (Ware & Thomson, 2005). Changes in body size will not only affect individual and population fitness, but may have an impact on feeding rates and alter food web connectivity given the size dependency of trophic processes (Hansen *et al.*, 1994; Rice *et al.*, 2015), as planktonic food webs are especially highly size-structured (Webb, 2012). Measuring and accounting for abundance in the field would also help to define the relationship between food availability per capita and adult body size under natural conditions. This may be particularly informative in light of the fact that the T–S response in the majority of ectotherms appears to compensate for only a small proportion of the predicted increase in metabolic rate with temperature, whilst metabolic rate in autotrophs (and thus primary productivity) increases substantially less with warming than metabolic rate in heterotrophs (Allen *et al.*, 2005).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Data S1. Seasonal body size data used in this study.
Table S1. AIC output comparing linear, quadratic, exponential and allometric fits for determining seasonal temperature–size responses.
Table S2. Mean 95% confidence intervals of individual T–S and C–S regressions for each taxonomic order.
Figure S1. Comparison of species-specific coefficients of determination for temperature and Chl-*a* concentration in explaining seasonal body mass changes (values averaged by order).

BIOSKETCH

The work presented here results from an ongoing collaboration between researchers from Queen Mary University of London, the University of Liverpool and the Centre for Ocean Life, Technical University of Denmark. We use a range of approaches such as meta-analysis, experimentation, conceptualization and modelling to establish and test governing rules of ecology and evolution. A major focus is the ecology, physiology and population processes of marine species, especially plankton, including the impact of a changing climate. Further information, including details of our latest research and publications, can be found at our research group websites: http://www.aquatic-ecology.co.uk/ and www.oceanlifecentre.dk

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APPENDIX 1: DATA SOURCES

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Equal temperature–size responses of the sexes are widespread within arthropod species

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Sexual size dimorphism (SSD) is often affected by environmental conditions, but the effect of temperature on SSD in ectotherms still requires rigorous investigation. We compared the plastic responses of size-at-maturity to temperature between males and females within 85 diverse arthropod species, in which individuals of both sexes were reared through ontogeny under identical conditions with excess food. We find that the sexes show similar relative (proportional) temperature–body size (T–S) responses on average. The high degree of similarity occurs despite an analysis that includes a wide range of animal body sizes, variation in degree of SSD and differences in the sign of the T–S response. We find no support for Rensch's rule, which predicts greater variation in male size, or indeed the reverse, greater female size variation. SSD shows no systematic temperature dependence in any of the 17 arthropod orders examined, five of which (Diptera, Orthoptera, Lepidoptera, Coleoptera and Calanoida) include more than six thermal responses. We suggest that the same proportional T–S response may generally have equivalent fitness costs and benefits in both sexes. This contrasts with effects of juvenile density, and food quantity/quality, which commonly result in greater size plasticity in females, suggesting these variables have different adaptive effects on SSD.

1. Introduction

Difference in body size between males and females within a species is termed sexual size dimorphism (SSD) and is widely observed throughout the plant and animal kingdoms [1]. Male-biased SSD, where males are the larger sex, is common among endothermic vertebrates and may in part relate to males competing for mates [2,3]. By contrast, female-biased SSD tends to predominate in ectothermic invertebrates and vertebrates [4,5]. The dimorphic niche hypothesis (reproductive role hypothesis) states that the differential reproductive roles of the sexes are associated with differential energetic costs [6] leading to different size optima [7,8], hence SSD. In most invertebrate species, there is considered to be selection for larger females, because this favours larger clutch size and/or offspring size. Males may benefit indirectly from a smaller body size associated with earlier adult emergence (protandry) because this may increase their chance of reaching maturity (important when they rove for females and suffer high mortality risk); and in populations with discrete generations protandry may increase the potential of males to be ready to fertilize females that reach sexual maturity later [9–11].

Although SSD has at times been assumed to be rather invariant within a species, studies have found this to change as a consequence of significantly greater variation in size of males [12] or females [13]. A variety of rules and theories have been formulated to explain variation in SSD, both between and within species [14–16]. Rensch's rule (RR) states that male body size varies more than female body size, irrespective of which sex is larger. RR was originally formulated to describe the pattern seen across species within a related clade, but has since been tested within species to see if similar drivers exist at the intra-specific level [13,17]. Within a species, it predicts an increase in SSD with increasing body size in species where males are the larger sex, and a decrease in SSD with body size in species where females are larger [14,16]. One prominent general hypothesis (i.e. evolutionary mechanism) potentially generating RR is when, over evolutionary time, directional (primarily sexual) selection for large male size is overall stronger than directional (primarily fecundity) selection for large female size [4]. Additionally, the fitness consequences of large versus small body size can differ between the sexes under different ecological and environmental conditions because the sexes differ in the degree of plasticity they exhibit in response to climatic or ecological variables [17–19].

