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

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Abstract

Two major intra-specific 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.

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; 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 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 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).

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 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 study 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 Response

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. All data and conversions are detailed in our Supplementary Information.

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 in Supporting Information). 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 $(\exp^{(\text{slope})} -1)*100$ = % 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 (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 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) (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 $^{o}C^{-1}$ (±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 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 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 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).

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 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) 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 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 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 (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 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).

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 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 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 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 (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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Figures Legends

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, categorized 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, categorized 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.

Figure 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 ($F_{1,112}$=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 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 ($F_{1,40}$=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.

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, categorized 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 L-S responses (t_{20}=-3.96, p<0.001). 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.

Figure 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 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; 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*.

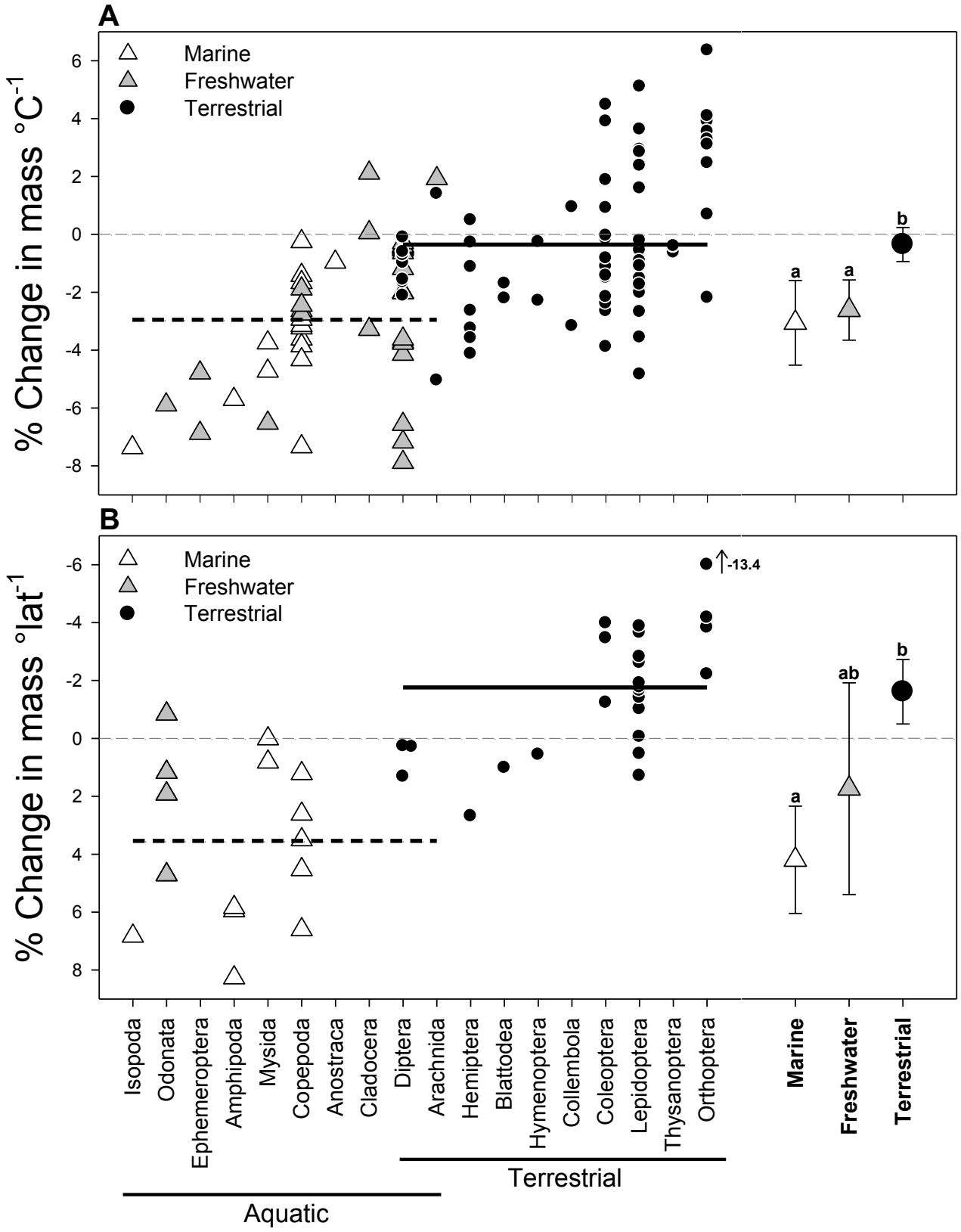
A
Marine
Freshwater
Terrestrial
% Change in mass °C-1
6
4
2
0
-2
-4
-6
-8
a
a
b
B
Marine
Freshwater
Terrestrial
% Change in mass °lat-1
-6
-4
-2
0
2
4
6
8
-13.4
a
ab
b
Isopoda
Odonata
Ephemeroptera
Amphipoda
Mysida
Copepoda
Anostraca
Cladocera
Diptera
Arachnida
Hemiptera
Blattodea
Hymenoptera
Collembola
Coleoptera
Lepidoptera
Thysanoptera
Orthoptera
Marine
Freshwater
Terrestrial
Aquatic
Terrestrial

