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Temperature variability and thermal performance in ectotherms: acclimation, behaviour, and experimental considerations

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ABSTRACT

Background: Among the predictions of the effect of future climate change, the impact of thermal conditions at local levels on the performance of individuals and their acclimation capacities is key to understanding animals' responses to global warming. Woodlice (the terrestrial isopod *Porcellio laevis*) exhibit a readily observed behaviour that may reflect their acclimation capacities. When they find themselves on their back, they sometimes roll over (i.e. right themselves). Whether they do, and how fast they do it, are measures of their behaviour and performance that vary with ambient temperature.

Goal: Measure experimentally the effect of acclimation to environmental thermal variability on the rollover behaviour of *Porcellio laevis*.

Methods: Maintain woodlice in 24°C laboratory environments. Each individual lived in one of three thermal treatments: constant temperature regime ($\delta = 0$); a regime with alternating temperatures of 27°C during daytime and 21°C at night ($\delta = 3$); and a regime with alternating temperatures of 32°C during daytime and 16°C at night ($\delta = 8$). After 3 months, measure the rollover behaviour (percentage that rolled over) and rollover speed of the woodlice at 5, 15, 25, 32, and 38°C. Rollover speed is the inverse of the time it took an individual to right itself.

Results: At 5 and 38°C, most woodlice seemed to be in thermal coma and had very slow rollover speed. At 15, 25, and 32°C, rollover speed was in the range 0.12–0.18 rollovers per second. At 15, 25, and 32°C, 52% of individuals righted themselves in ~4 s. At these temperatures, most individuals either rolled quickly or remained inactive for the entire 10-min test period. When we examined rollover speed in the cooler (night) and warmer (daytime) periods, individuals subjected to the three regimes of temperature variation differed: woodlice acclimated to $\delta = 8$ roll over very fast when temperature (T) is low and very slowly when T is high; woodlice acclimated to $\delta = 3$ roll over fairly fast when T is low and somewhat slowly when T is high; and woodlice acclimated to $\delta = 0$ roll over quite quickly when T is low and quite

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slowly when T is high. However, the higher and lower speeds at different regimes neutralized each other so that average rollover speed over the course of a whole day did not depend on the size of the daily fluctuation.

Conclusions: The impact of temperature variability must be taken into consideration. Behavioural responses that are seemingly costly in the very short term may be beneficial in the longer term.

Keywords: acclimation, global warming, performance variance, thermal variability.

INTRODUCTION

The Earth's climate is changing rapidly and there is increased variability in environmental conditions. Indeed, climate change is occurring at global, regional, and local scales (IPCC, 2014) and is one of the greatest threats to biodiversity (Dawson *et al.*, 2011; Leonard, 2014). Global warming impacts both the mean temperature of local environments, and the magnitude of diel and seasonal variation in temperature (Easterling *et al.*, 2000; Parmesan *et al.*, 2000; Burroughs, 2007; Paaajmans *et al.*, 2013; Thompson *et al.*, 2013). In addition to increases in mean temperatures on Earth, the IPCC (2014) predicts increases in extreme events such as heat waves. The predicted increase in the frequency of extreme environmental conditions is likely to have an even greater impact on biodiversity than the increase in average temperatures (Coumou and Rahmstorf, 2012). Although ecologists and evolutionary biologists recognize the importance of environmental variability (Levins, 1968; Gould, 1985; Bozinovic and Pörtner, 2015; Vasquez *et al.*, 2015), studies in global change biology have focused in the main on the ecological impact of changes in average climatic values. Under some climate change scenarios, temperature variability and extremes are expected to eclipse changes in average values (Meehl *et al.*, 2007).

Thermal variation, for example, appears to be an important selective force on organisms in the wild (Jentsch *et al.*, 2007; Lalouette *et al.*, 2007; Terblanche *et al.*, 2010; Bannerman *et al.*, 2011; Bozinovic *et al.*, 2011a; Thompson *et al.*, 2013). In this context, the study of the impact of thermal variability on the performance of individuals and their plastic responses to it, such as lethal and sub-lethal effects, is important for understanding the responses of organisms to different climatic scenarios (Somero, 2011; Bozinovic *et al.*, 2013a, 2013b; Estay *et al.*, 2014; Bozinovic and Pörtner, 2015; Vasquez *et al.*, 2015). Among ectotherms, the typically unimodal, asymmetric shape of the relationship between temperature and physiological performance implies that the impact of thermal variation on physiological performance will depend on the mean temperature at which performance peaks (Bozinovic *et al.*, 2011a, 2011b, 2013a, 2013b). When mean temperature is below the thermal optimum, thermal variation can either enhance or reduce physiological performance. In contrast, when mean temperature is equal to or greater than the thermal optimum, thermal variation will always impair performance. Thus, the mean and variance of temperature may interact predictably to determine fitness in a variable environment.