Variation in size at maturity has been widely documented for ectothermic species experiencing different thermal regimes during ontogeny [20–23]. This intra-specific phenotypically plastic response commonly leads to a reduction in size-at-stage with warming and has been called the temperature–size rule (TSR) [20]. Given the ubiquity of the TSR in taxonomic groups as diverse as bacteria, protists and metazoans [22,24,25], the proximate and ultimate causes have been explored in some detail [22,25–29]. The temperature–body size (T–S) response has been shown to vary in magnitude in relation to species body size, voltinism and taxonomic group, and between aquatic and terrestrial-living species [22,23]. Variation in SSD can result when males and females respond differently to their environment (differential-plasticity hypothesis [18]), and this may be an important contributor to the observed variation in SSD. Thus the degree to which different environmental factors such as juvenile density, food quality or quantity, and temperature elicit contrasting plastic body size responses between the sexes should be informative [12,19]. Here, we specifically focus on sex-dependent differences in T–S responses, as this may ultimately help to elucidate the underlying drivers of both SSD and T–S responses. Sex-based differences in T–S responses have been considered before; while most studies have been experimental and have considered single species, the syntheses and analyses of responses by Teder & Tammaru [13] and Stillwell *et al.* [19] on insects, have looked more broadly at differential changes in SSD with environmental conditions. We increase the amount of temperature response data considered by almost fourfold in our analysis and increase the range of species to include other Arthropoda. This allows greater power when testing variation in SSD with temperature. Furthermore, we explore variation in *absolute* sexual size differences across temperatures, and for the first time the degree to which SSD relates to the magnitude of the T–S response.

Most analyses of variation in body size responses (and comparisons between the sexes) have expressed this change in relative terms, for example, using regressions to derive the slope of log size of one sex versus the other, or comparing SSD as a proportion (see the varied approaches in Stillwell & Fox [12]; Teder & Tammaru [13]; and Stillwell *et al.* [19]). The use of relative size responses allows for the removal of bias caused by the differences in body size of the sexes and reduces statistical problems. Yet changes in absolute size differences between the sexes may hold important information relating to mate assessment and performance [30–32]. Consequently, although we focus our efforts mainly on exploring the thermal dependence of SSD within species using relative metrics, we also consider the implications of absolute differences in size between the sexes, and how this changes with temperature. We seek to test a range of interrelated questions using data from a diverse set of species from within the Arthropoda, specifically:

(i) Do the T–S responses differ systematically between the sexes intra-specifically, and is there any evidence to support RR?
(ii) Do differences in the T–S responses of the sexes relate to the magnitude of SSD?
(iii) How does the absolute difference in body mass between the sexes vary with temperature, and how does this differ from the relative responses?
(iv) How does the effect of temperature on SSD compare with that of other environmental influences: food quantity and quality, pathogen infection, photoperiod and larval crowding and competition, as quantified by Stillwell *et al.* [19]?

2. Material and methods

The data compilation of Horne *et al.* [23] was revisited; this provides a single extensive set of data on the size-at-maturity responses to temperature of a wide range of arthropod species, including marine, freshwater and terrestrial forms. Briefly, studies were systematically screened to include only laboratory studies where individuals were reared at a range of constant temperatures, with food concentrations at or above saturation, in order to remove the confounding impact of food limitation. Extreme, potentially stressful temperatures were excluded. Only adult size measurements were used for analysis from studies where males and females had been separated. In a small minority of cases, pupal size was considered to be a reliable correlate of size at maturity. We were careful to ensure that we only included measurements when data for both sexes had been provided and the same controlled experimental conditions were used for each sex. The minimum period of acclimation for the inclusion of adult size data was set so that only individuals that were raised from egg or first larval stage were included. Adult data were collected as a variety of metrics including lengths, volumes, and dry, wet or carbon mass. These measurements were subsequently converted to dry mass (mg) using intra-specific regressions. Where these were not available, regressions for closely related species, and occasionally more general interspecific regressions, were used. All data and conversions are detailed in our electronic supplementary material, table S1.

The sex-specific slopes of $\log_e$ dry mass versus temperature were used to examine thermal responses in body size for single species. This exponential form has the advantage of being a better fit than alternative transformations, as judged by Akaike weights (see electronic supplementary material, table S2). Moreover, it has the distinct advantage of allowing examination of relative change and is largely unbiased by absolute body size. We transformed the slopes into percentage change in dry mass per °C, using the formula $(\exp^{(\text{slope})} - 1) \times 100 = \%$ change in mass per °C [22]. A negative percentage indicates a decrease in size with increasing temperature (following the TSR) and a positive percentage an increase in size (converse TSR).

Differences in body size variation can be assessed in different ways. Blanckenhorn *et al.* [17] compared latitudinal clines in body size between the sexes and obtained different results depending on whether they examined clines in size ratios between the sexes (ratio-clines), or used an allometric approach plotting log body size of one sex versus that of the other. We therefore compared results derived using ratio-clines and allometric methods. First, we determined the degree of difference between T–S responses of the conspecific males and females (within single studies) as:

$$\text{T–S ratio} = \left(\frac{\text{larger T–S response}}{\text{smaller T–S response}}\right) - 1. \tag{2.1}$$

We defined the ratio as being positive when males had the greater T–S response, and negative when females had the greater response. This formulation has an advantage of providing symmetrical results around zero, regardless of which sex has the greater T–S response.

Second, we used the commonly applied method of performing a reduced major axis (RMA) regression of the $\log_{10}$ body size of one sex against the $\log_{10}$ body size of the other sex, where each individual data point represents the paired size measurements at one of the experimental temperatures. This allometric slope gives a quantitative expression of how the sizes of the sexes change together. In this regression, the more size-responsive sex was represented on the y-axis and the less size-responsive on the x-axis, so the slope was always more than or equal to 1. We subtracted 1 from the slope and again defined the value as being positive when male size was the more responsive, and negative when female size was the more responsive. This method once again ensures that results vary around zero, and there is symmetry based on equivalent differences between the sexes, regardless of which sex shows the greater response. We term this slope value the $\text{RMA}_{\text{Index}}$; zero indicates isometry, i.e. equal covariation in male and female size, while increasingly positive values indicate greater male size responses (variation), and increasingly large negative values signify greater female size responses.

