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Size and temperature effects on physiological traits in arthropods

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Abstract

Interest in the use of trait data in models has been growing, predominantly as a consequence of attempts to improve understanding of the effects of global temperature change on organisms. This interest has resulted in a rising need not only to provide ready access to data for a wide variety of taxa and traits, but also to understand how variability in environmental and in experimental conditions affects model predictions. Two factors lie at the heart of ectotherm diversity variation: body size and temperature. In consequence how traits scale with body size and how temperature affects traits are major questions in physiological ecology. Although scaling relationships for and temperature effects on arthropod ectotherms have been widely studied for decades two areas of research stand out as requiring further work. First no synthetic compilation exists of scaling relationships for arthropod ectotherms which would enable a firm understanding of the nature of variation of these relationships among taxa and traits. Second, understanding of the effects on fluctuating temperatures on physiological traits, relative to static temperature conditions, remains in its infancy. In this thesis these two questions are addressed in three ways. First, I synthesised scaling relationships for insects from the literature. I established a comprehensive dataset of scaling relationships which reveals how much is known about some aspects of scaling (such as of resting metabolic rate) and how little about others, in particular ontogenetic relationships. Second, I examined how experimental and environmental temperatures impact traits of the important soil-dwelling group the Collembola, or springtails. I selected this group due to the large effects environmental change is expected to have on soil systems and because data on thermal variability effects on the soil fauna is comparatively sparse. I determined whether differences in constant and fluctuating temperature conditions affect thermal tolerance and developmental trait values and thus if the many constant trait values available in the literature would be suitable for use in models. The outcomes reveal that the effects of fluctuations are inconsistent among traits. Almost no effect is observed for thermal

tolerance, and variable effects observed for developmental traits. Thus, the use of trait data from the literature – data which are typically collected under constant temperature conditions – in predictive models will be appropriate for some traits but not for others, indicating a need to examine such effects on a broader range of traits. Finally, I determined whether, in the case of biological invasions, thermal variation in an invasive species' original (native) environment might be the determining factor leading to thermal advantages of such species over indigenous species in the receiving (invaded) environment. The outcomes suggest that the effect of invasive species on biodiversity under climate change may be dependent on both the indigenous' and the invaders' native thermal environments. Thus, the generally predicted increase in the success of invasive species under future temperature conditions might not be general, but might rather be realised more prominently in polar and tropical environments relative to temperate ones. Overall, this work reveals that a focus on temperature and body size continues to provide much insight into the way the world works.

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes four original papers, one of which has been submitted for publication. The core theme of the thesis is the effect of temperature on the phenotypic plasticity of springtail traits. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the School of Biological Sciences under the supervision of Prof. Dr. Steven L. Chown and Dr. Keyne Monro. The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research. The table below outlines my contribution to the work involved for my four thesis chapters.

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author name(s), nature and % of contribution	Co-author(s) Monash Student
1	Scaling in insects-a comprehensive overview	Not submitted	80%, data collection, data analysis, writing first draft	1. Keyne Monro, analysis, input into manuscript 10%. 2. Steven Chown, concept, input into manuscript 10%	No No
2	Constant and fluctuating temperature acclimations have similar effects on phenotypic plasticity in springtails	Accepted - Journal of Thermal Biology	70%, concept, methodology, data collection, writing first draft	1. Charlene Janion Scheepers, data collection, input into manuscript 5% 2. Elise Ireland, data collection, input into manuscript 10% 3. Keyne Monro, input into manuscript 5% 4. Steven Chown, data analysis, input into manuscript 10%	No No No No
3	Variation in the development and survival of springtails exposed to constant and fluctuating temperatures	Not submitted	90%, concept, methodology, data collection, data analysis, writing first draft	1. Steven Chown, input into manuscript 10%	No

4	Thermal tolerance, disturbance and soil invasion	Not submitted	70%. Concept, methodology, data collection, data analysis, writing first draft	1. Laura Phillips, data collection 10% 2. Ian Aitkenhead, methodology 5% 3. Charlene Janion-Scheepers, data collection 5% 4. Steven L. Chown, concept, input into manuscript 10%	No No No No

I have not altered portions of submitted or published papers in order to create a consistent presentation of this thesis.

Student name: Jessica L. Hoskins

Date: 8th May 2020

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main supervisor name: Professor Steven L. Chown

Date: 8th May 2020

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General Introduction

In light of the recent impacts of climate change, understanding the effects of temperature on the traits of organisms has become increasingly important (e.g. Deutsch et al. 2008, 2018; Dillon et al. 2010; Diamond et al. 2018; Pinsky et al. 2019), particularly as the use of traits in models attempting to describe these effects becomes more common (e.g. Kearney 2012). In this regard there are a number of areas in which increased information on temperature, body size and the interaction these two factors have with other traits, would improve the ability of researchers to use traits in models. Temperature and body size are considered among the most important factors affecting the biodiversity of ectothermic organisms. Temperature is important because multiple aspects of ectotherm ecology and evolution are influenced by the state and variability of the external thermal environment (Sinclair et al. 2003; Angilletta et al. 2004; Angilletta 2009; Hoffmann et al. 2013; Kingsolver and Buckley 2017; Moretti et al. 2017). The relationship body size forms with other traits is important due to its link to the resilience of species to climate change (Gardener et al. 2011).

First, in regards to body size there is a large body of work available in the literature that could be synthesised. Trait data represent the raw material for which a wide range of research can be conducted and there are an increasing number of databases that have collated trait information for researchers to use (e.g. Jones et al. 2009; Kattge et al. 2011). However, one area for which data is yet to be synthesised in an updated form (acknowledging early works such as Peters 1986 and Calder 1996) across a variety of traits, is that of scaling relationships (i.e. the relationships that body size forms with other traits). In particular compilations of insect scaling relationships remain either somewhat outdated or limited, with prior work generally having a larger focus on birds and mammals (e.g. Peters 1986; Dodds et al. 2001; Savage et al. 2004; Glazier 2008). A dataset of scaling relationships would be a valuable resource as body size is an easily measurable trait that forms predictable relationships with almost every other trait (e.g.

Peters 1986; Honěk 1993; Chown and Gaston 2010). As such, scaling relationships can be used in a predictive capacity, with measurements of body size used to determine the value of other traits (e.g. Benke et al. 1999), something that would be invaluable in helping to monitor the effect of climate change induced reductions in ectotherm body size (Sheridan and Bickford 2011) on other traits, especially those relating to population dynamics and thus diversity. Furthermore, scaling relationships are potentially useful in terms of providing a greater understanding of the universal nature of relationships which might underpin patterns in biodiversity across multiple spatial scales (e.g. Brown et al. 2004). For example, one of the most prominent theories attempting to use scaling in this regard is the Metabolic Theory of Ecology (Brown et al. 2004; van de Meer 2006; Irlich et al. 2009; Price et al. 2012), a model that uses a universal metabolic scaling constant (e.g. $M^{0.75}$), body size and temperature to describe individual and population level changes. The MTE is based upon the premise that metabolic rate (and its relationship with body size and temperature) underpins all biological processes. These biological processes are affected by temperature (warmer temperatures equal faster processes) which in turn can alter traits and population dynamics, impacting biodiversity. As such, predicting the outcome of environmental change is made possible using a universal scaling relationship and measures of a species body size. A compilation of scaling relationships would be useful in determining if universal scaling values are similar to those predicted (i.e. metabolic scaling) or if these intrinsic values differ for insects. Despite the potential usefulness of scaling relationships, the availability of this information over a wide range of traits remains limited and difficult to access for insects. Thus, synthesising scaling relationships for this group, and others, stands to improve the ability of researchers to use body size, especially field measurements of body size, to predict the outcomes of environmental change on a variety of traits (see also Gallagher et al. 2020).

Second, as the incorporation of traits into ecological models attempting to describe the effects of climate change on biodiversity (e.g. species distribution models) becomes increasingly common, questions have arisen about the conditions under which traits are being measured, most prominently the effect of constant versus fluctuating temperatures (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). Specifically, can trait values collected under constant temperature conditions in laboratories be used to predict the effects of temperature on trait values in thermally variable field conditions? Answering this question has relevance not only in terms of accuracy of forecasts, but also in terms of whether or not the plethora of data currently available on traits in the literature and in databases can be used in accurately, or if new data measured under more variable temperature conditions are required (e.g. Niehaus et al. 2012; Vallardares et al. 2014; Dowd et al. 2015). This is important as there is evidence that the effect fluctuations have on traits varies from that of constant temperatures, especially at high or extreme temperatures (e.g. Ragland and Kingsolver 2008; Bozinovic et al. 2011; Colinet et al. 2015), and this may limit the ability to extend laboratory studies to the field (Ma et al. 2015). However, to date systematic investigations of the influence of fluctuating temperatures across a broad range of temperatures and traits remains limited (see examples in Bozinovic et al. 2011; Fischer et al. 2011; Sobek-Swant et al. 2012; Niehaus et al. 2012). Thus, improving the understanding of fluctuating temperatures across multiple traits will be vital to creating accurate forecasts of the effects of climate change on biodiversity.

Lastly, understanding the response of traits to temperature will also help in forecasting the effect invasive species have on indigenous biodiversity under climate change conditions (van Kleunen et al. 2010a; Nunez-Mir et al. 2019). In particular how the thermal variation in the invasive species' native environment might lead to advantages over indigenous species in

the receiving environment (Enders et al. 2020). Answering this question is vital as the establishment and spread of invasive species can result in major changes to biodiversity and ecosystem structure and function (Simberloff et al. 2013; Gallardo et al. 2016). This is why the use of trait-based approaches to examine the factors contributing to the success of invasive species have risen to prominence recently (e.g. Jones et al. 2009; Kattge et al. 2011; Parr et al. 2017). For example, studies comparing the thermal tolerance of invasive and indigenous species, show invasive species have greater tolerance to high temperatures (Braby and Somero 2006; Slabber et al. 2007; Chown et al. 2007), leading some to conclude that climate change is likely to advantage invasive species over indigenous species (e.g. Walther et al. 2009; Hulme 2017; Janion-Scheepers et al. 2018), which will ultimately alter community structure and function. Thus, a better understanding of the effect of environmental temperature on the traits of organisms in both indigenous and invasive species is essential for foreseeing the outcomes of invasions to indigenous biodiversity.

Thesis outline

With the recent increase in utilisation of trait data in models, brought about by attempts to predict the effects of global temperature change, has come a need to not only provide easy access to traits, as in online databases (e.g. Gallagher et al. 2020), but to assess how variability in environmental temperature in the field will affect outcomes (e.g. Niehaus et al. 2012). This is especially true in terms of using traits measured under constant laboratory conditions to predict effects in field conditions. Furthermore, as invasive species become more prevalent (Seebens et al. 2017), variability in the native environment of organisms becomes an important factor to consider in models, especially as invasive species typically come from areas of high thermal variability and have been shown to have an advantage over indigenous species in many cases (e.g. Janion-Scheepers et al. 2018).

In regards to this, research is lacking for soil organisms. Thus, in this thesis I focus on springtails (Collembola) as an exemplar organism to address these knowledge gaps (Chapters 2 to 4). Springtails are key components of the soil biota and are widely used as indicators of the impacts of changing conditions due to their sensitivity and responsiveness to environmental variation (Bahrndorff et al. 2009; Vandewalle et al. 2010; Everatt et al. 2013; van Dooremalen et al. 2013). Furthermore, due to the important role the soil biota play in providing ecosystem services and the effect this has on above ground ecosystems (Bardgett and van der Putten 2014), along with the large impact environmental change will have on soil systems (e.g. Bokhorst et al. 2012; Holmstrup et al. 2018), there has been a recent emphasis on understanding the ways in which the soil fauna are likely to respond to environmental change (Nielsen et al. 2015; Geisen et al. 2019). As such, I used springtails to assess the effects of fluctuating temperatures on the performance of springtail thermal tolerance traits (Chapters 2) and developmental traits (Chapter 3), with the aim of identifying if traits available in the literature collected under constant conditions would enable accurate forecasts of the effects of temperature change for these organisms. I also used springtails to investigate how thermal variability in the native environments of invasive and indigenous species is likely to affect the invaders' advantage in the indigenous environment (Chapter 4), with the aim of identifying if similarities between indigenous and invasive native environments could mitigate the effects of invasive species on indigenous biodiversity.

Given the value of springtails as model species, I wanted to use springtails for all my chapters. However, for Chapter 1, which draws together scaling relationships from the literature into a dataset and examines differences in values across interspecific, intraspecific and ontogenetic scaling levels, I focused on insects. A preliminary search of scaling relationships for springtails revealed that there was inadequate data available to create a dataset and compare scaling values between these levels. Knowledge of differences between scaling levels is

important due to the potential impact of misusing scaling relationships generated for different biological levels for predictive purposes. Thus, although a database of scaling values could not be developed for the Collembola, the current outcome continues to provide useful insights because, like springtails, insects are expected to be substantially affected by global temperature change (Deutsch et al. 2008; Duffy et al. 2015). In turn this is predicted to result in large effects on the delivery of ecosystem services, on human and animal health, and on the economy through effects on agriculture (Losey and Vaughan 2006; Bradshaw et al. 2016; Deutsch et al. 2018). This chapter therefore draws together insect scaling relationships with the aim of providing an extensive and accessible dataset for future research, to document the extent to which scaling has been studied in insects, and to determine if scaling values differ between the levels of scaling. My expectation is that it will stimulate investigations for other groups such as the Collembola.

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Chapter 1

Scaling in insects – a comprehensive systematic overview

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Abstract

Scaling relationships in insects have been the subject of many studies because they provide insights into a wide variety of biodiversity features, at the species, population and community levels. The predictive ability and mechanistic basis of scaling relationships can also provide insight into the outcomes of changing environmental conditions. As such, understanding how much is known about scaling in insects across multiple spatial scales is important. Yet, to date, our knowledge of these relationships in insects and easy access to such relationships remains limited. The aim of this review is therefore to develop an extensive synthetic compilation of open-access insect scaling relationships across multiple levels of organization in order to document the extent to which scaling has been studied in insects. A systematic review approach was used to identify studies on scaling relationships in insects from the published literature. A summary of available relationships revealed that scaling in insects focuses on particular traits and insect taxa. Most scaling relationships for insects concerned morphological or physiological traits, comprising ca. 83% of those examined. Likewise, 90% of scaling relationships are from taxa representing just eight insect Orders, with most representation from the Hymenoptera and Lepidoptera. An investigation of scaling levels showed that ontogenetic scaling relationships were underrepresented (6%), whilst intraspecific scaling was the most investigated across the widest variety of traits (n = 168), followed by interspecific scaling (n = 103). There was only one relationship for which we could compare scaling across the levels, metabolic rate v body size, and no significant difference in scaling between the levels for this trait was found. This overview of scaling in insects indicates that a well-established fundamental basis exists for understanding how body size affects morphological and physiological traits. Nonetheless, it may be difficult to understand broader patterns in scaling without additional data, especially on ontogenetic scaling and the extent of variation among taxa in scaling relationships.

1. Introduction

Body size is an important trait, being strongly related to or dependent on a suite of other traits, including morphological (Emlen and Nijhout 2000; Shingleton et al. 2008), physiological (D'Amico et al. 2001), life history and ecological traits (Peters 1986; Honek 1993; Chown and Gaston 2010). Relationships between body size and other traits (i.e. scaling relationships) have long been of interest in biology and related fields due to the fundamental insights and predictive capability these relationships confer (Marquet et al. 2014; Nijhout and Callier 2015; White and Kearney 2014), such as the development and use of a universal metabolic scaling exponent (West et al. 1997; Glazier 2005) to provide a unified theory of what drives processes in biology from individuals to populations (e.g. Brown et al. 2004).

Scaling relationships typically take the form of

$$y=ax^b \quad (1.)$$

or

$$y=a+bx \quad (2.)$$

where y = trait, a = normalisation constant, x = body size (usually a measure of body mass or length) and b = scaling exponent. Theory suggests that scaling exponents may be constrained to take a restricted variety of values (Peters 1986; West et al. 1997; Glazier 2005). These include, for example, the cube law for relationships between linear dimensions and mass (Froese 2006), and the widely-known quarter power scaling that lies at the heart of the much-discussed metabolic theory of ecology (Brown et al. 2004; van de Meer 2006; Irlich et al. 2009; Price et al. 2012). Scaling relationships may also vary among different biological levels, specifically among species (interspecific scaling), individuals within species (intraspecific scaling), and over the course of development (ontogenetic scaling) (Kozłowksi et al. 2003; Glazier 2005; Chown et al. 2007; Shingleton et al. 2007; Chown and Gaston 2010; Glazier and

Paul 2017). In consequence, empirical evidence on the form and variation of scaling relationships is of considerable interest for understanding the fundamental mechanisms underpinning biodiversity (Brown et al. 2004; Harrison 2017).

The predictive value and mechanistic basis of scaling relationships can also provide insight into the outcomes of changing environmental conditions. Of particular interest is how temperature affects body size in ectotherms (Sibly and Atkinson 1994; Angilletta et al. 2004), how warming climates may thus modify body size, and how in turn scaling relationships may be altered (Gardner et al. 2011; Sheridan and Bickford 2011). For example, evidence suggests that absolute metabolic rates should increase with warming in ectotherms (Dillon et al. 2010). However, because shifts in metabolic rate are typically greater for larger organisms, a simultaneous decline in body size due to warming might ameliorate to some degree increases in metabolic rate, as warming also typically reduces body size in ectotherms (Sibly and Atkinson 1994; Angilletta et al. 2004). Thus, even though warming is increasing metabolic rates (Dillon et al. 2020), and thereby increasing energy needs, competition for resources, and the risk of mortality which leads to demographic changes, a reduction in body size might limit this. This means the potential effect of increased metabolic rates due to warming might not be as large as predicted. Therefore, understanding the interactions between body size and traits is of considerable interest in ectotherms, as measures of body size determined under warmer conditions could be used to predict not only changes to a particular trait, but also larger scale demographic changes. This is especially relevant given the expectation of large impacts of warming on ectotherm populations (Lister and Garcia 2018), and in turn on the economy (Deutsch et al. 2018).

