Constant and fluctuating temperature acclimations have similar effects on phenotypic plasticity in springtails

Jessica L. Hoskinsa,[[1]](#footnote-1)\*, Charlene Janion-Scheepersb,c Elise Irelanda, Keyne Monroa, Steven L. Chowna

*aSchool of Biological Sciences, Monash University, Victoria 3800, Australia.*

b*Iziko Museums of* *South* *Africa, Cape Town, 8001, South Africa.*

*cDepartment of Biological Sciences, University of Cape Town, Rondebosch,7701, South Africa.*

[[2]](#footnote-2)

ABSTRACT

Much interest exists in the extent to which constant versus fluctuating temperatures affect thermal performance traits and their phenotypic plasticity. Theory suggests that effects should vary with temperature, being especially pronounced at more extreme low (because of thermal respite) and high (because of Jensen’s inequality) temperatures. Here we tested this idea by examining the effects of constant temperatures (10 to 30°C in 5°C increments) and fluctuating temperatures (means equal to the constant temperatures, but with fluctuations of ±5°C) temperatures on the adult (F2) phenotypic plasticity of three thermal performance traits – critical thermal minimum (*CTmin*), critical thermal maximum (*CTmax*), and upper lethal temperature (ULT50) in ten species of springtails (Collembola) from three families (Isotomidae 7 spp.; Entomobryidae 2 spp.; Onychiuridae 1 sp.). The lowest mean *CTmin* value recorded here was -3.56±1.0°C for *Paristoma notabilis* and the highest mean *CTmax* was 43.1±0.8°C for *Hemisotoma thermophila*. The Acclimation Response Ratio for *CTmin* was on average 0.12°C/°C (range: 0.04 to 0.21°C/°C), but was much lower for *CTmax* (mean: 0.017°C/°C, range: -0.015 to 0.047°C/°C) and lower also for ULT50 (mean: 0.05°C/°C, range: -0.007 to 0.14°C/°C). Fluctuating versus constant temperatures typically had little effect on adult phenotypic plasticity, with effect sizes either no different from zero, or inconsistent in the direction of difference. Previous work assessing adult phenotypic plasticity of these thermal performance traits across a range of constant temperatures can thus be applied to a broader range of circumstances in springtails.

Keywords: Critical thermal limits; fluctuating temperatures; field temperatures; soil animals; temperate Australia; thermal tolerance

**1. Introduction**

Environmental temperature is one of the most significant factors affecting ectotherms. Multiple aspects of ectotherm ecology and evolution are influenced by the state and variability of the external thermal environment. In turn these are mediated by a suite of traits such as those involving thermal tolerance and life history (Sinclair et al. 2003; Angilletta et al. 2004; Angilletta 2009; Hoffmann et al. 2013; Kingsolver and Buckley 2017; Moretti et al. 2017). Unveiling the extent of ectotherm thermal trait variation is thus critical for exploring the potential effects of environmental change on ectotherm diversity (e.g., Deutsch et al. 2008, 2018; Dillon et al. 2010; Diamond et al. 2018; Pinsky et al. 2019). Indeed, trait-temperature interactions are often incorporated into biophysical models that seek to predict the outcomes of environmental change for specific species (e.g. Kearney and Porter 2009). Recently, attention has turned towards a better understanding of trait variation in an environmental change context using different assessment circumstances, such as constant versus fluctuating temperatures, because they can influence trait values (Mitchell and Hoffmann 2010; Clusella-Trullas et al. 2011; Niehaus et al. 2012; Valladares et al. 2014; Dowd et al. 2015; Colinet et al. 2015; Lawson et al. 2015; Hoffmann and Sgrò 2018; Kovacevic et al. 2019; Salachan et al. 2019).

Constant temperatures have long been used in experiments seeking to identify variation in thermal trait values caused by factors other than environmental variation – for example, adaptive or non-adaptive differences among populations and/or species. By contrast, organisms typically face daily temperature variation, along with seasonal changes in the range of daily fluctuations. This daily and seasonal variation in temperature can have significant effects on the form of traits related to thermal performance (e.g., Gilchrist 1995; Angilletta et al. 2006; Scheiner et al. 2019; Torson et al. 2019). For example, substantial differences in trait values under fluctuating and constant temperatures, with the same mean value, may arise because of Jensen’s inequality (Ruel and Ayres 1999; Denny 2017), the effects of which on development are also sometimes known as the Kaufmann effect (Worner 1992). Jensen’s inequality is a mathematical property of nonlinear functions such as thermal performance, which typically follows a bell-shaped curve. Estimates of average performance at the same point on this curve will theoretically differ because the constant-temperature estimate only includes one value, whereas the fluctuating-temperature estimate also includes performance at higher and lower temperatures (Denny 2017). This can result in variable effects on trait values, depending on the mean temperature and the extent of the thermal fluctuations (Carrington et al. 2013a; Kjaersgaard et al. 2013; Colinet et al. 2015). Such differences in outcome have been demonstrated for many species, including flies, mosquitoes and butterflies, for development (Kjaersgaard et al. 2013; Carrington et al. 2013a), survival (Ragland and Kingsolver 2008), reproduction (Carrington et al. 2013b), and thermal tolerance traits (Bozinovic et al. 2011, Fischer et al. 2011). In consequence, results obtained under constant laboratory conditions may not reflect the situation in thermally-variable natural systems (Behrens et al. 1983; Brakefield and Kesbeke 1997), potentially limiting the extension of laboratory studies to the field (Ma et al. 2015). In this regard, limitations may also affect the accuracy of forecast models incorporating trait data (Niehaus et al. 2012; Valladares et al. 2014; Dowd et al. 2015).

Exploring the nature of trait variation in response to fluctuating and constant temperatures has been identified as a crucial requirement for thermal physiology (Sinclair et al. 2016; Morash et al. 2018) due to the growing use of trait data to understand community assembly (e.g. Start et al. 2018; Miller et al. 2019), and the need to model both species and system responses to environmental change (Deutsch et al. 2018; Pinsky et al. 2019). Moreover, it is of particular importance when estimating the extent to which short-term plasticity (in the form of thermal acclimation) may alter thermal tolerance responses (Sgrò et al. 2016; Salachan et al. 2019). Variation among upper and lower thermal limits in their responses to acclimation, and the extent to which phenotypic plasticity in response to high temperatures might mediate the effects of environmental change, have profound implications for assessing the drivers of variation in species abundances and ranges and for modelling future outcomes of environmental change (Valladares et al. 2014; Gunderson and Stillman 2015; Donelson et al. 2019; Scheiner et al. 2019). Yet systematic investigations of the influence of constant versus fluctuating temperatures on phenotypic plasticity across a broad range of temperatures remains limited (see examples in Bozinovic et al. 2011; Fischer et al. 2011; Sobek-Swant et al. 2012; Sørensen et al. 2020).

The aim of this study is, therefore, to test the hypothesis, based on theoretical expectations outlined above, that the short-term (non-developmental) phenotypic plasticity of thermal tolerance traits differs between constant and fluctuating acclimation temperature treatments. That is, whether fluctuating temperatures elicit different responses in thermal tolerance traits relative to constant temperatures. We do this to determine whether thermal tolerance traits measured under typical laboratory conditions with constant temperatures are suitable for describing how those traits respond to temperature under more natural circumstances where temperatures fluctuate. We measure three traits commonly used to document thermal tolerance in ectotherms – critical thermal minimum (*CTmin*), critical thermal maximum (*CTmax*), and upper lethal limits (ULT50) – and estimate their ability to tolerate environmental temperature change (Deutsch et al. 2008; Huey et al. 2009; Diamond et al. 2012; Chown et al. 2015; Gunderson and Stillman 2015; Pinsky et al. 2019). We examine acclimation temperatures between 10°C and 30°C (on average) at 5°C increments. Previous findings have indicated that the effects of constant versus fluctuating temperatures may vary with mean temperature (e.g. Carrington et al. 2013a). For example, at low mean temperatures fluctuating temperatures may allow for thermal respite and for cold hardening which improves cold tolerance, whilst the impacts of Jensen’s inequality at high mean temperatures can lower heat tolerance as fluctuating temperatures exceed thermal tolerance limits (Colinet et al. 2015). Given previous work and theory on trait responses to a range of temperatures (e.g. Denny 2017), and factors such as Jensen’s inequality and thermal respite, we expected fluctuating temperature effects to deviate from constant temperature effects at the high and low ends of the temperature range we tested. Thus, the expectation is that fluctuating temperatures should increase thermal tolerance at low temperatures, and decrease thermal tolerance at high temperatures, in comparison to constant temperatures.

Here we use Collembola (springtails) as exemplar organisms to examine the effect of fluctuations on thermal tolerance traits. Springtails are key components of the soil biota (Hopkin 1997; Rusek 1998; Bardgett and van der Putten 2014), responsive in terms of their thermal biology to environmental variation (Bahrndorff et al. 2009; Everatt et al. 2013; van Dooremalen et al. 2013), and show strong relationships between thermal tolerance trait variation and community composition (Ellers et al. 2018; Treasure et al. 2019). Springtails are also widely used as indicators of the impacts of changing environmental conditions (Vandewalle et al. 2010), and are expected to be profoundly impacted by anthropogenic global change (e.g. Bokhorst et al. 2012; Holmstrup et al. 2018; Janion-Scheepers et al. 2018).

Much research is available on thermal tolerance traits in springtails, in particular for critical thermal limits (e.g. Bahrndorff et al. 2006; Slabber et al. 2007; Allen et al. 2016; Alemu et al. 2017; Janion-Scheepers et al. 2018; Jensen et al. 2019). In these studies, typically it has been shown that *CTmin* and *CTmax* can both show quite substantial phenotypic plasticity (unlike in some other arthropods) (Alemu et al. 2017; Jensen et al. 2019; Liu et al. 2020), that different experimental rates can affect both thermal acclimation responses and trait values (Allen et al. 2016; Alemu et al. 2017), and that significant differences can be found in traits based on geography, climate, habitat and whether or not species are indigenous to a given area (Bahrndorff et al. 2006; Liefting and Ellers 2008; Janion-Scheepers et al. 2018; Jensen et al. 2019; Phillips et al. 2020). Yet, almost all of the work on phenotypic plasticity and other forms of variation in thermal tolerances undertaken to date has employed constant temperature acclimation treatments to measure trait values. As a result, to date there have been few studies that investigate the effects of temperature fluctuations on thermal tolerance traits in springtails. Indeed, even more broadly, doing so is now an active area of research because of the paucity of previous studies (e.g. Salachan et al. 2019). Thus, understanding if the constant temperature trait values available in the literature are an accurate reflection of the response of thermal traits to field temperature conditions is critically important. Doing so will provide insights into the fundamental variation of these traits, and its eco-evolutionary basis, and into forecasts of the response of springtail biodiversity to environmental change.

**2. Materials and methods**

*2.1 Collection and stock maintenance*

Springtails were extracted (using a Berlese-Tullgren funnel system (Southwood and Henderson 2009)) from soil and leaf litter samples collected from within 10 m of 10 sites within the Jock Marshall Reserve, Monash University Clayton Campus in Victoria Australia (S37.9096°, E145.1400°) (Fig. A.1). Twenty 1 L samples were collected every two weeks during the spring and early summer months (September to December) of 2016 to 2019, as springtails are most abundant at this time and allow colonies with a high genetic diversity to be maintained. The Jock Marshall Reserve comprises open woodlands with an understory of grasses and herbs. Mean annual precipitation is approximately 705 mm, with a mean summer (December to February) maximum of 25.3°C, and a mean winter (June to August) minimum of 6.6°C (1971-2019 data, [www.bom.gov.au](http://www.bom.gov.au)).

Springtail species were identified using keys (e.g. **Fjellberg 1998; Greenslade et al. 2014)** and via DNA barcoding (using the mitochondrial cytochrome oxidase subunit I gene) following previous approaches (described in full in Janion-Scheepers et al. 2018). Single species cultures (F0) were established for 10 springtail species belonging to the families Isotomidae, Entomobryidae and Onychiuridae. Seven species belonged to the Isotomidae: *Folsomia* sp.*; Cryptopygus* sp.; *Mucrosomia caeca* (Wahlgren 1906); *Parisotoma notabilis* (Schäffer 1896); *Desoria trispinata* (Mac Gillivray 1896); *Hemisotoma* *thermophila* (Axelson 1900); and *Isotopenola loftyensis* (Womersley 1934). Of the remaining three species *Sinella* sp. and *Lepidocyrtus* sp. belong to the family Entomobryidae and *Orthonychiurus* cf. *folsomi* to the Onychiuridae. Ten springtail species were used in this study to determine if variation in the response of thermal tolerance traits to constant and fluctuating temperatures is consistent across springtails as a group, at least in an initial assessment. Assessment of the influence of phylogeny was not undertaken because of previous indications of minimal phylogenetic signal in springtail thermal tolerance trait data (Janion-Scheepers et al. 2018; Liu et al. 2020).

Cultures were maintained in plastic vials (70 ml) containing a Plaster-of-Paris: charcoal mixture (9:1) that was kept moist to avoid desiccation. Cultures were maintained in a controlled-temperature room at 20°C (verified temperature via Thermochron iButtons™ **(**model DS1920G, **Maxim Integrated, San Jose, CA, USA)** as 20.3±0.4°C), provided a standard *ad libitum* diet of plane tree bark (*Plantanus* sp.) (see Hoskins et al. 2015; Janion-Scheepers et al. 2018), and maintained on a 12h light:12h dark photoperiod. We use this photoperiod because it is a reasonable average for the spring to early summer period when the majority of the springtail collections were made, and because it is typical of experiments on thermal tolerance in springtails (e.g. Janion-Scheepers et al. 2018), enabling us to compare outcomes with previous research. Eggs were collected from cultures three times per week and assigned to pots at a density of 50-100 eggs. At maturity, first-generation (F1) springtails were combined randomly to reduce inbreeding effects and maintained as above until adult second-generation (F2) springtails were achieved. F2 adults were used in all experiments (as in Janion-Scheepers et al. 2018) to reduce any extant environmental or parental effects and to mitigate lab adaptation, and were used within four weeks of maturity (the first egg laying event) to avoid age differences (see Hoffmann and Sgrò 2018 for discussion).

