The Temperature Size Rule in Arthropods: Independent of Macro-Environmental Variables but Size Dependent

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Synopsis

Temperature is a key factor that affects the rates of growth and development in animals, which ultimately determine body size. Although not universal, a widely documented and poorly understood pattern is the inverse relationship between the temperature at which an ectothermic animal is reared and its body size (temperature size rule [TSR]). The proximate and ultimate mechanisms for the TSR remain unclear. To explore possible explanations for the TSR, we tested for correlations between the magnitude/direction of the TSR and latitude, temperature, elevation, habitat, availability of oxygen, capacity for flight, and taxonomic grouping in 98 species/populations of arthropods. The magnitude and direction of the TSR was not correlated with any of the macro-environmental variables we examined, supporting the generality of the TSR. However, body size affected the magnitude and direction of the TSR, with smaller arthropods more likely to demonstrate a classic TSR. Considerable variation among species exists in the TSR, suggesting either strong interactions with nutrition, or selection based on microclimatic or seasonal variation not captured in classic macro-environmental variables.

Introduction

Body size is one of the most important biological parameters influencing life history, ecology, and evolution (Cossins and Bowler 1987; Bonner 2006; Angilletta 2009). It determines the timing and progression of individual development, affects fecundity and fitness, and strongly influences population dynamics (Chown and Gaston 1997; Kingsolver and Huey 2007, 2008; Chown and Gaston 2010). Body size is affected by many factors including sexual selection, predation selection (on both predators and prey), and availability of resources. However, at least for ectotherms, body temperature is often an extremely important abiotic factor affecting body size. The general negative relationship of ectotherms’ body size with environmental temperature, known as the temperature size rule (TSR), especially within taxonomic groups or across ecological assemblages, is one of the better documented macro-ecological and macro-physiological patterns in plants, animals, protozoans, and bacteria (Atkinson 1994; Karl and Fischer 2008; Kingsolver and Huey 2008; Chown and Gaston 2010). This rule applies to a large majority of taxa studied as long as the analysis is confined to temperature ranges over which higher temperatures lead to higher growth rates (Atkinson 1994; Angilletta and Dunham 2003). However, the mechanisms underlying the general pattern of the TSR are still matters of contention (Kingsolver and Huey 2008; Chown and Gaston 2010). Generally, higher temperatures decrease developmental times more than they increase growth rates, resulting in smaller body sizes in adults (van der Have and de Jong 1996; Walters and Hassall 2006). Thus, a key question is why duration of development is more sensitive to temperature than growth rate is. In one insect, *Manduca sexta*, Davidowitz et al. (2004) showed that thermal effects on duration of development were due to changes in the length of the time from the critical weight to molting, suggesting that the removal of juvenile hormone or the secretion of ecdysone might be strongly temperature-sensitive.
levels often affects body size in the predicted manner larger animals, rearing ectotherms at different oxygen explained by problems with the delivery of oxygen to certain increases in ventilation with temperature such a mechanism could explain the TSR in animals of oxygen relative to demand. von Bertalanffy–Perrin model predicted that growth efficiency should decline with temperature, and that in fact efficiency of growth most commonly increases with, or is insensitive to, temperature in ectotherms, providing strong evidence against the von Bertalanffy–Perrin model. Angilletta and Dunham (2003) followed a suggestion from Atkinson (1996) and proposed that a mechanism for the TSR could be a constraint on growth rate arising late in ontogeny. They pointed to several studies that have shown that larger individuals of a species tend to have lower optimal temperatures for growth efficiency, arising from larger individuals experiencing stronger thermal effects on growth efficiency relative to smaller individuals. A prominent, related hypothesis for the TSR is the Maintain Aerobic Scope—Regulate Oxygen Supply (MASROS) hypothesis of Atkinson et al. (2006). These authors point out that metabolic rate increases more dramatically with temperature than does diffusion-based oxygen supply, potentially leading to oxygen-limitations that would be more likely to occurred in larger individuals at higher temperatures (due to their greater need for oxygen). They interpret the TSR as potentially an acclimatory response, with the smaller body size at higher temperature both reducing oxygen need and diffusion distances (especially if accompanied by reduced cell sizes at higher temperatures). In support of this hypothesis, cell size does decrease with rearing temperature in some species (Atkinson et al. 2006). The MASROS hypothesis could link to Angilletta and Dunham (2003) hypothesis that some factor limits growth later in ontogeny, with this factor being a deficiency in the supply of oxygen relative to the demand for it. Angilletta and Dunham (2003) questioned whether such a mechanism could explain the TSR in animals that utilize convective ventilation for gas exchange; certainly increases in ventilation with temperature could allow larger animals to maintain the supply of oxygen relative to demand.