In this regard, insects are of particular significance. Not only are they essential for terrestrial and freshwater ecosystem functioning (Wallace and Webster 1996; Weisser and Siemann 2013), but they are also of considerable economic significance to agriculture, human and animal

health, and the delivery of ecosystem services (Losey and Vaughan 2006; Bradshaw et al. 2016). Moreover, insects are expected to be affected substantially by global temperature change (Menéndez 2007; Duffy et al. 2015; Deutsch et al. 2018). One way in which these effects will play out is through the joint impacts of changing biological rates (such as metabolic rate or growth rate) and body sizes. Size responses to climate change are also being complicated by other factors, including additional environmental change drivers (e.g. Babin-Fenske et al. 2008; Treasure and Chown 2014; Xi et al. 2016). Thus, comprehension of the likely outcomes of environmental change can be much improved by using empirical information on the relationship between size and other important features, such as physiological and life history characteristics (Dillon et al. 2010; Huey et al. 2012).

For these reasons and others (such as providing a means to estimate one measure of body size, such as mass, from another, such as length; e.g. Benke et al. 1999), much attention has focused on understanding scaling relationships in insects. Although many studies are directly interested in understanding the value that scaling relationships take (e.g. Peters 1986; Shingleton et al. 2007), others estimate scaling relationships *en route* to other goals, such as investigations of behaviour (e.g. Berrigen and Pepin 1995) or estimates of assemblage characteristics (e.g. Chown and Steenkamp 1996). In consequence, the literature on insect scaling is simultaneously extensive and difficult to access, being diverse and scattered.

The purpose of this study is therefore to draw together insect scaling relationships in an extensive and openly-accessible dataset and to document the extent to which scaling has been studied in insects. Additionally, due to interest in differences in scaling across biological levels of organisation (Kozłowksi et al. 2003; Glazier 2005; Chown et al. 2007; Shingleton et al. 2007; Chown and Gaston 2010; Glazier and Paul 2017), along with the potential misuse of universal scaling relationships in a predictive capacity when they do not accurately represent scaling

values for a particular trait (e.g. metabolic scaling), the nature of variation in scaling exponents among the interspecific, intraspecific and ontogenetic levels is also investigated.

2. Materials and Methods

2.1 Dataset compilation

A systematic review (Pullin and Stewart 2006) approach was used to identify studies on scaling relationships in insects from the published literature. Searches were conducted in the *Google Scholar* and *Web of Science* datasets. Structured key word searches were used, along with opportunistic searches using whole initial search strings, or parts thereof. Boolean search terms were: (size* OR length* OR mass*) AND (isometry* OR allometry* OR scaling*) AND (insect* OR arthropod*). From initial searches, reference lists of all subsequent works (including those in reviews by Peters 1986 and by Chown and Gaston 2010) were used to find additional works and to initiate a further set of searches. Searches included work published up to December 2017.

Each published work was assessed to determine its suitability for inclusion in the dataset, reducing the 774 studies initially identified to 278 studies. Studies were excluded if they: did not report the scaling relationship; did not include measurement units; reported different equations for the same set of data (apparently in error); did not differentiate among interspecific, intraspecific or ontogenetic scaling; or had small sample sizes (<3). Studies were not excluded on the basis of coefficient of determination (i.e. low R^2), but were omitted where the relationship was not significant. The scaling equations were not standardized to common units for a given trait, such as providing mass in grams and metabolic rate in Watts, (e.g. Peters 1986; Chown et al. 2007).

All details provided by the author about each scaling relationship were reported. Because the level of reporting by authors was inconsistent, the data available for each

relationship varies — for example, the scaling equation was reported in full (e.g. $y=ax^b$) for some relationships, whilst only the scaling exponent (b) was reported for others. Typically, body size (x) in each scaling relationship was a measure of body mass (~61%) or body length (~38%), although rarely (~1% of relationships) other measures (e.g. hind tibia length or head width) were used as proxies for body size. The statistical method used to estimate each scaling relationship was also documented (see a comprehensive overview of these methods in White and Kearney 2014). Ordinary least squares regression was the most common of the documented statistical methods (89%). The dataset also contains details of the authors' original taxonomic classification, along with updated classifications since publication, as in the case of Isoptera to Blattodea. Updated classifications were used in all analyses and were determined using the "Open Tree of Life" dataset (Hinchliff et al. 2015). The original traits as named by authors, the units of measurement, whether the relationship was log, linear, or log-linear, the full reference for each scaling relationship, life stage and gender were also documented, where available. The dataset is provided in full as an online resource at Monash Figshare doi:10.26180/5eb49c291aed4.

2.2 Analysis

Each scaling relationship was allocated to a level of organisation based on whether the relationship concerned interspecific, intraspecific or ontogenetic scaling (Shingleton et al. 2007, 2008; Chown and Gaston 2010), then further grouped into major category classes based on trait (y) characteristics. Major category classes were defined as Ecology, Locomotion, Morphology, Biochemistry, Physiology, and Behaviour (Table A1). Relationships that did not directly fit into these categories (e.g. mitochondrial volume in muscle) were classified as Undefined. Traits were then further categorised into minor trait categories (n = 59). For example, traits such as "standard metabolic rate" and "mass-specific metabolic rate" were first placed into the

Physiology major category and then into the "metabolic rate" minor category (see Table A1 for a detailed list of traits and the categories in which each were placed). This particular grouping was made to gain an overview of the focus of research in scaling for insects. To illustrate the distribution of scaling exponents across the diversity of insect taxa, categories, and scaling levels, frequency histograms and plots were used as generated in R v 3.5.2 (R core team 2018) using the R studio platform v1.1.463 (RStudio Team 2016). Due to the interest in universal scaling exponents, a summary table of the median and range for the most common relationships in the dataset (where n>9 exponents) is also provided (Table 1).

2.3 Variation among the interspecific, intraspecific and ontogenetic levels

Due to a low number of scaling exponents per trait and low numbers of ontogenetic scaling relationships, assessment of the variation in scaling between interspecific, intraspecific and ontogenetic scaling levels was limited to the relationship between log standard metabolic rate (y) and log body mass (x). Standard metabolic relationships were considered those that did not explicitly describe active and mass-specific metabolic rate. A phylogenetic linear mixed model (PLMM) was used to compare the scaling levels components for metabolic rate, whilst accounting for variation introduced by the relatedness of species. In the PLMM the scaling exponent (*b*) and scaling level (i.e. interspecific, intraspecific and ontogenetic) were fixed effects and a phylogeny for each scaling level was included as a random effect. To identify the model that best fit the data, alternative models with and without phylogenies were compared using likelihood-ratio tests based on a chi-squared distribution (Gilmour et al. 1995). As the statistical method used in generating a scaling exponent (i.e. ordinary least squares or reduced major axis regression) produces different values (see White and Kearney 2014), statistical method was initially added as a fixed effect, but was subsequently removed because no effect was observed ($p = 0.96$).

The "Open Tree of Life" (Hinchliff et al. 2015) and the R package "rotl" (Michonneau et al. 2016) were used to generate a phylogeny for each of the scaling levels, with branch lengths estimated using the Grafen method (Grafen 1989) in the R package "ape" (Paradis et al. 2004). The PLMM was run using the package ASReml-R v 3.0 (Gilmore et al. 1995). All analyses were run in R v 3.5.2 (R core team 2018) using the R studio platform v1.1.463 (RStudio Team 2016).

3. Results

3.1 Scaling relationships in insect taxa

3.1.1 Orders

Scaling relationships spanned 18 insect Orders, with ~92% of relationships attributed to taxa within eight Orders (Fig. 1; Table A2). The frequency of scaling exponents and the number of traits per Order illustrate that scaling in insects has been well researched in Hymenoptera (bees, ants and wasps), Lepidoptera (moths and butterflies), Diptera (flies) and Coleoptera (beetles) (Fig. 2A). The best-studied in terms of number of Families and species were Diptera and Hymenoptera (Figs. 2C, 2E; Table A3), although scaling in Diptera was investigated for a larger number of species whilst Hymenoptera had more scaling relationships. The least studied Orders were Microcoryphia (bristletails), Mantodea (mantises), and Thysanoptera (thrips), for which there are fewer than three scaling exponents in the dataset.

3.1.2 Families

There are 150 Families represented in the dataset (Fig. 1; Table A3). Those with the highest frequency of scaling exponents were Formicidae (ants, n = 261), Apidae (bees, n = 155), Acrididae (short-horned grasshoppers, n = 177), and Nymphalidae (brush-footed butterflies, n = 114). Scaling exponents were distributed unequally amongst Families, with one or two

Families representing the majority of scaling exponents within each Order. For example, 70% of scaling relationships for Hymenoptera were represented by two Families (Formicidae and Apidae), and for Orthoptera (grasshoppers), 85% of relationships were for Acrididae.

3.1.3 Species

In total, scaling relationships were available for 532 species. The most-studied species were *Locusta migratoria* (Migratory locust, n = 88) and *Manduca sexta* (Tobacco horn worm, n = 77) (Table A3), which are both common agricultural pests (e.g. Bullen 1966; Lingren et al. 1977) and frequently used as model species (e.g. Hinks and Erlandson 1994; Greenlee and Harrison 2005). Other common species in the dataset were *Leptophlebia cupida* (Early brown spinner (Mayfly) n = 50), *Osmia lignaria* (blue orchard bee; n = 46), *Schistocerca Americana* (American grasshopper n = 40), and *Achroia grisella* (Lesser wax moth, n = 20).

3.2 Categories and traits

Scaling relationships are reported for 265 traits in this dataset. The number of scaling relationships available for each trait was highly variable. Most traits (~84%) had less than 10 scaling exponents, with the number of exponents available ranging from 1 (44% of cases) to 624 (see Table 1 for a list of the most common scaling relationships in the dataset). Morphological traits were the most common and also had the highest frequency of scaling exponents when compared to other major trait categories (Figs. 2D, 2F, 3; Tables A5, A6). The most frequent morphological traits were those associated with measures of mass (n = 972) or length (n = 284). Physiological traits were also common, although the number of traits and the variety of insects investigated was about a third of that observed for morphological scaling (Figs. 2B, 3). Metabolic traits were the most frequent of the physiological traits (n = 456), making up ~75% of all scaling relationships within the Physiology category. Scaling

relationships associated with Life history and Locomotion were less common. Life history traits were fewer than those associated with Locomotion (most likely because life history focuses on a few traits, such as those involving growth, fecundity and mortality). The most frequent traits associated with Life History and Locomotion were those related to fecundity (n = 131) and speed (running, swimming or flight) (n = 57) (Fig. 3), respectively. There were few examples of scaling relationships associated with Biochemistry, Ecology, or Behaviour.

3.3 Interspecific, intraspecific and ontogenetic scaling

Intraspecific scaling (within species) was most common, followed by interspecific scaling (among species), whilst ontogenetic scaling (over the course of development) was rare, representing only 6.3% of relationships in the dataset (Table A6). Intraspecific scaling was investigated across the widest variety of traits (n = 168), followed by interspecific scaling (n = 103), and even though the frequency of ontogenetic relationships within the dataset was low, the variety of traits investigated was relatively large (n = 62) (Fig. 2H; Table A6). Interspecific scaling was studied over the widest range of taxa, covering 17 of the 18 Orders, whilst intraspecific and ontogenetic scaling covered 15 and 8 Orders, respectively. Interspecific and intraspecific relationships for Hymenoptera and Lepidoptera were most common. Within ontogenetic scaling, relationships for Orthoptera and Ephemeroptera were most common (Fig. 2G; Table A2), although this was due almost exclusively to the large amount of data available for *L. migratoria* (n = 79) and *L. cupida* (n = 40), which made up 84% of all ontogenetic data. For all biological levels morphological and physiological traits were the most common, although life history traits were also common for intraspecific scaling (Fig. 2H; Table A6).

3.4 Does metabolic scaling differ across biological levels?

A phylogenetic linear mixed model showed that interspecific, intraspecific and ontogenetic scaling relationships did not differ for standard metabolic scaling (Wald $\chi^2_{(2)} = 0.06$, $p = 0.94$). Whilst there was no significant difference in scaling between the levels, the addition of phylogeny to the models had an effect on the mean scaling exponents. For ontogenetic and intraspecific scaling, phylogeny decreased estimates of the mean slope. The phylogenetically-corrected mean slope estimated for ontogenetic scaling was 0.71±0.29 and the uncorrected slope was 0.81±0.03, whilst the phylogenetically-corrected slope for intraspecific scaling was 0.73±0.13 and the uncorrected slope was 0.76±0.15 (Table 2). The opposite was true for interspecific scaling for which the addition of a phylogeny increased the mean scaling exponent. The phylogenetically-corrected mean slope estimate for interspecific scaling was 0.77±0.07, and the uncorrected slope was 0.75±0.02) (Table 2). There was also no significant difference among biological levels without a phylogeny (Wald $\chi^2_{(2)} = 1.24$, $p = 0.23$), although there was a significant improvement to model fit when phylogeny was included (LRT $\chi^2_{(5)} = 45.89$, $P > 0.0001$). This indicates that the relatedness of species affects variation in scaling exponents more than the biological level at which they are measured. Furthermore, due to the change in mean scaling exponents when corrected for phylogeny, we expect that failure to consider the evolutionary history of insects when investigating scaling patterns or form, at least for metabolic scaling, has the potential to lead to misinterpretation or erroneous conclusions about scaling relationships.

4. Discussion

4.1 Overview of scaling in insects

Although this dataset provides scaling relationships for a large variety of insects, proportionally most relationships focus on a small number of taxa. This bias could potentially be linked to species diversity, in that the Orders with the largest number of species are consequently the

most studied. Species richness in insects is highest in Coleoptera followed by Lepidoptera, Diptera, Hymenoptera, Hemiptera and Orthoptera (Zhang 2013, Stork 2018). Whilst these Orders were among the most studied in the dataset, the proportion of scaling exponents per Order did not correspond to the proportion of described species in each Order. As such it seems unlikely that species diversity is responsible for which insects are studied the most. Another possible reason for the focus on particular insects could be the relevance or availability of insect species. For example, the most frequently occurring species in the dataset are either pest species that generally have a large negative impact on agriculture, such as *L. migratoria* (e.g. Bullen 1966), or are used extensively as model species, such as *M. sexta* (e.g. Greenlee and Harrison 2005; Woods 2010; Sears et al. 2012). Whatever the reason, investigation of broad scaling patterns or universal laws without considering a large variety of insect taxa is likely to present an incomplete picture, especially because of the influence of phylogenetic relatedness on trait values (e.g. Symonds and Elgar 2002; Duncan et al. 2007; Chown et al. 2007; Capellini et al. 2010; Ehnes et al. 2011) and the variation found among different higher taxa (White et al. 2012).

Just as particular taxa were overrepresented in the scaling relationships available, so too are particular traits. This focus on particular traits may be due to what information can be inferred from them, what they can be used for, or simply because the scaling relationship was not the original goal of the research. For example, scaling relationships for morphological traits, such as length or mass, are often used to describe shape changes and their implications (e.g. Koehl 1996; Hirst 2014; Glazier et al. 2015), and length is commonly used to generate mass measurements (e.g. Benke et al. 1999), as length is a more easily measurable trait. Metabolic scaling is often studied because of its potential to influence all other traits, which is consequently why metabolic scaling has been used extensively in models attempting to explain ecological processes (Brown et al. 2004; Brown and Sibly 2012; Kearney 2012; Sibly et al. 2012; Maino and Kearney 2014; Schramski et al. 2015). Metabolic scaling is also controversial

in terms of empirical arguments for or against geometric and quarter power scaling laws (e.g. Dodds et al. 2001; White and Seymour 2003; Glazier 2005, 2006; Farrell-Grey and Gotelli 2005; White et al. 2007; Kearney and White 2012; Maino and Kearney 2014). Although traits, such as metabolic rate and length, offer important insights into the effects of modifying body size, for a deeper understanding of scaling patterns in insects, and for scaling relationships to be a useful resource for predictive purposes — either to generate mean scaling exponents for traits, or to generate species-specific trait data — more information on a wider range of traits and insect taxa are needed. Especially as models incorporating trait values are increasingly becoming more sophisticated, often utilising multiple traits to predict broad biological impacts, such as those associated with biodiversity or abundance and climate change (e.g. Kearney et al. 2009, 2012; Pearson et al. 2014; Urban et al. 2016).

4.2 Interspecific, intraspecific and ontogenetic scaling

Limited data were available for ontogenetic scaling. Although the reasons for this are unclear, the lack of ontogenetic data may be associated with methodological considerations of taking measurements across all developmental stages, thus, measuring ontogenetic data may require insects to be reared in a laboratory setting (e.g. Greenlee and Harrison 2005; Callier and Nijhout 2012). This may be why the majority of ontogenetic data in this dataset comes from species with documented rearing protocols, such as *Locusta migratoria* (e.g. Hinks and Erlandson 1994). Whatever the reason, the lack of ontogenetic data is potentially problematic for understanding broad scale patterns in scaling, particularly as ontogenetic scaling typically deviates from patterns observed in interspecific and intraspecific scaling (Glazier 2006; Yagi et al. 2010; Sears et al. 2012; Maino and Kearney 2015). This is potentially because of changes in shape over ontogeny (Hirst et al. 2014; Glazier et al. 2015), or because traits during

embryonic stages of insect development can have different values regardless of mass (e.g. Maino and Kearney 2014).