*2.2 Experimental thermal environment*

Soil temperature data was collected from the Jock Marshall Reserve between December 2015 and February 2016 (the hottest months) using six Thermochron iButtons™ (**Maxim Integrated, San Jose, CA, USA**) placed level with soil surface below the leaf litter and humus layers ( see Fig A.1 for locations). Temperature data was recorded every half hour and mean field thermal variation and mean temperature were used to determine experimental acclimation temperatures because of differences between macroclimates and microclimates (see discussion in Woods et al. 2015). Five constant temperature (10°C, 15°C, 20°C, 25°C, 30°C) and five fluctuating temperature (10±5°C, 15±5°C, 20±5°C, 25±5°C, 30±5°C) acclimations were used to assess critical thermal limits and upper lethal limits based on the soil temperatures (Table A.1). Thus, constant temperature (Tconst) acclimations were selected as two temperatures below and two temperatures above the rearing temperature (the temperature closest to mean summer soil temperature (18.7°C, Dec 2015 to Feb 2016). Fluctuating temperature (Tfluct) acclimations varied ±5°C around the constant temperatures, consistent with the maximum daily variation documented in the field. Fluctuations followed diel thermal conditions observed in the field (as outlined above) with the highest temperature at 13h00 and the lowest at 06h00. Temperature increased from the lowest to the highest fluctuation over seven hours in increments of ~0.7°C every half an hour. Temperature decreased from the highest to the lowest fluctuation over seventeen hours at a rate of ~0.3°C every half hour. All acclimations were undertaken in controlled temperature incubators (MIR-154, SANYO, Japan, for constant temperature acclimations, and KB 115 (E3.1) Binder cooling incubator, Tuttlingen, Germany for fluctuating temperature acclimations) and were monitored using Thermochron iButtons™ **(Maxim Integrated, San Jose, CA, USA**), under 12h light:12h dark photoperiod. Between 25 and 35 springtails were acclimated for each species, acclimation temperature and treatment. During acclimation, springtails were kept in 70 ml plastic vials containing a Plaster-of-Paris: charcoal mixture (9:1) kept moist to avoid desiccation, and provided a standard *ad libitum* diet of plane tree bark (*Plantanus* sp.). Springtail adults were acclimated for seven days (see also Janion-Scheepers et al. 2018). This acclimation time was chosen because previous work on arthropods has demonstrated that seven days typically enables the development of a full short-term acclimation response (Hoffmann and Watson 1993; Weldon et al. 2011). Springtails were typically removed from acclimation for use in experiments between 05h00 and 07h00, the point at which the fluctuating temperatures were the lowest. *Mucrosomia caeca*, *Parisotoma notabilis*, *Desoria trispinata, Cryptopygus* sp.*, Orthonychiurus* cf. *folsomi*, and *Lepidocyrtus* sp. did not survive acclimation under the 30°C fluctuating temperature regime. Thus, critical thermal limits were not compared between constant and fluctuating acclimations with a mean of 30°C for these species. Likewise, upper lethal limits were not compared between constant and fluctuating acclimations at 30°C for any species, due to low or no replication numbers at this temperature.

*2.3 Critical thermal limits*

To assess critical thermal limits, springtails were placed into a custom-built, hollow metal stage (Monash University Instrument Facility, Clayton Campus, VIC, Australia), fitted with a covered 40 ml plastic vial containing a Plaster-of-Paris substrate that was moistened to prevent desiccation of the animals (following Janion-Scheepers et al. 2018). The stage was attached to a programmable water bath (Grant Instruments TFX200, Cambridge, UK) and the temperature was raised (*CTmax*) or lowered (*CTmin*) at a rate of 0.05°C per minute by running heated or cooled liquid (50:50 water/propylene glycol mix) through the stage. This ramping rate was chosen because it is among recorded rates of environmental (soil) temperature change for temperate sites (Allen et al. 2016). Ramping was initiated at the rearing temperature (20°C), which was maintained for 15 minutes prior to initiation to avoid any influence of start temperature on the results (Terblanche et al. 2007). During ramping, springtails were monitored every half hour until behavioural changes were observed (either moving faster for *CTmax* or slower for *CTmin*). At this point springtails were checked for righting ability every 10 minutes or increase of 0.5°C until righting ability was lost. This measure was defined as the minimum (*CTmin*) or maximum (*CTmax*) temperature at which springtails lost co-ordinated muscle function – that is, when they were no longer able to right themselves when tipped onto their side or back with a paintbrush (as in Janion-Scheepers et al. 2018). Lack of righting response in the springtails was measured after ~30 seconds, but was re-checked at the next test temperature (i.e. 10 minute interval or increase of 0.5°C) prior to righting response being tested at that temperature. Although for *CTmax* there is almost no recovery once righting response is lost, for *CTmin* righting response is often slower and springtails may recover in the time between one test temperature and another. Temperature of the vials was measured with thermocouples (type K) connected to a temperature data logger (RDXL 12SD, Omega Engineering, USA). Typically, critical thermal limits were measured for 40 to 60 individual springtails (replicates) for each species and acclimation. Springtails were separated into two trials of 20 to 30 individuals for *CTmin* and *CTmax* measurements. Individuals were only used once in either a *CTmin* or a *CTmax* trial. Trials were often run concurrently (up to eight trials). However, as the availability of F2 adults across all species was not consistent (some species reproduced faster and were more fecund than others) trials were run whenever sufficient adult springtails (25 to 35) were available to place into an acclimation.

*2.4 Upper lethal temperature*

Mortality assays were used to determine how constant and fluctuating acclimation temperatures affected the response of ULT50  – the point at which 50% mortality occurs. To obtain ULT50 values, % mortality was measured for a range of experimental temperatures to obtain mortality measures between 0% and 100%, and then these values were used to calculate the ULT50. The number of experimental temperatures required to obtain ULT50 varied between species due to differences in thermal sensitivity (i.e. species differed in the temperature range required to observe mortality between 0 and 100%). Overall, three to five experimental temperatures occurring between 32°C and 44°C were used (see Table A.2 for species specific temperatures). The range of experimental temperatures used for each species started at the temperature at which no mortality occurred and increased in 2°C increments until 100% mortality was observed. For *M. caeca*, for example, four experimental temperatures were used: the starting temperature (where 0% mortality occurred) was 32°C, and then two additional temperatures (34 and 36°C) were examined before the final temperature of 38°C (at which 100% mortality was observed). Each run of experimental temperatures was repeated three times for each combination of experimental temperature, acclimation and species.

Mortality assays were undertaken by placing adult F2 springtails into glass McCartney vials (28 ml) with a moistened Plaster-of-Paris substrate. Each replicate at each temperature included 20-30 individuals per glass vial, with the three glass vials representing the three replicates, leading to n = 3 per experimental temperature, and % mortality calculated using the 20-30 adults in each vial. Vials were submerged in a water bath (Grant Instruments TFX200, Cambridge, UK), allowed to reach experimental temperature (~5 minutes), maintained at the experimental temperature for one hour (Slabber et al. 2007), then removed and allowed to recover for 24 hours at 20°C after which mortality was documented. Mortality was considered as springtails that did not move or those without coordinated movement when stimulated with a paintbrush and was assessed separately for each replicate. A thermocouple (type K) attached to a temperature data logger (Omega Engineering, USA) was used to obtain an accurate measure of the temperature inside the vials placed in the water bath, which did not vary more than ±0.2°C.

Three ULT50 values were produced per acclimation temperature and species using the % mortality measurements obtained from the mortality assays. One replicate of the three from each experimental temperature run was used at a time to produce a ULT50. For example, for *M. caeca*, replicate one from each temperature from the experimental temperature range of 32, 34, 36, 38°C was used to produce the first ULT50 value, replicate two was used to produce the second ULT50 value, and replicate three the third ULT50 value.

*2.5 Statistical analysis*

Generalised linear models were fitted to replicates (i.e. individual) measures of critical thermal limits to assess the effect of acclimation temperature and the form of the acclimation treatment (constant vs. fluctuating) on both *CTmin* and *CTmax*. The slope of the relationship for the constant treatment was used as an estimate of the Acclimation Response Ratio in the form of °C change in critical thermal limit per °C change in acclimation temperature (see Gunderson and Stillman 2015). Estimation statistics and estimation plots (Cumming 2014; Ho et al. 2019), were then used to examine more closely the extent to which thermal limits varied significantly between the constant and fluctuating temperature treatments. Estimation statistics use effect sizes and confidence intervals to determine the magnitude of difference between variables. Estimation statistics are used alongside more typical statistical methods, as effect sizes can infer whether or not differences in thermal tolerance traits measured under constant and fluctuating temperatures are ecologically meaningful, in that the difference between these two temperature regimes may be significant but not meaningful if the effect size is very small.

Generalised linear models were fitted to ULT50 values to assess the effect of acclimation temperature and the form of the acclimation treatment (constant vs. fluctuating) on ULT50. As above, the slope of the relationship for the constant treatment was used as an estimate of the Acclimation Response Ratio in the form of °C change in ULT50 per °C change in acclimation temperature.

All statistical analyses were implemented in R v. 3.5.2 (R Core Team 2018), using RStudio release 1.2.1335-1 (RStudio Team 2016). All table-wide P-values were corrected for multiple comparisons using a Benjamini-Hochberg adjustment. Estimation plots (sometimes known as Cumming plots) were generated using the “dabestr” v0.2.2 package (Ho et al. 2019). Estimation plots show both the raw data and the estimated difference between the means of two variables, in this case the difference in thermal tolerance values for constant and fluctuating temperatures. ULT50 was calculated using a logit analysis in the package “ecotox” v1.3.3.

**3. Results**

*3.1 How do acclimations affect thermal traits?*

On average across species, springtails responded to constant temperature acclimations between 10°C and 30°C by increasing thermal tolerance as acclimation temperature increased (Fig 1; Table 1). This response was much greater in *CTmin* in comparison to *CTmax* and ULT50. *CTmin* was significantly influenced by acclimation temperature in all ten species investigated, while increasing acclimations affected *CTmax* in seven species and ULT50 in four species (Table 2). The mean Acclimation Response Ratio for *CTmin* was 0.123°C/°C (range 0.041 to 0.208°C/°C), whilst the influence of short-term acclimation on *CTmax* was much less pronounced, with a mean Acclimation Response Ratio of 0.017°C/°C (range -0.015 to 0.047°C/°C) (Table 2; Table A.3). For ULT50, acclimation treatments had slightly more effect than for *CTmax*, with a mean Acclimation Response Ratio of 0.049°C/°C and range from -0.007 to 0.135°C/°C (Table 2; Table A.3).

*3.2 Is there a difference between constant and fluctuating temperature conditions?*

The results of this study demonstrate that overall, the measurements of thermal tolerance traits under constant and fluctuating temperature conditions do not differ. Despite a significant effect of fluctuating temperature acclimations on the response of *CTmin* in seven species, *CTmax* in 8 species and ULT50 in 3 species (Table 2), responses to fluctuating temperature acclimations were overall small and inconsistent in direction, or not significantly different to responses to constant temperature acclimations. Furthermore, there was no consistent effect of fluctuations at low or high temperatures for any trait ­– that is, fluctuations did not lower thermal tolerance at high temperatures and raise thermal tolerance at low temperatures, as expected in comparison to constant temperatures. Instead, in comparison to constant temperatures, fluctuating temperatures produced four outcomes in thermal tolerance: a decrease at high temperatures and an increase at low temperatures (where “Treatment (Tfluct)” is negative and “Slope” is positive in the GLM); an increase at high temperatures and a decrease at low temperatures (where “Treatment (Tfluct)” is positive and “Slope” is negative in the GLM); an overall increase (where “Treatment (Tfluct) is positive and “Slope” is non-significant in the GLM); or no effect at all (Table 2; Table A.3).

Estimation statistics used to identify differences in *CTmin* and *CTmax* between fluctuating and constant temperatures for each species, also revealed that effect sizes for those differences were small, and in many instances their 95% confidence intervals overlapped with zero, indicating no effect of acclimation treatment type on critical thermal limits (Table 3). For *CTmin*, the mean estimated absolute difference between the fluctuating and constant treatments was 0.5°C (range: 0.02 to 1.63°C), with 16 of the 44 effect sizes being equivalent to zero and the remainder of the effect sizes being inconsistently different to zero in a positive or negative direction. For *CTmax*, 13 of the 44 effect sizes were no different from zero, and the remainder had a mean and range of differences similar to those for *CTmin* (mean: 0.5°C; range: 0.01 to 1.6°C), with values also being inconsistently different to zero in either direction. The estimation plots illustrate these small and inconsistent differences clearly, whether the data are considered on a per species or per acclimation treatment basis (Figs. 2, 3, Table A.4; Table A.5).

Fluctuating versus constant temperatures typically also had little influence on ULT50, (Fig. 1C; Table 2). Estimation plots showing the effect of fluctuating temperatures on ULT50 across acclimations (Fig. 3C; Table A.5), showed small mean differences between fluctuating and constant temperatures with the 95% confidence intervals of all acclimations overlapping with zero, indicating overall fluctuating temperatures did not elicit a different response in ULT50 than that of constant temperature acclimations.