We quantified the absolute degree of SSD using the Sexual Dimorphic Index (SDI) of Lovich & Gibbons [33], where:

$$\text{SDI} = \left(\frac{\text{mass of larger sex}}{\text{mass of smaller sex}}\right) - 1. \tag{2.2}$$

We followed the recent convention that the SDI index is given as a positive value when males are the larger sex, and as a negative value when females are larger. Mean mass values for each sex were predicted at 20°C from the T–S regressions, which in most cases did not involve any extrapolation.

In order to compare estimates of sex differences in size responses to temperature derived using the two methods, we plotted each T–S ratio value against its appropriate $\text{RMA}_{\text{Index}}$ value across all 116 T–S responses and performed a RMA regression (figure 1). Additionally, we performed a paired Wilcoxon signed-rank test to compare T–S ratio and $\text{RMA}_{\text{Index}}$ values. To determine whether the proportion of male- and female-biased T–S ratio and $\text{RMA}_{\text{Index}}$ values differed significantly from 0.5, we used a χ^2 proportionality test.

The independent effects of taxonomic order, environment type (terrestrial, freshwater and marine) and life cycle type (i.e. hemi- versus holometaboly) on the size and sign of the T–S ratio and $\text{RMA}_{\text{Index}}$ were determined using analysis of variance. Crustaceans were considered hemimetabolous, as these have direct development from larvae to juveniles to adults. *Post hoc* comparisons (Tukey HSD) were used to identify any significant differences in both the T–S ratio and $\text{RMA}_{\text{Index}}$ between taxonomic orders. Ordinary least-squares (OLS) regression analysis was used to determine whether the T–S ratio changes significantly with the absolute degree of SSD (indexed by SDI). All statistical analyses, with the exception of RMA analyses, were conducted using the free statistical software package *R* [34]. All RMA analyses were performed using the free statistical program *RMA for JAVA v. 1.21* [35].

To quantitatively assess the degree to which absolute sizes of males and females converge or diverge at increasing temperatures, we determined sex-specific slopes of the linear regressions of dry mass (mg) on temperature for each sex (as an estimate from what may be a somewhat curved relationship). The difference between these slopes gives the degree to which the two converge or diverge with increasing temperature; this difference was expressed as a percentage of the female size (at 20°C), hence the formula is

$$\left(\frac{\text{slope for larger sex} - \text{slope for smaller sex}}{\text{mass of the female at } 20^{\circ}\text{C}}\right) \times 100. \tag{2.3}$$

A negative value represents convergence, and a positive value divergence with increasing temperature. Normalizing to female mass at 20°C adjusts for any considerable differences in absolute size between different taxa and the sexes. Again, we used analysis of variance to determine the effect of the absolute degree of SSD (indexed by SDI) and taxonomic order on change in absolute size difference with temperature, and a *post hoc* Tukey HSD test to look for significant pairwise differences between individual taxonomic orders. In addition, a series of one-sample t-tests were used to identify which orders, if any, had a mean change in absolute size difference between sexes with temperature that differed significantly from zero.

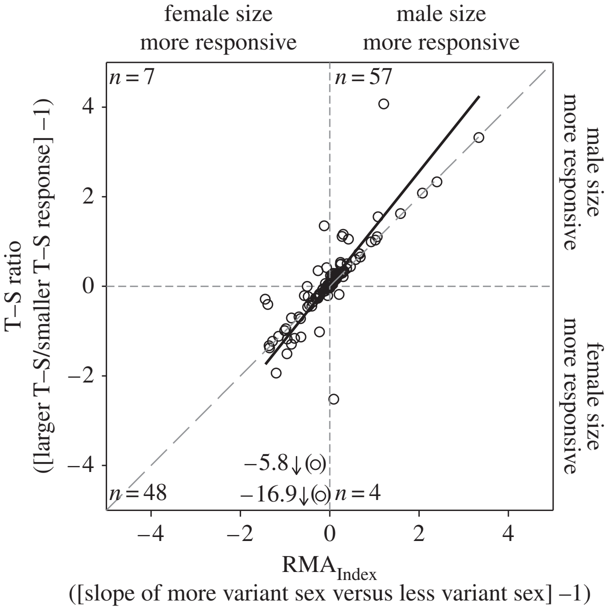

Figure 1. Comparison of two methods for estimating sex differences in body size response to rearing temperature: T–S ratio [(larger response % change in body mass $^{\circ}\text{C}^{-1}$/smaller % change in body mass $^{\circ}\text{C}^{-1}$) − 1] and $\text{RMA}_{\text{Index}}$ [slopes derived from the RMA regression of body mass values for the sex with the more thermally responsive body size on the y-axis (logged) versus the body size values for the less responsive sex on the x-axis (logged) − 1] for arthropod species, where each point represents a single species in a single study. Positive values represent greater response in male size than female, negative values greater response in female size. An RMA regression through the data (excluding the two extreme ratios given in brackets) is given by the solid line and has a slope of 1.25 (1.13–1.38, 95% CI range). The RMA slope is significantly different from 1. The diagonal dashed line indicates a 1 : 1 relationship. The number of values falling within each of the four quadrants (n) is indicated.