For metabolic scaling specifically, ontogenetic scaling exponents are typically observed to deviate from those of interspecific and intraspecific scaling (e.g. Glazier 2006; Maino and Kearney 2014). Typically the mean ontogenetic scaling exponent is expected to be higher than that of the other levels (e.g. Glazier 2006; Caruso et al. 2010; Sears et al. 2012). However, in this case, no significant differences were found among the levels, even though mean values showed an outcome different to the expectation, with the ontogenetic exponent (0.71) lower than both intraspecific (0.73) and interspecific exponents (0.77). This inconsistency with previous findings may be for a number of reasons. Firstly, the analysis in this study corrected for phylogenetic effects because the evolutionary history of species has been shown to affect scaling exponents (Symonds and Elgar 2002; Duncan et al. 2007; Chown et al. 2007; Capellini et al. 2010; Ehnes et al. 2011), where most scaling studies do not (e.g. Peters 1986; Glazier 2005). Exclusion of phylogenetic effects is especially problematic for the analysis of interspecific data in this study, since studies generating interspecific scaling exponents without phylogenies are likely to be misestimating values. This may be why, even though we considered phylogenetic non-independence in this study, the mean interspecific scaling exponent (0.77) was higher in comparison to results seen in previous research reporting a phylogenetically-corrected interspecific value of 0.75 (Chown et al. 2007; Ehnes et al. 2011). Secondly, the sample size and number of taxa analysed for each level varied substantially. For example, intraspecific scaling had two and half times the number of scaling exponents for interspecific scaling, and six times the number for ontogenetic scaling. Likewise, the number of species analysed for ontogenetic scaling was very low (11), and even the 52 species analysed for intraspecific metabolic scaling, might have been inadequate to properly test variation in scaling form across the biological levels, especially considering interspecific and ontogenetic scaling

are known to be highly variable (e.g. Glazier 2006; Caruso et al. 2010). Thus, the frequency and quality of metabolic scaling exponents available in this dataset may not allow us to fully understand the nature of variation in metabolic scaling among the biological levels.

5. Conclusion

Scaling relationships are of interest not only because they provide insights into the mechanisms underpinning biodiversity in organisms (Marquet et al. 2014; Nijhout and Callier 2015; White and Kearney 2014; Brown et al. 2004; Harrison 2017), but also because they offer a way for researchers to easily estimate trait values from body size (e.g. Benke et al. 1999). Such an approach is becoming increasingly important as models predicting biological or ecological outcomes, such as those involved with climate change, move towards incorporating trait values to improve the accuracy of forecasts (e.g. Kearney 2012; Pearson et al. 2014; Urban et al. 2016). Knowledge and potential application of insect scaling relationships is relevant in light of the influence of climate change on insect body size (Gardener et al. 2011; Sheridan and Bickford, 2011), and thus on insect biodiversity, especially as insects are often excluded from investigations of broad scaling patterns (e.g. Dodds et al. 2001; Savage et al. 2004; Glazier 2008).

This overview of scaling in insects indicates that there is a well-established fundamental basis for understanding how body size affects morphological and physiological traits, especially those associated with length and metabolic rate. However, there are also indications that it may be difficult to understand broader patterns in scaling without additional data, especially on ontogenetic scaling and the variety in relationships that may be found among Families and Orders. The dataset provided here offers a foundation on which to develop further research.

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Tables

Table 1

Median, range and number of scaling exponents for the most common relationships (n > 9) in the dataset. Relationships are sorted by level and category.

Interspecific

Category	Relationship	median	min	max	n	form
Life History	Number of eggs vs dry body mass	1.048	0.652	4.62	14	linear
Locomotion	Wing loading vs wet body mass	0.325	0.14	0.77	13	log-log
Morphology	Wing area vs wet body mass	0.809	0.63	1.05	10	log-log
	Femur length vs body length	1.105	0.67	2.03	14	log-log
	Leg length vs body length	1.09	0.63	1.35	14	log-log
	Tibia length vs body length	1.115	0.6	2.26	16	log-log
	Wing length vs wet body mass	0.41	-0.15	0.535	11	log-log
	Ash free dry mass vs body length	2.709	1.63	3.45	18	log-log
	Dry mass vs body length	2.3325	0.81	4.026	46	linear
	Dry mass vs body length	2.724	0.132	4.15	185	log-log
	Exoskeletal dry mass vs dry body mass	0.9935	0.92	1.109	12	log-log
	Wet mass vs body length	0.017	0.001	2.691	13	linear
	Wet mass vs body length	2.6985	1.89	3.219	28	log-log
	Egg volume vs body length	0.785	0.13	2.92	60	linear
	Egg volume vs body length	0.9	0.24	1.73	60	log-log
	Head width vs dry body mass	0.5175	0.16	3.51	16	log-log
Physiology	Mass-specific metabolic rate vs wet body mass	-0.37	-0.65	-0.22	13	log-log
	Metabolic rate vs dry body mass	0.99	0.67	2.65	13	log-log
	Metabolic rate vs wet body mass	0.78	0.083	1.06	133	log-log
	Ventilation volume vs wet body mass	0.71	0.499	1.196	13	log-log

Intraspecific

Category	Relationship	median	min	max	n	form
Biochemistry	Water content vs dry body mass	1.001	0.6	1.339	11	log-log
Life History	Number of eggs vs dry body mass	1.0535	0.255	5.145	80	linear
	Number of eggs vs wet body mass	1.92	0.21	9.47	22	linear
Locomotion	Running speed vs wet body mass	0.35	0.14	1.19	16	log-log
	Swimming speed vs dry body mass	0.346	0.166	0.42	16	log-log
Morphology	Wing area vs wet body mass	0.55	0.25	0.947	18	log-log
	Tracheal diameter vs wet body mass	0.21	0.11	0.46	13	log-log
	Length vs wet body mass	0.3	0.154	0.559	14	log-log
	Wing length vs dry body mass	1.369	0.676	2.321	17	linear
	Ash free dry mass vs body length	2.741	2.01	4.14	52	log-log
	Dry mass vs body length	2.79	0.51	4.69	321	log-log
	Dry mass vs wet body mass	0.33	0.232	2.286	10	linear
	Dry mass vs wet body mass	0.85	0.41	1.272	25	log-log
	Lamellae dry mass vs dry body mass	0.351	0.211	0.834	17	log-log
	Tracheal dry mass vs wet body mass	0.945	0.74	1.16	12	linear
	Wing dry mass vs dry body mass	0.765	0.58	0.9	12	log-log
	Wet mass vs body length	2.72	1.205	4.48	25	log-log
	Abdomen wet mass vs thorax length	3.59	2.063	4.9	12	log-log
	Abdomen wet mass vs wet body mass	1.365	1.14	1.95	22	log-log
	Testes wet mass vs wet body mass	1.303	0.74	8.858	15	log-log
	Eye span vs body length	1.034	0.199	3.027	60	linear
Physiology	Active metabolic rate vs wet body mass	0.76	0.37	0.92	12	log-log
	Mass-specific metabolic rate vs dry body mass	-0.22	-0.73	-0.14	16	linear
	Mass-specific metabolic rate vs dry body mass	-0.31	-0.99	-0.02	15	log-log
	Mass-specific metabolic rate vs wet body mass	-0.225	-0.88	-0.02	18	log-log

	Metabolic rate vs dry body mass	0.72	0.35	1.07	29	log-log
	Metabolic rate vs wet body mass	0.7875	0.11	1.09	148	log-log
	Ventilation volume vs wet body mass	1.045	0.576	1.59	10	log-log

Ontogenetic

Category	**Relationship**	**Median**	**Min**	**Max**	**N**	**form**
Physiology	Metabolic rate vs wet body mass	0.91	0.52	0.99	18	log-log

Table 2

Mean and phylogenetically estimated mean for each scaling level for the relationship of metabolic rate and body mass. Standard error and 95% confidence intervals are also provided.

Level	n	Uncorrected			Corrected		
		mean±s.e.	95% CI		mean±s.e.	95% CI	
			lower	upper		lower	upper
Interspecific	68	0.746 ±0.017	0.712	0.779	0.773±0.068	0.639	0.907
Intraspecific	162	0.764±0.152	0.734	0.794	0.727±0.132	0.466	0.988
Ontogenetic	25	0.811±0.033	0.742	0.88	0.715±0.285	0.153	1.277

Figures

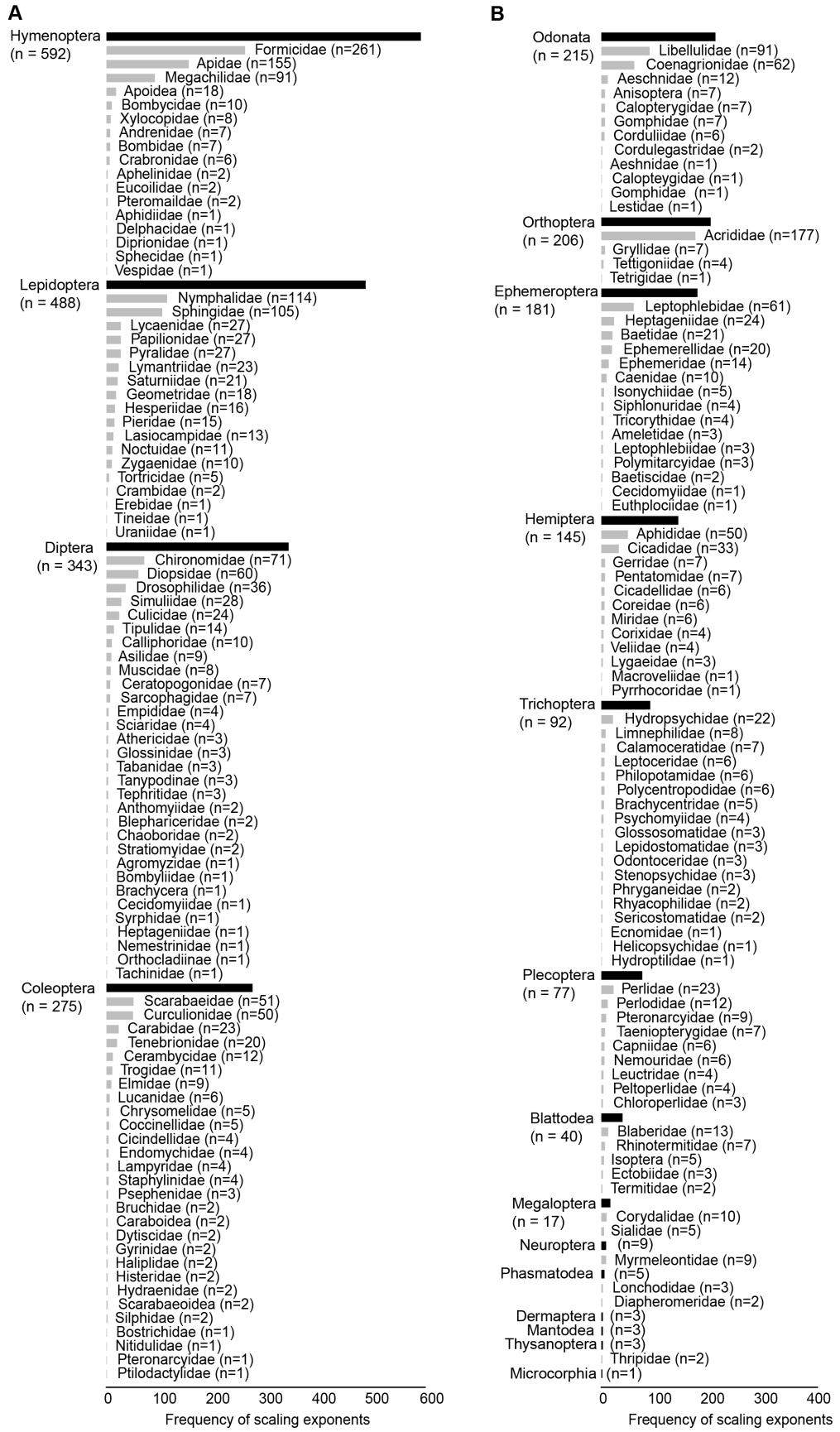
A
Hymenoptera (n = 592)
Formicidae (n=261)
Apidae (n=155)
Megachilidae (n=91)
Apoidea (n=18)
Bombycidae (n=10)
Xylocopidae (n=8)
Andrenidae (n=7)
Bombidae (n=7)
Crabronidae (n=6)
Aphelinidae (n=2)
Eucoilidae (n=2)
Pteromaildae (n=2)
Aphidiidae (n=1)
Delphacidae (n=1)
Diprionidae (n=1)
Sphecidae (n=1)
Vespidae (n=1)
Lepidoptera (n = 488)
Nymphalidae (n=114)
Sphingidae (n=105)
Lycaenidae (n=27)
Papilionidae (n=27)
Pyralidae (n=27)
Lymantriidae (n=23)
Saturniidae (n=21)
Geometridae (n=18)
Hesperiidae (n=16)
Pieridae (n=15)
Lasiocampidae (n=13)
Noctuidae (n=11)
Zygaenidae (n=10)
Tortricidae (n=5)
Crambidae (n=2)
Erebidae (n=1)
Tineidae (n=1)
Uraniidae (n=1)
Diptera (n = 343)
Chironomidae (n=71)
Diopsidae (n=60)
Drosophilidae (n=36)
Simuliidae (n=28)
Culicidae (n=24)
Tipulidae (n=14)
Calliphoridae (n=10)
Asilidae (n=9)
Muscidae (n=8)
Ceratopogonidae (n=7)
Sarcophagidae (n=7)
Empididae (n=4)
Sciaridae (n=4)
Athericidae (n=3)
Glossinidae (n=3)
Tabanidae (n=3)
Tanypodinae (n=3)
Tephritidae (n=3)
Anthomyiidae (n=2)
Blephariceridae (n=2)
Chaoboridae (n=2)
Stratiomyidae (n=2)
Agromyzidae (n=1)
Bombyliidae (n=1)
Brachycera (n=1)
Cecidomyiidae (n=1)
Syrphidae (n=1)
Heptageniidae (n=1)
Nemestrinidae (n=1)
Orthocladiinae (n=1)
Tachinidae (n=1)
Coleoptera (n = 275)
Scarabaeidae (n=51)
Curculionidae (n=50)
Carabidae (n=23)
Tenebrionidae (n=20)
Cerambycidae (n=12)
Trogidae (n=11)
Elmidae (n=9)
Lucanidae (n=6)
Chrysomelidae (n=5)
Coccinellidae (n=5)
Cicindellidae (n=4)
Endomychidae (n=4)
Lampyridae (n=4)
Staphylinidae (n=4)
Psephenidae (n=3)
Bruchidae (n=2)
Caraboidea (n=2)
Dytiscidae (n=2)
Gyrinidae (n=2)
Haliplidae (n=2)
Histeridae (n=2)
Hydraenidae (n=2)
Scarabaeoidea (n=2)
Silphidae (n=2)
Bostrichidae (n=1)
Nitidulidae (n=1)
Pteronarcyidae (n=1)
Ptilodactylidae (n=1)
0
100
200
300
400
500
600
Frequency of scaling exponents
B
Odonata (n = 215)
Libellulidae (n=91)
Coenagrionidae (n=62)
Aeschnidae (n=12)
Anisoptera (n=7)
Calopterygidae (n=7)
Gomphidae (n=7)
Corduliidae (n=6)
Cordulegastridae (n=2)
Aeshnidae (n=1)
Calopteygidae (n=1)
Gomphidae (n=1)
Lestidae (n=1)
Orthoptera (n = 206)
Acrididae (n=177)
Gryllidae (n=7)
Tettigoniidae (n=4)
Tetrigidae (n=1)
Ephemeroptera (n = 181)
Leptophlebidae (n=61)
Heptageniidae (n=24)
Baetidae (n=21)
Ephemerellidae (n=20)
Ephemeridae (n=14)
Caenidae (n=10)
Isonychiidae (n=5)
Siphlonuridae (n=4)
Tricorythidae (n=4)
Ameletidae (n=3)
Leptophlebiidae (n=3)
Polymitarcyidae (n=3)
Baetiscidae (n=2)
Cecidomyiidae (n=1)
Euthplociidae (n=1)
Hemiptera (n = 145)
Aphididae (n=50)
Cicadidae (n=33)
Gerridae (n=7)
Pentatomidae (n=7)
Cicadellidae (n=6)
Coreidae (n=6)
Miridae (n=6)
Corixidae (n=4)
Veliidae (n=4)
Lygaeidae (n=3)
Macroveliidae (n=1)
Pyrrhocoridae (n=1)
Trichoptera (n = 92)
Hydropsychidae (n=22)
Limnephilidae (n=8)
Calamoceratidae (n=7)
Leptoceridae (n=6)
Philopotamidae (n=6)
Polycentropodidae (n=6)
Brachycentridae (n=5)
Psychomyiidae (n=4)
Glossosomatidae (n=3)
Lepidostomatidae (n=3)
Odontoceridae (n=3)
Stenopsychidae (n=3)
Phryganeidae (n=2)
Rhyacophilidae (n=2)
Sericostomatidae (n=2)
Ecnomidae (n=1)
Helicopsychidae (n=1)
Hydroptilidae (n=1)
Plecoptera (n = 77)
Perlidae (n=23)
Perlodidae (n=12)
Pteronarcyidae (n=9)
Taeniopterygidae (n=7)
Capniidae (n=6)
Nemouridae (n=6)
Leuctridae (n=4)
Peltoperlidae (n=4)
Chloroperlidae (n=3)
Blattodea (n = 40)
Blaberidae (n=13)
Rhinotermitidae (n=7)
Isoptera (n=5)
Ectobiidae (n=3)
Termitidae (n=2)
Megaloptera (n = 17)
Corydalidae (n=10)
Sialidae (n=5)
Neuroptera (n=9)
Myrmeleontidae (n=9)
Phasmatodea (n=5)
Lonchodidae (n=3)
Diapheromeridae (n=2)
Dermaptera (n=3)
Mantodea (n=3)
Thysanoptera (n=3)
Thripidae (n=2)
Microcorphia (n=1)
0
100
200
300
400
Frequency of scaling exponents

Figure 1

Frequency of scaling exponents by Order and Family. Instances where multiple Orders were investigated (classified as “Insecta” n = 45) are not included. Data are presented from the most frequent Order to the least frequent Order and are separated in two panels for aesthetic purposes, with panel **A** showing the four most frequent Orders and panel **B** showing the remaining Orders.