Figure 1

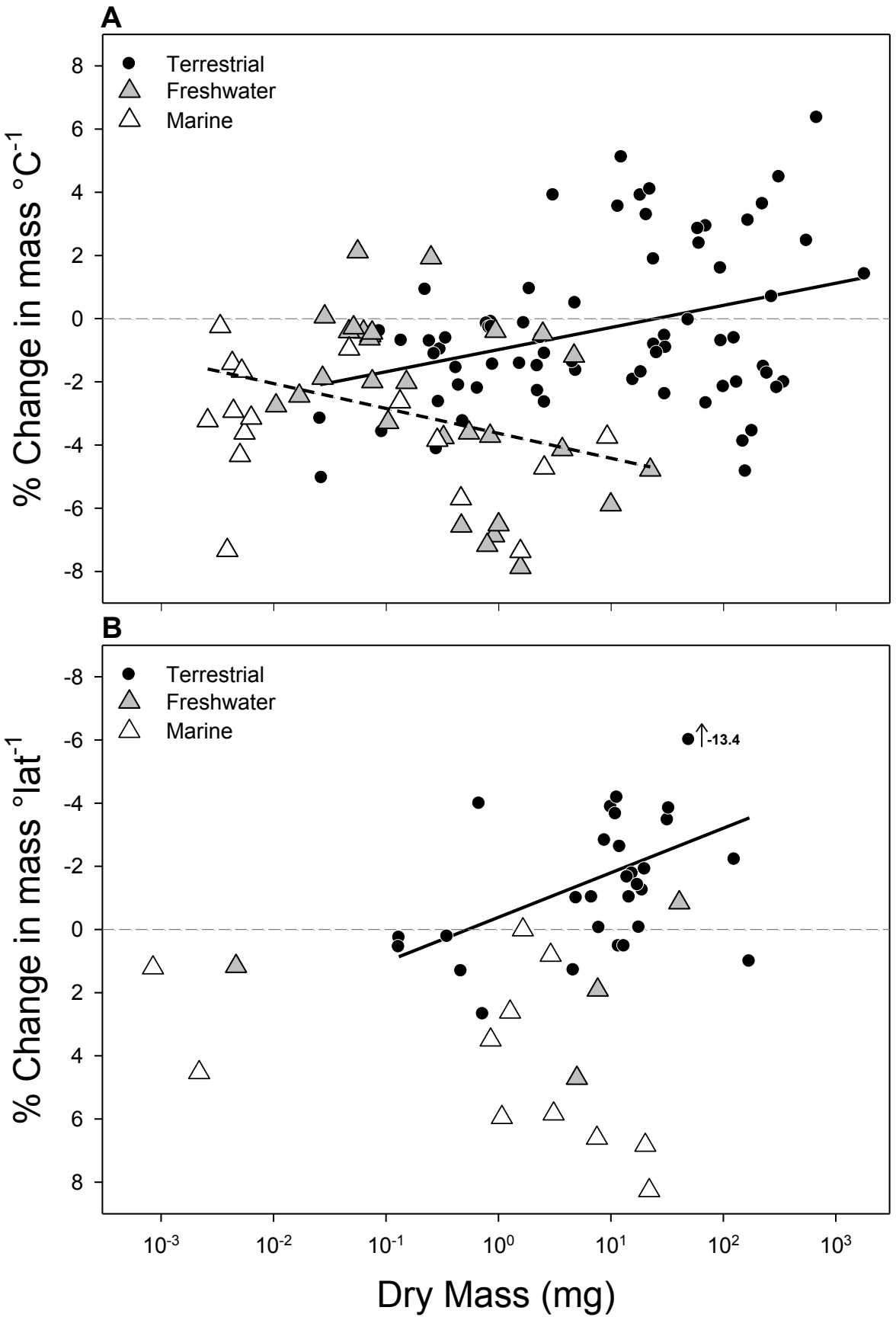
A
Terrestrial
Freshwater
Marine
% Change in mass °C-1
8
6
4
2
0
-2
-4
-6
-8
B
Terrestrial
Freshwater
Marine
-13.4
% Change in mass °lat-1
-8
-6
-4
-2
0
2
4
6
8
10-3
10-2
10-1
100
101
102
103
Dry Mass (mg)