3. Results

Our meta-analysis includes 116 paired male and female T–S responses [% change in dry mass (DM) $^{\circ}\text{C}^{-1}$] from 85 arthropod species, including representatives from 17 taxonomic orders from marine, freshwater and terrestrial environments. These responses have a negative slope in approximately 84% of cases, and only in two instances was the sign of the T–S response different between males and females within a species. There is wide variation in the strength and direction of the T–S response, ranging from $-8.15\%^{\circ}\text{C}^{-1}$ to $5.67\%^{\circ}\text{C}^{-1}$ in females. This variation across species can be largely accounted for by strong differences in responses between water-living versus air-breathing species [22,23], and, in terrestrial arthropods, between univoltine and multivoltine species [23].

In comparing the two methods used to assess which sex has the stronger size response to warming (T–S ratio versus $\text{RMA}_{\text{Index}}$; figure 1), we typically find a close agreement

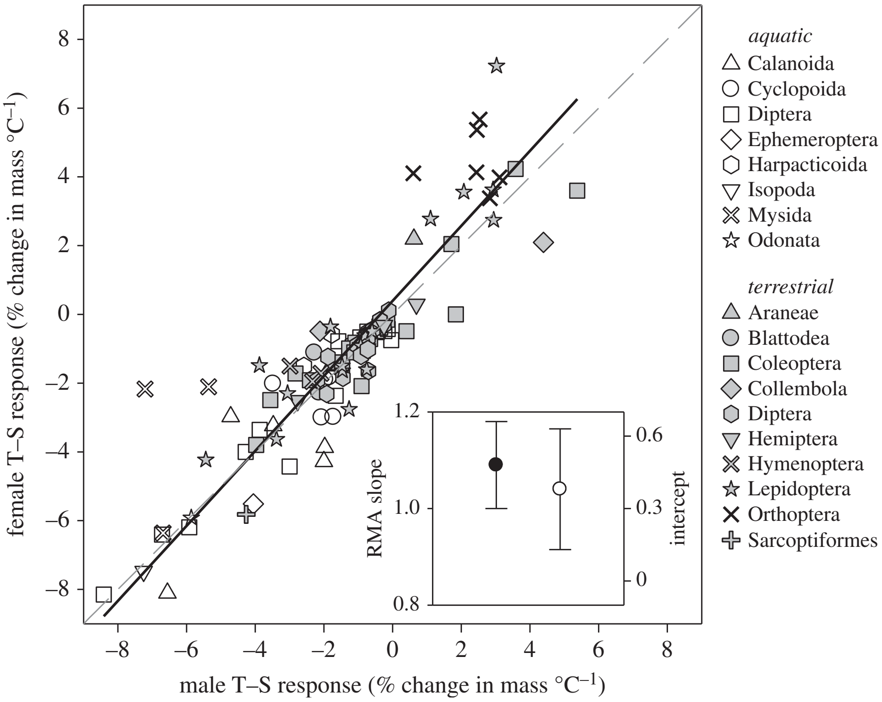

Figure 2. Female versus male T–S responses (% change in body mass $^{\circ}C^{-1}$). Each point represents a single study of a species and is classified by taxonomic order. An RMA regression through the data is given by the solid line and has a slope of 1.09 (1.00–1.18, 95% CI range) with an intercept of 0.38 (0.13–0.63, 95% CI range), denoted in the inset panel by the black and open circles, respectively. The RMA slope is not significantly different from 1. The diagonal dashed line indicates a 1 : 1 relationship.

between them. The RMA_{Index} suggests that male body size is more responsive to temperature in 61 out of 116 cases, which compares with 64 cases calculated using the T–S ratio. Only in 11 instances did the two disagree on which sex had the greater size response to temperature. Regressing the two metrics against one another (using RMA; figure 1), and excluding the two extreme values indicated in brackets, gives a slope of 1.25 (1.13 to 1.38, 95% CI range) and an intercept of 0.06 (−0.03 to 0.15, 95% CI range). This slope is significantly different from 1, while the intercept is not significantly different from zero, as inferred from the 95% CIs (figure 1). Including the two extreme values also results in a slope significantly greater than 1 (slope = 2.71, 2.25 to 3.16, 95% CI range). Comparing the T–S ratio and RMA_{Index} values using a paired Wilcoxon signed-rank test also reveals a significant difference between the two ($V = 3295$, $p = 0.01$). We conclude that although the two metrics produce on average very similar values, the T–S ratio tends to give somewhat more extreme values than does the RMA_{Index}. Because of the very close similarity in the results between these methods, we only present the T–S ratio data henceforth. However, all analyses have also been undertaken using the RMA_{Index} and are summarized in the electronic supplementary material: these further support the conclusions we present here.