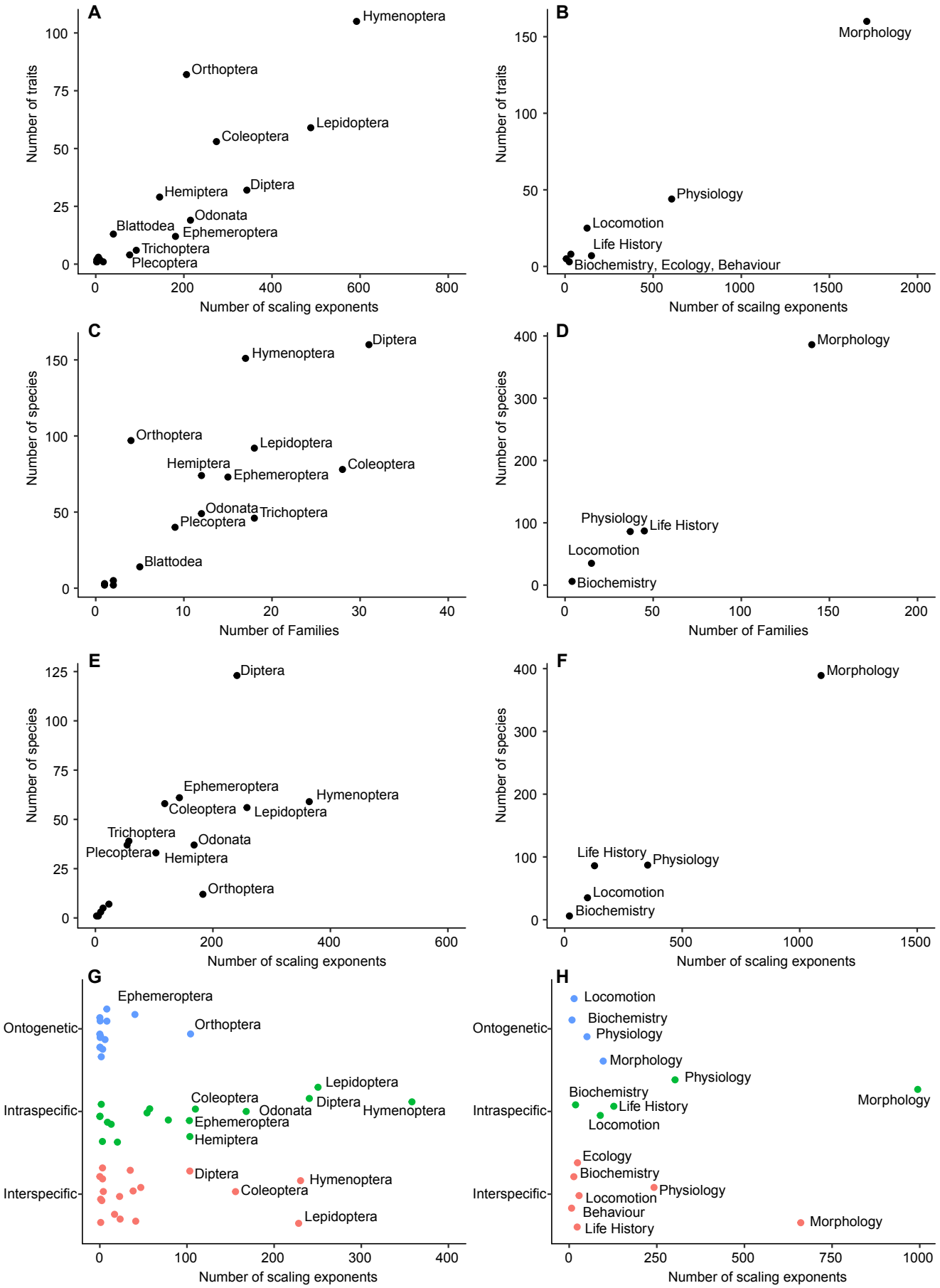
A
Number of traits
Number of scaling exponents
Hymenoptera
Orthoptera
Lepidoptera
Coleoptera
Hemiptera
Diptera
Odonata
Blattodea
Ephemeroptera
Trichoptera
Plecoptera
B
Number of traits
Number of scailng exponents
Morphology
Physiology
Locomotion
Life History
Biochemistry, Ecology, Behaviour
C
Number of species
Number of Families
Diptera
Hymenoptera
Orthoptera
Lepidoptera
Hemiptera
Coleoptera
Ephemeroptera
Odonata
Trichoptera
Plecoptera
Blattodea
D
Number of species
Number of Families
Morphology
Physiology
Life History
Locomotion
Biochemistry
E
Number of species
Number of scaling exponents
Diptera
Ephemeroptera
Hymenoptera
Coleoptera
Lepidoptera
Trichoptera
Odonata
Plecoptera
Hemiptera
Orthoptera
F
Number of species
Number of scaling exponents
Morphology
Life History
Physiology
Locomotion
Biochemistry
G
Ontogenetic
Intraspecific
Interspecific
Number of scaling exponents
Ephemeroptera
Orthoptera
Lepidoptera
Coleoptera
Diptera
Hymenoptera
Odonata
Ephemeroptera
Hemiptera
Diptera
Hymenoptera
Coleoptera
Lepidoptera
H
Ontogenetic
Intraspecific
Interspecific
Number of scaling exponents
Locomotion
Biochemistry
Physiology
Morphology
Physiology
Biochemistry
Life History
Morphology
Locomotion
Ecology
Biochemistry
Physiology
Locomotion
Behaviour
Life History
Morphology

Figure 2

Overview of the study of scaling in insects. Panels **A**, **C, E and G** show an overview of scaling across insect Orders and panels **B**, **D**, **F** and **H**, show an overview of scaling across categories. Specifically, panels **A** and **B** show the number of scaling exponents versus the number of traits, panels **C** and **D** show the number of Families versus the number of species, panels **E** and **F** show the number of scaling exponents versus the number of species (for intraspecific and ontogenetic scaling only), and **G** and **H** show the number of scaling exponents per scaling level (with interspecific values in red, intraspecific values in green and ontogenetic values in blue). Labels for Orders and categories with low frequencies were removed for aesthetic purposes. Values are provided in Tables A2, A3, for panels **A** – **F** and in Tables A5, A6 for panels **G** and **H**.

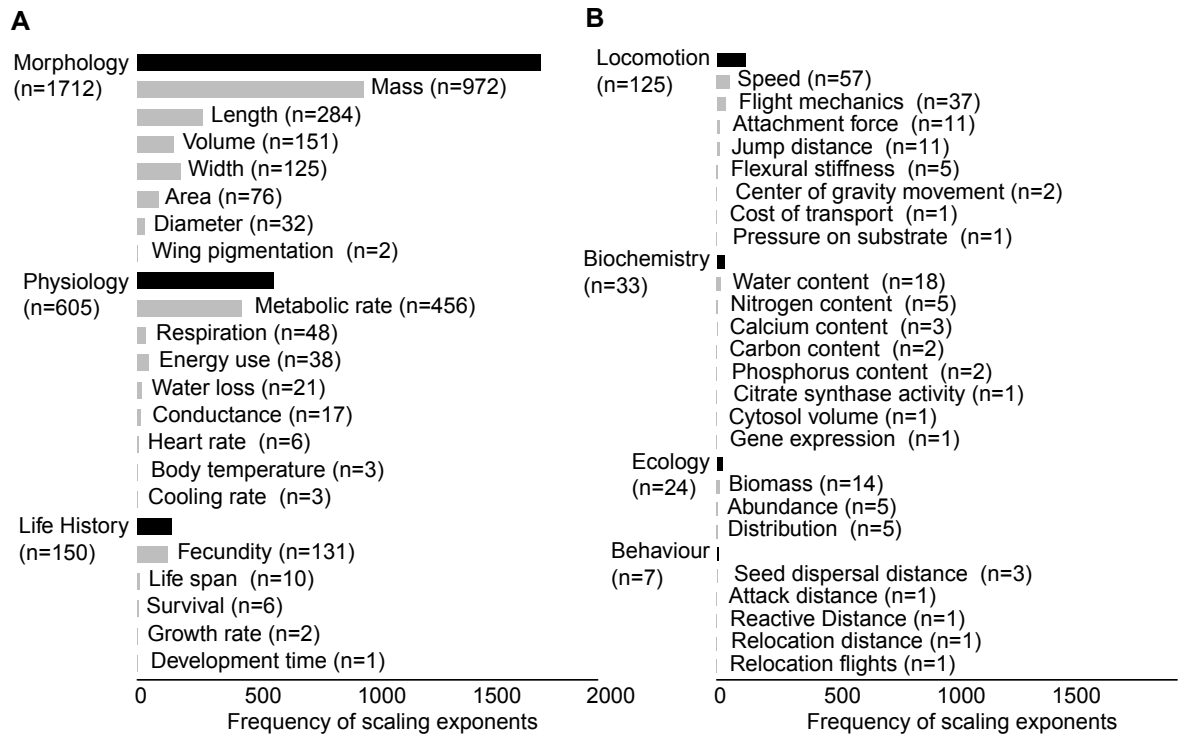

Figure 3

Frequency of scaling exponents for major and minor categories of scaling in insects. Minor categories were instances where measurements pertained to a common trait. For example the minor category "Metabolic rate" includes traits such as standard metabolic rate, active metabolic rate and mass-specific metabolic rate (see Table A1 for traits in each category). Data are presented from the most frequent category to the least frequent category and are separated into two panels for aesthetic purposes i.e. panel **A** shows data for the three most frequent categories whilst panel **B** shows the remaining categories.

Appendix A: Supplementary data

Table A1

Traits (y) in the dataset grouped by major and minor categories.

Major category	Minor category	Trait/s
Behaviour	Attack distance	attack distance
	Reactive Distance	reactive distance
	Relocation distance	relocation distance
	Relocation flights	relocation flights
	Seed dispersal distance	seed dispersal distance
Biochemistry	Calcium content	calcium content
	Carbon content	carbon content
	Citrate synthase activity	citrate synthase activity
	Cytosol volume	cytosol volume
	Gene expression	gene expression
	Nitrogen content	nitrogen content
	Phosphorus content	phosphorus content
	Water content	water content
Ecology	Abundance	abundance
	Biomass	biomass
	Distribution	distribution
Life History	Development time	development time
	Fecundity	number of nymphs, number of eggs, number of ovarioles
	Growth rate	growth rate
	Life span	life span
	Survival	survival
Locomotion	Attachment force	attachment force (per body weight), friction force (average), friction force (maximum), ground reaction force, vertical force (lifting load), vertical force (maximum)
Locomotion	Centre of gravity movement	centre of gravity movement
	Cost of transport	cost of transport
	Flexural stiffness	tensile elasticity, flexural storage stiffness
	Flight mechanics	gas density for hovering (minimal), stroke volume (estimated), wing loading, wingbeat frequency
	Jump distance	jump distance
	Pressure on substrate	pressure on substrate
	Speed	flight speed, running speed, speed lost, stride frequency, stride length, swimming speed

Morphology	Area	area (air sac abdomen), area (air sacs), area (body), area (abdomen), area (brain), area (cocoon), area (head orifice cross sectional area) , area (head trachea cross sectional area) , area (leg orifice cross sectional area) , area (leg trachea cross sectional area) , area (mitochondrial inner membrane), area (pulvillus), area (testis), area (thoracic air sac), area (thorax), area (total lamellar surface), area (trachea outer epidermal surface area), area (wing), area (egg), respiratory structures, wing aspect ratio
	Diameter	diameter (breaking joint), diameter (claw tip), diameter (diastolic), diameter (eye), diameter (systolic), diameter (thoracic), diameter (thorax diameter), diameter (tibia mesothoracic legs), diameter (tibia metathoracic legs), diameter (tracheal)
	Length	length (egg), length (body), length (10th dorsal transverse tracheae), length (6th dorsal transverse tracheae), length (abdomen), length (abdominal tergite 2), length (abdominal tergite 3), length (barsitarsus), length (elytron), length (embryo), length (eye), length (femur and tibia), length (femur), length (fifth segment), length (forceps), length (fore tibia), length (forewing), length (front leg), length (gena), length (gut), length (head capsule), length (head), length (hind tibia), length (horn), length (intertegular), length (leg), length (mandible), length (mesonotum), length (mesoscutal), length (proboscis), length (propodeal), length (scape), length (tergum 1), length (thorax), length (tibia), length (wing)
	Mass	dry mass (body, ash free), wet mass (cocoon), dry mass (body), dry mass (spermatophore), wet mass (ejaculate), dry mass (exoskeletal chitin), dry mass (fat), dry mass (femoral exoskeleton) , dry mass (flight muscle), dry mass (genitalia), dry mass (head), dry mass (lamellae), dry mass (lean), dry mass (muscle), dry mass (prothorax), dry mass (thoracic), dry mass (tracheal mass), dry mass (wing), wet mass (egg), mass (cuticle), mass (femur), mass (thoracic muscle), provision mass, wet mass, wet mass (abdomen), wet mass (adult 45 days after pupation), wet mass (at the end of wintering), wet mass (before wintering), wet mass (brain), wet mass (extensor tibia muscle), wet mass (femur), wet mass (testes), wet mass (thoracic)
	Volume	volume (air sac), volume (respiratory structures), volume (egg), volume (body), volume (brain), volume (femur connective tissue), volume (femur exoskeleton), volume (femur haemolymph), volume (femur muscle), volume (femur nerve), volume (femur tendon), volume (femur), volume (gut), volume (thoracic), volume (tracheal)

	Width	width (respiratory structures), width (cocoon), distance (intermandibular), distance (intertegular), distance (mandible/eye), width (egg), eye span, width (body), width (10th dorsal transverse tracheae), width (4th abdominal segment), width (6th dorsal transverse tracheae), width (8th abdominal segment), width (abdomen), width (abdominal tergite 2), width (abdominal tergite 3), width (clypeus), width (cuticle thickness), width (eye), width (forewing), width (head), width (mesonotum), width (mesoscutal), width (pronotum), width (propodeal), width (tergum 1), width (thoracic), width (tibia), width (wing)
	Wing pigmentation	wing pigmentation
Physiology	Body temperature	body temperature
	Conductance	conductance
	Cooling rate	cooling rate
	Energy use	energetic content, energy expenditure per wing stroke, energy use (cost of running),induced power, induced power (muscle, mass-specific), inertial power requirements, jump energy, kinetic energy, muscle efficiency, mechanical power output (zero elastic energy storage), metabolic energy, metabolic power (aerodynamic), overall efficiency (hovering, perfect elastic energy storage), overall efficiency (hovering, zero elastic energy storage), power (total for flight), power for flight (centre of body), power input, power output (average), power output (mass-specific), power output (mechanical, perfect elastic energy storage), power output (mechanical, zero elastic energy storage), power output (muscle mass-specific), power output (peak), power output (total), profile power, profile power requirements
	Heart rate	cardiac output (estimated), heartrate efficiency (hovering, perfect elastic energy storage), myofibrillar efficiency (apparent), myofibrillar efficiency (gross)
	Metabolic rate	metabolic rate (active), metabolic rate (mass- specific), metabolic rate, metabolic rate (hibernating), metabolic rate (resting)
	Respiration	ventilation frequency, ventilation rate, ventilation volume
	Water loss	water loss
Undefined	Undefined	egestion, food assimilation, food diameter, ingestion rate , load size , pit diameter , Reynolds number , density (volume density in muscle-lumen only), density (volume density in muscle), mitochondrial volume in muscle, myofibril + sarcoplasmic reticulum volume in muscle, nuclei volume in muscle

Table A2

Frequency of scaling exponents by scaling level (interspecific, intraspecific and ontogenetic) and Order.

Order*	Frequency of scaling exponents			
	Interspecific	**Intraspecific**	**Ontogenetic**	**Total**
Hymenoptera	228	358	6	592
Lepidoptera	230	250	8	488
Diptera	103	240	1	344
Coleoptera	156	110	8	274
Odonata	47	168	0	215
Orthoptera	23	79	104	206
Ephemeroptera	38	103	40	181
Hemiptera	41	103	0	144
Trichoptera	35	57	0	92
Plecoptera	23	54	0	77
Blattodea	17	20	3	40
Megaloptera	4	13	0	17
Neuroptera	0	9	0	9
Phasmatodea	1	3	2	6
Dermaptera	3	0	0	3
Mantodea	3	0	0	3
Thysanoptera	1	2	0	3
Microcoryphia	2	0	0	2
Total	**955**	**1569**	**172**	**2696**

*Does not include instances where scaling has been investigated for multiple insect orders, classified in the dataset as Insecta (n = 45)

Table A3

Number of Families and species per Order, along with the number of traits available per Order.