In support of the hypothesis that the TSR can be explained by problems with the delivery of oxygen to larger animals, rearing ectotherms at different oxygen levels often affects body size in the predicted manner (Table 1). In most species tested, rearing in hypoxia reduces body size while rearing in hyperoxia occasionally increases body size modestly, with other species showing no effect (Table 1). The varied effects of hyperoxia may be partly explained by the potential for hyperoxia to cause damaging oxidative stress, especially at higher levels (Rascon and Harrison 2010). Callier and Nijhout (2011) proposed that, at least in holometabolous insects, decreasing oxygen supply relative to demand leads to internal hypoxia that may trigger the hormonal cascade that leads to molt; given the exponential effect of temperature on metabolic rate, it is conceivable that this effect could be stronger at higher temperatures, thereby explaining the TSR. Multigenerational studies have shown that hyperoxia can drive the evolution of larger body size in Drosophila (Klok et al. 2009), and multigenerational exposure to hypoxia increases phenotypic plasticity—greater reduction in size in response to hypoxia (Harrison and Haddad 2011), consistent with a role for internal oxygen in determining body size. However, the critical studies necessary to determine whether oxygen can explain the TSR in any species have not yet been conducted. In particular, we do not yet know whether the supply of oxygen relative to demand decreases at higher temperatures, or whether resulting changes in internal PO2 are sensed and transduced by the neuroendocrine systems that control growth and developmental time. Frazier et al. (2001) have shown that the effect of oxygen on body size is greater at higher temperatures in Drosophila, consistent with this hypothesis.

Although the TSR is an example of phenotypic plasticity, it is important to recognize that there are data that suggest that the TSR is maintained by natural selection. Scheiner and Lyman (1991) were able to alter the TSR dramatically by 20 generations of family-level selection in Drosophila. Different populations of the butterfly Pieris rapae have different slopes of the TSR, demonstrating that the TSR can evolve (Kingsolver et al. 2007). These findings suggest that comparative studies of the TSR may be useful for exploring ultimate, and perhaps proximate, explanations for the TSR.

In this study, we utilize the relatively large body of literature on the TSR in arthropods to test for links between the magnitude of the TSR and a variety of environmental factors. One obvious possible correlate of the magnitude and direction of the TSR is habitat temperature. At any given body size, higher temperature leads to an exponentially greater metabolic rate (Gillooly et al. 2001); thus, higher habitat temperatures should be associated with greater changes in metabolic rate and oxygen demand for
any given change in body size. This effect (if uncompensated) would tend to decrease growth efficiency at higher temperatures and reduce the ratio of oxygen supply relative to demand. This general observation leads to the prediction that species from habitats with higher temperatures (or from lower latitudes and/or elevations) may exhibit a greater TSR (stronger effect of temperature on size), unless other factors such as increases in ventilation or respiratory structure provide compensation. Similarly, the MASROS hypothesis might predict that at higher elevations or at reduced levels of dissolved oxygen in aquatic habitats, there could be a greater TSR unless animals compensate for the reduced availability of oxygen. Habitat (terrestrial versus aquatic) is another variable that potentially could affect the magnitude and direction of the TSR. Availability of oxygen is much greater in terrestrial environments; thus, if the TSR is a consequence of a reduced supply of oxygen relative to demand at higher temperatures, it is plausible that aquatic species would decrease in body size more strongly in response to higher temperature. Indeed, in a recent comparative analysis, Forster et al. (2012) concluded that aquatic species exhibit a greater TSR than terrestrial species, and they suggested that this difference could be related to differences in the availability of oxygen across aquatic and terrestrial habitats. Body size might affect the magnitude of the TSR response because smaller arthropods are likely to be more dependent on diffusion than on convection for oxygen supply, and thus may have restricted options for matching supply of oxygen to demand across temperatures. We also compared arthropods capable of flight with those not capable of flight, reasoning that the higher resting metabolic rates of flying insects (Chown et al. 2007) might make them more temperature-sensitive. We restricted this study to arthropods because these all have similar developmental processes that are quite different from those of vertebrates and other invertebrates, with juveniles growing and molting repeatedly, using generally similar developmental hormones; we reasoned that this restriction might increase the power of finding correlations between TSR and environment. We also explored for taxonomic correlates of the magnitude of the TSR as a further assessment of the generality of the TSR. If certain taxa have different magnitudes or directions of TSR, this could provide a useful tool for exploring ultimate and proximate hypotheses for the TSR. For example, it has been suggested that Orthoptera exhibit positive or non-significant changes in body size with rising temperature (Mousseau 1997), with grasshoppers being one of the few arthropod taxa that have been shown to have body sizes independent of the oxygen level at which they were reared (Harrison et al. 2006), thereby providing some support for the MASROS hypothesis.