We observe similar body size plasticity to temperature in both the males and females of a species on average. We find a significant correlation between female and male T–S responses across species ($p < 0.001$, $R^2 = 0.81$), with an RMA regression slope of 1.09 (1.00–1.18, 95% CI range), and with an intercept of 0.38 (0.13–0.63, 95% CI range; figure 2). We find that male size responds to temperature more strongly than size of conspecific females in 64 out of 116 cases, as assessed using the T–S ratio. Hence, the null hypothesis that a stronger size response is observed equally often in each sex cannot be rejected (χ^2 proportionality test; $\chi^2 = 1.24$, $p = 0.27$). This finding is further supported when size response is measured using the RMA_{Index} ($\chi^2 = 0.22$, $p = 0.64$), in which male size responds more strongly than conspecific female size to temperature in 61 out of 116 cases.

Our analysis highlights a close similarity in the T–S responses of both sexes within the Arthropoda, and also within orders of Arthropoda (figure 3), although some orders have much fewer data than others in our study. Although within single taxonomic orders there is variation in the T–S ratio between species, analysis of variance and *post hoc* comparisons (Tukey HSD) show taxonomic order to have no significant effect on the size or sign of the T–S ratio ($F_{17,98} = 0.77$, $p = 0.72$), and there appear to be no systematic patterns, such that in all orders for which we have sufficient data to make the test, the mean value does not differ significantly from zero (figure 3). Similarly, we find no effect of environment type (marine, freshwater and terrestrial) on the thermal dependence of SSD ($F_{2,113} = 2.41$, $p = 0.09$), even though we know that the overall size and sign of T–S responses shown by animals relate strongly to these different environmental categories [22]. The effect of life cycle (hemi- versus holometaboly) was also non-significant, both when testing across the entire dataset ($F_{1,114} = 0.16$, $p = 0.69$) and exclusively within insects ($F_{1,92} = 0.47$, $p = 0.49$). Although the T–S ratio values for Orthoptera do not differ significantly from zero, in all six species considered female size responded (increased) most to warmer rearing temperatures (figure 3).

The T–S ratio does not change significantly with the degree of SSD (as indexed by SDI), inferred from the non-significant OLS regression between the two ($F_{1,114} = 0.28$, $p = 0.60$). Thus, across environments, orders, and for varying degrees of SDI, we find no evidence to suggest greater thermally induced variance in male size (i.e. no evidence to support an intra-specific version of RR) nor indeed the opposite, greater variance in female size. Neither sex exhibited

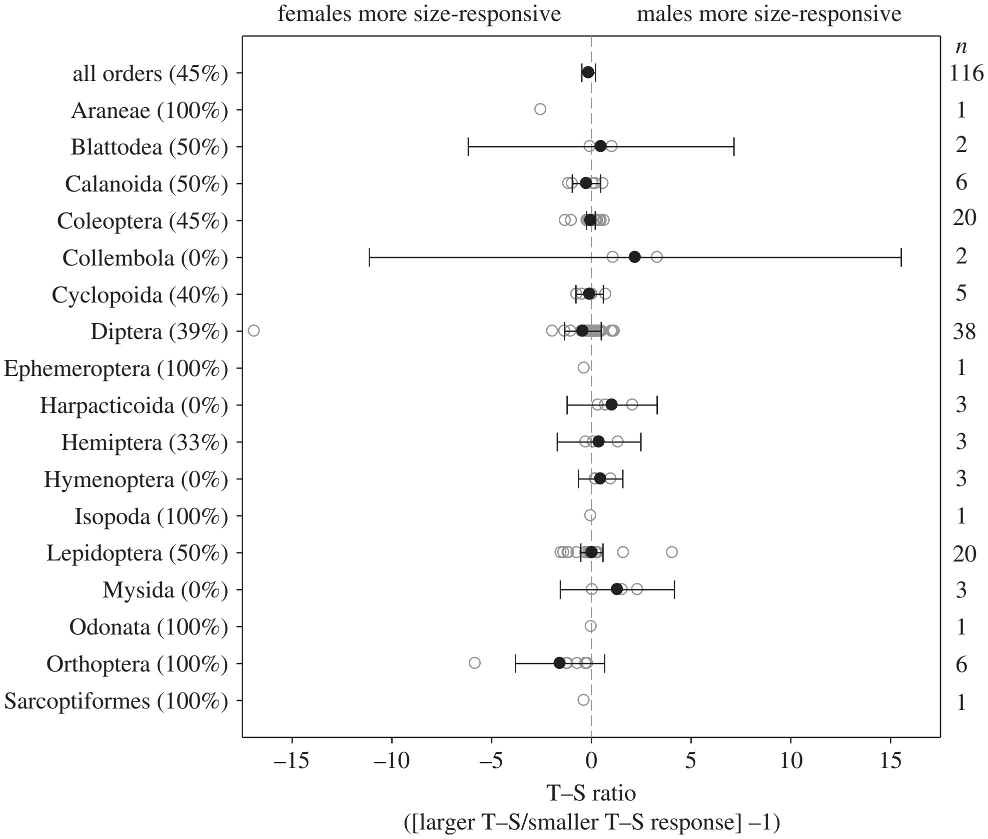

Figure 3. A comparison of the thermal dependence of male and female size responses (T–S ratio) within arthropod species, categorized by taxonomic order. Error bars denote 95% CI. The vertical dashed line denotes zero, i.e. no difference between male and female body size responses to temperature. Values greater than zero indicate more responsive male mass. Values less than zero indicate more responsive female mass. All order-specific T–S ratios do not differ significantly from zero. The percentage of cases in which female size was the more responsive is given in brackets on the left-hand side of the panel after each order. The sample size (*n*) of each order is given on the right-hand side of the panel.

significantly greater relative body size response to temperature than the other on average.