**4. Discussion**

On average, the sizes of the responses of *CTmin*, *CTmax* and ULT50 to the constant temperature acclimation treatments were in keeping with other investigations of the effects of adult (non-developmental) acclimation in springtails (Slabber et al. 2007; Everatt et al. 2013; Allen et al. 2016; Janion-Scheepers et al. 2018; Jensen et al. 2019). Effect sizes were typically much larger (by nearly 10x on average) for *CTmin* than for *CTmax*, and small for ULT50. In some species, effects for *CTmax* were as large as or larger than those found for *CTmin*, depending on the experimental conditions. Relatively small effects of altered thermal conditions have also been found over longer-term treatments (such as those of laboratory selection) in springtails (Janion-Scheepers et al. 2018). These differing responses of adult acclimation at the upper and lower ends of the thermal performance curve are similar to those found for insects, although exceptions (as was the case here) have been found in this group too (e.g. Kristensen et al. 2008; Overgaard et al. 2011; Hoffmann et al. 2013; Kellermann et al. 2017; Oyen and Dillon 2018). Just why such large interspecific differences in especially *CTmax* can be found among springtails, when responses to acclimation and laboratory selection are constrained, thus remains unexplained (Janion-Scheepers et al. 2018). One explanation could be phylogenetic relatedness, in that the *CTmax* of closely related species should be more similar than those that are distantly related. The low number of species in this study make phylogenetic analysis difficult. However, a study by Janion-Scheepers and colleagues (2018) on critical thermal limits in springtails indicates phylogenetic relatedness has little impact on *CTmax* values. Thus, the variation observed in *CTmax* for this study is unlikely to be related to phylogeny (see also Liu et al. 2020). Another explanation may be that constant versus fluctuating temperatures, at different mean temperatures, have very different influences on ectotherms both in the field and in laboratory assessments (Carrington et al. 2013a; Kjaersgaard et al. 2013; Colinet et al. 2015; Kingsolver and Buckley 2017).

Several studies have shown that short term changes in thermal tolerance may differ in invertebrates when exposure is to fluctuating rather than constant temperatures (Bahrndorff et al. 2009; Fischer and Karl 2010; Terblanche et al. 2010; Fischer et al. 2011; Sobek-Swant et al. 2012; Paaijmans et al. 2013; Manenti et al. 2014; Torson et al. 2019; see also Sgrò et al. 2016). These works have often found that exposure to fluctuations proves beneficial to thermal tolerance under temperature conditions that do not induce stress (for a review see Colinet et al. 2015). The opposite may be true under extreme conditions, especially high temperatures. Thus, fluctuations at low or intermediate temperatures should be potentially beneficial, in the sense of improving thermal tolerance, with those at high temperatures proving detrimental, and in particular because of the asymmetric nature of thermal performance curves (Huey et al. 2012; Colinet et al. 2015; Denny 2017).

By contrast with these findings, we found very limited effects of constant versus fluctuating temperatures on *CTmin*, *CTmax* and ULT50. Even in the four species which survived the 30°C fluctuating temperature treatment, the influences on *CTmax* were variable, with fluctuating temperatures having either no significant effect (*H. thermophila*), a decline in *CTmax* (*I. loftyensis*) as theory predicts and other studies have found, or conversely an increase in *CTmax* (*Cryptopygus* sp.; *Sinella* sp.). Such outcomes may not be especially surprising for *CTmax* and ULT50 where, generally, trait variation with acclimation is relatively limited (e.g. Terblanche et al. 2010; Allen et al. 2016; Kellermann et al. 2017), as was the case here. By contrast, even in the case of the relatively responsive *CTmin* (Allen et al. 2016; Janion-Scheepers et al. 2018) the influence of constant versus fluctuating temperatures was relatively small on average, often having an effect size no different from zero, or effects that were inconsistent. The largest absolute effect of fluctuating versus constant temperature treatments on *CTmin* was 1.63°C, compared with the largest acclimation effect of 4.2°C for *CTmin* overall.

The relatively limited effects found here may be a consequence of the fact that we examined adult acclimation treatments rather than assessing developmental plasticity, where effects may have been more pronounced. Certainly, developmental plasticity responses can often be much more pronounced than those of adult acclimation (see e.g. Terblanche and Chown 2006; Kellermann et al. 2017), although this is not always the case for thermal tolerance traits (Zeilstra and Fischer 2005; Slotsbo et al. 2016). Nonetheless, what the case is for springtails is not well understood. A longer-term exposure to constant versus fluctuating temperatures may well have a more pronounced effect given expectations from theory (Huey et al. 2012; Denny 2017). Alternatively, the most pronounced effects may come from occasional extreme temperatures, which are only now starting to be investigated (Kingsolver and Buckley 2017; Liu et al. 2020). In particular, the level of exposure to extreme events, i.e. one prolonged event or many short events, may be important to thermal tolerances (e.g. Ghaedi and Andrew 2016). Perhaps a further explanation may be sought in the duration of the acclimation treatment, which lasted for seven days. Typically, such an exposure is more than sufficient to result in a full response to the treatment temperature in a range of insects (Weldon et al. 2011; Kellermann et al. 2017). However, thermal acclimation response in a wide range of springtail species is yet to be examined.

Irrespective of the reasons for the outcomes found here, what they demonstrate is that for a variety of Collembola species (from three families), acclimation to fluctuating temperatures that reflect field conditions have little effect on the estimates of adult (non-developmental) phenotypic plasticity in critical thermal limits and ULT50 relative to acclimation to constant temperature conditions. Thus, previous work using constant temperature conditions to assess adult phenotypic plasticity across a range of temperatures can be considered more broadly applicable for the conditions experienced by springtails under natural conditions in the field. Thus, they can also be considered reliable for investigating the likely responses of these organisms to changing environments.

**Acknowledgments**

We thank Ian Aitkenhead for assistance with laboratory equipment, Rebecca Hallas for managing the laboratory in which the work was undertaken, and Mikhail Potapov and Wanda Weiner for taxonomic advice. Four reviewers provided helpful comments on a previous draft of this work.

**Declaration of Competing Interests**

The authors declare they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Funding**

This work was funded by **an Australian Government Research Training Program (RTP) Scholarship and by the** Holsworth Wildlife Research Endowment – Equity Trustees Charitable Foundation & the Ecological Society of Australia, **awarded to JLH,** and by Australian Research Council Discovery Project DP170101046.

**References**

Alemu, T., Alemneh, T., Pertoldi, C., Ambelu, A., and Bahrndorff, S., 2017. Costs and benefits of heat and cold hardening in a soil arthropod. Biol. J. Linn. Soc. 122, 765-773.

Allen, J. L., Chown, S. L., Janion-Scheepers, C., and Clusella-Trullas, S., 2016. Interactions between rates of temperature change and acclimation affect latitudinal patterns of warming tolerance. Conserv. Physiol. 4, cow053.

Angilletta Jr, M. J., and Angilletta, M. J., 2009. Thermal adaptation: a theoretical and empirical synthesis. UK, Oxford. Oxford University Press.

Angilletta Jr, M. J., Bennett, A. F., Guderley, H., Navas, C. A., Seebacher, F., and Wilson, R. S., 2006. Coadaptation: a unifying principle in evolutionary thermal biology. Physiol. Biochem. Zool. 79, 282-294.

Angilletta Jr, M. J., Steury, T. D., and Sears, M. W., 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. Integr. Comp. Biol. 44, 498-509.

Bahrndorff, S., Holmstrup, M., Petersen, H., and Loeschcke V. 2006.Geographic variation for climatic stress resistance traits in the springtail *Orchesella cincta*. Journal of Insect Physiology 52, 951–959.

Bahrndorff, S., Mariën, J., Loeschcke, V., and Ellers, J., 2009. Dynamics of heat‐induced thermal stress resistance and hsp70 expression in the springtail, *Orchesella cincta*. Funct. Ecol. 23, 233-239.

Bardgett, R. D., and Van Der Putten, W. H., 2014. Belowground biodiversity and ecosystem functioning. Nature. 515, 505-511.

Behrens, W., Hoffmann, K.-H., Kempa, S., Gäßler, S., and Merkel-Wallner, G., 1983. Effects of diurnal thermoperiods and quickly oscillating temperatures on the development and reproduction of crickets, *Gryllus bimaculatus*. Oecologia. 59, 279-287.

Bokhorst, S., Phoenix, G., Bjerke, J., Callaghan, T., Huyer‐Brugman, F., and Berg, M., 2012. Extreme winter warming events more negatively impact small rather than large soil fauna: shift in community composition explained by traits not taxa. Glob. Change Biol. 18, 1152-1162.

Bozinovic, F., Bastías, D. A., Boher, F., Clavijo-Baquet, S., Estay, S. A., and Angilletta Jr, M. J., 2011. The mean and variance of environmental temperature interact to determine physiological tolerance and fitness. Physiol. Biochem. Zool. 84, 543-552.

Brakefield, P. M., and Kesbeke, F., 1997. Genotype–environment interactions for insect growth in constant and fluctuating temperature regimes. Proc. R. Soc. Lond. B. 264, 717-723.

Carrington, L. B., Armijos, M. V., Lambrechts, L., Barker, C. M., and Scott, T. W., 2013a. Effects of fluctuating daily temperatures at critical thermal extremes on *Aedes aegypti* life-history traits. PloS one. 8, e58824.

Carrington, L. B., Seifert, S. N., Willits, N. H., Lambrechts, L., and Scott, T. W., 2013b. Large diurnal temperature fluctuations negatively influence *Aedes aegypti* (Diptera: Culicidae) life-history traits. J. Med. Entomol. 50, 43-51.

Chown, S. L., Duffy, G. A., and Sørensen, J. G., 2015. Upper thermal tolerance in aquatic insects. Curr. Opin. Insect Sci. 11, 78-83.

Clusella-Trullas, S., Blackburn, T. M., and Chown, S. L., 2011. Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. Am. Nat. 177, 738-751.

Colinet, H., Sinclair, B. J., Vernon, P., and Renault, D., 2015. Insects in fluctuating thermal environments. Annu. Rev. Entomol. 60,

Cumming, G., 2014. The new statistics: Why and how. Psychol. Sci. 25, 7-29.

Denny, M., 2017. The fallacy of the average: on the ubiquity, utility and continuing novelty of Jensen's inequality. J. Exp. Biol. 220, 139-146.

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., and Martin, P. R., 2008. Impacts of climate warming on terrestrial ectotherms across latitude. PNAS. 105, 6668-6672.

Deutsch, C. A., Tewksbury, J. J., Tigchelaar, M., Battisti, D. S., Merrill, S. C., Huey, R. B., and Naylor, R. L., 2018. Increase in crop losses to insect pests in a warming climate. Science. 361, 916-919.

Diamond, S. E., Chick, L. D., Perez, A., Strickler, S. A., and Martin, R. A., 2018. Evolution of thermal tolerance and its fitness consequences: parallel and non-parallel responses to urban heat islands across three cities. Proc. R. Soc. Lond. B. 285, 20180036.

Diamond, S. E., Sorger, D. M., Hulcr, J., Pelini, S. L., Toro, I. D., Hirsch, C., Oberg, E., and Dunn, R. R., 2012. Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. Glob. Change Biol. 18, 448-456.

Dillon, M. E., Wang, G., and Huey, R. B., 2010. Global metabolic impacts of recent climate warming. Nature. 467, 704.

Donelson, J. M., Sunday, J. M., Figueira, W. F., Gaitán-Espitia, J. D., Hobday, A. J., Johnson, C. R., Leis, J. M., Ling, S. D., Marshall, D., and Pandolfi, J. M., 2019. Understanding interactions between plasticity, adaptation and range shifts in response to marine environmental change. Philos. Trans. R. Soc. B. 374, 20180186.

Dowd, W. W., King, F. A., and Denny, M. W., 2015. Thermal variation, thermal extremes and the physiological performance of individuals. J. Exp. Biol. 218, 1956-1967.

Ellers, J., Berg, M. P., Dias, A. T., Fontana, S., Ooms, A., and Moretti, M., 2018. Diversity in form and function: Vertical distribution of soil fauna mediates multidimensional trait variation. J. Anim. Ecol. 87, 933-944.

Everatt, M., Bale, J., Convey, P., Worland, M., and Hayward, S., 2013. The effect of acclimation temperature on thermal activity thresholds in polar terrestrial invertebrates. J. Insect Physiol. 59, 1057-1064.

Fischer, K., and Karl, I., 2010. Exploring plastic and genetic responses to temperature variation using copper butterflies. Clim. Res. 43, 17-30.

Fischer, K., Kölzow, N., Höltje, H., and Karl, I., 2011. Assay conditions in laboratory experiments: is the use of constant rather than fluctuating temperatures justified when investigating temperature-induced plasticity? Oecologia. 166, 23-33.

Fjellberg, A., 1998. The Collembola of Fennoscandia and Denmark. Part 1: Poduromorpha fauna. Entomologica Scandinavica. Brill Academic, Leiden.

Ghaedi, B., and Andrew, N. R. 2016. The physiological consequences of varied heat exposure events in adult *Myzus persicae*: a single prolonged exposure compared to repeated shorter exposures. PeerJ. 4, e2290.

Gilchrist, G. W., 1995. Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. Am. Nat. 146, 252-270.

Greenslade, P., Ireson, J., and Skarżyński, D. 2014. Biology and key to the Australian species of *Hypogastrura* and *Ceratophysella* (Collembola: Hypogastruridae). Austral Entomol. 53: 53–74.

Gunderson, A. R., and Stillman, J. H., 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc. R. Soc. Lond. B. 282, 20150401.