### Methods

#### Body size data related to temperature

To explore possible environmental correlates of the TSR, we compiled a dataset of studies in which arthropods completed development in a controlled environment setting (usually the laboratory) at various constant temperatures; from the size ranges reported, we calculated indices of the response of body size to temperature. A specific hierarchical regime was followed for deciding which data to include. First, the source had to include clear and unambiguous size measurements across ranges of non-stressful temperatures (data in which higher or lower temperatures
affected survival or completion of development were excluded). We also took care not to include results from studies that were conducted under conditions with other potential constraints on size, such as nutritional challenges. Additionally, the references had to provide sufficient geographical information to allow us to obtain data on latitude and elevation. If latitude and elevation were not provided, we obtained these, when possible, from data on locality information provided by the authors, using Google Earth (earth.google.com). From these data, it was possible to calculate partial pressures of atmospheric oxygen (aPO2) for terrestrial studies based on standard physico-chemical equations for the earth’s atmospheric composition, taking into account the fractional content of oxygen in the present-day atmosphere (0.2095) and the effect of elevation (in meters above sea level—masl) on atmospheric pressure (Reid et al. 1977). Similarly, levels of dissolved ambient oxygen (in μMol l−1) in freshwater habitats were calculated, accounting for aPO2 for the given elevation, salinity and temperature, and for marine habitats accounting for aPO2, salinity, and temperature at sea level. The references we used mostly noted the temperatures under which animals were reared. We needed more generally applicable indicators of environmental temperature that would also account for the inherent thermal divergence between the northern and southern hemispheres (Sinclair and Chown 2005). Using a polynomial estimation of the relationship of temperature to latitude (Fig. 4 in Addo-Bediako et al. [2000]—the “crooked bowtie plot”), compiled from macro-ecological temperatures (Gaston and Chown 1999; Addo-Bediako et al. 2000), we extracted absolute maxima, absolute minima, and absolute ranges of latitudinally related temperatures to test possible relationships of size–temperature responses to global temperature indicators.

The data were coded according to taxonomic ranks (class, order, family), flight status (given the effect of flight on the standard metabolic rate of flying insects) (Chown et al. 2007), size classes (0–5 mm, 5–10 mm, and >10 mm in body length), and type of habitat (terrestrial, freshwater, marine). For insects, we also coded for type of development (hemimetabolic and holometabolic) and for species with aquatic developmental stages, we coded for air breathing (using atmospheric oxygen) versus gill breathing (using dissolved oxygen). The data were from 83 species, mostly insects (Table 2). However, there were sufficient data available for the classes Malacostraca and Arachnida to include analyses within and across the higher taxonomic groups.

### Normalization of data

The source literature reported a multitude of varying size metrics (body length; head capsule width; leg, wing, thorax, or lengths of other body parts; wing areas; and various measures of mass). This made the data inherently incomparable since there is a dearth of morphometric information allowing for the reliable conversion of these various metrics to one uniform size metric such as body length. As such we could not analyze the data according to variations in body size per se. However, that was not the focus of this study. In this case, we were only interested in the relative size-responses of species and/or populations to temperature, irrespective of their absolute sizes. Hence, we normalized the various size metrics, setting the size index to a value of 1 at a given normalization temperature. The normalization temperatures used were either the standard maintenance (common-garden) temperatures for laboratory-reared experimental populations as reported by the authors or the mean field temperatures reported from field surveys. If neither of these were reported, we used the median temperature in the range reported. For example, if a species were experimentally reared at 10°C, 15°C, 20°C, 25°C, and 30°C, and attained mean sizes of 5.5, 5.0, 4.5, 4.0, and 3.5 (here the actual size metric is irrelevant), while the laboratory population is usually maintained at 25°C, we normalized the sizes using the size associated with 25°C as the denominator and the all other sizes as numerators in our normalizations. In this case, the normalization temperature was 25°C and the size was 4.0; thus, the normalized sizes would be 1.375, 1.25, 1.125, 1.0, and 0.875 at rearing temperatures of 10°C, 15°C, 20°C, 25°C, and 30°C, respectively. To correct for dimensional variation in the size indices used, we took the square roots of areas and the cube roots of volumes (or masses) prior to normalization.