Several studies provide experimental evidence for the influence of thermal variation on individual performance (Siddiqui *et al.*, 1973; Pétavy *et al.*, 2004; Terblanche *et al.*, 2010; Bozinovic *et al.*, 2011b, 2013a, 2013b; Folguera *et al.*, 2011; Kjaersgaard *et al.*, 2013; Clavijo-Baquet *et al.*, 2014). In 1972, Siddiqui and Barlow conducted a now classic study that showed that thermal fluctuations, within the range of temperatures favourable for reproduction, enhanced the rate of population growth. In addition, Orcutt and Porter (1983) observed a positive effect of thermal variation on the population growth rate of water fleas. More recently, Estay *et al.* (2011) found that

thermal variation had a negative effect on fitness of flour beetles when mean temperature was in the decelerating part of the performance curve. Similar interactions between the mean and the variance of temperature have been observed in other studies ([Dallwitz, 1984](#); [Paaijmans et al., 2010](#)). Thus, adaptation as well as acclimation to varying thermal environments depends on the temporal pattern of environmental changes and the physiological tolerance of each phenotype.

Recently, different groups have attempted to formally include the effect of thermal variation in the physiological performance of individuals in nature and how the temporal and spatial variation in body temperature may impact physiological performance ([Martin and Huey, 2008](#); [Paaijmans et al., 2010](#); [Foray et al., 2013](#); [Vasseur et al., 2014](#); [Dowd et al., 2015](#)). The fundamental problem, however, is that the incorporation of temperature variation involves a temporal component that can affect both the type and magnitude of any change in performance (see [Schulte et al., 2011](#)). Whereas acute changes in temperature should affect performance while the performance curve itself remains unchanged, prolonged exposure to different levels of thermal variation may give rise to acclimatory adjustments that alter the shape of the performance curve – and, as a result, the predictions of recently proposed models ([Vasseur et al., 2014](#); [Dowd et al., 2015](#)). We argue that discriminating between these two responses can be enlightening: not only can one quantify how thermal variation might impact mean performance across temperatures ([Vasseur et al., 2014](#); [Dowd et al., 2015](#)), but also determine how acclimatory responses to a thermally variable environment might ameliorate this impact.

Consequently, in this study we experimentally tested for the effect of acclimation to environmental thermal variability on the performance curve of the terrestrial isopod *Porcellio laevis*. We wished to determine whether observed responses could in any way be adaptive by explicitly incorporating thermal variability in the analysis. Terrestrial isopods or woodlice are good models to test hypotheses in global change biology, since they exhibit a broad distribution and cope with extreme abiotic conditions ([Schultz, 1961, 1972](#); [Miller and Cameron, 1987](#); [Helden and Hassall, 1998](#)). *Porcellio laevis*, in particular, is a good animal model, since it is cosmopolitan and shows plasticity in physiological and life-history traits in response to different geographic-climatic conditions ([Warburg et al., 2001](#); [Castañeda et al., 2004](#); [Lardies and Bozinovic, 2006, 2008](#); [Bozinovic et al., 2014](#)).

METHODS

Localities and laboratory experiment

This study was performed with woodlice (*Porcellio laevis*) collected in San Carlos de Apoquindo [33°23'S, 70°31'W, 1230 m above sea level ([Lardies et al., 2004](#))]. Woodlice were collected by hand from under stones, pieces of wood, and soil litter. Specimens were placed in containers with vegetable soil and carrot slices, and transported to the laboratory. Animals were maintained in plastic boxes with a base layer of plaster of Paris to maintain humidity. Dry spinach and water was provided *ad libitum*.

Based on our previous experience ([Folguera et al., 2009](#)), for 3 months individuals were randomly assigned to one of three thermal treatments in climatic chambers: (1) a constant temperature regime of 24°C ($\delta = 0$); (2) a regime with alternating temperatures of 27°C during daytime and 21°C at night ($\delta = 3$); and (3) a regime with alternating temperatures of 32°C during daytime and 16°C at night ($\delta = 8$). These experimental temperatures

were chosen because they are within the thermal range and daily variability of *P. laevis* (Folguera *et al.*, 2011) during their active season.

We used rollover speed as a measure of performance in the three temperature treatments. Following Folguera *et al.* (2009), rollover speed was measured at 5, 15, 25, 32, and 38°C using the following protocol. At each temperature, approximately 50 woodlice were placed individually in plastic boxes measuring $2.2 \times 2.2 \times 2.4$ cm. The boxes were then placed inside an aluminium incubator within a water bath ($\pm 0.5^\circ\text{C}$). After 30 min, we evaluated rollover speed for 10 min as a measure of righting response. If an animal was unable to right itself within this time, it was considered to have null performance (rollover speed = 0). These woodlice were used for all experimental temperatures except for 38°C, for which we used a different set of woodlice.