Order	Frequency		
	Traits	**Families**	**Species**
Hymenoptera	105	14	59
Lepidoptera	59	17	56
Diptera	32	24	123
Coleoptera	54	20	58
Odonata	19	12	37
Orthoptera	67	4	12
Ephemeroptera	12	14	61
Hemiptera	29	11	33
Trichoptera	6	15	39
Plecoptera	4	9	37
Blattodea	13	4	7
Megaloptera	1	2	5
Neuroptera	2	1	3
Phasmatodea	3	2	1
Dermaptera	2	0	-
Mantodea	1	0	-
Thysanoptera	2	1	1
Microcoryphia	1	0	-

Table A4

The insect species for which scaling relationships were most commonly investigated. Frequency of scaling exponents and number of traits investigated are included for species with >9 scaling exponents, in order from highest number of scaling exponents to lowest.

Species	**Order**	**Frequency**	
		Scaling exponents	**Traits**
Locusta migratoria	Orthoptera	88	29
Manduca sexta	Lepidoptera	77	17
Leptophlebia cupida	Ephemeroptera	50	9
Osmia lignaria propinqua	Hymenoptera	46	8
Scaptotrigona poscitca	Hymenoptera	45	15
Bombus impatiens	Hymenoptera	42	17
Osmia cornuta	Hymenoptera	41	11
Schistocerca americana	Orthoptera	40	24
Erythemis simplicicollis	Odonata	25	1
Pachydiplax longipennis	Odonata	25	2
Achroia grisella	Lepidoptera	20	1
Melanoplus sanguinipes	Orthoptera	19	3
Pararge aegeria	Lepidoptera	18	5
Aedes albopictus	Diptera	17	1
Schistocerca gregaria	Orthoptera	17	14
Libellula incesta	Odonata	16	1
Drepanosiphum platanoidis	Hemiptera	14	7
Solenopsis invicta	Hymenoptera	14	4
Atta colombica	Hymenoptera	13	5
Atta sexdens rubropilosa	Hymenoptera	13	2
Drosophila melanogaster	Diptera	12	7
Atta laevigata	Hymenoptera	12	1
Libellula lydia	Odonata	12	1
Trypoxylus dichotomus septentrionalis	Coleoptera	11	3
Argia translata	Odonata	11	4
Pyrrhosoma nymphula	Odonata	11	3
Bombyx mori	Hymenoptera	10	2
Malacosoma neustria	Lepidoptera	10	2
Zygaena trifolii	Lepidoptera	10	1

Table A5

Frequency of scaling exponents per category and scaling level.

Category	Frequency of scaling exponents			
	Interspecific*	**Intraspecific**	**Ontogenetic**	**Total**
Morphology	661	994	97	1752
Physiology	242	302	51	595
Life History	23	127	0	150
Locomotion	28	89	8	125
Biochemistry	13	18	2	33
Ecology	24	0	0	24
Behaviour	7	0	0	7
Undefined	2	39	14	55
Total	**1000**	**1569**	**172**	**2741**

* Includes instances where scaling has been investigated for multiple insect orders, classified in the dataset as Insecta (n = 45).

Table A6

Number of traits, Orders, Families per category.

Category	Frequency			
	Traits	**Orders**	**Families**	**Species**
Morphology	150	18	142	389
Physiology	43	11	47	87
Life History	8	8	48	86
Locomotion	22	8	18	35
Biochemistry	8	5	7	6
Ecology	3	3	4	0
Behaviour	5	3	3	0
Undefined	16	7	11	16

Chapter 2

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

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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 examined the effects of constant (10 to 30°C in 5°C increments) and fluctuating (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 (CT_{min}), critical thermal maximum (CT_{max}), and upper lethal temperature (ULT_{50})) in ten species of springtails (Collembola) from three families (Isotomidae 7 spp.; Entomobryidae 2 spp.; Onychiuridae 1 sp.). The lowest mean CT_{min} value recorded here was -3.56±1.0°C for *Paristoma notabilis* and the highest mean CT_{max} was 43.1±0.8°C for *Hemisotoma thermophila*. The Acclimation Response Ratio for CT_{min} was on average 0.12°C/°C (range: 0.04 to 0.21°C/°C), but was much lower for CT_{max} (mean: 0.017°C/°C, range: -0.015 to 0.047°C/°C) and lower also for ULT_{50} (mean: 0.05°C/°C, range: -0.007 to 0.14°C/°C). Fluctuating versus constant temperature treatments 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 using constant temperature conditions to assess adult phenotypic plasticity of these thermal performance traits across a range of temperatures can thus be applied to a broader range of circumstances in springtails.

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, mediated by a suite of traits (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, however, attention has been drawn to the need for better understanding of trait variation in an environmental change context because of the influence on trait values of different assessment circumstances, including constant versus fluctuating temperatures (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).

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), possibly limiting the extent to which conclusions from laboratory studies can be extended to the field (Ma et al. 2015). Such limitations may also affect the accuracy of forecast models incorporating trait data (Niehaus et al. 2012; Valladares et al. 2014; Dowd et al. 2015). Because of the growing use of trait data to understand community assembly (e.g. Start et al. 2018; Miller et al. 2019), and to model both species and system responses to environmental change (Deutsch et al. 2018; Pinsky et al. 2019), 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). 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).

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 (CT_{min}), critical thermal maximum (CT_{max}), and upper lethal limits (ULT_{50}) – 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 CT_{min} and CT_{max} 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 from soil and leaf litter samples collected from the Jock Marshall Reserve (JMR), Monash University Clayton Campus in Victoria Australia (S37.9096°, E145.1400°) using a Berlese-Tullgren funnel system (Southwood and Henderson 2009). The JMR comprises open woodlands with an understory of grasses and herbs. Mean annual precipitation is approximately 705 mm, with a mean summer (Dec-Feb) maximum of 25.3°C, and a mean winter (Jun-Aug) minimum of 6.6°C (1971-2019 data, 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) with the assistance of taxonomic experts following previous approaches (described in full in

Janion-Scheepers et al. 2018). Single species cultures (F0) were established of 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.

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), were 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. Eggs were collected from cultures three times per week and assigned to pots at a density of 50-100 eggs. At maturity, first generation 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 JMR between December 2015 and February 2016 (the hottest months) using Thermochron iButtons™ (model DS1920G, Maxim Integrated, San Jose, CA, USA) placed level with soil surface below the leaf litter and humus layers. 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) acclimation treatments were used to assess critical thermal limits and upper lethal limits based on the soil temperatures (Table B1). Thus, constant temperature acclimation treatments 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 acclimation treatments 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 7 hours in increments of ~0.7°C every half an hour. Temperature decreased from the highest to the lowest fluctuation over 17 hours at a rate of ~0.3°C every half hour. All acclimation treatments were undertaken in controlled temperature incubators (MIR-154, SANYO, Japan, for constant temperature acclimation treatments, and KB 115 (E3.1) Binder cooling incubator, Tuttlingen, Germany for fluctuating temperature acclimation treatments) and were monitored using Thermochron iButtons™ (model:DS1920G, Maxim Integrated, San Jose, CA, USA), under 12h light:12h dark photoperiod. F2 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). *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, comparisons of constant and fluctuating acclimation

treatments with a mean of 30°C were not undertaken for these species for examination of critical thermal limits. In the examination of upper lethal limits, 30°C acclimation treatments (FT and CT) were not assessed 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) that was 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 (CT_{max}) or lowered (CT_{min}) 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 CT_{max} or slower for CT_{min}). 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 (CT_{min}) or maximum (CT_{max}) temperature at which springtails lost co-ordinated muscle function, i.e. when they were no longer able to right themselves when tipped over onto their side or back with a paintbrush (as in Janion-Scheepers et al. 2018). 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

40-60 individual springtails for each species and acclimation. Springtails were separated into two replicates of 20-30 individuals for CT_{min} and CT_{max} measurements. Individuals were only used once in either a CT_{min} or a CT_{max} trial.

2.4 Upper lethal temperature

Mortality assays were used to determine how constant and fluctuating acclimation temperatures affected the response of upper lethal temperature on springtails. Mortality was measured over a range of experimental temperatures for each acclimation (Table B2). Experimental temperatures varied between species as the temperature range required to observe mortality between 0 and 100% was different depending on the thermal sensitivity of the species being tested. However, in general, experimental temperatures occurred between 32°C and 44°C and increased in 2°C increments from when no mortality was observed until complete mortality was observed. Three replicates of 20-30 springtails were used for each experimental temperature and acclimation, and springtails were not used more than once for each temperature. Adult F2 springtails were placed into glass McCartney vials (28ml) with a moistened Plaster-of-Paris substrate. 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, then removed and allowed to recover for 24 hours at 20°C after which mortality was documented. Mortality was considered as springtails which 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 (RDXL 12SD, Omega Engineering, USA) was used to obtain an accurate measure of water bath temperature, which did not vary more than ±0.2°C.

2.5 Statistical analysis

Generalised linear models were used to assess the effect of acclimation temperature and the form of the acclimation treatment (constant vs. fluctuating) on both CT_{min} and CT_{max} implemented in R v. 3.5.2 (R Core Team 2018), using the software platform RStudio release 1.2.1335-1 (RStudio Team 2016). The slope of the relationship for the constant treatment was used as an estimate of the acclimation response ratio (ARR) in the form of °C change in critical thermal limit per °C change in acclimation temperature (see Gunderson and Stillman 2015). Estimation statistics (Cumming 2014; Ho et al. 2019), which use effect sizes and confidence intervals to determine the magnitude of difference between variables, were then used to examine more closely the extent to which thermal limits varied significantly between the constant and fluctuating thermal regimes. Estimation plots (sometimes known as Cumming plots) were generated in R v. 3.5.2 (R Core Team 2018), using the RStudio platform (RStudio Team 2016) and the "dabestr" v0.2.2 package (Ho et al. 2019).

The upper lethal temperature at which 50% mortality occurred (ULT_{50}) was calculated from the mortality assays, using a logit analysis in the package "ecotox" v1.3.3 implemented in R v. 3.5.2 (R Core Team 2018), using the RStudio platform (RStudio Team 2016). Three ULT_{50} values were calculated for each of the 10-springtail species from the three replicates of mortality data obtained for each of the experimental temperatures and acclimation treatments. Generalised linear models were used to assess the effect of acclimation temperature and the form of the acclimation treatment (constant vs. fluctuating) on ULT_{50} implemented in R v. 3.5.2 (R Core Team 2018), using the software platform RStudio (RStudio Team 2016). As above, the slope of the relationship for the constant treatment was used as an estimate of the acclimation response ratio (ARR) in the form of °C change in ULT_{50} per °C change in acclimation temperature.

3. Results

3.1 How do acclimation treatments affect thermal traits?

Critical thermal minima were significantly influenced by acclimation temperature in all 10 of the species investigated. On average, across all species, springtails responded to constant temperature acclimation treatments between 10°C and 30°C by increasing thermal limits as acclimation temperature increased, with the mean ARR equal to 0.123°C/°C (range 0.041 to 0.208°C/°C) (Fig. 1; Table 1; Table B3; Table B4). By contrast, the influence of short-term acclimation on CT_{max} was much less pronounced, with a mean ARR of 0.017°C/°C (range -0.015 to 0.047°C/°C) across the 10°C to 30°C constant acclimation treatments, with no significant effect of acclimation treatment in three of the species (*Desoria trispinata, Mucrosomia caeca,* and *Sinella* sp.) (Fig. 1; Table 1; Table B3; Table B4). In the case of ULT_{50}, acclimation treatments showed slightly more effect than for CT_{max}, with a mean ARR of 0.049°C/°C and range from -0.007 to 0.135°C/°C (Fig. 2; Table 1), but also showed no significant response to acclimation in three of the species (*Cryptopygus* sp., *Folsomia* sp.*,* and *Lepidocyrtus* sp.).

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

Even though there was a significant effect of fluctuating temperature acclimation treatments on the response of CT_{min}, CT_{max} and ULT_{50} (Table 1), in general the response to fluctuating temperature acclimation treatments was either not significantly different to that of the constant temperature acclimation treatments, or small and inconsistent in direction. Estimation statistics, which were used to identify differences between fluctuating and constant temperatures for CT_{min} and CT_{max}, revealed that effect sizes for the difference between the two acclimation regimes 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 2). The mean estimated absolute difference between the fluctuating and constant treatments for CT_{min}

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 CT_{max}, 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 CT_{min} (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. 3, 4, Table B5; Table B6).

Fluctuating versus constant temperatures typically also had little influence on ULT_{50}, with only three species showing a significant effect (Fig. 2; Table 1). Furthermore, estimation plots showing the effect of fluctuating temperatures on ULT_{50} across acclimation treatments (Fig. 5; Table B6), showed small mean differences between fluctuating and constant temperatures with the 95% confidence intervals of all acclimation treatments overlapping with zero, indicating overall fluctuating temperatures did not elicit a different response in ULT_{50} than constant temperature acclimation treatments.

4. Discussion

On average, the sizes of the responses of CT_{min}, CT_{max} and ULT_{50} 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 CT_{min} than for CT_{max}, and small for ULT_{50}, though with some species showing effects for CT_{max} as large as or larger than those found for CT_{min}, 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 CT_{max} can be found among springtails, when responses to acclimation and laboratory selection are constrained, thus remains unexplained (Janion-Scheepers et al. 2018). One 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).

Indeed, 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). Moreover, 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), whereas 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 CT_{min}, CT_{max} and ULT_{50}. Even in the four species which survived the 30°C fluctuating temperature treatment, the influences on CT_{max} were variable, with fluctuating temperatures having either no significant effect (*H. thermophila*), a decline in CT_{max} (*I. loftyensis*) as theory predicts and other studies have found, or conversely an increase in

CT_{max} (*Cryptopygus* sp.; *Sinella* sp.). Such outcomes may not be especially surprising for CT_{max} and ULT_{50} 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 CT_{min} (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 CT_{min} was 1.63°C, compared with the largest acclimation effect of 4.2°C for CT_{min} 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), though 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, and 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). 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 ULT_{50} 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, and thus also reliable for investigating the likely responses of these organisms to changing environments.

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Tables

Table 1

Summary outcomes of generalised linear models (Gaussian distribution, identity link) comparing the effects of constant (CT) and fluctuating (FT) temperature acclimation treatments on thermal tolerance traits in 10 springtail species. Table includes results for critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}), and upper lethal temperature (ULT_{50}). Results with a significantly negative Treatment (boldface) and significantly positive Slope indicate that thermal traits for fluctuating temperatures are higher at high temperature acclimation treatments and lower at low temperature acclimation treatments in comparision to constant temperature treatments. Results with a positive Treatment and negative Slope (boldface) indicate that thermal traits at constant temperatures are higher at high temperature acclimation treatments and lower at low temperature acclimation treatments in comparison to fluctuating temperature treatments. Results with a positive Treatment and non-significant Slope indicate that thermal traits are higher for fluctuating temperature treatments than constant temperature treatments. Full outcomes provided in Supplementary Table B4.

CT_{min}

Species	**Acclimation**		**Treatment (FT)**		**Slope**	
	Estimate	**P-value**	**Estimate**	**P-value**	**Estimate**	**P-value**
Cryptopygus sp.	0.134	***	**-1.197**	***	0.053	***
Desoria trispinata	0.071	***	-0.426	ns	0.009	ns
Folsomia sp.	0.154	***	0.553	*	**-0.045**	***
Hemisotoma thermophila	0.138	***	**-1.177**	***	0.048	**
Isotopenola loftyensis	0.095	***	0.122	ns	0.003	ns
Lepidocyrtus sp.	0.041	***	**-0.716**	***	0.051	***
Mucrosomia caeca	0.175	***	1.88	***	**-0.111**	***
Orthonychiurus cf. *folsomi*	0.208	***	0.543	*	**-0.039**	**
Parisotoma notabilis	0.115	***	0.813	**	0.002	ns
Sinella sp.	0.106	***	0.458	**	-0.01	ns

CT_{max}

Species	**Acclimation**		**Treatment (FT)**		**Slope**	
	Estimate	**P-value**	**Estimate**	**P-value**	**Estimate**	**P-value**
Cryptopygus sp.	0.022	***	**-0.565**	**	0.048	***
Desoria trispinata	0.004	ns	0.136	ns	-0.001	ns
Folsomia sp.	0.03	***	**-0.588**	**	0.031	***
Hemisotoma thermophila	0.047	***	0.562	**	-0.013	ns
Isotopenola loftyensis	0.023	**	0.495	*	-0.036	**

Lepidocyrtus sp.	-0.015	*	**-1.054**	***	0.066	***
Mucrosomia caeca	-0.004	ns	**-1.878**	***	0.104	***
Orthonychiurus cf. *folsomi*	0.04	***	0.356	ns	-0.024	*
Parisotoma notabilis	0.026	***	**-1.294**	***	0.07	***
Sinella sp.	0	ns	**-0.736**	***	0.03	***

ULT_{50}

Species	**Acclimation**		**Treatment (FT)**		**Slope**	
	Estimate	**P-value**	**Estimate**	**P-value**	**Estimate**	**P-value**
Cryptopygus sp.	0.025	ns	-1.025	ns	0.041	ns
Desoria trispinata	0.038	*	-0.506	ns	0.027	ns
Folsomia sp.	-0.002	ns	**-1.616**	***	0.089	***
Hemisotoma thermophila	0.068	*	-0.201	ns	0.009	ns
Isotopenola loftyensis	0.054	*	0.882	ns	-0.046	ns
Lepidocyrtus sp.	-0.007	ns	-0.732	ns	0.051	ns
Mucrosomia caeca	0.135	***	1.868	*	-0.076	ns
Orthonychiurus cf. *folsomi*	0.046	***	**-1.576**	**	0.069	*
Parisotoma notabilis	0.070	***	-0.260	ns	0.001	ns
Sinella sp.	0.061	**	0.289	ns	-0.007	ns

* 0.01 **0.001 *** <0.001

Table 2

Estimated differences between means (es) (Cumming 2014; Ho et al. 2019) of fluctuating and constant temperature acclimation for critical thermal minimum (CT_{min}) and critical thermal maximum (CT_{max}), per acclimation (10°C to 30°C) and springtail species. Negative es values indicate fluctuating temperature acclimation treatments reduced thermal limits. Boldface values show cases where fluctuating acclimation temperatures were not significantly different from constant temperature acclimation treatments i.e. where 95% confidence intervals overlap with zero.