To obtain an index of the magnitude and direction of the TSR, we conducted linear regression analyses on the normalized sizes versus temperature (°C)

### Table 2 Taxonomic groupings of arthropods included in this study

<table>
<thead>
<tr>
<th>Classes</th>
<th>Orders</th>
<th>Families</th>
<th>Species</th>
<th>Populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insecta</td>
<td>11</td>
<td>43</td>
<td>66</td>
<td>1–5</td>
</tr>
<tr>
<td>Malacostraca</td>
<td>5</td>
<td>7</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Branchiopoda</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Entognatha</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Arachnida</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
for each species or population (Fig. 1). We termed the slopes of these regressions as the “normalized size response to temperature” (NSRT) indices, and then used the NSRT index for each species/population as the dependent variable in our subsequent statistical analyses. If the linear regression of normalized size on temperature was not statistically significant in these analyses, we set the NSRT index to a very low non-zero value (0.000001) to reflect the neutral relationship and allow for inclusion in subsequent analyses. The frequency distribution of these NSRT indices indicated that the data were not normally distributed (Fig. 2). Thus, we used non-parametric tests for all subsequent analyses. Spearman Rank correlations were used to test for latitudinal effects on the NSRT, testing northern and southern hemispheres separately since hemispheres often show different macro-ecological patterns (Sinclair and Chown 2005). The effects of elevation, calculated $a\text{PO}_2$, dissolved oxygen, or temperature (normalization temperature and absolute temperatures—maximum, minimum, and range) on NSRT indices were also tested with Spearman Rank correlations. If we found a significant correlation, we then tested whether the correlation could be explained by phylogeny by conducting a linear regression of the environmental variable on the phylogenetically independent contrasts, using the ape package in R. To test for effects of discontinuous traits (e.g., habitat, flight capability, and size classes), we used Kruskal–Wallis multiple comparisons. Throughout, statistical analyses were performed using Statistica 10 (www.statsoft.com, Tulsa, OK), with $P<0.05$ required for statistical significance.

Results

Distribution of NSRT indices

The linear regression analyses of the normalized sizes on temperature of each individual species or population yielded 56 species/populations having a statistically significant negative relationship with the temperature range tested (consistent with TSR), 11 were statistically significant positive (counter TSR) and the remaining 28 were non-significant (Fig. 1; see Table 3 for a taxonomic breakdown). Four species had extremely high or extremely low NSRT indices, falling well outside of the distribution of the other NSRT index values and as such were excluded as outliers from all subsequent analyses. A frequency distribution of the NSRT indices, excluding the aforementioned outliers, show that in the majority of cases, there was a change in body size of 2–15% for each 10°C change in temperature—in both negative and positive directions (Fig. 2). These NSRT indices were also not normally distributed (Shapiro–Wilk’s $W=0.955$, $P<0.0026$), necessitating the use of non-parametric tests for subsequent analyses.

Should one include populations, or just species, in the dataset?

We analyzed the data both with and without including the populations within species as separate data points, and this did not affect any of our conclusions. For several reasons, we decided to include the data for individual populations of species as separate data points even though these data are not as independent as a dataset with only one population per species. Most importantly, in many cases, the populations showed different NSRT indices, suggesting to us that deciding on a single value for a species
would be difficult, and also that the within-species data might be useful for discerning correlations with environmental variables. We had eight species with more than one population. Of these, five species had populations varying between negative and neutral NSRT indices. *Acyrthosiphon pisum* (Aphididae) had one population with a neutral NSRT index and four populations with negative ones. *Thrips tabaci* and *Thrips major* (Thripidae), each had two populations with neutral NSRT indices and one population with a negative one. *Frankliniella occidentalis* (Thripidae) had one population with a negative index and one population that was neutral. *Lycaena hypoothe* (Lycaenidae) had one neutral population and two with negative NSRT indices (see Supplementary Material).

### Correlations between macro-environmental variables and NSRT indices

In general, when data for all arthropods are pooled, or when analyses of correlation are limited to the class Insecta, there were no significant relationships between geographic/environmental variables and the NSRT indices (Table 4).

#### Significant NSRT index patterns in the Malacostraca

One exception to the general lack of correlation between the NSRT indices and macro-environmental variables was the Malacostraca, all of which are marine. In this class, the NSRT indices were negatively correlated with latitude and the annual temperature range of the habitat and positively correlated both with absolute minimum and absolute maximum temperatures. Thus, higher latitude species living in habitats with a broader annual thermal range tended to show a more negative NSRT index, while the lower latitude species showed either no size-temperature response or a positive NSRT index (Fig. 3). When a linear model was run on the phylogenetically independent contrasts, the trend toward a negative relationship between NSRT and latitude remained, but became non-significant ($F_{1,5} = 5.04$, $p = 0.04$)....

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**Table 3** The numbers of species or populations within taxonomic classes that had statistically significant negative or positive NSRT indices, and those that showed no statistically significant size response to temperatures at which the animals were reared.