Statistics

We first tested whether the righting response time at each temperature measured differed between acclimation groups using a conventional one-way ANOVA. Analyses used righting response time rather than rollover speed because the latter, a ratio (1/response time), inflates the contribution of fast responses and the associated measurement error (at 15, 25, and 32°C, righting response was ~ 4 s for 52% of individuals). In these analyses, we conservatively assigned a righting response time of 10 min for individuals that were unresponsive during this period.

Interestingly, the distribution of righting response time and rollover speed differed considerably between the thermal extremes (5 and 38°C) and the remaining temperatures. At 5 and 38°C, most woodlice appeared to be in thermal coma, resulting in a unimodal distribution of rollover speed with a very small variance (Fig. 1). In contrast, at 15, 25, and 32°C, the distribution of rollover speed was skewed towards the extremes because most individuals either rolled over within a relatively short time or remained inactive for the entire 10 min. Given these contrasting responses and the main differences between thermal performance curves within the range 15–32°C (see below), we focused on the putative impact of the different thermal regimes within this range. The proportion of responsive (rollover speed > 0) and unresponsive individuals (rollover speed = 0) for each temperature was compared across thermal regimes ($\delta = 0, 3, \text{ and } 8^\circ\text{C}$) using chi-square tests. We then tested whether the impact of temperature on the righting response time changed across thermal regimes using an ANCOVA that included the interaction between measurement temperature and acclimation. This approach can accommodate the high dispersion of rollover speed observed within each measurement temperature and is justified because, within the range 15–32°C, the relationship between rollover speed and temperature for each group is quasi-linear (Fig. 1). Results of the ANOVAs and ANCOVA were compared with bootstrap analyses to ensure that they were reliable (for each test, we randomly sampled 1000 replicates from the original distribution with replacement). Results were virtually identical; hence we report the results of the parametric tests.

We analysed the performance curves as a function of temperature ($P(T)$) for each thermal regime by means of fourth-order polynomial regression analysis. Because subsequent analyses incorporating thermal variation rely on thermal functions that adequately describe the empirical data, we employed this approach for heuristic reasons to maximize the goodness of fit of the curves in spite of the increased number of parameters. In contrast, other studies have employed the Akaike Information Criterion (AIC), which penalizes the

incorporation of extra parameters, to find a balance between the goodness of fit and the complexity of the models (e.g. Angilletta, 2006; Niehaus *et al.*, 2012).

Having tested whether thermal performance curves respond to acclimation to different levels of thermal variability, we estimated how fluctuations around a mean temperature \bar{T} are expected to affect average performance ($\overline{P(\bar{T})}$) based on these curves. For clarity, hereafter we refer to the thermal variability associated with the acclimation treatments as δ ($\delta = 0, 3, \text{ and } 8^\circ\text{C}$) and the variation inherent to the performance measurement as σ_T ($\sigma_T = 0 \text{ and } 8^\circ\text{C}$). Following Dowd *et al.* (2015), we obtained the first and second derivative of $P(T)$, $P'(T)$ and $P''(T)$, and estimated the mean performance at 24°C using:

$$\overline{P(\bar{T})} \approx P'(\bar{T}) + \frac{1}{2} P''(\bar{T}) \sigma_T^2, \quad (1)$$

where T is the average temperature and σ_T^2 is the temperature variance. We also estimated the standard deviation of $\overline{P(\bar{T})}$:

$$\sigma_P = \sqrt{[P'(\bar{T})]^2 \sigma_T^2 + \frac{1}{4} [P''(\bar{T})]^2 (\sigma_T^2)^2}, \quad (2)$$

and the coefficient of variation:

$$CV_P = \frac{\sigma_P}{\overline{P(\bar{T})}}. \quad (3)$$

Subsequently, we expanded these analyses to the entire range of \bar{T} employing numerical simulations (Dowd *et al.*, 2015). Statistical analyses were conducted with Statistica v.5.0 (StatSoft, Inc., 2001), and all computer simulations were performed in R.