Ho, J., Tumkaya, T., Aryal, S., Choi, H., and Claridge-Chang, A., 2019. Moving beyond P values: Everyday data analysis with estimation plots. BioRxiv. 377978.

Hoffmann, A. A., Chown, S. L., and Clusella‐Trullas, S., 2013. Upper thermal limits in terrestrial ectotherms: how constrained are they? Funct. Ecol. 27, 934-949.

Hoffmann, A. A., and Sgrò, C. M., 2018. Comparative studies of critical physiological limits and vulnerability to environmental extremes in small ectotherms: How much environmental control is needed? Integr. Zool. 13, 355-371.

Hoffmann, A. A., and Watson, M., 1993. Geographical variation in the acclimation responses of Drosophila to temperature extremes. Am. Nat. 142, S93-S113.

Holmstrup, M., Ehlers, B. K., Slotsbo, S., Ilieva‐Makulec, K., Sigurdsson, B. D., Leblans, N. I., Ellers, J., and Berg, M. P., 2018. Functional diversity of Collembola is reduced in soils subjected to short‐term, but not long‐term, geothermal warming. Funct. Ecol. 32, 1304-1316.

Hopkin, S. P. 1997. Biology of the springtails (Insecta: Collembola). Oxford University Press, Oxford, UK.

Hoskins, J. L., Janion-Scheepers, C., Chown, S. L., and Duffy, G. A., 2015. Growth and reproduction of laboratory-reared neanurid Collembola using a novel slime mould diet. Sci. Rep. 5, 11957.

Huey, R. B., Deutsch, C. A., Tewksbury, J. J., Vitt, L. J., Hertz, P. E., Álvarez Pérez, H. J., and Garland Jr, T., 2009. Why tropical forest lizards are vulnerable to climate warming. Proc. R. Soc. Lond. B. 276, 1939-1948.

Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A., Jess, M., and Williams, S. E., 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. Philos. Trans. R. Soc. B. 367, 1665-1679.

Janion-Scheepers, C., Phillips, L., Sgrò, C. M., Duffy, G. A., Hallas, R., and Chown, S. L., 2018. Basal resistance enhances warming tolerance of alien over indigenous species across latitude. PNAS. 115, 145-150.

Jensen, A., Alemu, T., Alemneh, T., Pertoldi, C., and Bahrndorff, S., 2019. Thermal acclimation and adaptation across populations in a broadly distributed soil arthropod. Funct. Ecol. 33, 833-845.

Kearney, M., and Porter, W., 2009. Mechanistic niche modelling: combining physiological and spatial data to predict species’ ranges. Ecol. Lett. 12, 334-350.

Kellermann, V., van Heerwaarden, B., and Sgrò, C. M., 2017. How important is thermal history? Evidence for lasting effects of developmental temperature on upper thermal limits in Drosophila melanogaster. Proc. R. Soc. Lond. B. 284, 20170447.

Kingsolver, J. G., and Buckley, L. B., 2017. Quantifying thermal extremes and biological variation to predict evolutionary responses to changing climate. Philos. Trans. R. Soc. B. 372, 20160147.

Kjærsgaard, A., Pertoldi, C., Loeschcke, V., and Blanckenhorn, W. U., 2013. The effect of fluctuating temperatures during development on fitness-related traits of *Scatophaga stercoraria* (Diptera: Scathophagidae). Environ. Entomol. 42, 1069-1078.

Kovacevic, A., Latombe, G., and Chown, S. L., 2019. Rate dynamics of ectotherm responses to thermal stress. Proc. R. Soc. Lond. B. 286, 20190174.

Kristensen, T. N., Hoffmann, A. A., Overgaard, J., Sørensen, J. G., Hallas, R., and Loeschcke, V., 2008. Costs and benefits of cold acclimation in field-released Drosophila. PNAS. 105, 216-221.

Lawson, C. R., Vindenes, Y., Bailey, L., and van de Pol, M., 2015. Environmental variation and population responses to global change. Ecol. Lett. 18, 724-736.

Liefting M, and Ellers J. 2008.Habitat-specific differences in thermal plasticity in natural populations of a soil arthropod. Biological Journal of the Linnean Society 94,265–271.

Liu, W.P.A., Phillips, L.M., Terblanche, J.S., Janion-Scheepers, C., and Chown, S.L. 2020.Strangers in a strange land: Globally unusual thermal tolerance in Collembola from the Cape Floristic Region. Funct. Ecol. Doi: 10.1111/1365-2435.13584

Ma, G., Rudolf, V. H., and Ma, C. s., 2015. Extreme temperature events alter demographic rates, relative fitness, and community structure. Glob. Change Biol. 21, 1794-1808.

Manenti, T., Sørensen, J. G., Moghadam, N. N., and Loeschcke, V., 2014. Predictability rather than amplitude of temperature fluctuations determines stress resistance in a natural population of Drosophila simulans. J. Evol. Biol. 27, 2113-2122.

Miller, J. E., Damschen, E. I., and Ives, A. R., 2019. Functional traits and community composition: A comparison among community‐weighted means, weighted correlations, and multilevel models. Methods Ecol. Evol. 10, 415-425.

Mitchell, K. A., and Hoffmann, A. A., 2010. Thermal ramping rate influences evolutionary potential and species differences for upper thermal limits in *Drosophila*. Funct. Ecol. 24, 694-700.

Morash, A. J., Neufeld, C., MacCormack, T. J., and Currie, S., 2018. The importance of incorporating natural thermal variation when evaluating physiological performance in wild species. J. Exp. Biol. 221, jeb164673.

Moretti, M., Dias, A. T., De Bello, F., Altermatt, F., Chown, S. L., Azcárate, F. M., Bell, J. R., Fournier, B., Hedde, M., and Hortal, J., 2017. Handbook of protocols for standardized measurement of terrestrial invertebrate functional traits. Funct. Ecol. 31, 558-567.

Niehaus, A. C., Angilletta, M. J., Sears, M. W., Franklin, C. E., and Wilson, R. S., 2012. Predicting the physiological performance of ectotherms in fluctuating thermal environments. J. Exp. Biol. 215, 694-701.

Overgaard, J., Kristensen, T. N., Mitchell, K. A., and Hoffmann, A. A., 2011. Thermal tolerance in widespread and tropical *Drosophila* species: does phenotypic plasticity increase with latitude? Am. Nat. 178, S80-S96.

Oyen, K. J., and Dillon, M. E., 2018. Critical thermal limits of bumblebees (*Bombus impatiens*) are marked by stereotypical behaviors and are unchanged by acclimation, age or feeding status. J. Exp. Biol. 221, jeb165589.

Paaijmans, K. P., Heinig, R. L., Seliga, R. A., Blanford, J. I., Blanford, S., Murdock, C. C., and Thomas, M. B., 2013. Temperature variation makes ectotherms more sensitive to climate change. Glob. Change Biol. 19, 2373-2380.

Phillips, L.M., Aitkenhead, I., Janion-Scheepers, C., King, C.K., McGeoch, M.A., Nielsen, U.N., Terauds, A. Liu, W.P.A., and Chown, S.L. 2020. Basal tolerance but not plasticity gives invasive springtails the advantage in an assemblage setting. Conservation Physiology 8, coaa049.

Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L., and Sunday, J. M., 2019. Greater vulnerability to warming of marine versus terrestrial ectotherms. Nature. 569, 108-111.

R Core Team**,** 2018. R: A language and environment for statistical computing*.* R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ragland, G. J., and Kingsolver, J. G., 2008. The effect of fluctuating temperatures on ectotherm life-history traits: comparisons among geographic populations of *Wyeomyia smithii*. Evol. Ecol. Res. 10, 29-44.

RStudio Team,2016. Rstudio: Integrated Development for R. RStudio, Inc., Boston, MA. URL http://www.rstudio.com/.

Ruel, J. J., and Ayres, M. P., 1999. Jensen’s inequality predicts effects of environmental variation. Trends Ecol. Evol. 14, 361-366.

Rusek, J., 1998. Biodiversity of Collembola and their functional role in the ecosystem. Biodivers. Conserv. 7, 1207-1219.

Salachan, P. V., Burgaud, H., and Sørensen, J. G., 2019. Testing the thermal limits: Non-linear reaction norms drive disparate thermal acclimation responses in Drosophila melanogaster. J. Insect Physiol. 118, 103946.

Scheiner, S. M., Barfield, M., and Holt, R. D., 2020. The genetics of phenotypic plasticity. XVII. Response to climate change. Evol. Appl. 13, 388-399.

Sgrò, C. M., Terblanche, J. S., and Hoffmann, A. A., 2016. What can plasticity contribute to insect responses to climate change? Annu. Rev. Entomol. 61, 433-451.

Sinclair, B. J., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., Slotsbo, S., Dong, Y., Harley, C. D., Marshall, D. J., and Helmuth, B. S., 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? Ecol. Lett. 19, 1372-1385.

Sinclair, B. J., Vernon, P., Klok, C. J., and Chown, S. L., 2003. Insects at low temperatures: an ecological perspective. Trends Ecol. Evol. 18, 257-262.

Slabber, S., Worland, M. R., Leinaas, H. P., and Chown, S. L., 2007. Acclimation effects on thermal tolerances of springtails from sub-Antarctic Marion Island: indigenous and invasive species. J. Insect Physiol. 53, 113-125.

Slotsbo, S., Schou, M. F., Kristensen, T. N., Loeschcke, V., and Sørensen, J. G., 2016. Reversibility of developmental heat and cold plasticity is asymmetric and has long-lasting consequences for adult thermal tolerance. J. Exp. Biol. 219, 2726-2732.

Sobek-Swant, S., Crosthwaite, J. C., Lyons, D. B., and Sinclair, B. J., 2012. Could phenotypic plasticity limit an invasive species? Incomplete reversibility of mid-winter deacclimation in emerald ash borer. Biol. Invasions. 14, 115-125.

Sørensen, J. G., Winther, M. L., Salachan, P. V., & MacLean, H. J., 2020. Drawing the line: Linear or non-linear reaction norms in response to adult acclimation on lower thermal limits. J. Insect Physiol. 104075.

Southwood, T. R. E., and Henderson, P. A. 2009. Ecological methods. John Wiley and Sons.

Start, D., McCauley, S., and Gilbert, B., 2018. Physiology underlies the assembly of ecological communities. PNAS. 115, 6016-6021.

Terblanche, J. S., and Chown, S. L., 2006. The relative contributions of developmental plasticity and adult acclimation to physiological variation in the tsetse fly, *Glossina pallidipes* (Diptera, Glossinidae). J. Exp. Biol. 209, 1064-1073.

Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C., and Chown, S. L., 2007. Critical thermal limits depend on methodological context. Proc. R. Soc. Lond. B. 274, 2935-2943.

Terblanche, J. S., Nyamukondiwa, C., and Kleynhans, E., 2010. Thermal variability alters climatic stress resistance and plastic responses in a globally invasive pest, the Mediterranean fruit fly (*Ceratitis capitata*). Entomol. Exp. Appl. 137, 304-315.

Torson, A. S., Yocum, G. D., Rinehart, J. P., Nash, S. A., and Bowsher, J. H., 2019. Fluctuating thermal regimes prevent chill injury but do not change patterns of oxidative stress in the alfalfa leafcutting bee, *Megachile rotundata*. J. Insect Physiol. 118, 103935.

Treasure, A. M., le Roux, P. C., Mashau, M. H., and Chown, S. L., 2019. Species-energy relationships of indigenous and invasive species may arise in different ways–a demonstration using springtails. Sci. Rep. 9, 1-12.

Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M. B., Balaguer, L., Benito‐Garzón, M., Cornwell, W., Gianoli, E., van Kleunen, M., and Naya, D. E., 2014. The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. Ecol. Lett. 17, 1351-1364.

Van Dooremalen, C., Berg, M. P., and Ellers, J., 2013. Acclimation responses to temperature vary with vertical stratification: implications for vulnerability of soil‐dwelling species to extreme temperature events. Glob. Change Biol. 19, 975-984.

Vandewalle, M., de Bello, F., Berg, M. P., Bolger, T., Dolédec, S., Dubs, F., Feld, C. K., Harrington, R., Harrison, P. A., Lavorel, S., da Silva, P. M., Moretti, M., Niemelä, J., Santos, P., Sattler, T., Sousa, J. P., Sykes, M. T., Vanbergen, A. J., and Woodcock, B. A., 2010. Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. Biodivers. Conserv. 19, 2921-2947.

Weldon, C. W., Terblanche, J. S., and Chown, S. L., 2011. Time-course for attainment and reversal of acclimation to constant temperature in two *Ceratitis* species. J. Therm. Biol. 36, 479-485.

Woods, H. A., Dillon, M. E., and Pincebourde, S., 2015. The roles of microclimatic diversity and of behavior in mediating the responses of ectotherms to climate change. J. Therm. Biol. 54, 86-97.

Worner, S. P., 1992. Performance of phenological models under variable temperature regimes: consequences of the Kaufmann or rate summation effect. Environ. Entomol. 21, 689-699.

Zeilstra, I., and Fischer, K., 2005. Cold tolerance in relation to developmental and adult temperature in a butterfly. Physiol. Entomol. 30, 92-95.

**Table 1**

Mean *CTmin*, *CTmax* and ULT50, and its standard deviation for 10 springtail species acclimated to Tconst and Tfluct ranging from 10 to 30°C (±5°C for Tfluct). For ULT50 n = 3 for all means (s.d.).

