

## Research



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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

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 intra-specific 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  $3 \times 10^5$  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

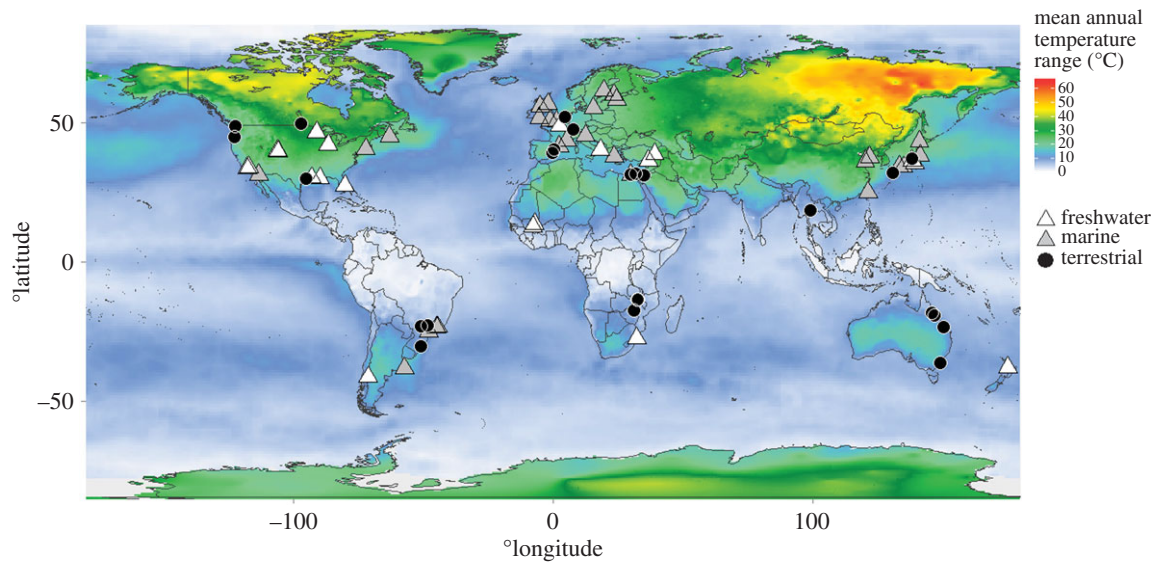
ecologists, including macro-ecologists, in understanding and predicting individual and community level responses to climate change [40]. This is critical given the link between decadal-scale changes in the body sizes of ectotherms and shifts in climate [41–43].

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  $(\exp^{(\text{slope})} - 1) \times 100 = \% \text{ change in mass per } ^\circ\text{C}$  [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;  $1.0^\circ \text{ latitude} \times 1.0^\circ \text{ longitude}$  global grid) and long-term monthly mean air temperature data from 1981 to 2010 (University of Delaware Air Temperature and Precipitation dataset;  $0.5^\circ \text{ latitude} \times 0.5^\circ \text{ longitude}$  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), log<sub>10</sub>-transformed species body mass (at  $15^\circ\text{C}$  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). Log<sub>10</sub>-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.  $\Delta\text{AICc}$ ) 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  $(\exp^{(\text{slope} \times \text{ATR})} - 1) \times 100 = \text{total \% change in mass}$ .



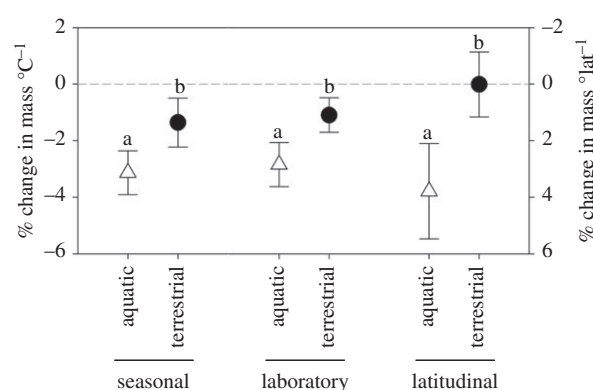
We compared total percentage change in body size between aquatic-developing and terrestrial species using a two-sample *t*-test. For both groups, an OLS regression of total percentage change in mass against ATR was used to determine whether species from more or less thermally variable environments exhibited 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 °C<sup>-1</sup>). 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.