RESULTS

Figure 1 shows rollover speed as a function of T for woodlice acclimated in the three thermal regimes ($\delta = 0, 3, \text{ and } 8^\circ\text{C}$). Fitted curves were as follows:

$$\overline{P(\bar{T})} = -0.169 + 0.051 T - 0.0029 T^2 + 7.93 \times 10^{-5} T^3 - 8.76 \times 10^{-7} T^4 \quad (\delta = 0^\circ\text{C})$$

$$R^2 = 0.268, F_{4,161} = 14.73, P \ll 0.001,$$

$$\overline{P(\bar{T})} = -0.222 + 0.066 T - 0.0041 T^2 + 1.12 \times 10^{-4} T^3 - 1.20 \times 10^{-6} T^4 \quad (\delta = 3^\circ\text{C})$$

$$R^2 = 0.307, F_{4,168} = 18.58, P \ll 0.001,$$

and

$$\overline{P(\bar{T})} = -0.368 + 0.109 T - 0.0074 T^2 + 2.09 \times 10^{-4} T^3 - 2.12 \times 10^{-6} T^4 \quad (\delta = 8^\circ\text{C})$$

$$R^2 = 0.329, F_{4,99} = 12.12, P \ll 0.001.$$

Visual inspection shows that the curves adequately describe the variation in average rollover speed across temperatures, and suggest that woodlice exposed to the most variable thermal environment ($\delta = 8$) tend to perform better in cooler temperatures than in the warm (Fig. 1). Results of ANOVAs comparing thermal regimes partly support this finding, and suggest that mean rollover speed for $\delta = 8$ tends to be lower at 32°C ($F_{2,299} = 2.64, P = 0.076$). Importantly, this difference borders significance under the conservative assumption of a righting response time of 10 min for woodlice that were unresponsive during this period, and becomes statistically significant ($P < 0.05$) assuming response times of 13 min or longer. The ANCOVA pooling measurements at 15, 25, and 32°C also indicates that temperature effects on rollover speed vary depending on the thermal regime ($F_{1,299} = 9.39, P_{\text{temp}} = 0.002$ and

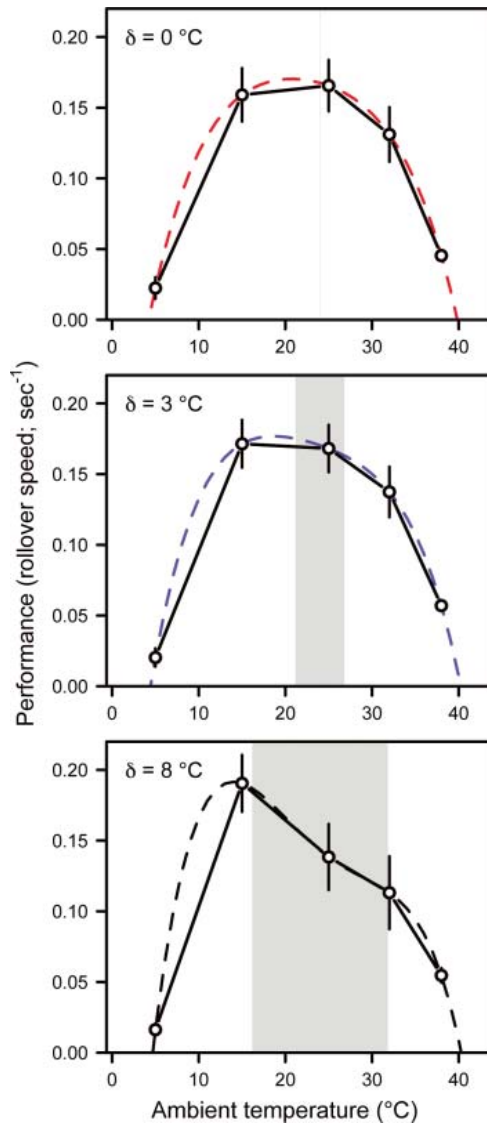


Fig. 1

Fig. 1. Performance (rollover speed) in woodlice acclimated to three thermal variability regimes ($\delta = 0$, $\delta = 3$, and $\delta = 8^\circ\text{C}$). Temperature variability highlighted in grey for the different acclimation treatments. Measurements were performed at constant temperature conditions of ambient daily temperature variability within a period of 10 min, hence these curves describe thermal performance when $\sigma_T = 0^\circ\text{C}$. Mean values \pm standard error are shown in black and the fitted curves are depicted in red ($\delta = 0^\circ\text{C}$), blue ($\delta = 3^\circ\text{C}$), and black ($\delta = 8^\circ\text{C}$), respectively.