Figure 2

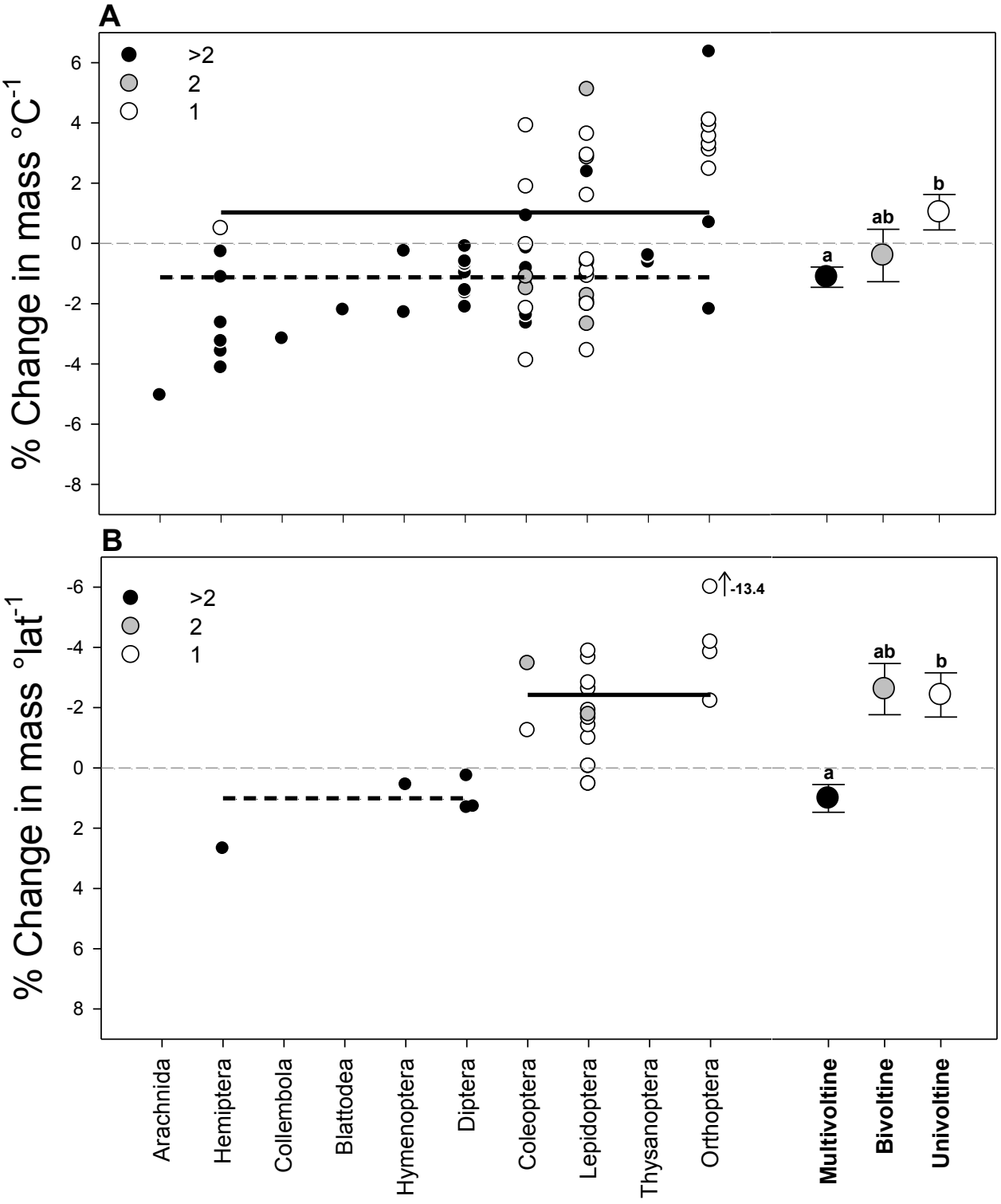
A
% Change in mass °C-1
>2
2
1
6
4
2
0
-2
-4
-6
-8
a
ab
b
B
% Change in mass °lat-1
>2
2
1
-13.4
-6
-4
-2
0
2
4
6
8
a
ab
b
Arachnida
Hemiptera
Collembola
Blattodea
Hymenoptera
Diptera
Coleoptera
Lepidoptera
Thysanoptera
Orthoptera
Multivoltine
Bivoltine
Univoltine