<table>
<thead>
<tr>
<th>Classes</th>
<th>Negative NSRT</th>
<th>Positive NSRT</th>
<th>No relation</th>
<th>% consistent with TSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insecta</td>
<td>48</td>
<td>8</td>
<td>21</td>
<td>61%</td>
</tr>
<tr>
<td>Malacostraca</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>70%</td>
</tr>
<tr>
<td>Entognatha</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0%</td>
</tr>
<tr>
<td>Arachnida</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0%</td>
</tr>
</tbody>
</table>

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**Fig. 2** Frequency distributions of the NSRT index values of arthropod populations.
P = 0.07). Malacostracans’ NSRT indices were not significantly correlated with either normalization temperature or dissolved oxygen (Spearman R = 0.07, n = 10, P = 0.536).

**Effects of habitat, taxonomic group, flight status, development type, mode of breathing, and size**

Kruskal–Wallis multiple comparisons testing for differences in the magnitudes of NSRT indices among, and within, the taxonomic groups, between habitats (terrestrial, fresh water, marine), and flight status mostly showed no significant differences (Table 5). One important exception was size class. Arthropods in size class 10 (>10 mm in body length) differed significantly from size classes 0 and 5 (0–5 mm and 5–10 mm in body length, respectively). Size class 10 had generally higher ranked NSRT indices, while the distributions for both size classes 0 and 5 had a majority of negative NSRT indices (Fig. 4). This result was maintained when data for size class were selected only for Insecta (Kruskal–Wallis H = 7.613, df = 2, n = 77, P = 0.0222). Similarly, a Spearman Rank correlation test shows a highly significant positive relationship between arthropod size class and NSRT (Insecta: Spearman R = 0.294; n = 90, P = 0.0049). Furthermore, within habitat types, the terrestrial and marine arthropods showed significant positive relationships between size class and NSRT (Terrestrial: Spearman R = 0.327, n = 58, P = 0.013. Marine: Spearman R = 0.638, n = 10, P = 0.048). By contrast, the fresh water arthropods showed no relationship (Spearman R = 0.03, n = 22, P = 0.89).

Despite prior reports of possible cross-order variation in the TSR among insects, there was no significant variation among insect orders in the NSRT indices (Fig. 5). However, power of our test was low for some key groups. The NSRT indices were all non-significant for Orthoptera (n = 2) and Heteroptera (n = 3), so additional data are needed for those orders.
Discussion

Our data strongly support the generality of the TSR by demonstrating that the magnitude of the normalized body size response to temperature (NSRT) is not significantly affected by taxonomic group, latitude, elevation, temperatures, availability of oxygen, flight status, or habitat (aquatic versus terrestrial) among arthropods. This result supports the hypothesis that the proximate mechanism for the TSR is a general, evolutionarily primitive physiological mechanism, a hypothesis also supported by the prevalence of the TSR in protozoa and bacteria. However, we have also shown that there is substantial variation in the magnitude of the TSR among species and among populations. Since we attempted to exclude studies with nutritional or other limitations, this suggests that the variation in the magnitude and direction of this response may be driven by natural selection.

Critique of the data and of the approach

While we were able to pull together considerable data for this analysis (88 species/populations and over 696 data points), the power of some of these analyses was relatively low, weakening our confidence in the results. This was particularly true for the within-Malacostraca and within-Arachnida tests, and for analyses at the lower taxonomic levels within the Insecta. Our test of the effect of elevation was not particularly rigorous as the range of elevations in this study was relatively small (Table 3). It would be extremely useful to conduct tests of the effect of NSRT indices within taxa across greater ranges of elevation.

Another critique of our approach is that some prior studies have demonstrated that different body parts respond differently to the temperature at which the animals were reared (e.g., Atkinson et al. 2006; Frazier et al. 2008). If such disparities in the thermal responses among body parts are large and common, this could seriously flaw our analysis, as we used classic geometric corrections for normalizing the relative responses of whatever metric of size was reported. Fortunately, only one species included in our analyses used wing area as a metric (the fruit fly, Ceratitis capitata: Diptera: Tephritidae—see the dataset in the Supplementary Material) and is unlikely to change the major outcome of the analyses, while none of our studies used size of genitalia as the metric for size, a body part whose size has been

Table 5 Kruskal–Wallis multiple comparison tests for significant differences in the normalized temperature size response (NSRT) indices within taxonomic groups, across habitat types (terrestrial, freshwater, and marine), with capability for flight, type of development, and mode of breathing in aquatic insects