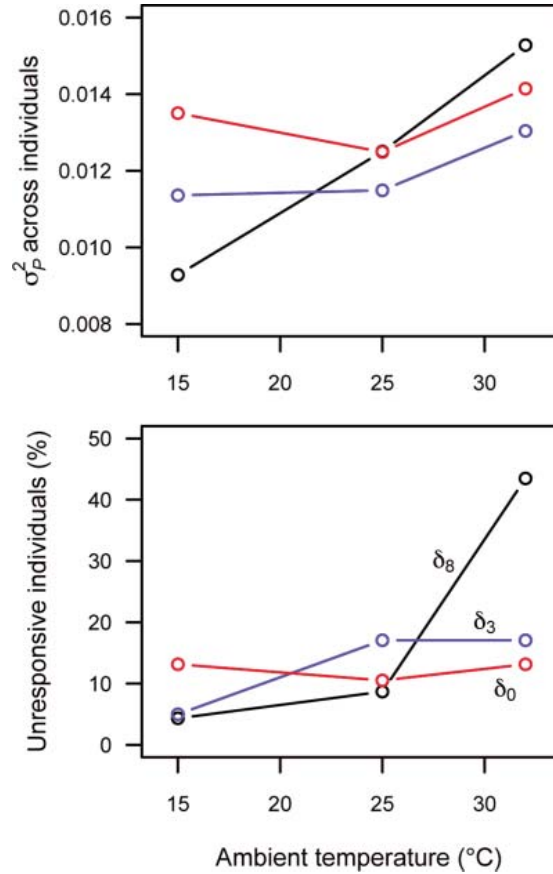


Fig. 2

Fig. 2. Individual variation in performance (rollover speed) in woodlice acclimated to three thermal variability regimes ($\delta = 0$, $\delta = 3$, and $\delta = 8^\circ\text{C}$). Performance is expressed as the fraction of individuals that remained inactive (i.e. did not right themselves) or as the variance in rollover speed (s^{-1}) across individuals within each measurement temperature.

$F_{2,299} = 2.72$, $P_{\text{temp} \times \text{acclim}} = 0.067$), with the interaction effect attaining statistical significance ($P < 0.05$) when response times for unresponsive individuals are set to 12 min or longer.

What accounts for the decreased performance of the highly variable $\delta = 8$ thermal regime at warmer temperatures? Interestingly, the variance in rollover speed increases with temperature primarily in this group, with a 64.6% increase in variance from 15°C to 32°C versus a 4.7% and 14.7% increase in the $\delta = 0$ and $\delta = 3$ thermal regime, respectively (Fig. 2). The increased variance in performance of $\delta = 8$ reflects a substantial fraction of the individuals in this group being unresponsive during trials at 32°C (Fig. 2), which is significantly higher than the number of unresponsive individuals in the $\delta = 0$ and $\delta = 3$ thermal regimes at this temperature according to a chi-square test ($\chi^2 = 8.60$, d.f. = 2, $P = 0.013$). In contrast, no differences were detected at 15 and 25°C ($\chi^2 < 2.3$ and $P > 0.32$ in both cases). This result is counterintuitive because woodlice in the $\delta = 8$ thermal regime were exposed to a temperature of 32°C for 12 h during their daily cycle, and therefore the unresponsiveness observed in a substantial fraction of these individuals cannot possibly result from a thermal coma – i.e. if an acute exposure to 32°C results in thermal coma, then the 12 h exposure to this temperature should have had substantially higher detrimental effects (see Rezende *et al.*, 2014). Taken together, these results suggest that the decreased performance and the higher fraction of unresponsive individuals in the $\delta = 8$ thermal regime at 32°C reflect to a large degree voluntary behaviour rather than physiological constraints.

Having assessed how rollover speed in the three thermal regimes is affected by temperature in the short term (i.e. within 10 min or less), we now quantify how thermal fluctuations around a mean value are expected to impact long-term average performance. Whereas in our setting the thermal variation in the short term is $\sigma_T \approx 0^\circ\text{C}$ because temperatures remain almost constant within minutes, average performance over the course of 24 h can be calculated assuming $\sigma_T = 8^\circ\text{C}$ for a thermal regime involving daily fluctuations between 16 and 32°C (Fig. 3).

Analyses suggest that the highly asymmetric thermal performance curve at $\delta = 8$ results primarily in more extreme daily fluctuations in thermal performance and, as a consequence, a higher σ_P and CV_P than $\delta = 0$ and $\delta = 3$ (Table 1). In contrast, daily average performance $\overline{P(T)}$ was strikingly similar across treatments with overlapping $\pm \sigma_P$, suggesting that these estimates are statistically indistinguishable at 24°C (Table 1). Notably, $\delta = 8$ was the only thermal regime in which $\overline{P(T)}$ increased compared with short-term $\overline{P(T)}$ at 24°C (an 11.1% increase for $\delta = 8$ vs. a 10.1% and 5.7% decrease for $\delta = 0$ and $\delta = 3$, respectively), even though the latter never fell outside the 95% confidence interval ($\pm 1.96 \sigma_P$) required for differences to be deemed significant (Table 1). Therefore, woodlice exposed to a highly variable thermal environment ($\delta = 8$) seem to modulate their short-term performance to attain in the long term the same levels of performance across \overline{T} than their $\delta = 0$ and $\delta = 3$ counterparts (Fig. 4).