Figure 3

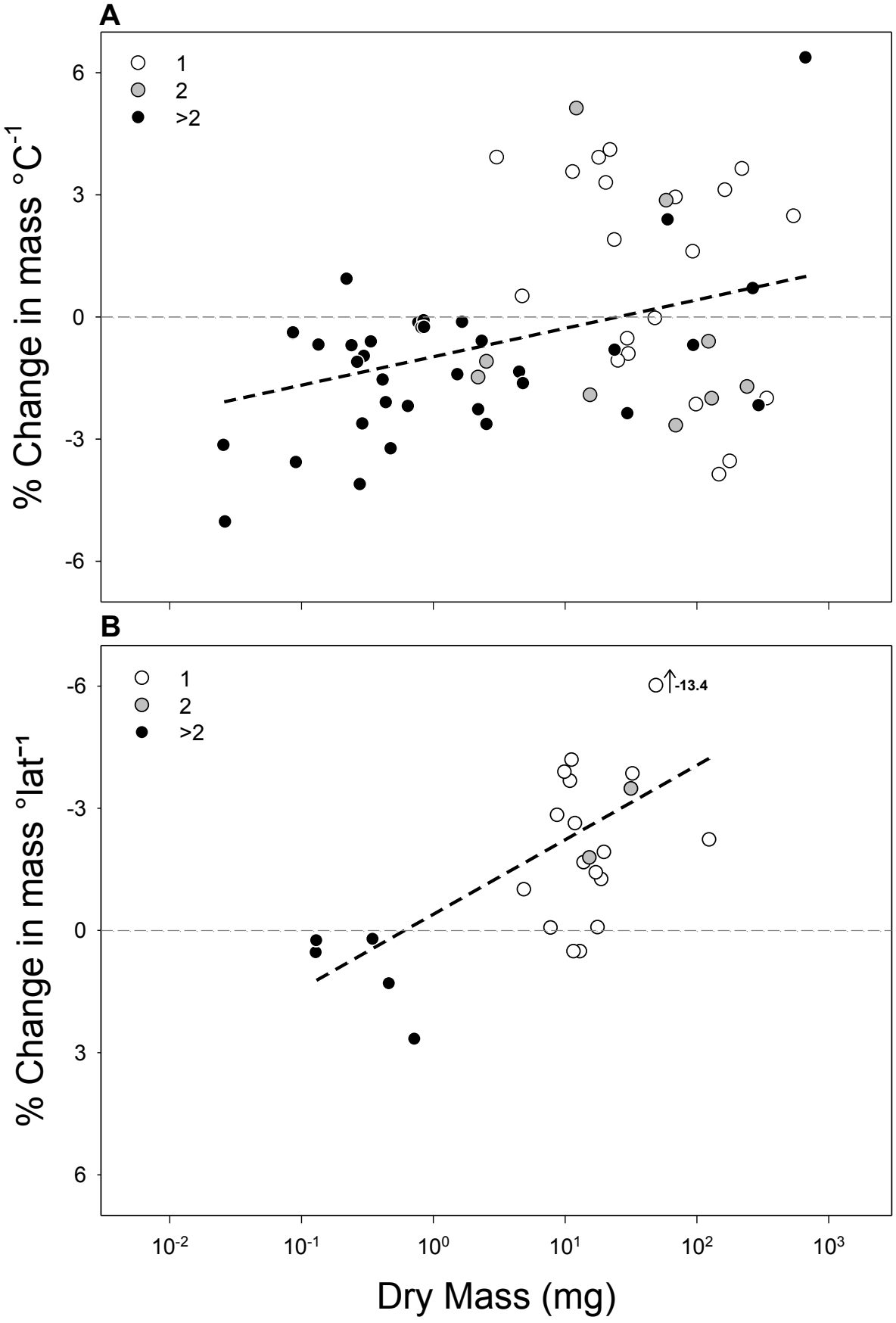
A
1
2
>2
6
3
0
-3
-6
% Change in mass °C-1
B
1
2
>2
-13.4
-6
-3
0
3
6
% Change in mass °lat-1
10-2
10-1
100
101
102
103
Dry Mass (mg)