<table>
<thead>
<tr>
<th>Categories</th>
<th>KW: H</th>
<th>df</th>
<th>N</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td>2.698</td>
<td>3</td>
<td>90</td>
<td>0.441</td>
</tr>
<tr>
<td>Orders—across all arthropods</td>
<td>23.733</td>
<td>18</td>
<td>90</td>
<td>0.164</td>
</tr>
<tr>
<td>Orders—within Insecta</td>
<td>12.693</td>
<td>10</td>
<td>77</td>
<td>0.241</td>
</tr>
<tr>
<td>Orders—within Malacostraca</td>
<td>6.673</td>
<td>4</td>
<td>10</td>
<td>0.155</td>
</tr>
<tr>
<td>Habitats</td>
<td>2.016</td>
<td>2</td>
<td>90</td>
<td>0.365</td>
</tr>
<tr>
<td>Fight capability—within arthropods</td>
<td>1.354</td>
<td>1</td>
<td>90</td>
<td>0.245</td>
</tr>
<tr>
<td>Flight capability—within Insecta</td>
<td>3.379</td>
<td>1</td>
<td>77</td>
<td>0.066</td>
</tr>
<tr>
<td>Holometabolic versus Hemimetabolic—Insecta</td>
<td>0.044</td>
<td>1</td>
<td>77</td>
<td>0.833</td>
</tr>
<tr>
<td>Air-breathing versus Gill-breathing—aquatic Insecta</td>
<td>0.255</td>
<td>1</td>
<td>22</td>
<td>0.614</td>
</tr>
</tbody>
</table>

No significant differences were found.

Fig. 3 (A) In the Malacostraca, Spearman Rank correlation tests showed the NSRTs to be significantly negatively related to latitude (±95% CI: −0.625 to −0.991). (B) The NSRTs are significantly positively related to absolute maximum (±95% CI: 0.993–0.714) and minimum temperatures (±95% CI: 0.989–0.576), while negatively related to the absolute temperature range (±95% CI: −0.460 to −0.985). The data were plotted with fitted trend lines to indicate the polarity of the relationships.
reported to be quite thermally-independent in Drosophila (Shingleton et al. 2009).

Is the MASROS hypothesis supported?

These data did not support many of the predictions we made based on the MASROS hypothesis, i.e., that decreases in oxygen supply relative to demand drive changes in body size. We predicted that if the MASROS hypothesis was correct, one would observe a stronger NSRT index (1) at higher environmental temperatures, (2) under reduced availability of oxygen, (3) in aquatic compared with terrestrial environments, and (4) in animals capable of flight. None of these patterns were observed.

In a recent study, Forster et al. (2012) took a very similar approach but reached the different conclusion that aquatic species show a stronger TSR than terrestrial species. Perhaps surprisingly, our two meta-analyses were primarily based on different data-sets, and thus were largely independent. Examination of Foster et al.’s dataset (available online as Supplementary Material) showed only 20 source papers in common between our two studies (Foster et al. referenced 126 papers of metazoans; we referenced 71 papers of arthropods). Partly the differences in sources related to our choice to utilize studies that reported multiple size metrics which we then normalized (see “Methods” section); in the Foster et al. study, all the species’ size metrics were converted to dry mass as their size index. Their study did have more power, with 169 species versus our 84.

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**Fig. 4** Frequency distributions of NSRT values within size classes (A, 0: 0–5 mm; B, 5: 5–10 mm; C, 10: >10 mm) show that while all three classes have several species/populations showing neutral value NSRT indices (13, 8, and 5, respectively), species/populations in size class 10 (>10 mm) have small but equal numbers of negative and positive NSRT values (C)—differing significantly from both size classes 0 (A) and 5 (B). Species/populations in size class 0 mostly have negative NSRT indices, but some have neutral ones, while size class 5 has more negative than positive NSRT indices. Kruskal–Wallis $H = 9.928$, df = 2, $n = 90$, $P < 0.007$.

**Fig. 5** A scatter plot of the NSRT indices of all species/populations tested within the insect orders. Note that the majority of populations show negative NSRT indices—consistent with the TSR—although two orders with limited data show zero values. The extreme positive NSRT outlier in the Coleoptera is a dermestid species—Attagenus megatoma—a cosmopolitan species known as the black carpet beetle.
A fundamental difference in our two approaches was that we restricted our analyses to arthropods, while Foster et al. used data from 20 non-arthropod animal studies, mostly dealing with vertebrate species, and more than a third of their organisms were unicellular species. In our data, with 90 species and populations considered, there was no evidence that aquatic and terrestrial arthropods differ in their body size responses to rearing temperature (Table 5). Thus, we suggest that Foster et al.’s conclusion, that aquatic species have stronger reduction in body size in response to temperature, results from pooling taxonomic groups that have different size responses to temperature. Foster et al. argued that oxygen is more challenging to obtain in aquatic relative to terrestrial habitats and concluded that their finding that aquatic species show a stronger reduction in body size at higher temperatures supported the MASROS hypothesis. Our data do not support this line of reasoning, since we found no differences in size responses to temperature between aquatic and terrestrial arthropods.