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Family** | **Species** | **Acclimation (°C)** | ***CTmin*** | | | | ***CTmax*** | | | | **ULT50** | |
|  |  |  | **Tconst** |  | **Tfluct** |  | **Tconst** |  | **Tfluct** |  | **Tconst** | **Tfluct** |
|  |  |  | **mean (s.d.) (°C)** | **n** | **mean (s.d.) (°C)** | **n** | **mean (s.d.) (°C)** | **n** | **mean (s.d.) (°C)** | **n** | **mean (s.d.) (°C)** | **mean (s.d.) (°C)** |
| Entomobryidae | *Lepidocyrtus* sp. | 10 | -1.17 (0.44) | 55 | -1.81 (0.82) | 46 | 36.78 (0.67) | 54 | 36.53 (0.37) | 43 | 35.9 (0.29) | 35.21 (0.9) |
|  |  | 15 | -1.63 (0.41) | 42 | -0.82 (0.37) | 49 | 36.83 (0.34) | 40 | 36.76 (0.56) | 44 | 34.69 (0.56) | 35.31 (0.32) |
|  |  | 20 | -1.3 (0.52) | 51 | -1.12 (0.25) | 42 | 37.34 (0.54) | 44 | 37.21 (0.54) | 44 | 34.88 (0.91) | 35.38 (0.34) |
|  |  | 25 | -0.52 (0.53) | 47 | -0.18 (0.49) | 44 | 36.6 (0.84) | 58 | 37.23 (0.52) | 43 | 35.73 (0.28) | 35.91 (0.51) |
|  |  | 30 | -0.66 (1.02) | 23 |  |  | 36.53 (0.95) | 55 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Sinella* sp. | 10 | -0.55 (0.44) | 45 | -0.03 (0.34) | 52 | 40.52 (0.33) | 45 | 39.4 (0.16) | 48 | 37.21 (0.36) | 37.75 (0.65) |
|  |  | 15 | 0.27 (0.26) | 46 | 0.43 (0.47) | 54 | 39.19 (0.45) | 45 | 40.05 (0.43) | 51 | 37.97 (0.51) | 37.71 (0.62) |
|  |  | 20 | 0.01 (0.25) | 48 | 0.39 (0.42) | 56 | 40.15 (0.43) | 69 | 39.99 (0.35) | 52 | 37.94 (0.18) | 38.01 (0.14) |
|  |  | 25 | 1.16 (0.57) | 40 | 0.88 (0.47) | 46 | 40.33 (0.26) | 47 | 39.77 (0.62) | 49 | 38.24 (0.21) | 38.56 (0.36) |
|  |  | 30 | 1.64 (0.37) | 51 | 2.14 (0.55) | 51 | 39.96 (0.42) | 48 | 40.33 (0.78) | 44 |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| Isotomidae | *Cryptopygus* sp. | 10 | -3.16 (1.17) | 48 | -3.08 (1.62) | 49 | 40.89 (0.7) | 49 | 41.55 (0.72) | 52 | 39.16 (0.8) | 38.11 (0.43) |
|  |  | 15 | -1.92 (0.94) | 48 | -2.56 (0.83) | 49 | 41.41 (0.71) | 48 | 40.87 (0.4) | 51 | 38.51 (0.15) | 39.03 (0.25) |
|  |  | 20 | 0.33 (1.36) | 46 | -1.3 (0.51) | 45 | 41.58 (0.67) | 49 | 41.25 (0.57) | 44 | 39.44 (0.38) | 38.68 (0.69) |
|  |  | 25 | -0.88 (0.54) | 45 | -0.22 (0.75) | 54 | 40.91 (0.69) | 43 | 42.02 (0.45) | 44 | 39.26 (0.19) | 39.33 (0.33) |
|  |  | 30 | -0.32 (0.35) | 50 | 0.4 (0.6) | 47 | 41.65 (0.39) | 55 | 42.8 (0.8) | 43 |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Desoria trispinata* | 10 | -1.86 (1.03) | 45 | -3.04 (0.7) | 43 | 38.02 (0.83) | 46 | 38.49 (0.35) | 49 | 35.66 (0.58) | 35.53 (0.49) |
|  |  | 15 | -3.38 (0.78) | 49 | -3.07 (0.4) | 47 | 38.39 (0.58) | 52 | 37.86 (0.31) | 50 | 35.91 (0.42) | 35.75 (0.12) |
|  |  | 20 | -3.06 (0.41) | 52 | -2.74 (0.61) | 49 | 37.8 (0.26) | 51 | 38.12 (0.32) | 47 | 36.2 (0.4) | 36.09 (0.13) |
|  |  | 25 | -1.71 (0.76) | 43 | -1.82 (0.61) | 48 | 38.14 (0.43) | 58 | 38.47 (0.38) | 46 | 36.2 (0.34) | 36.49 (0.43) |
|  |  | 30 | -0.96 (0.61) | 46 |  |  | 38.27 (1.2) | 44 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Folsomia* sp. | 10 | -2.22 (0.69) | 22 | -2.67 (0.69) | 49 | 38.22 (0.88) | 47 | 38.22 (0.48) | 49 | 35.81 (0.79) | 34.85 (0.42) |
|  |  | 15 | -2.09 (0.51) | 50 | -2.28 (0.7) | 47 | 38.44 (0.39) | 48 | 38.08 (0.4) | 47 | 35.51 (0.28) | 35.74 (0.39) |
|  |  | 20 | -1.77 (0.7) | 50 | -1.74 (0.47) | 48 | 39.06 (0.65) | 56 | 38.84 (0.59) | 49 | 35.69 (0.72) | 35.57 (0.27) |
|  |  | 25 | -0.67 (0.57) | 53 | -1.04 (0.41) | 51 | 38.62 (0.41) | 49 | 38.98 (0.43) | 47 | 35.71 (0.43) | 36.35 (0.38) |
|  |  | 30 | 0.61 (0.66) | 45 |  |  | 38.89 (0.46) | 44 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Hemisotoma thermophila* | 10 | -1.65 (0.75) | 44 | -2.33 (1.01) | 43 | 41.91 (0.79) | 58 | 42.4 (0.58) | 46 | 39.38 (0.73) | 39.25 (0.35) |
|  |  | 15 | -2.06 (0.89) | 54 | -2.18 (0.64) | 48 | 41.9 (1.05) | 40 | 42.47 (0.95) | 40 | 39.17 (0.39) | 39.42 (0.39) |
|  |  | 20 | -0.48 (1.52) | 52 | -1.63 (0.62) | 45 | 42.66 (0.62) | 52 | 42.49 (0.77) | 50 | 39.85 (0.39) | 39.25 (0.45) |
|  |  | 25 | -1.31 (0.72) | 44 | -0.59 (0.9) | 44 | 42.23 (0.74) | 57 | 42.8 (0.81) | 40 | 40.28 (0.58) | 40.59 (0.34) |
|  |  | 30 | 1.3 (0.73) | 47 | 1.51 (1.11) | 43 | 42.97 (0.7) | 50 | 43.07 (0.76) | 56 |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Isotopenola loftyensis* | 10 | -0.52 (0.34) | 50 | 0.1 (0.81) | 43 | 41.31 (0.93) | 47 | 41.09 (1.02) | 54 | 38.28 (0.65) | 38.57 (0.37) |
|  |  | 15 | -0.03 (0.33) | 51 | -0.54 (0.47) | 48 | 41.2 (0.81) | 60 | 41.33 (0.82) | 45 | 38.37 (0.38) | 39 (0.58) |
|  |  | 20 | 0.44 (1.06) | 94 | 0.6 (0.74) | 50 | 40.73 (1.21) | 44 | 41.3 (0.9) | 56 | 39.14 (0.13) | 38.66 (0.33) |
|  |  | 25 | 0.18 (0.47) | 53 | 0.85 (0.79) | 44 | 41.59 (0.58) | 60 | 40.7 (0.89) | 49 | 38.93 (0.48) | 38.83 (0.76) |
|  |  | 30 | 1.85 (1.26) | 44 | 1.81 (0.71) | 47 | 41.61 (0.88) | 78 | 41.07 (0.9) | 42 |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Mucrosomia caeca* | 10 | -0.39 (0.77) | 65 | -0.23 (0.72) | 56 | 36.01 (0.57) | 75 | 35.1 (0.44) | 49 | 34.09 (0.3) | 32.7 (0.42) |
|  |  | 15 | -0.74 (0.65) | 48 | 0.15 (0.67) | 47 | 35.52 (0.36) | 51 | 35.42 (0.61) | 47 | 33.86 (0.13) | 33.2 (0.7) |
|  |  | 20 | -0.24 (0.46) | 45 | -0.07 (0.6) | 57 | 35.09 (0.52) | 51 | 35.52 (0.59) | 57 | 34.05 (0.01) | 34.41 (0.5) |
|  |  | 25 | 1.23 (0.59) | 48 | 0.94 (0.75) | 51 | 36.21 (0.59) | 54 | 36.76 (0.24) | 50 | 35.02 (0.59) | 34.55 (0.56) |
|  |  | 30 | 3.34 (1.13) | 43 |  |  | 35.68 (1.2) | 77 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Parisotoma notabilis* | 10 | -3.2 (0.58) | 47 | -2.48 (1.11) | 44 | 37.07 (0.41) | 49 | 37.48 (0.38) | 43 | 34.63 (0.62) | 34.57 (0.36) |
|  |  | 15 | -2.9 (0.4) | 43 | -1.31 (0.82) | 46 | 36.91 (0.37) | 56 | 37.76 (0.36) | 47 | 34.18 (0.15) | 34.75 (0.41) |
|  |  | 20 | -3.56 (1.02) | 45 | -0.94 (0.69) | 50 | 36.84 (0.3) | 45 | 36.67 (0.64) | 52 | 34.99 (0) | 35.94 (0.07) |
|  |  | 25 | -2.06 (0.75) | 48 | 0.21 (0.47) | 47 | 37.13 (0.37) | 45 | 38.08 (0.64) | 51 | 35.53 (0.01) | 36.09 (0.36) |
|  |  | 30 | -0.7 (0.77) | 44 |  |  | 37.55 (0.65) | 65 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| Onychiuridae | *Orthonychiurus* cf. *folsomi* | 10 | -2.33 (0.87) | 48 | -2.98 (0.49) | 47 | 37.5 (0.41) | 52 | 36.13 (0.43) | 49 | 35.71 (0.26) | 34.45 (0.12) |
|  |  | 15 | -1.76 (1.02) | 53 | -1.68 (0.76) | 46 | 37.63 (0.64) | 47 | 36.93 (0.48) | 41 | 34.99 (0) | 34.33 (0.05) |
|  |  | 20 | -0.38 (1.19) | 43 | -2.26 (0.72) | 42 | 37.22 (0.59) | 111 | 37.33 (0.27) | 42 | 35.93 (0.14) | 35.17 (0.2) |
|  |  | 25 | 0.43 (0.67) | 59 | -0.86 (0.79) | 48 | 38.27 (0.47) | 47 | 37.58 (0.39) | 42 | 36.16 (0.14) | 35.37 (0.23) |
|  |  | 30 | 1.77 (0.63) | 46 |  |  | 38.18 (0.73) | 50 |  |  |  |  |