Species	**Acclimation (°C)**	CT_{min}		CT_{max}	
		es (°C)	**95% CI**	**es (°C)**	**95% CI**
Cryptopygus sp.	10	0.08	**-0.41, 0.72**	0.65	0.39, 0.94
	15	-0.64	-0.99, -0.29	-0.54	-0.75, - 0.29
	20	-1.63	-2.09, -1.26	-0.32	-0.56, -0.06
	25	0.67	0.41, 0.92	1.11	0.885, 1.36
	30	0.72	0.53, 0.92	1.15	0.87, 1.39
Desoria trispinata	10	-1.17	-1.57, -0.84	0.47	0.23, 0.76
	15	0.32	0.06, 0.55	-0.52	-0.69, -0.33
	20	0.32	0.12, 0.52	0.32	0.21, 0.44
	25	-0.11	**-0.38, 0.20**	0.33	0.16, 0.48
	30				
Folsomia sp.	10	-0.45	-0.82, -0.13	-0.01	**-0.25, 0.33**
	15	-0.20	**-0.43, 0.07**	-0.36	-0.52, -0.20
	20	0.02	-0.23, 0.24	-0.21	**-0.44, 0.03**
	25	-0.37	-0.58, -0.18	0.37	0.19, 0.53
	30				
Hemisotoma thermophila	10	-0.70	-1.03, -0.29	0.49	0.21, 0.74
	15	-0.12	-0.42, 0.18	0.57	0.14, 1.0
	20	-1.15	-1.66, -0.74	-0.17	**-0.44, 0.11**
	25	0.72	0.39, 1.07	0.57	0.251, 0.88
	30	0.21	**-0.18, 0.6**	0.10	**-0.19, 0.38**
Isotopenola loftyensis	10	0.62	0.37, 0.89	-0.22	**-0.60, 0.16**
	15	-0.51	-0.67, -0.35	0.13	-0.18, 0.44
	20	0.15	**-0.15, 0.46**	0.57	0.20, 1.08
	25	0.67	0.42, 0.954	-0.90	-1.17, -0.60
	30	-0.04	**-0.49, 0.35**	-0.54	-0.87, -2.1
Lepidocyrtus sp.	10	-0.63	-0.89, -0.37	-0.25	-0.45, -0.02
	15	0.80	0.64, 0.97	-0.06	**-0.27, 0.12**
	20	0.18	**-0.02, 0.31**	-1.30	**-0.37, 0.08**
	25	0.34	0.14, 0.56	0.60	0.40, 0.92
	30				
Mucrosomia caeca	10	0.16	**-0.11, 0.42**	-0.91	-1.09, -0.73

	15	0.88	0.62, 1.14	-0.09	**-0.28, 0.12**
	20	0.18	**-0.2, 0.39**	0.44	0.23, 0.65
	25	-0.30	-0.56, -0.03	0.56	0.38, 0.73
	30				
Orthonychiurus cf. *folsomi*	10	-0.15	**-0.57, 0.24**	-0.02	**-0.18, 0.13**
	15	0.46	0.07, 0.80	0.12	**-0.06, 0.36**
	20	-0.56	-1.07, -0.24	-0.55	-0.76, -0.35
	25	-0.22	**-0.43, 0**	-0.12	**-0.40, 0.04**
	30				
Parisotoma notabilis	10	0.22	**-0.01, 0.42**	-0.94	-1.1, -0.77
	15	1.22	1.00, 1.51	0.02	**-0.16, 0.20**
	20	1.30	0.93, 1.66	0.49	0.37, 0.6
	25	1.19	0.87, 1.48	0.45	0.29, 0.61
	30				
Sinella sp.	10	0.53	0.38, 0.69	1.12	-1.23, -1.02
	15	0.16	0.02, 0.51	0.87	0.68, 1.03
	20	0.37	0.25, 0.51	-1.60	-0.30, -0.02
	25	-0.28	-0.51, -0.07	-0.55	-0.74, -0.37
	30	0.50	0.31, 0.68	0.36	0.05, 0.58

Figures

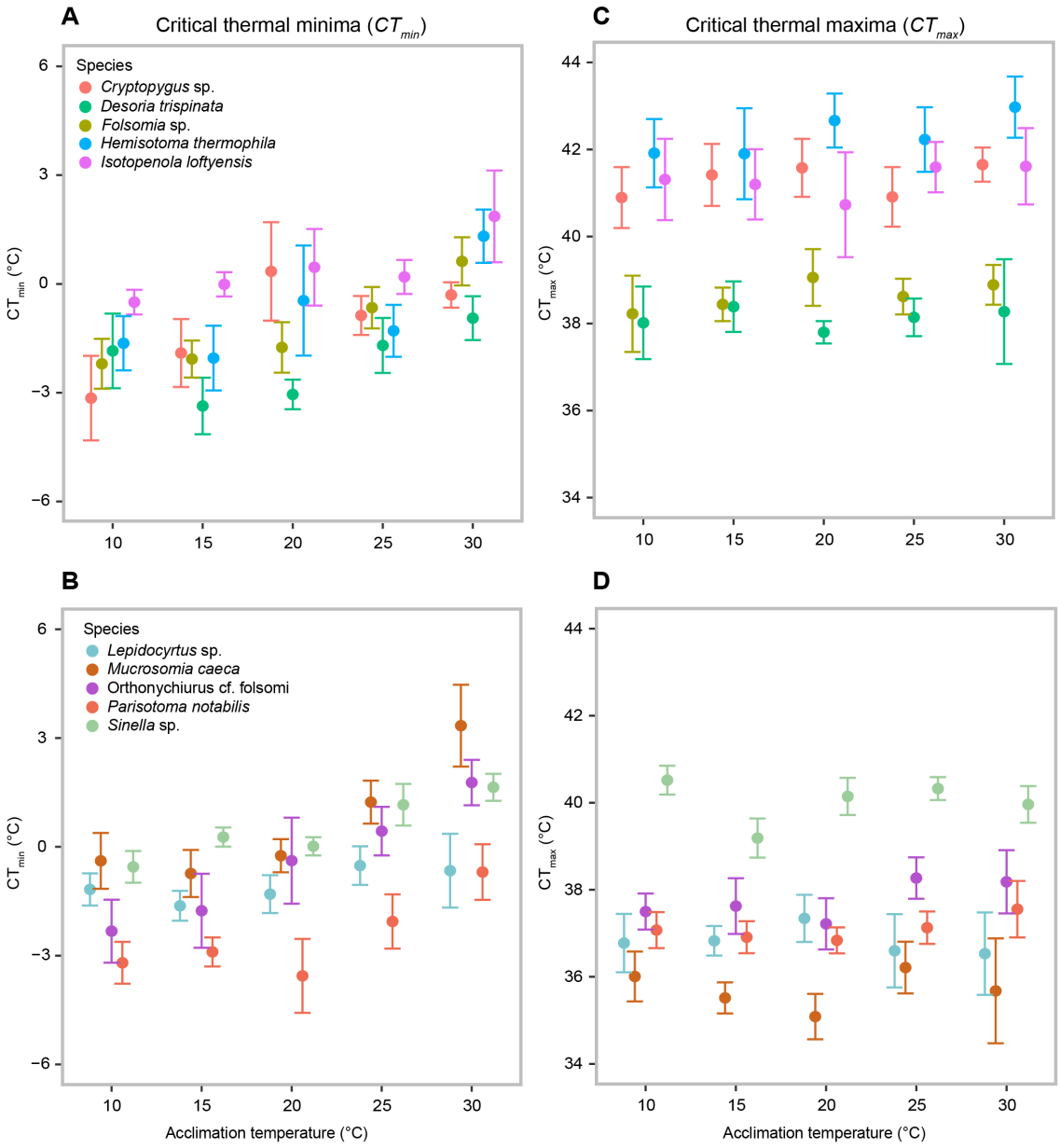

Figure 1

The mean response of critical thermal limits to constant temperature acclimation treatments between 10 and 30°C for 10 springtail species. Plots **A** and **B** show the mean and standard deviation for critical thermal minimum (CT_{min}) and plots **C** and **D** show the mean and standard deviation for critical thermal maximum (CT_{max}). Species are divided between two plots for conveneince, with each colour representing a species. Colours attributed to species are the same for plots **A** and **C** and the same for plots **B** and **D**. See Table B3 for mean, standard deviation and sample size values for each species, acclimation and critical thermal limit (values for fluctuating temperature acclimation treatments are also available here).

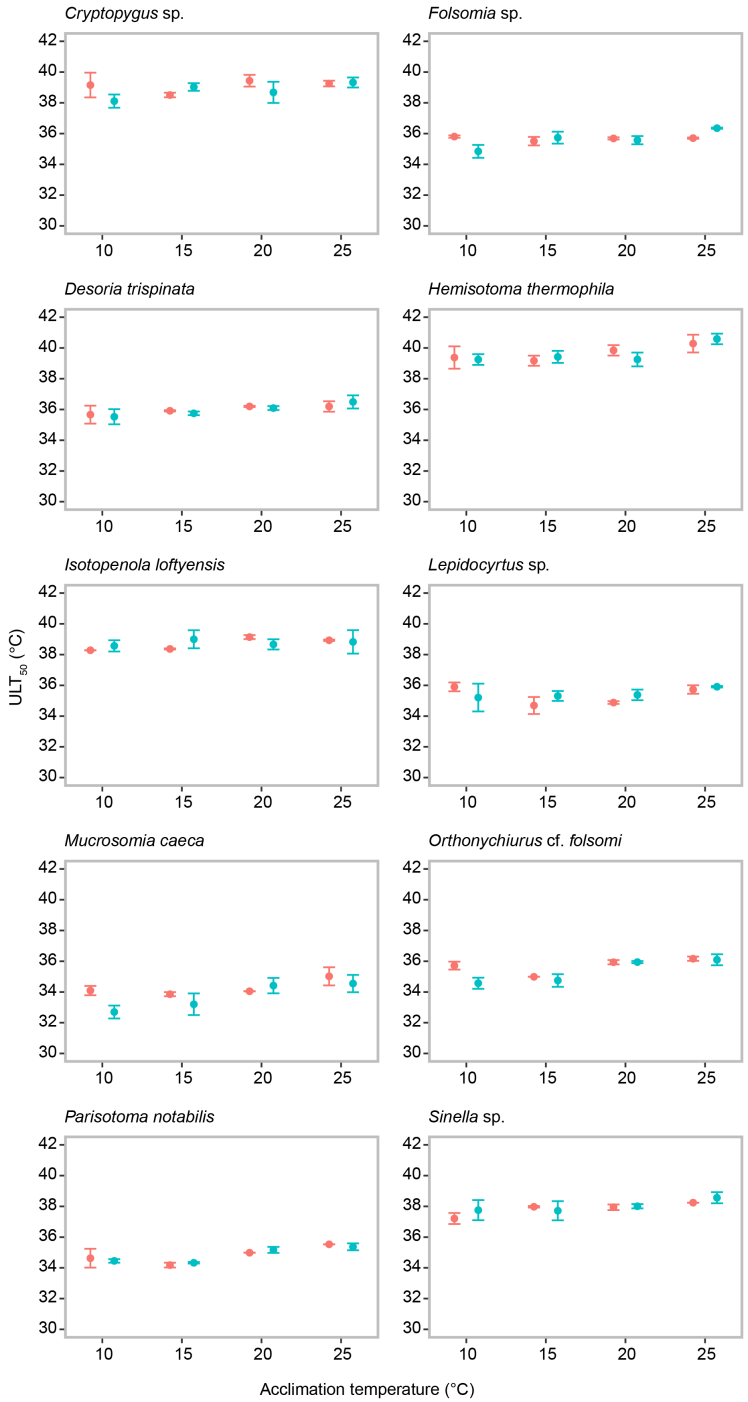

Figure 2

Mean response of upper lethal limits (ULT_{50}) to constant and fluctuating temperature acclimation treatments ranging from 10 to 25°C in 10 springtail species. For each acclimation the mean and standard deviation are shown. Fluctuating temperatures (FT) increased and decreased by 5°C around the constant temperature (CT) acclimation treatments i.e. if the CT acclimation is 10°C the corresponding FT acclimation is 10±5°C. Constant temperature acclimation treatments are represented here as red, and fluctuating temperature acclimation

treatments are represented as blue. See Table B5 for mean, standard deviation and sample size values for each species and acclimation.

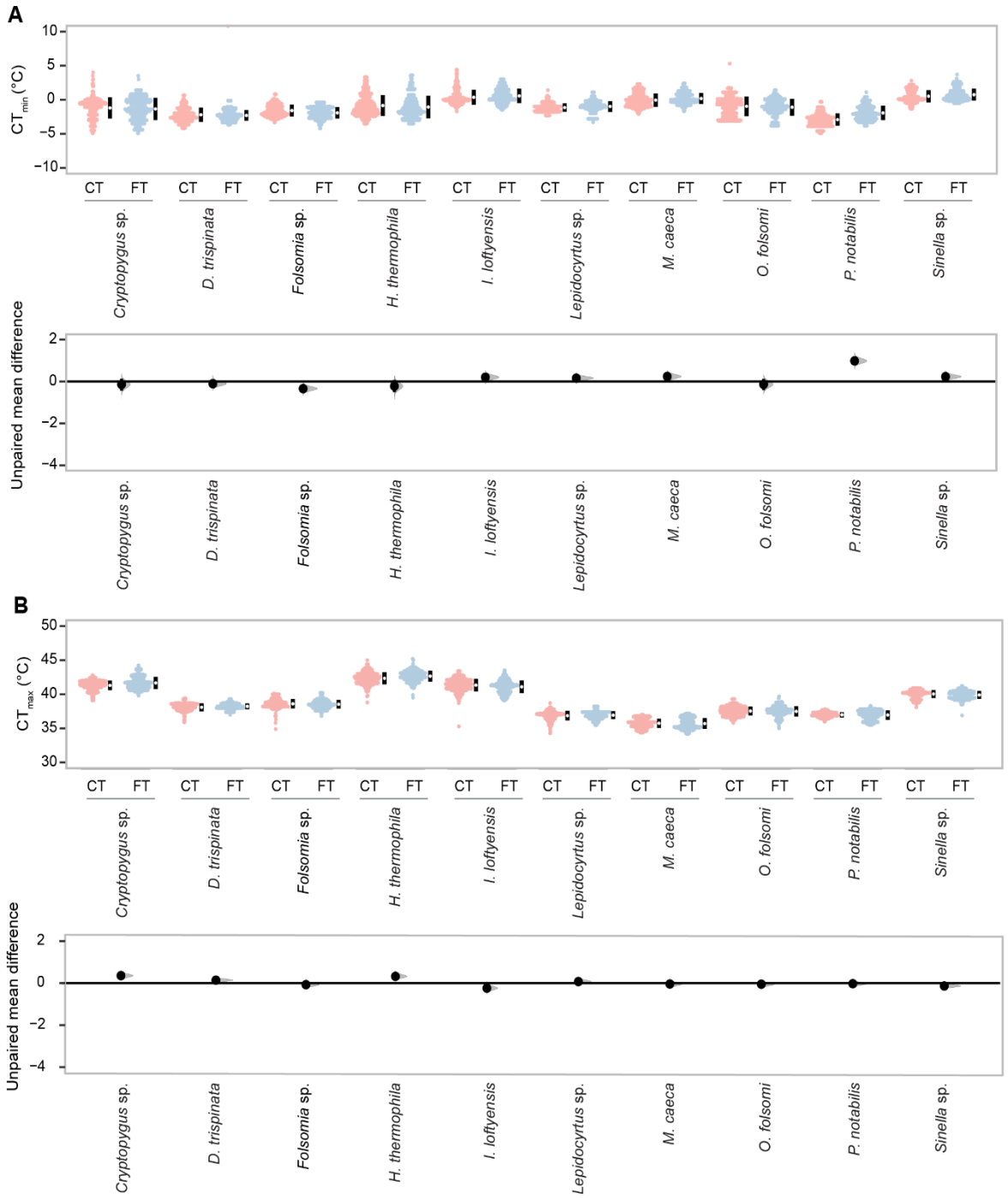

Figure 3

Estimation plots (Cumming 2014; Ho et al. 2019) indicating by how much, on average, critical thermal limits measured under constant temperatures differ from those measured under fluctuating temperatures, on a per species basis. Plot **A** represents results for critical thermal minimum (CT_{min}) and plot **B** represents results for critical thermal maximum (CT_{max}). The upper section of each plot shows the raw data (in °C) along with the mean and standard deviation for each species and acclimation regime. Constant temperature (CT) data are

coloured red, and fluctuating temperature (FT) data are coloured blue. The lower section of each plot shows the estimated mean difference, plus the 95% confidence intervals, between FT and CT acclimation treatments for each species. Where the 95% CI of the estimated mean difference overlaps with zero, there is no difference between critical thermal limits of springtails exposed to FT and CT. Negative estimated mean differences indicate fluctuating temperature acclimation treatments reduced thermal tolerance, whilst positive values indicate fluctuating temperature acclimation improved thermal tolerance. See Table B6 for estimated mean difference and 95% confidence interval values for each species and Table 2 for the results on a per acclimation basis for each species. Note only four species (*H. thermophila*, *Sinella* sp., *I. loftyensis*, and *Cryptopygus* sp.) were investigated for the 30°C acclimation.