We found that smaller insects, or arthropods in general, were more likely to exhibit the TSR, and this finding differed in some respects from the conclusions of Foster et al. (2012). We showed significant positive correlation between size class and NSRT indices across all arthropod species, with those in the smaller size classes showing stronger and most consistently negative NSRT values (Fig. 4). This pattern, with smaller species showing a stronger reduction in body size with temperature was also statistically shown within terrestrial and marine arthropods considered separately. Forster et al. found a similar relationship within their terrestrial metazoans, with smaller species tending to decrease in size more strongly at higher temperatures (Foster et al. 2012, Fig. 2). By contrast, Foster et al. reported that aquatic metazoans (marine and fresh water data combined) showed the opposite trend, with larger species showing greater reductions in body size at higher temperatures (Foster et al. 2012, Fig. 3). We speculate that the differences between the findings of our two studies may be due to the constituents of the datasets. The terrestrial metazoans in the Foster et al. study were mostly insects and other arthropods, as in our study. However, as noted above, the Foster et al. study included vertebrates, and these were primarily aquatic, large, and showed strong reductions in body size at higher temperatures (Foster et al. 2012, Fig. 3); thus pooling of the vertebrate data with the arthropods may have driven the finding that larger species showed a stronger TSR.

Despite the differences in results between our study and that of Foster et al. (2012), we concur with those authors in finding some support for the MASROS hypothesis. While we lack good experimental verification that smaller arthropods are more dependent on diffusion for gas exchange, this possibility is supported by the observed increase in mass-specific tracheal investment in beetles (Kaiser et al. 2007) and grasshoppers (Greenlee et al. 2009) and by the increase in mass-specific ventilation with size documented for grasshoppers (Greenlee and Harrison 2004; Greenlee et al. 2004). Very small insects have been reported to have reduced tracheal systems (Schmitz and Perry 1999), consistent with diffusion being sufficient for gas exchange. It does seem plausible that a greater dependence on diffusion for gas exchange would make it more likely for oxygen to become limiting later in development. Arthropods that utilize primarily diffusion for gas exchange could still alter their respiratory morphology with respect to the temperature they were reared, thereby increasing the capacity for delivering oxygen in response to exponentially increasing metabolic rates; to our knowledge, there are no studies yet that have investigated that possibility. Arthropods that have significant capacities for convective ventilation have an additional mechanism that matches the capacity for gas exchange to tissues’ needs as temperature and metabolism rises. We hypothesize that some arthropods (those that do not show the TSR) instead utilize convective ventilation to cope with the exponentially increasing need for gas exchange as temperature rises, thereby preventing the need for a decrease in size. There are some data showing that ventilation increases at higher temperatures in arthropods (Lighton and Wehner 1993), although we are unaware of studies that quantitatively have compared convective ventilation with metabolism across temperatures in this group. This is at least partly due to the technical difficulties of quantifying ventilation in arthropods. In vertebrates, there are many studies showing that acute exposure to higher temperatures increases convective ventilation (Jackson 1971; Giordano and Jackson 1973). Some of these vertebrates do show the TSR, so clearly use of convective ventilation does not preclude the TSR. Interestingly, ventilation generally does not increase as strongly as metabolism in terrestrial vertebrates, leading to a rise in blood PCO₂ with temperature, and potentially a fall in PO₂ (Reeves 1977), consistent with MASROS. It is important to note that it has also been shown that smaller species are more likely to demonstrate clines in body size with latitude (Chown and Gaston 1999;
Temperature size rule in arthropods

Blanckenhorn and Demont, 2004). Chown and Gaston (2010) have suggested that smaller species with shorter developmental times may be less likely to encounter nutritional constraints associated with length of season, providing an alternate explanation for the association between body size and the magnitude of the TSR found here.

The lack of a relationship between the NSRT index and latitude, temperature, elevation, habitat, and capacity for flight could simply be due to animals evolving systems of supplying oxygen that match their demand and their environment, such that the ratio of the supply of oxygen to demand is independent of the environment. In support of this hypothesis, ventilation rates have been shown to scale in proportion to metabolic rate across body sizes and environmental temperatures in ectotherms (Schmidt-Nielsen, 1984), and insects of different body sizes have similar critical PO2 values (Lease et al., 2012). If this is the case, then comparative studies like these are inappropriate for testing the MASROS hypothesis, and studies of the mechanisms for the TSR during plasticity are required.

The Malacostraca provided some interesting exceptions to these general conclusions. Within this group, there were strong effects of latitude and absolute temperatures on the NSRT indices, with high-latitude species showing the typical TSR, with size in mid-latitude and lower-latitude species being unaffected by temperature or being larger at higher temperature (Fig. 3), though this relationship was not significant after correcting for phylogeny. This pattern was opposite to our predicted responses based on the MASROS hypothesis.