Warmer rearing conditions cause male and female absolute sizes to converge in 62 cases, and diverge in 54 cases. We find a significant positive relationship between the extent of convergence/divergence in body size with warming and the strength of the female T–S response, such that species that exhibit a strong negative T–S response also show the greatest convergence in absolute body size with warming, whereas those that exhibit a strong positive T–S response show the greatest divergence in absolute body size with warming ($F_{1,113} = 98.2$, $p < 0.001$, $r^2 = 0.46$). The corresponding regression lies predominantly within the range of values predicted given the range in SDI observed across our dataset, and based on an assumption that both males and females have identical proportional T–S responses (see electronic supplementary material, figures S2a and S2b, for a conceptual and quantitative examination of this). This leads us to the simple explanation that this strongly significant relationship is an outcome of similar T–S responses between males and females, which causes a greater absolute degree of size convergence and divergence as relative response increases; an outcome expected simply from mathematics, but one with possible ecological implications. Taxonomic order has no significant effect on convergence or divergence ($F_{16,99} = 1.51$, $p = 0.11$), and the mean degree of size convergence or divergence does not differ significantly from zero, with the exception of three orders: Cyclopoida ($-1.94\%\ ^{\circ}\mathrm{C}^{-1} \pm 1.28$ CI; $t_4 = -4.20, p = 0.01$), Diptera ($-0.24\%\ ^{\circ}\mathrm{C}^{-1} \pm 0.19$ CI; $t_{37} = -2.58$, $p = 0.01$) and Orthoptera ($4.61\%\ ^{\circ}\mathrm{C}^{-1} \pm 2.47$ CI; $t_5 = 4.79$, $p < 0.01$; figure 4). Hence there is strong divergence in the absolute size of the sexes with warming in Orthoptera, but convergence in the Cyclopoida and Diptera.

4. Discussion

In our examination of the T–S responses of a wide range of arthropod species, we find that male size responds to temperature more strongly than size of conspecific females in 64 out of 116 cases, as assessed using the T–S ratio (figure 2). We cannot statistically reject the null hypothesis that a stronger size response is observed equally often in each sex. Indeed, this finding is also supported when size response is measured using the $\mathrm{RMA}_{\mathrm{Index}}$. Furthermore, our analysis highlights a close similarity in the T–S responses of both sexes within orders of Arthropoda (figure 3). However, we must highlight that our conclusions are phylogenetically limited, in that only five orders (Diptera, Orthoptera, Lepidoptera, Coleoptera and Calanoida) included six or more datasets, while most orders (11 out of 17) were represented by just three or fewer. Moreover, orders for which there are more data are still taxonomically restricted. For example, the majority of dipteran species are from just two families (Drosophilidae and Culicidae), while more than 50% of lepidopteran species are from two families (Lycaenidae and Nymphalidae), although these two butterfly families have the greatest number of species worldwide.

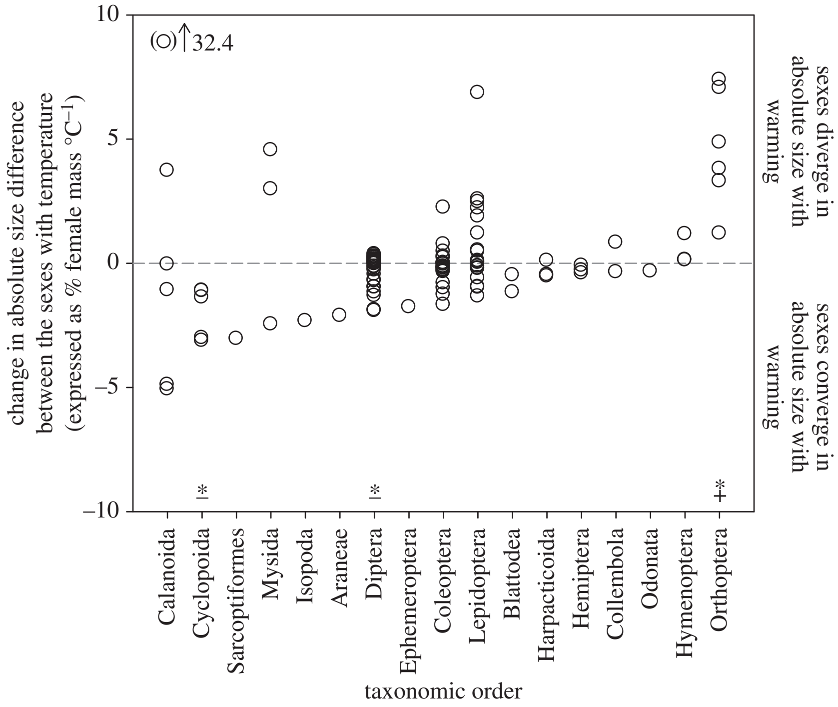

Figure 4. Change in the absolute mass difference between the sexes of a species with increasing temperature, expressed as a percentage of the female size (at 20°C) per degree Celsius. Data are categorized by taxonomic order. The overall effect of taxonomic order on the degree of convergence or divergence is not significant ($F_{16,99} = 1.51$, $p = 0.11$). The mean for each order does not differ significantly from zero, with the exception of Cyclopoida ($-1.94\%\ ^{\circ}\mathrm{C}^{-1} \pm 1.28$ CI; $t_4 = -4.20$, $p = 0.01$), Diptera ($-0.24\%\ ^{\circ}\mathrm{C}^{-1} \pm 0.19$ CI; $t_{35} = -2.24$, $p = 0.03$) and Orthoptera ($4.61\%\ ^{\circ}\mathrm{C}^{-1} \pm 2.47$CI; $t_5 = 4.79$, $p < 0.01$). These orders are marked with an (*) and + or − to indicate that these have divergence or convergence, respectively.