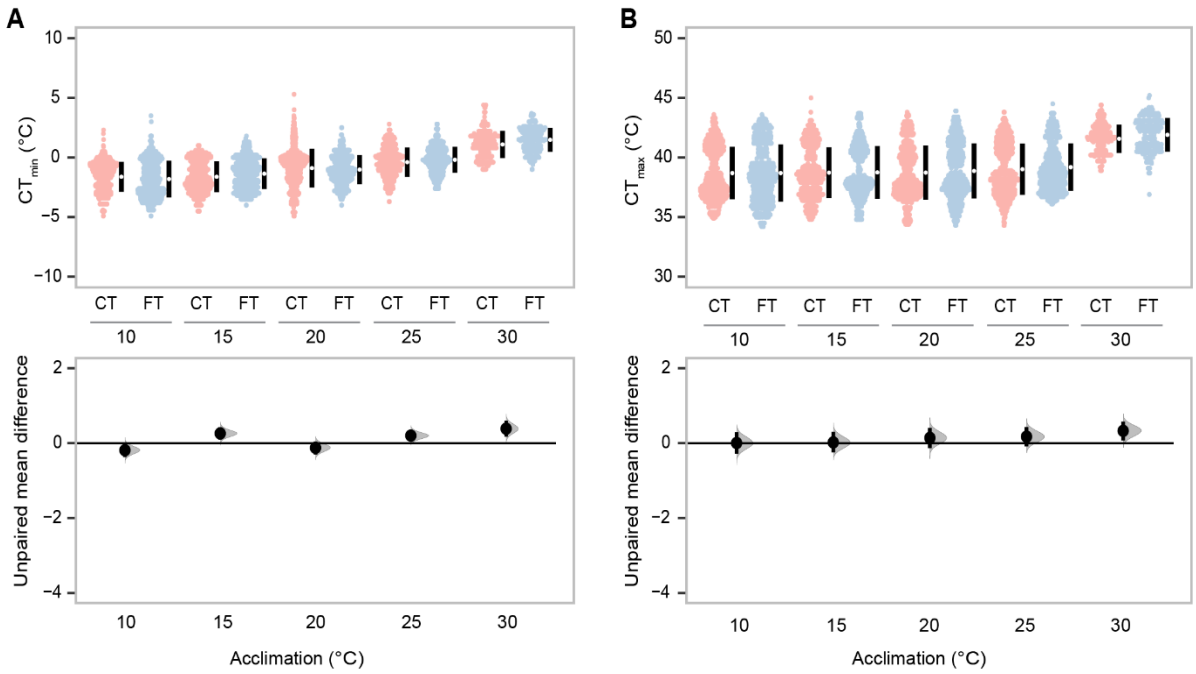

Figure 4

Estimation plots indicating by how much, on average, critical thermal limits measured under constant temperatures differ from those measured under fluctuating temperatures, on a per acclimation basis. Plot **A** represents results for critical thermal minimum (CT_{min}) and plot **B** represents results for critical thermal maximum (CT_{max}). Constant temperature (CT) acclimation treatments ranged from 10°C to 30°C and fluctuating temperature (FT) acclimation treatments varied ±5°C around the CT acclimation treatments. The upper section of each plot shows the raw data (in °C) along with the mean and standard deviation for each acclimation and regime. CT data are coloured red, and FT data are coloured blue. The lower section of each plot shows the estimated mean difference (or effect size), plus the 95% confidence intervals, between CT and FT regimes for each acclimation temperature. Where the 95% CI of the estimated mean difference overlaps with zero, there is no difference between critical thermal limits of springtails exposed to CT and FT. Negative estimated mean differences indicate FT acclimation treatments lowered thermal tolerance, whilst positive values indicate FT acclimation treatments improved thermal tolerance. See Table 2 for the full suite of estimated differences and Table B6 for the effect sizes, 95% CI and mean and standard deviation values for these plots. Note only four species (*H. thermophila*, *Sinella* sp., *I. loftyensis*, and *Cryptopygus* sp.) were investigated for the 30°C acclimation.

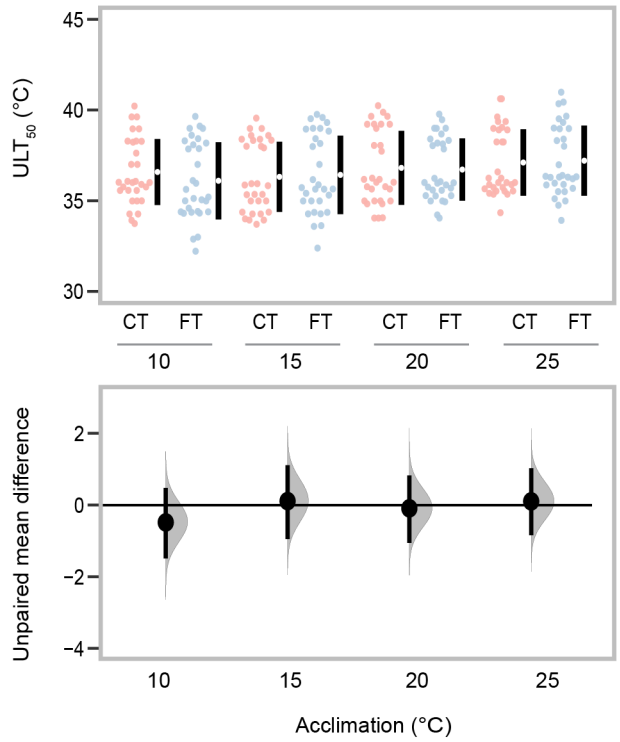

Figure 5

Estimation plot indicating by how much, on average, upper thermal limits (ULT_{50}) measured under constant temperatures differ from those measured under fluctuating temperatures, on a per acclimation basis. Constant temperature (CT) acclimation treatments ranged from 10°C to 25°C and fluctuating temperature (FT) acclimation treatments varied ±5°C around the CT acclimation treatments. The upper section of the plot shows the raw data (in °C) along with the mean and standard deviation for each acclimation. CT data are coloured red, and FT data are coloured blue. The lower section of the plot shows the estimated mean difference, plus the 95% confidence interval, between critical thermal limits measured under CT and FT acclimation treatments. Where the 95% CI of the estimated mean difference overlaps with zero, there is no difference between ULT_{50} of springtails exposed to fluctuating and constant temperatures. Negative estimated mean differences indicate FT acclimation treatments increased mortality i.e. the temperature at which 50% mortality occurred was lower, whilst positive values indicate fluctuating temperature acclimation decreased mortality. See Table B6 for the effect sizes and 95% confidence intervals for this plot.

Appendix B: Supplementary data

Table B1

Mean daily soil temperature, its standard deviation (s.d.), and maximum and minimum soil temperatures, from the study site (the Jock Marshall Reserve) for the December 2015 to February 2016 period. Data were collected using six Thermochron iButtons™ (model:DS1920G Maxim Integrated, San Jose, CA, USA) 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 B2

The range of temperatures 10 springtail species were exposed to in order to generate upper lethal temperatures at which 50% mortality occurred (ULT_{50}) for acclimation treatments between 10 and 25°C. Springtails were exposed to temperatures increasing from the lowest temperature, at which 0% morality was observed to the highest, at which 100% mortality was observed, in 2°C increments.

Species	**Experimental temperature (°C)**	
	Lowest	**Highest**
Cryptopygus sp.	36	44
Desoria trispinata	32	40
Folsomia sp.	32	40
Hemisotoma thermophila	36	44
Isotopenola loftyensis	36	42
Lepidocyrtus sp.	32	38
Mucrosomia caeca	32	38
Orthonychiurus cf. *folsomi*	32	40
Parisotoma notabilis	32	40
Sinella sp.	36	40

Table B3

Mean critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}) and upper lethal temperature (ULT_{50}), and its standard deviation for 10 springtail species acclimated to constant (CT) and fluctuating (FT) temperatures ranging from 10 to 30°C. Sample size is given for CT_{min} and CT_{max}. The sample size for ULT_{50} is n = 3, which are an estimate of ULT_{50} calculated from three replicates of mortality assays run over temperatures between 32 and 44°C.

Constant temperature

Species	**Acclimation (°C)**	CT_{min}		CT_{max}		UTL_{50}
		mean (s.d.) (°C)	**n**	**mean (s.d.). (°C)**	**n**	**mean (s.d.) (°C)**
Cryptopygus sp.	10	-3.16 (1.17)	48	40.89 (0.7)	49	39.16 (0.8)
	15	-1.92 (0.94)	48	41.41 (0.71)	48	38.51 (0.15)
	20	0.33 (1.36)	46	41.58 (0.67)	49	39.44 (0.38)
	25	-0.88 (0.54)	45	40.91 (0.69)	43	39.26 (0.19)
	30	-0.32 (0.35)	50	41.65 (0.39)	55	
Desoria trispinata	10	-1.86 (1.03)	45	38.02 (0.83)	46	35.66 (0.58)
	15	-3.38 (0.78)	49	38.39 (0.58)	52	35.91 (0.42)
	20	-3.06 (0.41)	52	37.8 (0.26)	51	36.2 (0.4)
	25	-1.71 (0.76)	43	38.14 (0.43)	58	36.2 (0.34)
	30	-0.96 (0.61)	46	38.27 (1.2)	44	
Folsomia sp.	10	-2.22 (0.69)	22	38.22 (0.88)	47	35.81 (0.79)
	15	-2.09 (0.51)	50	38.44 (0.39)	48	35.51 (0.28)
	20	-1.77 (0.7)	50	39.06 (0.65)	56	35.69 (0.72)
	25	-0.67 (0.57)	53	38.62 (0.41)	49	35.71 (0.43)
	30	0.61 (0.66)	45	38.89 (0.46)	44	
Hemisotoma thermophila	10	-1.65 (0.75)	44	41.91 (0.79)	58	39.38 (0.73)
	15	-2.06 (0.89)	54	41.9 (1.05)	40	39.17 (0.39)
	20	-0.48 (1.52)	52	42.66 (0.62)	52	39.85 (0.39)
	25	-1.31 (0.72)	44	42.23 (0.74)	57	40.28 (0.58)
	30	1.3 (0.73)	47	42.97 (0.7)	50	
Isotopenola loftyensis	10	-0.52 (0.34)	50	41.31 (0.93)	47	38.28 (0.65)
	15	-0.03 (0.33)	51	41.2 (0.81)	60	38.37 (0.38)
	20	0.44 (1.06)	94	40.73 (1.21)	44	39.14 (0.13)
	25	0.18 (0.47)	53	41.59 (0.58)	60	38.93 (0.48)
	30	1.85 (1.26)	44	41.61 (0.88)	78	
Lepidocyrtus sp.	10	-1.17 (0.44)	55	36.78 (0.67)	54	35.9 (0.29)
	15	-1.63 (0.41)	42	36.83 (0.34)	40	34.69 (0.56)

	20	-1.3 (0.52)	51	37.34 (0.54)	44	34.88 (0.91)
	25	-0.52 (0.53)	47	36.6 (0.84)	58	35.73 (0.28)
	30	-0.66 (1.02)	23	36.53 (0.95)	55	
Mucrosomia caeca	10	-0.39 (0.77)	65	36.01 (0.57)	75	34.09 (0.3)
	15	-0.74 (0.65)	48	35.52 (0.36)	51	33.86 (0.13)
	20	-0.24 (0.46)	45	35.09 (0.52)	51	34.05 (0.01)
	25	1.23 (0.59)	48	36.21 (0.59)	54	35.02 (0.59)
	30	3.34 (1.13)	43	35.68 (1.2)	77	
Orthonychiurus cf. *folsomi*	10	-2.33 (0.87)	48	37.5 (0.41)	52	35.71 (0.26)
	15	-1.76 (1.02)	53	37.63 (0.64)	47	34.99 (0)
	20	-0.38 (1.19)	43	37.22 (0.59)	111	35.93 (0.14)
	25	0.43 (0.67)	59	38.27 (0.47)	47	36.16 (0.14)
	30	1.77 (0.63)	46	38.18 (0.73)	50	
Parisotoma notabilis	10	-3.2 (0.58)	47	37.07 (0.41)	49	34.63 (0.62)
	15	-2.9 (0.4)	43	36.91 (0.37)	56	34.18 (0.15)
	20	-3.56 (1.02)	45	36.84 (0.3)	45	34.99 (0)
	25	-2.06 (0.75)	48	37.13 (0.37)	45	35.53 (0.01)
	30	-0.7 (0.77)	44	37.55 (0.65)	65	
Sinella sp.	10	-0.55 (0.44)	45	40.52 (0.33)	45	37.21 (0.36)
	15	0.27 (0.26)	46	39.19 (0.45)	45	37.97 (0.51)
	20	0.01 (0.25)	48	40.15 (0.43)	69	37.94 (0.18)
	25	1.16 (0.57)	40	40.33 (0.26)	47	38.24 (0.21)
	30	1.64 (0.37)	51	39.96 (0.42)	48	

Fluctuating temperature

Species	**Acclimation (°C)**	CT_{min}		CT_{max}		UTL_{50}
		mean (s.d.) (°C)	**n**	**mean (s.d.) (°C)**	**n**	**mean (s.d.) (°C)**
Cryptopygus sp.	10	-3.08 (1.62)	49	41.55 (0.72)	52	38.11 (0.43)
	15	-2.56 (0.83)	49	40.87 (0.4)	51	39.03 (0.25)
	20	-1.3 (0.51)	45	41.25 (0.57)	44	38.68 (0.69)
	25	-0.22 (0.75)	54	42.02 (0.45)	44	39.33 (0.33)
	30	0.4 (0.6)	47	42.8 (0.8)	43	
Desoria trispinata	10	-3.04 (0.7)	43	38.49 (0.35)	49	35.53 (0.49)
	15	-3.07 (0.4)	47	37.86 (0.31)	50	35.75 (0.12)
	20	-2.74 (0.61)	49	38.12 (0.32)	47	36.09 (0.13)
	25	-1.82 (0.61)	48	38.47 (0.38)	46	36.49 (0.43)
	30					

Folsomia sp.	10	-2.67 (0.69)	49	38.22 (0.48)	49	34.85 (0.42)
	15	-2.28 (0.7)	47	38.08 (0.4)	47	35.74 (0.39)
	20	-1.74 (0.47)	48	38.84 (0.59)	49	35.57 (0.27)
	25	-1.04 (0.41)	51	38.98 (0.43)	47	36.35 (0.38)
	30					
Hemisotoma thermophila	10	-2.33 (1.01)	43	42.4 (0.58)	46	39.25 (0.35)
	15	-2.18 (0.64)	48	42.47 (0.95)	40	39.42 (0.39)
	20	-1.63 (0.62)	45	42.49 (0.77)	50	39.25 (0.45)
	25	-0.59 (0.9)	44	42.8 (0.81)	40	40.59 (0.34)
	30	1.51 (1.11)	43	43.07 (0.76)	56	
Isotopenola loftyensis	10	0.1 (0.81)	43	41.09 (1.02)	54	38.57 (0.37)
	15	-0.54 (0.47)	48	41.33 (0.82)	45	39 (0.58)
	20	0.6 (0.74)	50	41.3 (0.9)	56	38.66 (0.33)
	25	0.85 (0.79)	44	40.7 (0.89)	49	38.83 (0.76)
	30	1.81 (0.71)	47	41.07 (0.9)	42	
Lepidocyrtus sp.	10	-1.81 (0.82)	46	36.53 (0.37)	43	35.21 (0.9)
	15	-0.82 (0.37)	49	36.76 (0.56)	44	35.31 (0.32)
	20	-1.12 (0.25)	42	37.21 (0.54)	44	35.38 (0.34)
	25	-0.18 (0.49)	44	37.23 (0.52)	43	35.91 (0.51)
	30					
Mucrosomia caeca	10	-0.23 (0.72)	56	35.1 (0.44)	49	32.7 (0.42)
	15	0.15 (0.67)	47	35.42 (0.61)	47	33.2 (0.7)
	20	-0.07 (0.6)	57	35.52 (0.59)	57	34.41 (0.5)
	25	0.94 (0.75)	51	36.76 (0.24)	50	34.55 (0.56)
	30					
Orthonychiurus cf. *folsomi*	10	-2.48 (1.11)	44	37.48 (0.38)	43	34.57 (0.36)
	15	-1.31 (0.82)	46	37.76 (0.36)	47	34.75 (0.41)
	20	-0.94 (0.69)	50	36.67 (0.64)	52	35.94 (0.07)
	25	0.21 (0.47)	47	38.08 (0.64)	51	36.09 (0.36)
	30					
Parisotoma notabilis	10	-2.98 (0.49)	47	36.13 (0.43)	49	34.45 (0.12)
	15	-1.68 (0.76)	46	36.93 (0.48)	41	34.33 (0.05)
	20	-2.26 (0.72)	42	37.33 (0.27)	42	35.17 (0.2)
	25	-0.86 (0.79)	48	37.58 (0.39)	42	35.37 (0.23)
	30					
Sinella sp.	10	-0.03 (0.34)	52	39.4 (0.16)	48	37.75 (0.65)

15	0.43 (0.47)	54	40.05 (0.43)	51	37.71 (0.62)
20	0.39 (0.42)	56	39.99 (0.35)	52	38.01 (0.14)
25	0.88 (0.47)	46	39.77 (0.62)	49	38.56 (0.36)
30	2.14 (0.55)	51	40.33 (0.78)	44	

Table B4

Full outcomes of generalised linear models (Gaussian distribution, identity link) comparing the effects of constant and fluctuating temperature acclimation treatments on thermal tolerance traits in 10 springtail species.