**Future directions**

The finding that the magnitude of the TSR is affected by body size in arthropods calls for new attention to interactions between absolute body size and temperature on physiology and development. Does the importance of diffusion and convection actually vary with body size? If so, how does this alter the developmental responses of arthropods to temperature and other environmental factors?

The fact that many of our predictions based on the MASROS hypothesis were not supported by the data suggests that alternate explanations for the TSR deserve attention. At the proximate level, one outstanding question remaining about the TSR is the extent to which it is driven by regulation at the level of the cell versus at the level of the organism. In some species, the TSR is associated with changes in cell sizes, while in others it is not (Blanckenhorn and Llaurens, 2005; Atkinson et al., 2006). Investigation of the effects of temperature on the mechanisms that control cell size (e.g., cyclins) has the potential to reveal pathways that could explain the TSR observed in species that change cell size with temperature. Since smaller sizes at higher temperatures are usually driven by shorter developmental times, key to understanding the TSR will be investigations of the effect of temperature on the neuroendocrine control of developmental timing. Insulin-signaling is emerging as a major pathway controlling both cell size and body size (Sterner 2003; Wu and Brown, 2006), but as yet we lack good studies of thermal effects on the functioning of the insulin-signaling pathway. At the organismal level, the findings of Davidowitz et al. (2004) that the interval to cessation of growth changes with temperature suggest an urgent need to examine patterns of the regulation of juvenile hormone and ecdysone across temperatures. Thermal effects on these hormones may explain the TSR within arthropods, all of which use similar developmental hormones to regulate moulting. At the ultimate level, the suggestions of Angilletta and Dunham (2003), that the optimal temperature for growth shifts lower in larger animals, should be further investigated. Other constraints on growth late in ontogeny (e.g., spatial or mechanical constraints) may be exacerbated at higher temperatures, explaining decreases in efficiency of growth later in ontogeny. For example, higher temperatures tend to increase muscle rate processes (Rome and Swank, 1992) while reducing the toughness of some biological materials (Yang et al., 2005; Willett et al., 2008); perhaps, the TSR is driven in part by the need to conserve mechanical safety margins across temperatures.

Although the TSR is generalized, there remains significant variation in the magnitude and direction of the response of body size to temperature across species, and the explanation for this variation is an open question. One possible explanation for the lack of correlation between the TSR and macro-environmental variables among arthropods is that macro-environmental variables are poor predictors of the micro-environments relevant to small arthropods (Willmer, 1982; Unwin and Corbet, 1991). Thus, more empirical investigations of factors such as temperature, availability of water, and level of ambient oxygen at the micro-scale (e.g., Holter, 1991; Holter and Spangenberg, 1997; Klok and Chown, 1998; Gibbs et al., 2003), and compilation of such micro-climatic data into databases, may be critical for rigorously testing the environmental effects on the TSR.

Ecological factors are likely to create variation in the TSR, both via plasticity and natural selection.
One likely source of variation in the TSR among species might be interactions with nutrition. While we attempted to exclude studies with nutritional limitations, it is quite likely that dietary quality varied across species. Recently, it has been shown that low quality of diet can reverse the direction of the TSR (Diamond and Kingsolver 2010), and that quality of diet affects the efficiency of growth and the preferred temperature of grasshoppers (Miller et al. 2009). A plausible source of variation in the TSR might be ecological factors that select life-history traits such as sexual selection and predation pressure. Such biotic factors are known to drive the evolution of body size in many species (Chown and Gaston 2010), and it would be surprising if such factors did not affect the TSR. Since the TSR is driven by a high responsiveness of developmental time to temperature (relative to growth rate), the duration of the growth period relative to length of the season seems likely to affect the TSR of a species. Short growing seasons select for rapid development (Chown and Gaston 2010), and this seems a likely abiotic variable that could drive the variation in TSR observed here, since the length of season may be only weakly correlated with latitude. It has been suggested that interactions between size and temperature related to starvation resistance might cause, or influence, the TSR, with larger body sizes allowing greater starvation resistance during longer periods of death that are more plausible at higher latitudes (Stillwell et al. 2007). Although our finding that latitude does not correlate with the magnitude of the TSR argues against this hypothesis, ecologically-relevant periods of death may not be strongly correlated with latitude or with average habitat temperature. Testing such ultimate mechanisms for variation in the TSR will likely require renewed attention to micro-environmental conditions. However, a renewed focus on broad collection of such data will be critical to develop predictive models of the thermal effects on body size and on arthropod populations.

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Supplementary data

The body sizes with related data on temperature, latitude, elevation and oxygen for the arthropod species included in the analyses are available at ICB online.

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