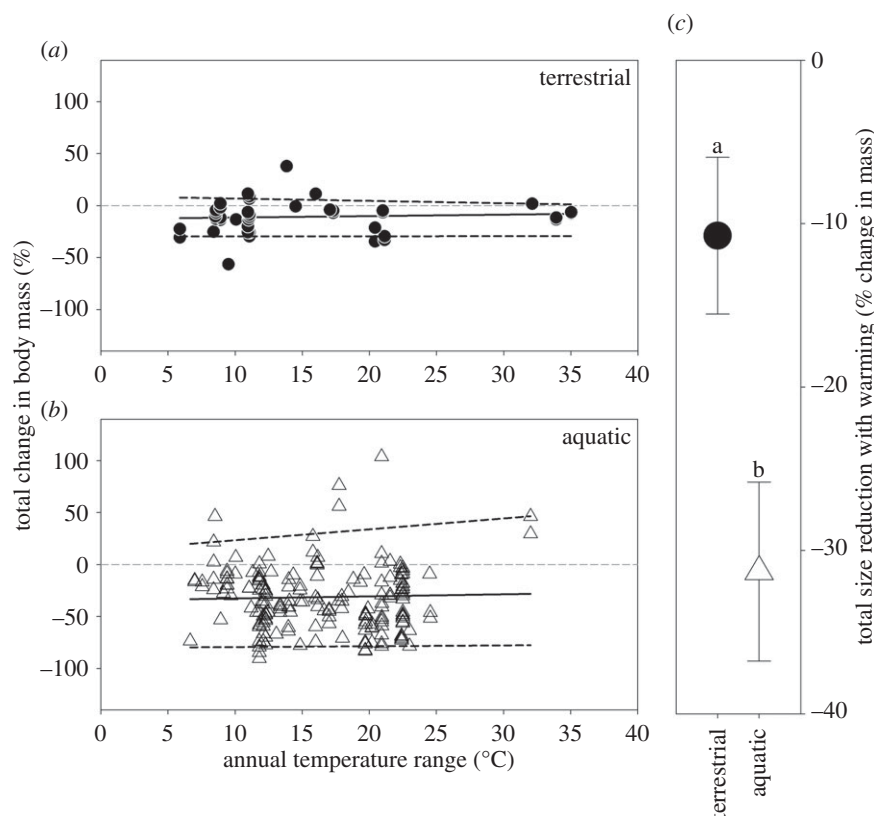


**Figure 2.** Comparison of mean aquatic- and terrestrial-developing seasonal temperature-body size gradients (% change in mass per °C ± 95% CI, left-hand y-axis) in arthropods with laboratory T-S responses (% change in mass per °C ± 95% CI, left-hand y-axis) and L-S clines (% change in mass per °latitude ± 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 °C<sup>-1</sup> ± 0.8; 95% CI) than terrestrial species (-1.4% body mass °C<sup>-1</sup> ± 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 °C<sup>-1</sup>; 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 ( $\pm$  95% CI)) for terrestrial and aquatic-developing species. Different letters above data points indicate significant differences.