Although there is a considerable range of T–S responses, from a large increase in size with warming (especially large-bodied univoltine terrestrial species), to marked reductions in size with warming (in many large-bodied aquatic species) [23], we find no systematic sex-based differences in response size within arthropods. This outcome occurs despite the often radically different behaviour, physiology, life history and body size between the sexes [36–38]. Furthermore, the average lack of sex differences in the T–S response also appears to be unaffected by the large variation in the degree of SSD, or taxonomic order. Finally, the T–S ratio does not significantly correlate with the absolute degree of SSD (indexed by SDI), which contrasts with the findings of Teder & Tammaru [13], in which females typically showed stronger phenotypic plasticity responses with varying environmental quality when SSD was more female-biased.

Blanckenhorn *et al.* [17], in an analysis that included both vertebrates and invertebrates, examined latitudinal clines in male and female body size and found somewhat contradictory outcomes depending upon the form of analysis used. Similar to the approach employed here, they calculated both an RMA regression of the size of one sex against the other, as well as comparing the ratio of latitudinal-size clines of each sex (termed slope ratio). Male size was found to vary more than female size in 66 out of 98 species when examining datasets based on latitudinal-size gradients. Thus, intra-specifically, the results conformed to greater male size variation (RR), suggesting a connection between Bergmann's and RRs. However, when using a conventional RMA regression, Blanckenhorn *et al.* [17] found that the size of neither sex was significantly more variable than the other (male size was found to vary more than female size in 55 out of 98 species). Notably, in both their study and our own, regressing the T–S ratio (or slope ratio) against the $\mathrm{RMA}_{\mathrm{Index}}$ generates a slope significantly greater than 1, suggesting that the former produces the more extreme values of the two metrics. This statistical effect has not manifested itself to the same extent in our own study. We note that because the T–S response of the less variable sex can be zero (i.e. the denominator in the T–S ratio equation), the T–S ratio can be infinite. A very low denominator value compared with the numerator will also generate very large ratios, so generating large variability, hence the apparent contradiction that can occur between the two methods. Although the different results generated by these two metrics do not alter the major outcomes and conclusions presented here, they did lead Blanckenhorn *et al.* [17] to present contrasting findings between methodologies. Unlike Blanckenhorn *et al.* [17], we find no significant differences in the body size responses of males or females, regardless of whether we use the $\mathrm{RMA}_{\mathrm{Index}}$ or T–S ratio. We note that whilst T–S responses are measured in controlled laboratory conditions using individuals from the same population, a great variety of influences can potentially select for changes in SSD across latitudes, which may not necessarily be linked directly with temperature. These factors may include, but are not limited to, the increased likelihood of genetic variation between populations, as well as size-dependent mortality and environmental factors such as food and season length.

The contrasting proximate mechanisms by which T–S responses are generated in organisms [39], and the extent to which the magnitude and direction of these responses correlate with life history (voltinism) [23]—with a possible trade-off between numbers of generations in a year and size at maturity—lead to the suggestion that these responses relate to fitness and hence are adaptive. Further, as the thermal dependence of size at maturity is so similar (in relative terms) between the sexes in individual species, this may mean that the responses have similar fitness costs and benefits in both

Table 1. Comparison of sex-specific plasticity in body mass in relation to environmental variables. We followed the methodology of Stillwell *et al.* [19] for the T–S data when undertaking RMA regressions for this table, such that $\log_{10}$ male size is plotted on the *y*-axis, and $\log_{10}$ female size on the *x*. Hence when the RMA slope is less than one, females are the more size-responsive sex, and when the RMA slope is more than 1, males are more size-responsive. CV is the coefficient of variation of body size across the data within each study. Asterisks denote a significant difference between the sexes ($*p < 0.05$, $**p < 0.01$). Table modified from Stillwell *et al.* [19], with additions from this study.

	which sex is more plastic			average degree of plasticity (CV among environments)			
environmental variable (taxonomic group)	females (no. studies with RMA slope less than 1)	males (no. studies with RMA slope more than 1)	χ^2	female	male	*t*	source
temperature (Arthropoda)	55 (47.4%)	61 (52.6%)	0.22	12.3%	12.1%	0.41	this study
temperature (Insecta)	46 (48.9%)	48 (51.1%)	0.01	11.6%	11.0%	1.14	this study
larval density/larval competition/ diet quantity (Insecta)	18 (72.0%)	7 (28.0%)	4.84*	16.0%	12.2%	3.42**	[19]
pathogenic infection (Insecta)	3 (50.0%)	3 (50.0%)	0.00	6.9%	7.2%	0.34	[19]
photoperiod (Insecta)	1 (16.7%)	5 (83.3%)	2.67	8.6%	10.7%	2.18	[19]
diet quality (Insecta)	83 (61.9%)	51 (39.1%)	7.64**	12.5%	11.5%	2.47*	[19]

males and females. While it is conceivable that a very high genetic correlation between the sexes of a species may help to explain the similar size response of males and females to temperature [40], this potential constraint seems to be overridden in cases of other environmental factors (e.g. changed diet or juvenile density) that generate systematic differences in size responses between males and females [19] (table 1).