**Table 2**

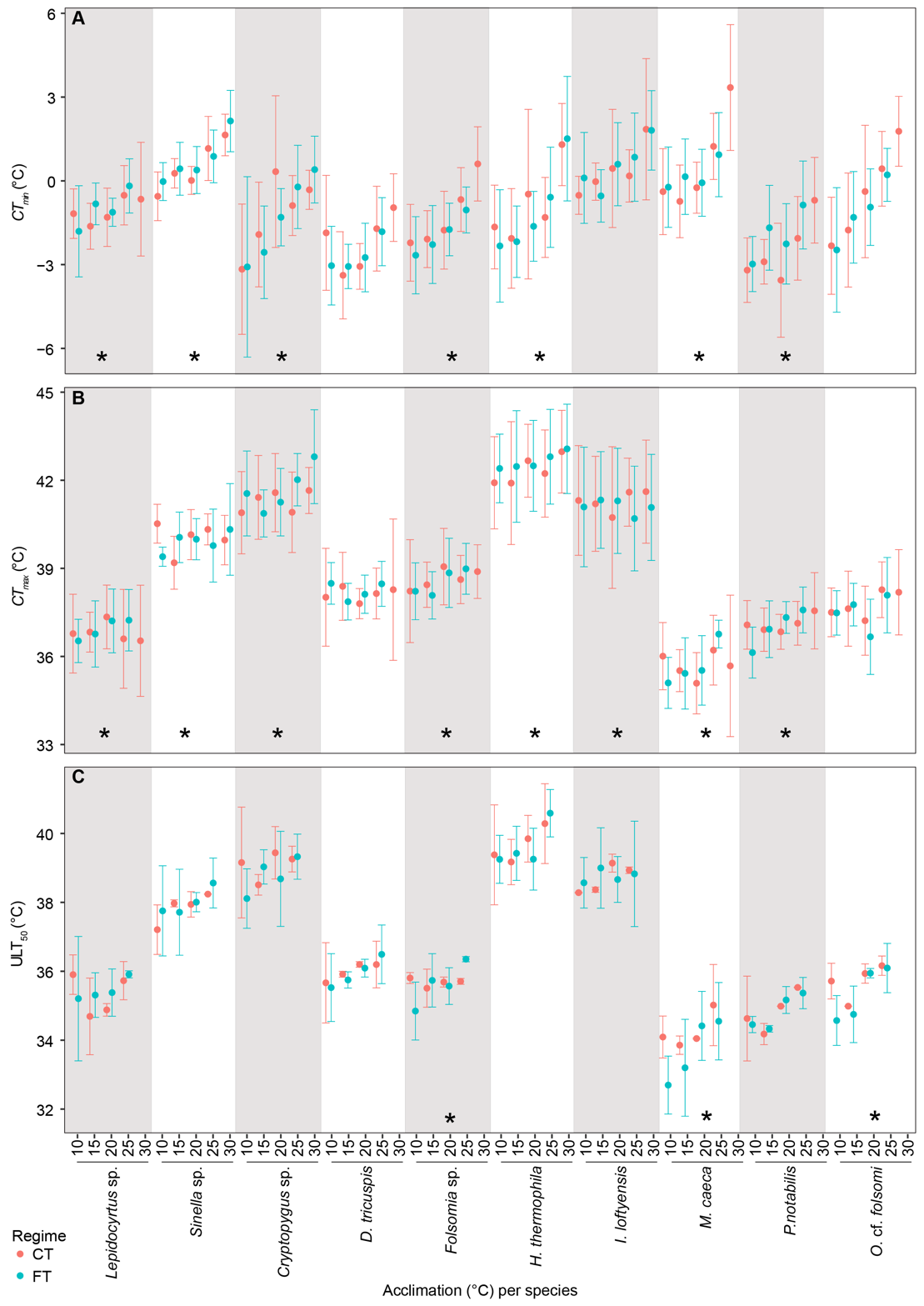
Summary outcomes of generalised linear models (Gaussian distribution, identity link) comparing the effects of Tconst and Tfluct acclimations on thermal tolerance traits (CTmin, CTmax and ULT50) in 10 springtail species. Significant relationships are in boldface. P-values have been corrected for multiple comparisons using a Benjamini-Hochberg adjustment. Full outcomes are provided in Supplementary Table A.3.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ***CTmin*** |  |  |  |  |  |  |  |
| **Family** | **Species** | **Acclimation** | | **Treatment (Tfluct)** | | **Slope** | |
|  |  | **Estimate** | **P-value** | **Estimate** | **P-value** | **Estimate** | **P-value** |
| Entomobryidae | *Lepidocyrtus* sp. | 0.041 | **<0.001** | -0.716 | **<0.001** | 0.051 | **<0.001** |
|  | *Sinella* sp. | 0.106 | **<0.001** | 0.458 | **0.002** | -0.01 | 0.158 |
| Isotomidae | *Cryptopygus* sp. | 0.134 | **<0.001** | -1.197 | **<0.001** | 0.053 | **<0.001** |
|  | *Desoria trispinata* | 0.071 | **<0.001** | -0.426 | 0.150 | 0.009 | 0.558 |
|  | *Folsomia* sp. | 0.154 | **<0.001** | 0.553 | **0.013** | -0.045 | **<0.001** |
|  | *Hemisotoma thermophila* | 0.138 | **<0.001** | -1.177 | **<0.001** | 0.048 | **0.002** |
|  | *Isotopenola loftyensis* | 0.095 | **<0.001** | 0.122 | 0.638 | 0.003 | 0.823 |
|  | *Mucrosomia caeca* | 0.175 | **<0.001** | 1.88 | **<0.001** | -0.111 | **<0.001** |
|  | *Parisotoma notabilis* | 0.115 | **<0.001** | 0.813 | **0.004** | 0.002 | 0.909 |
| Onychiuridae | *Orthonychiurus* cf. *folsomi* | 0.208 | **<0.001** | 0.543 | 0.052 | -0.039 | **0.006** |
|  |  |  |  |  |  |  |  |
| ***CTmax*** |  |  |  |  |  |  |  |
| **Family** | **Species** | **Acclimation** | | **Treatment (Tfluct)** | | **Slope** | |
|  |  | **Estimate** | **P-value** | **Estimate** | **P-value** | **Estimate** | **P-value** |
| Entomobryidae | *Lepidocyrtus* sp. | -0.015 | **0.015** | -1.054 | **<0.001** | 0.066 | **<0.001** |
|  | *Sinella* sp. | 0 | 0.930 | -0.736 | **<0.001** | 0.03 | **<0.001** |
| Isotomidae | *Cryptopygus* sp. | 0.022 | **0.001** | -0.565 | **0.005** | 0.048 | **<0.001** |
|  | *Desoria trispinata* | 0.004 | 0.488 | 0.136 | 0.516 | -0.001 | 0.930 |
|  | *Folsomia* sp. | 0.03 | **<0.001** | -0.588 | **0.002** | 0.031 | **0.002** |
|  | *Hemisotoma thermophila* | 0.047 | **<0.001** | 0.562 | **0.012** | -0.013 | 0.234 |
|  | *Isotopenola loftyensis* | 0.023 | **0.004** | 0.495 | **0.050** | -0.036 | **0.002** |
|  | *Mucrosomia caeca* | -0.004 | 0.543 | -1.878 | **<0.001** | 0.104 | **<0.001** |
|  | *Parisotoma notabilis* | 0.026 | **<0.001** | -1.294 | **<0.001** | 0.07 | **<0.001** |
| Onychiuridae | *Orthonychiurus* cf. *folsomi* | 0.04 | **<0.001** | 0.356 | 0.118 | -0.024 | **0.038** |
|  |  |  |  |  |  |  |  |
| **ULT50** |  |  |  |  |  |  |  |
| **Family** | **Species** | **Acclimation** | | **Treatment (Tfluct)** | | **Slope** | |
|  |  | **Estimate** | **P-value** | **Estimate** | **P-value** | **Estimate** | **P-value** |
| Entomobryidae | *Lepidocyrtus* sp. | -0.007 | 0.886 | -0.732 | 0.450 | 0.051 | 0.333 |
|  | *Sinella* sp. | 0.061 | **0.015** | 0.289 | 0.699 | -0.007 | 0.886 |
| Isotomidae | *Cryptopygus* sp. | 0.025 | 0.463 | -1.025 | 0.243 | 0.041 | 0.388 |
|  | *Desoria trispinata* | 0.038 | 0.058 | -0.506 | 0.333 | 0.027 | 0.333 |
|  | *Folsomia* sp. | -0.002 | 0.924 | -1.616 | **0.002** | 0.089 | **0.002** |
|  | *Hemisotoma thermophila* | 0.068 | **0.044** | -0.201 | 0.886 | 0.009 | 0.886 |
|  | *Isotopenola loftyensis* | 0.054 | **0.036** | 0.882 | 0.189 | -0.046 | 0.210 |
|  | *Mucrosomia caeca* | 0.135 | 0.064 | 1.868 | **0.030** | -0.076 | 0.091 |
|  | *Parisotoma notabilis* | 0.070 | **0.003** | -0.260 | 0.958 | 0.001 | 0.958 |
| Onychiuridae | *Orthonychiurus* cf. *folsomi* | 0.046 | 0.073 | -1.576 | **0.023** | 0.069 | 0.058 |

**Table 3**

Estimated differences between means (es) of Tconst and Tfluct for CTmin and CTmax, per acclimation (10°C to 30°C) and species. Boldface values show instances where Tfluct critical thermal limits were significantly different from Tconst i.e. where 95% confidence intervals do not overlap with zero.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Family** | **Species** | **Acclimation** | ***CTmax*** | | ***CTmin*** | |
|  |  |  | **es** | **95% CI** | **es** | **95% CI** |
| Entomobryidae | *Lepidocyrtus* sp. | 10 | -0.25 | **-0.45, -0.02** | -0.63 | **-0.89, -0.37** |
|  |  | 15 | -0.06 | -0.27, 0.12 | 0.80 | **0.64, 0.97** |
|  |  | 20 | -1.30 | -0.37, 0.08 | 0.18 | -0.02, 0.31 |
|  |  | 25 | 0.60 | **0.40, 0.92** | 0.34 | **0.14, 0.56** |
|  |  | 30 |  |  |  |  |
|  |  |  |  |  |  |  |
|  | *Sinella* sp. | 10 | 1.12 | **-1.23, -1.02** | 0.53 | **0.38, 0.69** |
|  |  | 15 | 0.87 | **0.68, 1.03** | 0.16 | **0.02, 0.51** |
|  |  | 20 | -1.60 | **-0.30, -0.02** | 0.37 | **0.25, 0.51** |
|  |  | 25 | -0.55 | **-0.74, -0.37** | -0.28 | **-0.51, -0.07** |
|  |  | 30 | 0.36 | **0.05, 0.58** | 0.50 | **0.31, 0.68** |
|  |  |  |  |  |  |  |
| Isotomidae | *Cryptopygus* sp. | 10 | 0.65 | **0.39, 0.94** | 0.08 | -0.41, 0.72 |
|  |  | 15 | -0.54 | **-0.75, - 0.29** | -0.64 | **-0.99, -0.29** |
|  |  | 20 | -0.32 | **-0.56, -0.06** | -1.63 | **-2.09, -1.26** |
|  |  | 25 | 1.11 | **0.885, 1.36** | 0.67 | **0.41, 0.92** |
|  |  | 30 | 1.15 | **0.87, 1.39** | 0.72 | **0.53, 0.92** |
|  |  |  |  |  |  |  |
|  | *Folsomia* sp. | 10 | -0.01 | -0.25, 0.33 | -0.45 | **-0.82, -0.13** |
|  |  | 15 | -0.36 | **-0.52, -0.20** | -0.20 | -0.43, 0.07 |
|  |  | 20 | -0.21 | -0.44, 0.03 | 0.02 | -0.23, 0.24 |
|  |  | 25 | 0.37 | **0.19, 0.53** | -0.37 | **-0.58, -0.18** |
|  |  | 30 |  |  |  |  |
|  |  |  |  |  |  |  |
|  | *Desoria trispinata* | 10 | 0.47 | **0.23, 0.76** | -1.17 | **-1.57, -0.84** |
|  |  | 15 | -0.52 | **-0.69, -0.33** | 0.32 | **0.06, 0.55** |
|  |  | 20 | 0.32 | **0.21, 0.44** | 0.32 | **0.12, 0.52** |
|  |  | 25 | 0.33 | **0.16, 0.48** | -0.11 | -0.38, 0.20 |
|  |  | 30 |  |  |  |  |
|  |  |  |  |  |  |  |
|  | *Hemisotoma thermophila* | 10 | 0.49 | **0.21, 0.74** | -0.70 | **-1.03, -0.29** |
|  |  | 15 | 0.57 | **0.14, 1.0** | -0.12 | -0.42, 0.18 |
|  |  | 20 | -0.17 | -0.44, 0.11 | -1.15 | **-1.66, -0.74** |
|  |  | 25 | 0.57 | **0.251, 0.88** | 0.72 | **0.39, 1.07** |
|  |  | 30 | 0.10 | -0.19, 0.38 | 0.21 | -0.18, 0.6 |
|  |  |  |  |  |  |  |
|  | *Isotopenola loftyensis* | 10 | -0.22 | -0.60, 0.16 | 0.62 | **0.37, 0.89** |
|  |  | 15 | 0.13 | -0.18, 0.44 | -0.51 | **-0.67, -0.35** |
|  |  | 20 | 0.57 | **0.20, 1.08** | 0.15 | -0.15, 0.46 |
|  |  | 25 | -0.90 | **-1.17, -0.60** | 0.67 | **0.42, 0.954** |
|  |  | 30 | -0.54 | **-2.1, -0.87** | -0.04 | -0.49, 0.35 |
|  |  |  |  |  |  |  |
|  | *Mucrosomia caeca* | 10 | -0.91 | **-1.09, -0.73** | 0.16 | -0.11, 0.42 |
|  |  | 15 | -0.09 | -0.28, 0.12 | 0.88 | **0.62, 1.14** |
|  |  | 20 | 0.44 | **0.23, 0.65** | 0.18 | -0.2, 0.39 |
|  |  | 25 | 0.56 | **0.38, 0.73** | -0.30 | **-0.56, -0.03** |
|  |  | 30 |  |  |  |  |
|  |  |  |  |  |  |  |
|  | *Parisotoma notabilis* | 10 | -0.94 | **-1.1, -0.77** | 0.22 | -0.01, 0.42 |
|  |  | 15 | 0.02 | -0.16, 0.20 | 1.22 | **1.00, 1.51** |
|  |  | 20 | 0.49 | **0.37, 0.6** | 1.30 | **0.93, 1.66** |
|  |  | 25 | 0.45 | **0.29, 0.61** | 1.19 | **0.87, 1.48** |
|  |  | 30 |  |  |  |  |
|  |  |  |  |  |  |  |
| Onychiuridae | *Orthonychiurus* cf. *folsomi* | 10 | -0.02 | -0.18, 0.13 | -0.15 | -0.57, 0.24 |
|  |  | 15 | 0.12 | -0.06, 0.36 | 0.46 | **0.07, 0.80** |
|  |  | 20 | -0.55 | **-0.76, -0.35** | -0.56 | **-1.07, -0.24** |
|  |  | 25 | -0.12 | -0.40, 0.04 | -0.22 | -0.43, 0 |
|  |  | 30 |  |  |  |  |

****

**Figure 1**

The mean response and standard deviation of *CTmin* (A), *CTmax* (B) and ULT50 (C) to Tconst (red) and Tfluct (blue) acclimations between 10 and 25 (ULT50) or 30°C (*CTmin* and *CTmax*) in 10 springtail species. Asterisks show instances of significant difference. Shaded columns are for aesthetic purposes only. See Table 1 and Table 2 for data.

**A close up of text on a black background

Description automatically generated**

**Figure 2**

Estimation plots showing the per species difference for *CTmin* (A) and *CTmax* (B)measured under Tconst (red) and Tfluct (blue) conditions. The upper section of each plot shows the raw data (in °C) along with the mean and standard deviation for each species and acclimation treatment. The lower panel of each plot shows the estimated difference between the means of Tconst and Tfluct plus the 95% confidence intervals. Where the 95% CI overlap with zero, there is no significant difference between Tconst and Tfluct. See Table A.4 for values.