DISCUSSION

Our results indicate that the thermal performance curve of woodlice changes significantly in response to acclimation to different levels of thermal variability, with individuals acclimated to a more variable thermal regime seemingly shifting their thermal optimum to lower temperatures (Fig. 1). Even though this shift seems to be accompanied by a cost of reduced performance at higher temperatures, there is circumstantial evidence that this response reflects behavioural modulation rather than detrimental thermal effects on performance. For instance, significant differences were observed at intermediate (32°C)

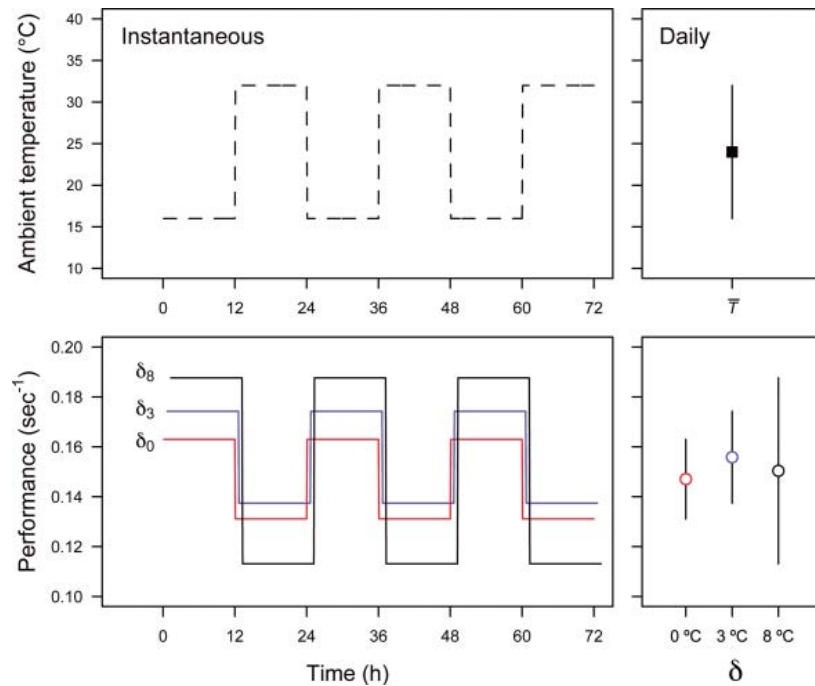


Fig. 3. Temporal variation in ambient temperature, corresponding to $\sigma_T = 8^\circ\text{C}$, and its predicted impact on rollover speed of woodlice acclimated to three thermal variability regimes ($\delta = 0$, $\delta = 3$, and $\delta = 8^\circ\text{C}$). For simplicity, we assume that the transition between thermal extremes is instantaneous, when in reality it would take a couple of hours. Note that, within short periods of time, animals would be experiencing either 16 or 32°C and their performance would change accordingly based on the thermal performance curves estimated for $\sigma_T = 0^\circ\text{C}$ (see Fig. 1). However, mean performance over longer periods (here, daily averages \pm standard deviation) is affected by the inherent temporal variation in temperature (see Table 1).

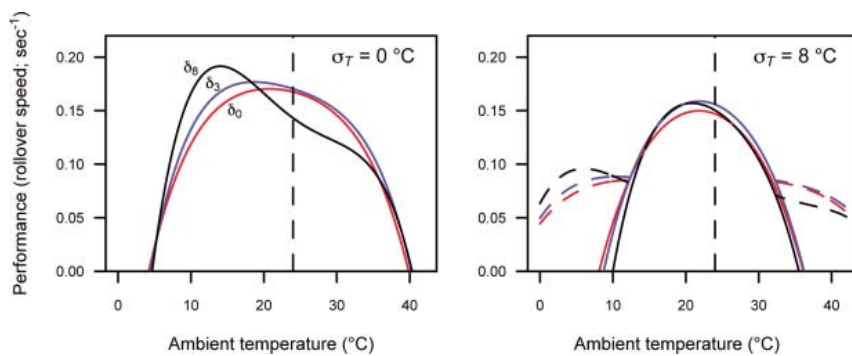


Fig. 4. Rollover speed in woodlice acclimated to three thermal variability regimes ($\delta = 0$, $\delta = 3$, and $\delta = 8^\circ\text{C}$), estimated for $\sigma_T = 0$ and 8°C . Average performance for $\sigma_T = 8^\circ\text{C}$ was estimated with computer simulations by incorporating variation around each temperature mean, as described for 24°C in Fig. 3. The costs of temporarily exceeding the bounds of the performance curve (for details, see Dowd *et al.*, 2015) were assumed to be either zero (dotted line) or to follow the polynomial equation fitted for $\sigma_T = 0$ (continuous line). Note that predictions in this range of temperatures are highly dependent on this underlying assumption. Estimates for 24°C are shown in Table 1.