Figure 4

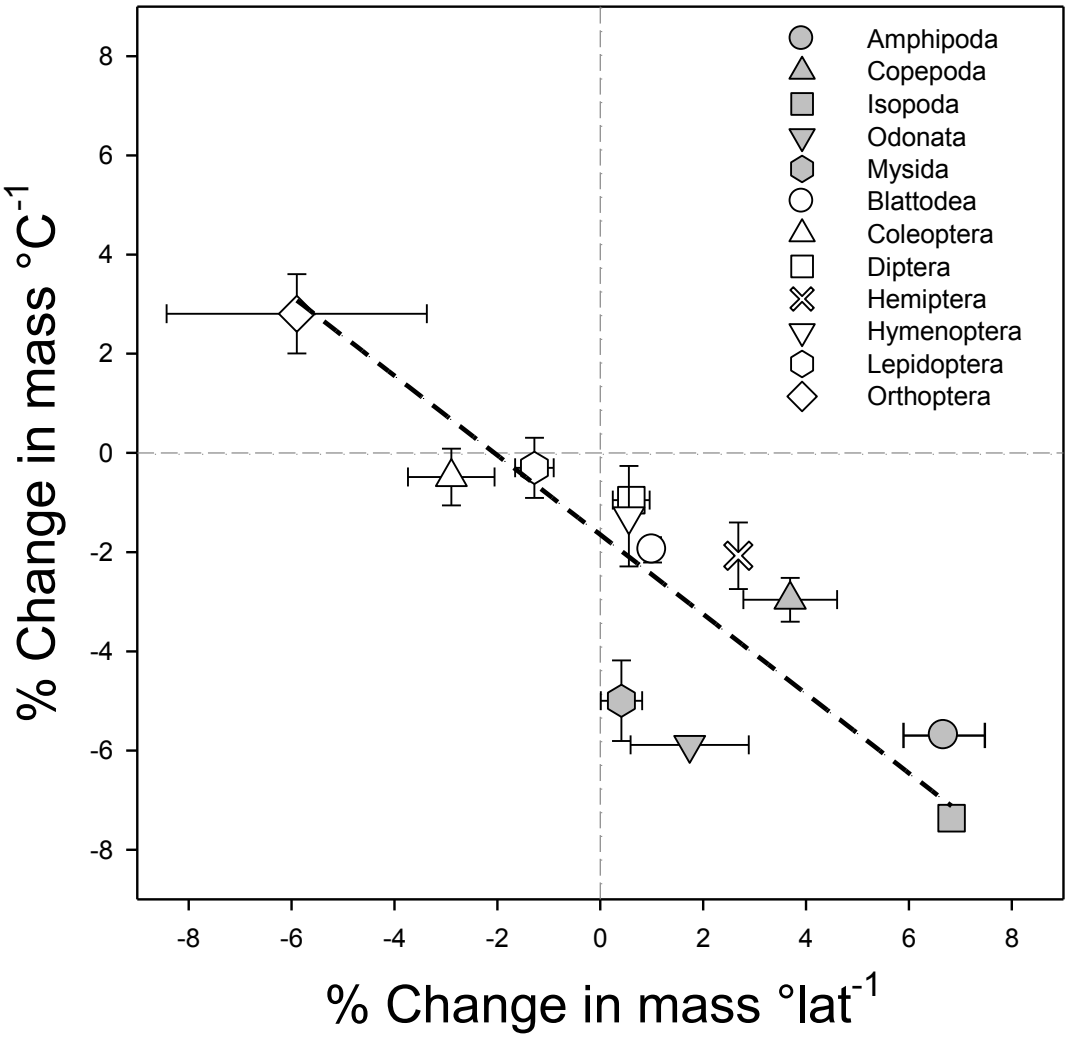
Amphipoda
Copepoda
Isopoda
Odonata
Mysida
Blattodea
Coleoptera
Diptera
Hemiptera
Hymenoptera
Lepidoptera
Orthoptera
% Change in mass °C-1
% Change in mass °lat-1
8
6
4
2
0
-2
-4
-6
-8
-8
-6
-4
-2
0
2
4
6
8

Figure 5