A screenshot of a cell phone

Description automatically generated

**Figure 3**

Estimation plots showing the per acclimation difference for *CTmin* (A), *CTmax* (B) and ULT50 (C) measured under Tconst (red) and Tfluct (blue) conditions. The upper panel of each plot shows the raw data (in °C) along with the mean and standard deviation for each species and acclimation treatment. The lower panel of each plot shows the estimated difference between the means of Tconst and Tfluct plus the 95% confidence intervals. Where the 95% CI overlap with zero, there is no significant difference between Tconst and Tfluct. See Table A.5 for values.

**Appendix A. Supplementary data**

**Table A.1**

Mean daily soil temperature (°C), its standard deviation (s.d.), and maximum and minimum soil temperatures (°C), from the study site (the Jock Marshall Reserve) for the December 2015 to February 2016 period. Data were collected using six Thermochron iButtons™set level with the soil surface, below the leaf litter. Mean soil temperature determined by averaging all temperature data for each day across the six iButtons. Minimum and maximum were the lowest and highest recorded soil temperature on that day, respectively.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Month/Year** | **Day** | **Soil temperature (°C)** | | | |
|  |  | **mean** | **s.d.** | **maximum** | **minimum** | |
| Dec-15 | 1 | 16.0 | 1.3 | 18.8 | 13.7 | |
| Dec-15 | 2 | 14.2 | 1.6 | 17.0 | 11.8 | |
| Dec-15 | 3 | 15.8 | 2.1 | 19.2 | 13.3 | |
| Dec-15 | 4 | 17.6 | 3.6 | 22.6 | 12.3 | |
| Dec-15 | 5 | 19.1 | 2.3 | 22.9 | 15.9 | |
| Dec-15 | 6 | 18.7 | 2.2 | 22.5 | 15.7 | |
| Dec-15 | 7 | 19.0 | 0.9 | 20.8 | 17.4 | |
| Dec-15 | 8 | 19.9 | 1.8 | 23.4 | 17.2 | |
| Dec-15 | 9 | 17.9 | 1.9 | 21.3 | 15.4 | |
| Dec-15 | 10 | 17.3 | 2.3 | 20.9 | 14.3 | |
| Dec-15 | 11 | 15.1 | 1.1 | 17.3 | 12.8 | |
| Dec-15 | 12 | 13.8 | 1.3 | 16.3 | 12.2 | |
| Dec-15 | 13 | 15.6 | 2.9 | 19.8 | 11.6 | |
| Dec-15 | 14 | 16.9 | 2.7 | 21.3 | 13.0 | |
| Dec-15 | 15 | 17.8 | 2.5 | 21.6 | 14.6 | |
| Dec-15 | 16 | 19.5 | 2.8 | 23.7 | 15.3 | |
| Dec-15 | 17 | 21.2 | 3.3 | 26.3 | 16.7 | |
| Dec-15 | 18 | 22.7 | 3.5 | 28.7 | 18.3 | |
| Dec-15 | 19 | 23.5 | 3.4 | 28.4 | 18.9 | |
| Dec-15 | 20 | 22.3 | 2.9 | 27.7 | 17.3 | |
| Dec-15 | 21 | 16.7 | 1.0 | 18.7 | 14.9 | |
| Dec-15 | 22 | 16.9 | 2.5 | 20.4 | 13.3 | |
| Dec-15 | 23 | 18.8 | 2.5 | 22.5 | 15.2 | |
| Dec-15 | 24 | 20.4 | 3.1 | 25.1 | 15.9 | |
| Dec-15 | 25 | 21.9 | 2.6 | 26.4 | 18.5 | |
| Dec-15 | 26 | 16.5 | 2.5 | 21.0 | 13.4 | |
| Dec-15 | 27 | 14.7 | 1.8 | 17.4 | 12.3 | |
| Dec-15 | 28 | 15.9 | 2.0 | 18.9 | 13.3 | |
| Dec-15 | 29 | 16.9 | 2.3 | 20.4 | 13.5 | |
| Dec-15 | 30 | 18.7 | 3.1 | 22.8 | 14.3 | |
| Dec-15 | 31 | 21.6 | 2.7 | 25.8 | 18.2 | |
| Jan-15 | 1 | 20.4 | 1.5 | 22.9 | 18.3 | |
| Jan-15 | 2 | 19.6 | 1.8 | 22.9 | 17.6 | |
| Jan-15 | 3 | 19.2 | 1.7 | 22.6 | 17.1 | |
| Jan-15 | 4 | 18.8 | 1.5 | 21.9 | 17.3 | |
| Jan-15 | 5 | 18.9 | 1.4 | 21.4 | 17.1 | |
| Jan-15 | 6 | 19.2 | 1.7 | 22.0 | 17.3 | |
| Jan-15 | 7 | 18.4 | 1.9 | 22.1 | 15.9 | |
| Jan-15 | 8 | 18.1 | 2.1 | 22.1 | 15.8 | |
| Jan-15 | 9 | 18.4 | 1.9 | 21.8 | 16.3 | |
| Jan-15 | 10 | 20.3 | 3.3 | 25.4 | 16.3 | |
| Jan-15 | 11 | 21.1 | 3.3 | 27.8 | 17.3 | |
| Jan-15 | 12 | 20.2 | 2.6 | 25.3 | 16.8 | |
| Jan-15 | 13 | 23.2 | 5.0 | 31.5 | 16.6 | |
| Jan-15 | 14 | 17.2 | 2.2 | 22.4 | 14.1 | |
| Jan-15 | 15 | 15.9 | 1.5 | 18.5 | 13.9 | |
| Jan-15 | 16 | 17.2 | 3.1 | 22.3 | 12.8 | |
| Jan-15 | 17 | 20.3 | 3.8 | 26.3 | 15.2 | |
| Jan-15 | 18 | 22.5 | 3.8 | 28.7 | 17.5 | |
| Jan-15 | 19 | 21.9 | 3.2 | 27.6 | 17.4 | |
| Jan-15 | 20 | 21.0 | 1.8 | 24.2 | 18.5 | |
| Jan-15 | 21 | 20.3 | 1.4 | 23.2 | 18.3 | |
| Jan-15 | 22 | 18.7 | 0.8 | 20.7 | 17.6 | |
| Jan-15 | 23 | 18.1 | 0.9 | 20.3 | 16.8 | |
| Jan-15 | 24 | 17.9 | 1.0 | 19.7 | 16.8 | |
| Jan-15 | 25 | 17.9 | 1.2 | 20.3 | 16.3 | |
| Jan-15 | 26 | 19.5 | 2.6 | 23.4 | 15.8 | |
| Jan-15 | 27 | 20.3 | 2.1 | 24.8 | 18.1 | |
| Jan-15 | 28 | 19.9 | 1.2 | 21.9 | 18.2 | |
| Jan-15 | 29 | 16.7 | 0.7 | 18.1 | 15.3 | |
| Jan-15 | 30 | 17.0 | 1.9 | 19.8 | 14.3 | |
| Jan-15 | 31 | 17.1 | 1.0 | 19.0 | 15.1 | |
| Feb-16 | 1 | 17.0 | 2.0 | 19.7 | 14.2 | |
| Feb-16 | 2 | 19.4 | 2.5 | 22.8 | 15.8 | |
| Feb-16 | 3 | 18.3 | 0.8 | 19.8 | 16.4 | |
| Feb-16 | 4 | 17.7 | 1.2 | 19.8 | 16.2 | |
| Feb-16 | 5 | 18.9 | 2.0 | 22.3 | 16.2 | |
| Feb-16 | 6 | 19.9 | 2.6 | 23.8 | 16.3 | |
| Feb-16 | 7 | 20.8 | 2.2 | 24.7 | 17.8 | |
| Feb-16 | 8 | 19.4 | 1.2 | 22.1 | 17.9 | |
| Feb-16 | 9 | 19.3 | 1.7 | 22.8 | 17.3 | |
| Feb-16 | 10 | 19.3 | 1.6 | 22.4 | 17.7 | |
| Feb-16 | 11 | 19.2 | 1.9 | 22.8 | 16.9 | |
| Feb-16 | 12 | 19.6 | 2.3 | 23.6 | 16.8 | |
| Feb-16 | 13 | 20.3 | 2.4 | 24.6 | 17.3 | |
| Feb-16 | 14 | 17.9 | 1.0 | 19.6 | 16.3 | |
| Feb-16 | 15 | 17.9 | 1.5 | 20.4 | 15.7 | |
| Feb-16 | 16 | 15.8 | 0.7 | 17.3 | 14.8 | |
| Feb-16 | 17 | 16.2 | 1.2 | 18.3 | 14.5 | |
| Feb-16 | 18 | - | - | - | - | |
| Feb-16 | 19 | - | - | - | - | |
| Feb-16 | 20 | 16.6 | 1.1 | 18.6 | 15.3 | |
| Feb-16 | 21 | 17.2 | 2.4 | 21.0 | 13.8 | |
| Feb-16 | 22 | 18.5 | 1.5 | 20.7 | 16.3 | |
| Feb-16 | 23 | 21.2 | 2.9 | 26.3 | 17.3 | |
| Feb-16 | 24 | 21.5 | 1.6 | 25.3 | 19.9 | |
| Feb-16 | 25 | 20.3 | 1.1 | 22.7 | 18.6 | |
| Feb-16 | 26 | 18.3 | 1.3 | 20.5 | 16.3 | |
| Feb-16 | 27 | 18.8 | 1.3 | 21.6 | 17.4 | |
| Feb-16 | 28 | 18.7 | 1.3 | 21.6 | 17.4 | |
| Feb-16 | 29 | 18.9 | 2.0 | 22.6 | 16.5 | |

**Table A.2**

Temperatures 10 springtail species were exposed to in order to generate upper lethal temperatures at which 50% mortality occurred (ULT50) for acclimations between 10 and 25°C.

|  |  |  |  |
| --- | --- | --- | --- |
| **Family** | **Species** | **Experimental temperature** | |
|  |  | **Lowest** | **Highest** |
| Entomobryidae | *Lepidocyrtus* sp. | 32 | 38 |
|  | *Sinella* sp. | 36 | 40 |
| Isotomidae | *Cryptopygus* sp. | 36 | 44 |
|  | *Desoria trispinata* | 32 | 40 |
|  | *Folsomia* sp. | 32 | 40 |
|  | *Hemisotoma thermophila* | 36 | 44 |
|  | *Isotopenola loftyensis* | 36 | 42 |
|  | *Mucrosomia caeca* | 32 | 38 |
|  | *Parisotoma notabilis* | 32 | 40 |
| Onychiuridae | *Orthonychiurus* cf. *folsomi* | 32 | 40 |