Table 1. Parameters obtained from polynomial regressions and equations (1–3) (see Methods)

Parameters	Thermal treatments (°C)		
	$\delta = 0$	$\delta = 3$	$\delta = 8$
$T = 24, \sigma_T = 0$			
$P(\bar{T})$	0.167	0.170	0.143
$\overline{P(T)}$	0.167	0.170	0.143
σ_P	0	0	0
CV_P	0	0	0
$T = 24, \sigma_T = 8$			
$P(\bar{T})$	0.167	0.170	0.143
$\overline{P(T)}$	0.151	0.161	0.159
σ_P	0.022	0.019	0.043
CV_P	0.144	0.120	0.270

Note: \bar{T} = mean temperature, σ_T = standard deviation of temperature, $P(\bar{T})$ = performance at mean temperature, $\overline{P(T)}$ = daily mean performance, σ_P = standard deviation of daily mean performance, CV_P = coefficient of variation of daily mean performance.

rather than extreme temperatures (38°C) and we found no evidence to suggest that the upper critical limit might have changed. Additionally, woodlice subjected to the high thermal variability regime encountered 32°C for roughly 12 h of their daily cycle (Fig. 3), and would likely not have survived this prolonged thermal challenge if comatose or physiologically impaired. And, finally, when taking daily temperature fluctuations into account, average performance did not differ across thermal regimes (Table 1), and therefore the reduced performance observed in the high variability treatment $\delta = 8$ in the short term ($\sigma_T = 0^\circ\text{C}$) is unlikely to translate into differences in a 24 h cycle ($\sigma_T = 8^\circ\text{C}$).

Comparisons between treatments suggest that thermal variation may alter the thermal performance curve, as originally hypothesized, even though this response seems to reflect primarily a compensatory behaviour rather than a physiological response (see Rojas *et al.*, 2014). This result highlights the need to discriminate between the impact of thermal variability on behaviour and functional capacity, which at its core involves discerning when measured performance is submaximal or maximal (Losos *et al.*, 2002; Head *et al.*, 2012). The incorporation of submaximal measurements adds a component of motivation (or lack thereof) that may obscure the relationship between any given variable of interest (e.g. temperature or morphology) and performance *sensu stricto* (Losos *et al.*, 2002; Careau and Garland, 2012; Astley *et al.*, 2013), which could have been the case in the present study. In principle, responses at these two levels could be disentangled in experiments involving multiple measurements per individual (Adolph and Pickering, 2008), in which analyses can be repeated with or without submaximal measurements to estimate how temperature affects both the behavioural and the physiological component that might underlie a performance curve. In practice, however, this can be logistically complicated because it is often difficult to identify submaximal measurements, and also philosophically problematic since this procedure may bias subsequent analyses (Losos *et al.*, 2002; but see Head *et al.*, 2012). Furthermore, the fact that we effectively estimated righting response time, which sets a limit to maximum performance because

responses can be nearly instantaneous (and this was indeed the case for a large fraction of the sample; see Statistics), also decreased the degree of resolution in maximum performance estimates across measurement temperatures and thermal regimes. Consequently, our experimental design cannot fully discriminate between behavioural and physiological responses to acclimation, even though it is clear that performance was submaximal at least in a fraction of unresponsive individuals (Fig. 2). Nonetheless, and we quote, ‘if differences in performance in the laboratory reflect behavioural differences in the field, such findings are informative, even if they do not represent estimates of maximal capabilities’ (Losos *et al.*, 2002, p. 58).

An important assumption that underlies our interpretation is that prolonged exposure to 32°C imposes some degree of physiological stress, which must be accommodated behaviourally. Previous studies have shown that desiccation is a major factor affecting survival and locomotor performance in woodlice (Edney, 1951, 1977; Dailey *et al.*, 2009), and we speculate that individuals in the $\delta = 8$ thermal regime were more often inactive at 32°C so as to reduce evaporative water loss. In *P. laevis* exposed to dry air at 30°C, Quinlan and Hadley (1983) reported a 40% reduction in evaporation from 3.24 to 1.96 $\text{mg} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$ as activity decreased in the course of the experiment, which translates to roughly 18.4% and 11.1% of initial body mass (143.3 mg in their study) during a 12 h period at 80% relative humidity. These estimates are expected to be higher at 32°C and might put individuals exposed to the highly variable thermal regime ($\delta = 8$) in jeopardy, because *P. laevis* loses its righting response and locomotor function, on average, at 83.6% and 70% of its hydrated mass (Dailey *et al.*, 2009). These calculations show that the reduction in water loss due to decreased activity may be important and may explain not only the behavioural response observed in this treatment. In contrast, the risk of desiccation in woodlice exposed to moderate variability ($\delta = 3$) with thermal extremes between 21 and 27°C was likely lower, which might explain why they did not exhibit any obvious behavioural response compared with the control ($\delta = 0$). Nonetheless, observed differences in mortality across thermal regimes, with mortality rates increasing with temperature variability (Folguera *et al.*, 2011), support our contention that physiological stress takes place at thermal extremes. Indeed, Rojas *et al.* (2014) demonstrated that *P. laevis* acclimated to a thermally variable environment reduce their exploratory behaviour. This scenario, as well as the long behavioural latency of isopods acclimated to variable environments, is consistent with this idea and our results. As a caveat, even though we interpret observed differences between treatments as a result of acclimation (i.e. plasticity), we cannot dismiss the potential contribution of selection during the course of the experiment.