The contrast between lack of effect of temperature on SSD observed here and the systematic effect of food quantity and also larval competition on SSD in insects [13,19] implies that there is generally a sex-dependent effect on body size caused by food resources but not by temperature (see table 1 for a quantitative comparison of the degree of plastic variation in the sexes with changes in different environmental conditions). A useful distinction is whether food supply or quality, or increased competition, acts primarily on reducing the size of the larger sex, which is consistent with energetic restrictions acting to a greater degree on larger bodies, or whether it acts primarily on just females, even in species in which males are the larger sex (e.g. some odonates). Female size was usually affected most by food supply, but in the few species with larger males than females, no consistent sex-dependent size response was observed [13]. Thus, the relative contribution of sex versus body size to the degree of size plasticity was not clearly distinguished.

The metrics used to study the allometric scaling of SSD commonly examine relative (proportional) change in body size (e.g. Blanckenhorn *et al.* [17]). Here, we also used two methods that examine change in relative body size within species, and that account for differences in size between species. In both cases we obtained the same major conclusions. In contrast to these quantitative analyses of relative size change, analyses of absolute size differences between sexes of conspecifics, and how these respond to environmental factors, have received much less attention. Yet measuring the extent to which increased rearing temperature causes absolute body sizes to converge or diverge between the sexes may be biologically informative. For example, females are very commonly the larger sex in arthropods [19,41], yet if both sexes show similar negative T–S responses (using the metrics described herein), then their absolute body sizes will converge with increasing temperature.

We observe considerable variation in both convergence and divergence in absolute mass across species that exhibit both a normal and converse TSR (represented conceptually and quantitatively in electronic supplementary material, figures S2a and S2b, respectively). Within the Orthoptera, absolute body sizes of the sexes significantly diverge with warming (figure 4). This divergence arises partly from the fact that Orthopterans generally follow the converse TSR [23] and have larger females than males, so that a similar T–S response of males and females will cause divergence in absolute size between the sexes with warming (a similar proportional size increase in the larger sex makes the absolute difference greater). But in addition, although the mean T–S ratio value for this order is not significantly different from zero (figure 3), in all six orthopteran species the females have a stronger T–S ratio than conspecific males (figure 3). The relatively strong variation in female body size with temperature observed in Orthoptera exerts an important influence on the mean T–S ratio and $\mathrm{RMA}_{\mathrm{Index}}$ of species that follow the converse TSR. Specifically, when comparing the thermal dependence of SSD between species that follow either the normal or converse TSR, we observe that females are the more variant sex in species that increase their size with warming, whilst there is no significant difference between the sexes in those that exhibit a normal T–S response. We report these observations with caution; Orthoptera account for nearly a third of positive T–S responses in our dataset, for which the sample size is already comparatively small ($n = 19$ for positive T–S responses versus $n = 97$ for negative T–S responses). Indeed, we find no significant difference in body size sensitivity between the sexes in either group when Orthoptera are excluded. Orthoptera have strongly positive T–S responses and strongly negative latitude-size clines, with larger species often being univoltine and hence potentially affected by season-length constraints on size-at-maturity [23], especially in the larger sex, which typically reaches adulthood later [42,43]. If such constraints are indeed greater in the larger (more season-length constrained) sex, warming may favour increased body size especially in females, hence the strong divergence in orthopteran absolute body sizes between the sexes at increased rearing temperatures. When there are more data available, an analysis of whether these sex-based patterns in thermal body size sensitivity extend more generally to other large univoltine species would be particularly informative.

A potential selection pressure is to reduce extreme size-divergence if it can lead to incompatibility between the sexes. Could such selection lead to very similar absolute changes in size between the sexes? In a species of Jerusalem cricket (Orthoptera: Stenopelmatinae: *Stenopelmatus*), copulatory-size incompatibility was observed even when the male was as little as 2 mm longer than their conspecific female, resulting in misalignment that prolonged or completely impeded copulation [31]. The absolute difference in mass or specific body lengths between the sexes can impact many size-dependent mating and reproduction events, including courtship, mate choice, copulation, mating behaviours and success of offspring when parental care is shared. The fact that many studies on size dimorphism focus on a single linear dimension of body size or total mass may be problematic to interpretation if critically important body dimensions do not change isomorphically to one another [18].

In conclusion, while previous comparisons of plastic body size responses of the sexes in relation to larval density and food quality in insects find greater relative variation in female size, especially when females are the larger sex [13,19], we find that plastic T–S responses under excess food show no consistent inter-sex differences in size response on average when examined across a wide range of arthropod orders. Indeed, our more comprehensive analysis for this variable concurs with the lack of consistent temperature effects on SSD detected by Stillwell *et al*. [19]. Consequently, we propose that in arthropods, temperature, unlike food supply, does not consistently affect optimal body size of one sex more than the other.

Authors' contributions. A.G.H., C.R.H. and D.A. designed the study, wrote the paper and defined the statistical approaches. C.R.H. collected the data and performed the statistical analyses.

Competing interests. We declare we have no competing interests.

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