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

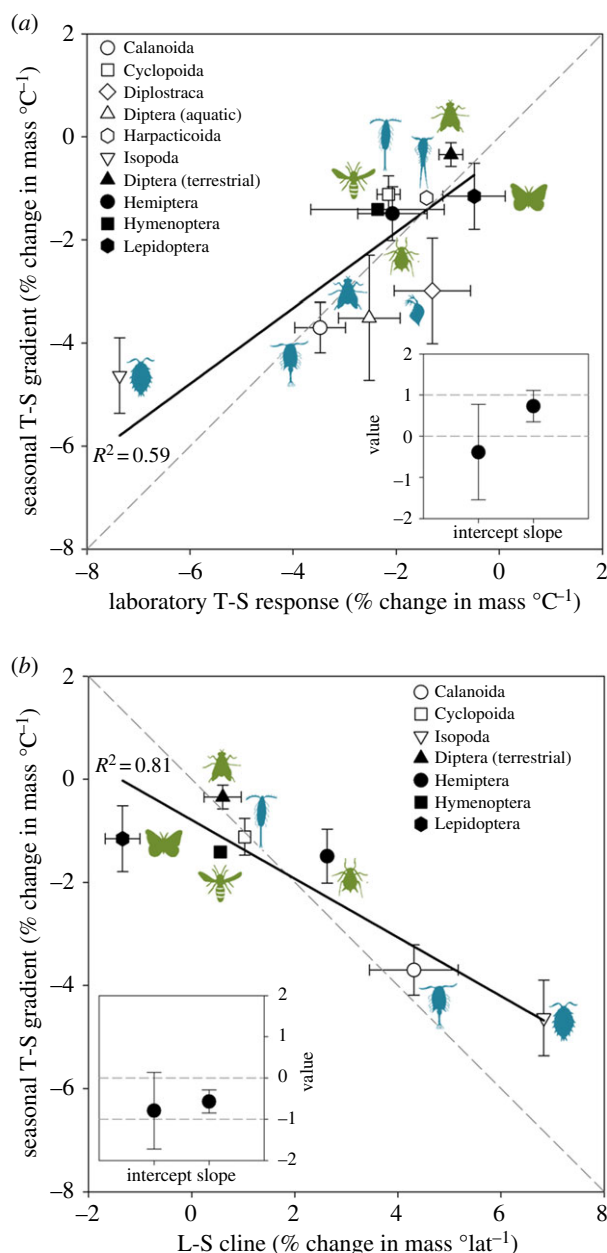
Total percentage change in body size over the annual cycle differed significantly between aquatic-developing and terrestrial species ( $t_{223} = -3.52$ ,  $p < 0.001$ ), but did not vary with mean annual temperature range in either group, such that, on average, total size change appeared relatively 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  $-10.7 \pm 4.8\%$  (95% CI), whereas overall size reduction in aquatic-developing species was almost threefold greater at  $-31.3 \pm 5.5\%$  (95% CI) (figure 3c). 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  $-80.2 \pm 22.6\%$  (95% CI), which is more than 2.5-fold greater than in terrestrial-developing species at  $-29.7 \pm 24.9\%$  (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 4a). This 1:1 match was supported by the RMA slope differing significantly from zero but not from 1 ( $0.73 \pm 0.38$ ; 95% CI), whilst the intercept did not differ significantly from zero ( $-0.39 \pm 1.16$ ; 95% CI) (inferred from 95% CIs; see inset panel in figure 4a). 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 =  $1.31 \pm 0.90$ ; intercept =  $0.56 \pm 1.85$ ;  $R^2 = 0.41$ ). 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 ( $1.51 \pm 0.61$ ; 95% CI), whereas the intercept did not differ significantly from zero ( $1.80 \pm 2.28$ ; 95% CI).

Seasonal T-S gradients negatively correlated with L-S clines at the level of taxonomic order ( $R^2 = 0.81$ ; figure 4b): 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 ( $1^{\circ}$  increase in latitude does not equal  $1^{\circ}\text{C}$  change in temperature), the gradient of the RMA slope did differ significantly from zero ( $-0.57 \pm 0.28$ ; 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  $\pm$  s.e.) in arthropods with (a) laboratory T-S responses (% change in body mass per °C  $\pm$  s.e.) and (b) L-S clines (% change in body mass per °lat  $\pm$  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 ( $\pm$  95% CI). (Online version in colour.)

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

## 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 3c). 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 4a) 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].

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