Results show that behavioural accommodation in combination with stressful thermal extremes can lead to a mismatch between \bar{T} and observed optimal temperatures at which performance is maximized (Fig. 4). This response is predicted by Jensen’s inequality and, as discussed in detail elsewhere (see Martin and Huey, 2008; Vercken *et al.*, 2012), might explain why ectothermic organisms often select temperatures that are seemingly suboptimal for performance (Huey and Bennett, 1987). According to the framework proposed by Martin and Huey (2008), this mismatch should increase with thermal variability, which is supported by our data. The polynomial fitted curves for $\sigma_T = 0^\circ\text{C}$ suggest that performance is maximized at $T = 20.7$, 18.6, and 14.0°C for $\delta = 0$, 3, and 8 respectively, resulting in a difference between these estimates and $\bar{T} = 24^\circ\text{C}$ of 3.3, 5.4, and 10°C and a significant relationship between the degree of mismatch and δ in spite of the reduced sample size ($r^2 = 99.5$, $F_{1,1} = 210.4$, one-tailed $P = 0.022$). Interestingly, calculation of optimal temperatures including thermal variability ($\sigma_T = 8^\circ\text{C}$) results in optimal temperatures of

21.8°C for both $\delta = 0$ and 3, and 20.8°C for $\delta = 8$, which are closer to $\bar{T} = 24^\circ\text{C}$, suggesting that the impact of behavioural responses observed in short-term assays is reduced when daily fluctuations in temperature, and therefore performance, are taken into account (Figs. 3 and 4). Consequently, our study unravels not only how woodlice acclimated to a highly variable thermal regime ($\delta = 8$) ameliorate the impact of repeated exposure to stressful thermal extremes by behavioural means (i.e. measurements at $\sigma_T = 0^\circ\text{C}$), but also the compensatory consequences of observed responses in the long term (estimates at $\sigma_T = 8^\circ\text{C}$).

Importantly, conclusions regarding these putative compensatory effects rely on how adequately thermal performance curves estimated at constant temperatures ($\sigma_T = 0^\circ\text{C}$) can be extrapolated to fluctuating environments ($\sigma_T = 8^\circ\text{C}$), which will depend on the trait, the range of temperatures, and the time-scale of the study. Our analyses discriminate between acute temperature effects, which occur within minutes or hours, and acclimatory responses, which occur within days or weeks (Schulte *et al.*, 2011; Niehaus *et al.*, 2012; Dowd *et al.*, 2015) and, around $\bar{T} = 24^\circ\text{C}$, calculations are likely realistic because the crucial assumption that performance curves estimated for $\sigma_T = 0^\circ\text{C}$ remain unchanged during the 24 h period over which performance is averaged presumably holds (Dowd *et al.*, 2015). For other \bar{T} , predicted values may also depend on the relative costs of temporarily exceeding the bounds of the performance curve (Fig. 4), a topic that remains virtually unexplored (see Dowd *et al.*, 2013). Consequently, we believe that our analyses provide an ecologically relevant portrayal of how *P. laevis* may respond to the thermal variability they encounter in the field (Folguera *et al.*, 2011). Nonetheless, because of the non-linear nature of performance curves, the effects of temperature averages and extremes cannot be disentangled (see Beuchat and Ellner, 1987; Ruel and Ayers, 1999; Martin and Huey, 2008) and the responses to different levels of thermal variation reported here are expected to change with other acclimation temperatures. Consequently, laboratory studies focusing on the impact of changing thermal conditions in natural populations must strive to reproduce the conditions these populations encounter in the field. Alternatively, increasingly complex designs combining different mean temperatures and temperature variability are required for a more general picture of how acclimation responses and acute temperature effects ultimately interact.

ACKNOWLEDGEMENTS

All experimental procedures were approved by the Animal Care Committee, Universidad Católica. S.I. Martel provided experimental assistance. Funded by FONDECYT-1130015 to F.B., FONDECYT-1120276 to P.S., and CAPES FB002 line 3 to F.B.

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