**Table A.3**

Full outcomes of generalised linear models (Gaussian distribution, identity link) comparing the effects of Tconst and Tfluct acclimation treatments on thermal tolerance traits in 10 springtail species. Significant relationships are in boldface. P-values have been corrected for multiple comparisons using a Benjamini-Hochberg adjustment.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***CTmin*** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Family** | **Species** | **Acclimation** | | | | **Treatment (Tfluct)** | | | | **Slope** | | | |
|  |  | **Estimate** | **se** | **t** | **P-value** | **Estimate** | **se** | **t** | **P-value** | **Estimate** | **se** | **t** | **P-value** |
| Entomobryidae | *Lepidocyrtus* sp. | 0.041 | 0.006 | 6.493 | **<0.001** | -0.716 | 0.194 | -3.688 | **<0.001** | 0.051 | 0.01 | 4.91 | **<0.001** |
|  | *Sinella* sp. | 0.106 | 0.005 | 22.199 | **<0.001** | 0.458 | 0.14 | 3.279 | **0.002** | -0.01 | 0.007 | -1.471 | 0.158 |
| Isotomidae | *Cryptopygus* sp. | 0.134 | 0.01 | 13.483 | **<0.001** | -1.197 | 0.299 | -4.006 | **<0.001** | 0.053 | 0.014 | 3.745 | **<0.001** |
|  | *Desoria trispinata* | 0.071 | 0.008 | 8.509 | **<0.001** | -0.426 | 0.282 | -1.511 | 0.150 | 0.009 | 0.015 | 0.65 | 0.558 |
|  | *Folsomia* sp. | 0.154 | 0.007 | 22.362 | **<0.001** | 0.553 | 0.215 | 2.569 | **0.013** | -0.045 | 0.011 | -4.22 | **<0.001** |
|  | *Hemisotoma thermophila* | 0.138 | 0.01 | 13.159 | **<0.001** | -1.177 | 0.317 | -3.718 | **<0.001** | 0.048 | 0.015 | 3.207 | **0.002** |
|  | *Isotopenola loftyensis* | 0.095 | 0.008 | 12.18 | **<0.001** | 0.122 | 0.237 | 0.516 | 0.638 | 0.003 | 0.011 | 0.251 | 0.823 |
|  | *Mucrosomia caeca* | 0.175 | 0.008 | 22.08 | **<0.001** | 1.88 | 0.26 | 7.241 | **<0.001** | -0.111 | 0.014 | -8.141 | **<0.001** |
|  | *Parisotoma notabilis* | 0.115 | 0.008 | 13.859 | **<0.001** | 0.813 | 0.277 | 2.941 | **0.004** | 0.002 | 0.014 | 0.114 | 0.909 |
| Onychiuridae | *Orthonychiurus* cf. *folsomi* | 0.208 | 0.008 | 26.475 | **<0.001** | 0.543 | 0.27 | 2.016 | 0.052 | -0.039 | 0.014 | -2.834 | **0.006** |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ***CTmax*** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Family** | **Species** | **Acclimation** | | | | **Treatment (Tfluct)** | | | | **Slope** | | | |
|  |  | **Estimate** | **se** | **t** | **P-value** | **Estimate** | **se** | **t** | **P-value** | **Estimate** | **se** | **t** | **P-value** |
| Entomobryidae | *Lepidocyrtus* sp. | -0.015 | 0.006 | -2.533 | **0.015** | -1.054 | 0.211 | -5.004 | **<0.001** | 0.066 | 0.011 | 6.073 | **<0.001** |
|  | *Sinella* sp. | 0 | 0.005 | 0.088 | 0.930 | -0.736 | 0.157 | -4.693 | **<0.001** | 0.03 | 0.007 | 4.071 | **<0.001** |
| Isotomidae | *Cryptopygus* sp. | 0.022 | 0.006 | 3.472 | **0.001** | -0.565 | 0.193 | -2.924 | **0.005** | 0.048 | 0.009 | 5.242 | **<0.001** |
|  | *Desoria trispinata* | 0.004 | 0.006 | 0.774 | 0.488 | 0.136 | 0.191 | 0.711 | 0.516 | -0.001 | 0.01 | -0.09 | 0.930 |
|  | *Folsomia* sp. | 0.03 | 0.005 | 5.6 | **<0.001** | -0.588 | 0.178 | -3.299 | **0.002** | 0.031 | 0.009 | 3.331 | **0.002** |
|  | *Hemisotoma thermophila* | 0.047 | 0.007 | 6.764 | **<0.001** | 0.562 | 0.215 | 2.618 | **0.012** | -0.013 | 0.01 | -1.271 | 0.234 |
|  | *Isotopenola loftyensis* | 0.023 | 0.007 | 3.041 | **0.004** | 0.495 | 0.242 | 2.045 | **0.050** | -0.036 | 0.011 | -3.172 | **0.002** |
|  | *Mucrosomia caeca* | -0.004 | 0.006 | -0.65 | 0.543 | -1.878 | 0.211 | -8.88 | **<0.001** | 0.104 | 0.011 | 9.54 | **<0.001** |
|  | *Parisotoma notabilis* | 0.026 | 0.004 | 6.677 | **<0.001** | -1.294 | 0.141 | -9.19 | **<0.001** | 0.07 | 0.007 | 9.573 | **<0.001** |
| Onychiuridae | *Orthonychiurus* cf. *folsomi* | 0.04 | 0.006 | 6.3 | **<0.001** | 0.356 | 0.216 | 1.647 | 0.118 | -0.024 | 0.011 | -2.167 | **0.038** |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **ULT50** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Family** | **Species** | **Acclimation** | | | | **Treatment (Tfluct)** | | | | **Slope** | | | |
|  |  | **Estimate** | **se** | **t** | **P-value** | **Estimate** | **se** | **t** | **P-value** | **Estimate** | **se** | **t** | **P-value** |
| Entomobryidae | *Lepidocyrtus* sp. | -0.007 | 0.029 | -0.231 | 0.886 | -0.732 | 0.763 | -0.960 | 0.450 | 0.051 | 0.042 | 1.217 | 0.333 |
|  | *Sinella* sp. | 0.061 | 0.020 | 3.124 | **0.015** | 0.289 | 0.509 | 0.567 | 0.699 | -0.007 | 0.028 | -0.248 | 0.886 |
| Isotomidae | *Cryptopygus* sp. | 0.025 | 0.027 | 0.916 | 0.463 | -1.025 | 0.699 | -1.467 | 0.243 | 0.041 | 0.038 | 1.085 | 0.388 |
|  | *Desoria trispinata* | 0.038 | 0.016 | 2.355 | 0.058 | -0.506 | 0.414 | -1.221 | 0.333 | 0.027 | 0.023 | 1.207 | 0.333 |
|  | *Folsomia* sp. | -0.002 | 0.015 | -0.156 | 0.924 | -1.616 | 0.394 | -4.105 | **0.002** | 0.089 | 0.021 | 4.174 | **0.002** |
|  | *Hemisotoma thermophila* | 0.068 | 0.027 | 2.532 | **0.044** | -0.201 | 0.696 | -0.289 | 0.886 | 0.009 | 0.038 | 0.240 | 0.886 |
|  | *Isotopenola loftyensis* | 0.054 | 0.021 | 2.654 | **0.036** | 0.882 | 0.533 | 1.655 | 0.189 | -0.046 | 0.029 | -1.572 | 0.210 |
|  | *Mucrosomia caeca* | 0.135 | 0.026 | 5.202 | 0.064 | 1.868 | 0.677 | 2.761 | **0.030** | -0.076 | 0.037 | -2.063 | 0.091 |
|  | *Parisotoma notabilis* | 0.070 | 0.018 | 3.751 | **0.003** | -0.260 | 0.481 | -0.054 | 0.958 | 0.001 | 0.026 | 0.053 | 0.958 |
| Onychiuridae | *Orthonychiurus* cf. *folsomi* | 0.046 | 0.021 | 5.531 | 0.073 | -1.576 | 0.542 | -2.910 | **0.023** | 0.069 | 0.029 | 2.358 | 0.058 |

**Table A.4**

Estimated difference between means (es), along with the mean and its standard deviation of *CTmin* and *CTmax* measured under Tconst and Tfluct conditions for 10 springtail species. Where the 95% CI of the estimated mean difference do not overlap with zero (those in boldface), there is a significant difference between the critical thermal limits of springtails exposed to Tconst andTfluct.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ***CTmin*** |  |  |  |  |  |  |  |
| **Family** | **Species** | **Tconst** | | **Tfluct** | | **es (°C)** | **95% CI** |
|  |  | **mean (s.d)** | **n** | **mean (s.d)** | **n** |  |  |
| Entomobryidae | *Lepidocyrtus* sp. | -1.1 (0.68) | 218 | -0.99 (0.79) | 181 | 0.16 | **0.01, 0.3** |
|  | *Sinella* sp. | 0.51 (0.89) | 230 | 0.75 (0.87) | 259 | 0.23 | **0.07, 0.39** |
| Isotomidae | *Cryptopygus* sp. | -1.2 (1.55) | 237 | -1.34 (1.63) | 244 | -0.14 | -0.43, 0.14 |
|  | *Desoria trispinata* | -2.24 (1.17) | 235 | -2.66 (0.77) | 187 | -0.1 | -0.29, 0.09 |
|  | *Folsomia* sp. | -1.13 (1.21) | 220 | -1.92 (0.84) | 195 | -0.34 | -0.51, -0.16 |
|  | *Hemisotoma thermophila* | -0.85 (1.54) | 241 | -1.07 (1.65) | 223 | -0.22 | -0.5, 0.08 |
|  | *Isotopenola loftyensis* | 0.36 (1.08) | 292 | 0.56 (1.06) | 232 | 0.2 | **0.02, 0.39** |
|  | *Mucrosomia caeca* | 0.53 (1.63) | 249 | 0.18 (0.82) | 211 | 0.24 | **0.06, 0.42** |
|  | *Parisotoma notabilis* | -2.49 (1.25) | 227 | -1.93 (1.05) | 183 | 0.99 | **0.78, 1.19** |
| Onychiuridae | *Orthonychiurus* cf. *folsomi* | -0.46 (1.71) | 249 | -1.1 (1.24) | 187 | -0.14 | -0.41, 0.13 |
|  |  |  |  |  |  |  |  |
| ***CTmax*** |  |  |  |  |  |  |  |
| **Family** | **Species** | **Tconst** | | **Tfluct** | | **es (°C)** | **95% CI** |
|  |  | **mean (s.d)** | **n** | **mean (s.d)** | **n** |  |  |
| Entomobryidae | *Lepidocyrtus* sp. | 36.79 (0.77) | 251 | 36.93 (0.58) | 174 | 0.07 | -0.06, 0.21 |
|  | *Sinella* sp. | 40.04 (0.58) | 254 | 39.9 (0.59) | 244 | -0.14 | **-0.24, -0.04** |
| Isotomidae | *Cryptopygus* sp. | 41.31 (0.71) | 244 | 41.66 (0.89) | 234 | 0.36 | **0.21, 0.51** |
|  | *Desoria trispinata* | 38.12 (0.74) | 251 | 38.23 (0.43) | 192 | 0.14 | **0.04, 0.25** |
|  | *Folsomia* sp. | 38.66 (0.66) | 244 | 38.53 (0.62) | 192 | -0.07 | -0.2, 0.06 |
|  | *Hemisotoma thermophila* | 42.34 (0.88) | 257 | 42.66 (0.81) | 232 | 0.32 | **0.17, 0.47** |
|  | *Isotopenola loftyensis* | 41.34 (0.93) | 289 | 41.1 (0.93) | 246 | -0.24 | **-0.4, -0.08** |
|  | *Mucrosomia caeca* | 35.73 (0.84) | 308 | 35.7 (0.8) | 203 | -0.04 | -0.18, 0.1 |
|  | *Parisotoma notabilis* | 37.13 (0.52) | 260 | 36.96 (0.69) | 174 | -0.03 | -0.15, 0.08 |
| Onychiuridae | *Orthonychiurus* cf. *folsomi* | 37.65 (0.71) | 307 | 37.49 (0.76) | 193 | -0.05 | -0.18, 0.09 |

**Table A.5**

Estimated difference between means (es), along with the mean and its standard deviation of *CTmin*, *CTmax* and ULT50 measured under Tconst and Tfluct conditions per acclimation. Boldface values show cases where Tfluct was significantly different from Tconst i.e. where 95% confidence intervals do not overlap with zero. For *CTmax* and *CTmin* only 4 species were investigated at the 30°C acclimation.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***CTmin*** |  |  |  |  |  |  |
| **Acclimation (°C)** | **Tconst** | | **Tfluct** | | **es (°C)** | **95% CI** |
|  | **mean (s.d) (°C)** | **n** | **mean (s.d) (°C)** | **n** |  |  |
| 10 | -1.62 (1.26) | 469 | -1.81 (1.53) | 472 | -0.19 | -0.37, -0.01 |
| 15 | -1.62 (1.29) | 484 | -1.36 (1.29) | 481 | 0.26 | **0.09, 0.43** |
| 20 | -0.89 (1.63) | 526 | -1.02 (1.21) | 484 | -0.13 | -0.31, 0.04 |
| 25 | -0.39 (1.23) | 480 | -0.19 (1.09) | 477 | 0.2 | **0.05, 0.36** |
| 30 | 0.85 (1.53) | 439 | 1.48 (1.01) | 188 | 0.37 | **0.17, 0.6** |
|  |  |  |  |  |  |  |
| ***CTmax*** |  |  |  |  |  |  |
| **Acclimation (°C)** | **Tconst** | | **Tfluct** | | **es (°C)** | **95% CI** |
|  | **mean (s.d) (°C)** | **n** | **mean (s.d) (°C)** | **n** |  |  |
| 10 | 38.69 (2.2) | 522 | 38.7 (2.4) | 482 | 0.00 | -0.28, 0.29 |
| 15 | 38.73 (2.14) | 487 | 38.75 (2.2) | 463 | 0.02 | -0.25, 0.30 |
| 20 | 38.73 (2.27) | 572 | 38.87 (2.3) | 493 | 0.14 | -0.13, 0.40 |
| 25 | 39.01 (2.15) | 518 | 39.19 (1.98) | 461 | 0.17 | -0.09, 0.43 |
| 30 | 39.05 (2.49) | 566 | 41.9 (1.41) | 185 | 0.33 | 0.07, 0.57 |
|  |  |  |  |  |  |  |
| **ULT50** |  |  |  |  |  |  |
| **Acclimation (°C)** | **Tconst** | | **Tfluct** | | **es (°C)** | **95% CI** |
|  | **mean (s.d) (°C)** | **n** | **mean (s.d) (°C)** | **n** |  |  |
| 10 | 36.58 (1.82) | 30 | 36.1 (2.13) | 30 | -0.49 | -1.49, 0.47 |
| 15 | 36.31 (1.93) | 30 | 36.42 (2.16) | 30 | 0.108 | -0.95, 1.11 |
| 20 | 36.81 (2.04) | 30 | 36.72 (1.71) | 30 | -0.09 | -1.06, 0.82 |
| 25 | 37.1 (1.83) | 30 | 37.21 (1.93) | 30 | 0.102 | -0.84, 1.02 |
| 30 |  |  |  |  |  |  |

A picture containing toy, skiing

Description automatically generated

**Figure A.1**

Picture of the Jock Marshal Reserve and springtail collection sites. Sites 4-5 and 7-10 were where the iButtons (temperature data loggers) were placed for the collection of microclimate data. Map generated using Google Earth.

1. \* Corresponding author. School of Biological Sciences, Monash University, Clayton Campus, Wellington Road, Clayton, 3800, Victoria, Australia

   *E-mail addresses:* [jess.hoskins@monash.edu](mailto:jess.hoskins@monash.edu) (J.L. Hoskins), [Charlene.janion-scheepers@uct.ac.za](mailto:Charlene.janion-scheepers@uct.ac.za) (C. Janion-Scheepers), [elise.c.ireland@gmail.com](mailto:elise.c.ireland@gmail.com) (E. Ireland), [keyne.monro@monash.edu](mailto:keyne.monro@monash.edu) (K. Monro), [steven.chown@monash.edu](mailto:steven.chown@monash.edu) (S.L. Chown). [↑](#footnote-ref-1)
2. *Abbreviations*: CTmax, critical thermal maximum; CTmin, critical thermal minimum; ULT50, upper lethal temperature at which 50% mortality occurs; Tconst, constant temperature; Tfluct, fluctuating temperature. [↑](#footnote-ref-2)