CT_{min}

Species	Acclimation				Treatment (FT)				Slope			
	Estimate	Std. Error	t value	P-value	Estimate	Std. Error	t value	P-value	Estimate	Std. Error	t value	P-value
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	0.132	0.009	0.015	0.65	0.516
Folsomia sp.	0.154	0.007	22.362	<0.001	0.553	0.215	2.569	0.011	-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	0.001
Isotopenola loftyensis	0.095	0.008	12.18	<0.001	0.122	0.237	0.516	0.606	0.003	0.011	0.251	0.802
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
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
Orthonychiurus cf. *folsomi*	0.208	0.008	26.475	<0.001	0.543	0.27	2.016	0.044	-0.039	0.014	-2.834	0.005
Parisotoma notabilis	0.115	0.008	13.859	<0.001	0.813	0.277	2.941	0.003	0.002	0.014	0.114	0.909
Sinella sp.	0.106	0.005	22.199	<0.001	0.458	0.14	3.279	0.001	-0.01	0.007	-1.471	0.142

CT_{max}

Species	Acclimation				Treatment (FT)				Slope			
	Estimate	Std. Error	t value	P-value	Estimate	Std. Error	t value	P-value	Estimate	Std. Error	t value	P-value
Cryptopygus sp.	0.022	0.006	3.472	0.001	-0.565	0.193	-2.924	0.004	0.048	0.009	5.242	<0.001
Desoria trispinata	0.004	0.006	0.774	0.439	0.136	0.191	0.711	0.477	-0.001	0.01	-0.09	0.929
Folsomia sp.	0.03	0.005	5.6	<0.001	-0.588	0.178	-3.299	0.001	0.031	0.009	3.331	0.001
Hemisotoma thermophila	0.047	0.007	6.764	<0.001	0.562	0.215	2.618	0.009	-0.013	0.01	-1.271	0.204
Isotopenola loftyensis	0.023	0.007	3.041	0.002	0.495	0.242	2.045	0.041	-0.036	0.011	-3.172	0.002
Lepidocyrtus sp.	-0.015	0.006	-2.533	0.012	-1.054	0.211	-5.004	<0.001	0.066	0.011	6.073	<0.001

Mucrosomia caeca	-0.004	0.006	-0.65	0.516	-1.878	0.211	-8.88	<0.001	0.104	0.011	9.54	<0.001
Orthonychiurus cf. *folsomi*	0.04	0.006	6.3	<0.001	0.356	0.216	1.647	0.1	-0.024	0.011	-2.167	0.031
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
Sinella sp.	0	0.005	0.088	0.93	-0.736	0.157	-4.693	0	0.03	0.007	4.071	<0.001

ULT_{50}

Species	**Acclimation**				**Treatment (FT)**				**Slope**			
	Estimate	**Std. Error**	**t value**	**P-value**	**Estimate**	**Std. Error**	**t value**	**P-value**	**Estimate**	**Std. Error**	**t value**	**P-value**
Cryptopygus sp.	0.025	0.027	0.916	0.370	-1.025	0.699	-1.467	0.158	0.041	0.038	1.085	0.291
Desoria trispinata	0.038	0.016	2.355	0.029	-0.506	0.414	-1.221	0.236	0.027	0.023	1.207	0.242
Folsomia sp.	-0.002	0.015	-0.156	0.877	-1.616	0.394	-4.105	0.001	0.089	0.021	4.174	4.68E-04
Hemisotoma thermophila	0.068	0.027	2.532	0.020	-0.201	0.696	-0.289	0.775	0.009	0.038	0.240	0.813
Isotopenola loftyensis	0.054	0.021	2.654	0.015	0.882	0.533	1.655	0.114	-0.046	0.029	-1.572	0.132
Lepidocyrtus sp.	-0.007	0.029	-0.231	0.820	-0.732	0.763	-0.960	0.349	0.051	0.042	1.217	0.238
Mucrosomia caeca	0.135	0.026	5.202	4.32E-05	1.868	0.677	2.761	0.012	-0.076	0.037	-2.063	0.052
Orthonychiurus cf. *folsomi*	0.046	0.021	5.531	0.04	-1.576	0.542	-2.910	0.009	0.069	0.029	2.358	0.029
Parisotoma notabilis	0.070	0.018	3.751	0.001	-0.260	0.481	-0.054	0.957	0.001	0.026	0.053	0.958
Sinella sp.	0.061	0.020	3.124	0.005	0.289	0.509	0.567	0.577	-0.007	0.028	-0.248	0.806

Table B5

Estimated difference between means (es), along with the mean and its standard deviation of fluctuating and constant temperature acclimation treatments for critical thermal minimum (CT_{min}) and critical thermal maximum (CT_{max}) for 10 springtail species. Where the 95% CI of the estimated mean difference overlaps with zero (those in boldface), there is no difference between the critical thermal limits of springtails exposed to FT and CT. Negative estimated mean differences indicate fluctuating temperature acclimation treatments reduced thermal tolerance, whilst positive values indicate fluctuating temperature acclimation treatments improved thermal tolerance.

CT_{min}

Species	**Constant temperature**		**Fluctuating temperature**		**es (°C)**	**95% CI**
	mean (s.d.) (°C)	**n**	**mean (s.d.) (°C)**	**n**		
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
Lepidocyrtus sp.	-1.1 (0.68)	218	-0.99 (0.79)	181	0.16	0.01, 0.3
Mucrosomia caeca	0.53 (1.63)	249	0.18 (0.82)	211	0.24	0.06, 0.42
Orthonychiurus cf. *folsomi*	-0.46 (1.71)	249	-1.1 (1.24)	187	-0.14	**-0.41, 0.13**
Parisotoma notabilis	-2.49 (1.25)	227	-1.93 (1.05)	183	0.99	0.78, 1.19
Sinella sp.	0.51 (0.89)	230	0.75 (0.87)	259	0.23	0.07, 0.39

CT_{max}

Species	**Constant temperature**		**Fluctuating temperature**		**es (°C)**	**95% CI**
	mean (s.d.) (°C)	**n**	**mean (s.d.) (°C)**	**n**		
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
Lepidocyrtus sp.	36.79 (0.77)	251	36.93 (0.58)	174	0.07	**-0.06, 0.21**
Mucrosomia caeca	35.73 (0.84)	308	35.7 (0.8)	203	-0.04	**-0.18, 0.1**
Orthonychiurus cf. *folsomi*	37.65 (0.71)	307	37.49 (0.76)	193	-0.05	**-0.18, 0.09**
Parisotoma notabilis	37.13 (0.52)	260	36.96 (0.69)	174	-0.03	**-0.15, 0.08**
Sinella sp.	40.04 (0.58)	254	39.9 (0.59)	244	-0.14	-0.24, -0.04

Table B6

Estimated difference between means (es), along with the mean and its standard deviation of fluctuating and constant temperature acclimation treatments for critical thermal minimum (CT_{min}) and critical thermal maximum (CT_{max}). Boldface values show cases where fluctuating temperatures were not significantly different from constant temperature acclimation treatments i.e. where 95% confidence intervals overlap with zero. For CT_{max} and CT_{min} only 4 species were investigated for the 30°C acclimation.

CT_{min}

Acclimation (°C)	**Constant temperature**		**Fluctuating temperature**		**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

CT_{max}

Acclimation (°C)	**Constant temperature**		**Fluctuating temperature**		**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

ULT_{50}

Acclimation (°C)	**Constant temperature**		**Fluctuating temperature**		**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						

Chapter 3

Variation in the development and survival of springtails exposed to constant and fluctuating temperatures

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Abstract

Much recent focus has been given to the potential differences between trait values generated under constant and fluctuating temperatures. Fluctuating temperatures may affect the accuracy of forecast models attempting to incorporate trait data, and thus, using trait values collected under constant temperatures are likely to produce biased estimates. How important these differences are for soil invertebrates remains poorly understood. This study therefore examines the effect of constant temperatures (10°C, 15°C, 20°C, 25°C, 30°C) and fluctuating temperatures (10±5°C, 15±5°C, 20±5°C, 25±5°C, 30±5°C) on development rate, lower development threshold (LDT), sum of effective temperatures (SET), survival across two developmental stages (egg to juvenile and juvenile to adult), and survival across the entire lifecycle (egg to adult), of ten springtail (Collembola) species from three families (Isotomidae: 7 spp.; Entomobryidae: 2 spp.; Onychiuridae: 1 sp.). Fluctuating temperatures affected development rate differently to constant temperatures in six species over at least one life stage, but no one species was affected consistently across all stages. The overall effect of fluctuations was to increase development rate at low temperature and to decrease development rate at high temperature. Fluctuating temperatures affected survival differently to constant temperatures in three species, but only during egg to juvenile and juvenile to adult stages. Thus, egg to adult survival was not affected by fluctuating temperatures. The overall effect of temperature on survival was inconsistent, with fluctuations decreasing survival in some species and increasing it in others. Fluctuating temperatures increased LDT and lowered SET, but this effect was only significant in the egg to juvenile developmental stage. High temperatures under constant conditions allowed survival, and so development, to continue in situations where fluctuations did not. Therefore, when building models, care should be taken to consider whether development traits were measured under constant or fluctuating temperatures.

1. Introduction

Understanding how temperature affects ectothermic organisms throughout their entire lifecycle is important. Thermal variation throughout ontogeny not only directly influences traits associated with demographic parameters, such as development and survival, but also indirectly influences many adult traits such as thermal tolerance and body size (e.g. Sibly and Atkinson 1994; Bowler and Terblanche 2008; Arias et al. 2011; Cavieres et al. 2016). In light of the substantial effect that climate change is predicted to have on ectothermic organisms (e.g. Deutsch et al. 2008, 2018; Dillon et al. 2010; Diamond et al., 2018; Pinsky et al. 2019), focus has recently been directed towards developing a better understanding of the influence that fluctuations in temperature have on 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). In particular, attention to has turned to what differences in trait values estimated under fluctuating and constant temperatures mean in terms of the ability to accurately predict effects under field conditions (e.g. Huey et al. 2012; Estay et al. 2014; Vasseur et al. 2014; Bozinovic and Pörtner 2015; Bozinovic et al. 2016; Bartheld et al. 2017).

Typically, constant temperatures are used to determine the effect of temperature on traits, usually by generating thermal performance curves (Huey and Stevenson 1979). However, theory predicts that fluctuating temperatures are likely to produce different trait values to those expressed under constant temperatures. This is, firstly, because the response of traits to temperature (or thermal performance) is typically a non-linear function following a bell-shaped curve, and secondly, because of Jensen's inequality (Ruel and Ayres 1999; Denny 2017; see also Worner 1992), a mathematical property of non-linear functions. Jensen's inequality results in different estimates of average performance at the same point on a thermal performance curve for constant and fluctuating temperatures, simply because the fluctuating-

temperature estimate also includes performance measures at higher and lower temperatures whereas the constant-temperature estimate does not (Denny 2017). This effect has been demonstrated in many studies and for a variety of traits (e.g. Ragland and Kingsolver 2008; Bozinovic et al. 2011; Fischer et al. 2011; Kjaersgaard et al. 2013; Carrington et al. 2013; Colinet et al. 2015). The outcome of exposure to fluctuations generally results in increasing performance at high temperatures up to a point of swift decline (Martin and Huey 2008), and, depending on the circumstances (e.g. whether or not thermal respite is involved (see Colinet et al. 2015)), variable performance at lower temperatures (in comparison to constant temperatures). Thus, trait values expressed under constant temperature conditions might not accurately reflect those expressed by ectothermic organisms under more thermally-variable natural conditions (e.g. Behrens et al. 1983; Brakefield and Kesbeke 1997). As a result, the utility of trait values collected under constant thermal environments to predict performance under natural conditions may be limited (Niehaus et al. 2012; Valladares et al. 2014; Dowd et al. 2015; Ma et al. 2015).

Variation in trait data collected under fluctuating and constant conditions may also affect the accuracy of forecast models attempting to incorporate trait data (Niehaus et al. 2012; Valladares et al. 2014; Dowd et al. 2015). With the recent increase in the use of trait data to understand community assembly (e.g. Start et al. 2018; Miller et al. 2019), and to model responses to environmental change (Deutsch et al. 2018; Pinsky et al. 2019), the need to provide accurate estimates of the nature of trait variation under natural conditions is growing. This is of particular importance in regards to the effect that fluctuations have on performance at various life stages, which may differ in their responses to temperature (e.g. Jensen et al. 2007). In particular, differences in the response of development and survival at various life stages may have significant consequences for population growth or decline, especially in terms of variation in the resilience of different life stages (e.g. Arias et al. 2011). Moreover, the

temperature experienced through ontogeny has been shown to affect phenotypic variation in adult traits, such as thermal tolerance and body size (e.g. Bowler and Terblanche 2008; Arias et al. 2011), which are traits frequently used to describe the effects of climate change on biodiversity (e.g. Sheridan and Bickford 2011; Gunderson and Stillman 2015). Despite the fact that there is a long history of studying the effects of temperature on developmental traits in ectothermic organisms, details on variation of the effect of fluctuations on each developmental stage over a broad range of temperatures remain relatively limited (for examples see Radmacher and Strohm 2011; Niehaus et al. 2012; Bayu et al. 2017).

The aim of this study, is therefore, to test the hypothesis that fluctuating temperatures elicit a different response in development and survival relative to constant temperatures. The purpose of which is to determine if development and survival measured under constant laboratory conditions can accurately describe the response of these traits under more natural thermally variable conditions. Temperatures ranging from 10 to 30ºC in 5ºC increments are examined, because the response of traits at high and low temperatures may differ (Denny 2017). Variation in these traits is also determined for multiple life stages, as previous research indicates fluctuations may have different effects on performance depending on life stage (e.g. Jensen et al. 2007).

Collembola (springtails), small soil invertebrates, were used as exemplar organisms due to their responsiveness to, and use as indicators of, changing environmental conditions (Bahrndorff et al. 2009; Vandewalle et al. 2010; Everatt et al. 2013; van Dooremalen et al. 2013). Furthermore, because springtails form an important part of the soil biota (Hopkin 1997; Rusek 1998; Bardgett and van der Putten 2014), and recent studies have indicated that soil systems will be impacted greatly by anthropogenic global change (e.g. Bokhorst et al. 2012; Holmstrup et al. 2018; Janion-Scheepers et al. 2018), a greater understanding of the responses

of the soil fauna to environmental change is necessary (Nielsen et al. 2015; Coyle et al. 2017; Cameron et al. 2018; Geisen et al. 2019).

2. Materials and methods

2.1 Springtail collection and stock maintenance

Springtail collection and stock maintenance was conducted as per the methods described in full in Chapter 2. Here, an abridged version of the methods is provided. Cultures were developed using springtails collected from Jock Marshall Reserve (JMR), a small urban reserve located on the Monash University, Clayton Campus in Victoria Australia (37.9096° S, 145.1400° E). Springtails were extracted from soil and leaf litter using a Berlese-Tullgren funnel system (Southwood and Henderson 2009) and 10 common, and abundant, springtail species from three families (Isotomidae; Entomobryidae; Onychiuridae) were identified and cultured. Species were identified as per methods in Janion-Scheepers et al. (2018) using DNA barcoding (using the mitochondrial cytochrome oxidase subunit I gene) and identification keys (e.g. Fjellberg 1998; Greenslade et al. 2014), and were confirmed by specialist springtail taxonomists. Species were identified as: *Folsomia* sp., *Cryptopygus* sp., *Mucrosomia caeca* (Wahlgren 1906), *Parisotoma notabilis* (Schäffer 1896), *Desoria trispinata* (Mac Gillivray 1896), *Hemisotoma thermophila* (Axelson 1900), *Isotopenola loftyensis* (Womersley 1934) from the family Isotomidae; *Sinella* sp. and *Lepidocyrtus* sp. from the family Entomobryidae; and *Orthonychiurus* cf. *folsomi* from the family Onychiuridae.

Springtail monocultures were maintained in plastic vials (70 ml) containing a moistened plaster-of-Paris:charcoal mixture (9:1), provided a standard (*ad libitum*) diet of plane tree bark (*Plantanus* sp.) (see Hoskins et al. 2015; Janion-Scheepers et al. 2018) and kept at 20°C in a controlled-temperature room (confirmed as 20.3±0.4°C by Thermochron iButtons™, model DS1920G, Maxim Integrated, San Jose, CA, USA) on a 12h light:12h dark

photoperiod. First-generation (F1) springtails were collected and reared to adults by removing eggs from cultures three times per week. At maturity (first egg laying event), adult springtails of the same species were combined randomly to mitigate inbreeding effects, and to reduce the potential impact of extant environmental or parental effects on results (as in Janion-Scheepers et al. 2018; and see Hoffmann and Sgrò 2018 for discussion). Second-generation eggs (F2) were used in experiments.

2.2 Experimental thermal environment

Five constant temperatures (10°C, 15°C, 20°C, 25°C, 30°C) and five fluctuating temperatures (10±5°C, 15±5°C, 20±5°C, 25±5°C, 30±5°C) were used to assess development rate, lower development threshold (LDT), sum of effective temperatures (SET), and survival in springtails. The amplitude of fluctuations was based upon the maximum mean daily variation in summer soil temperature conditions estimated from data collected from the field site (during the summer of 2015/16) using Thermochron iButtons™ (model DS1920G, Maxim Integrated, San Jose, CA, USA**)** (see Chapter 2 Materials and Methods and Appendix Table B1 for more information). Fluctuations in temperature followed field diel thermal conditions, with the highest temperature occurring at 13h00 and the lowest at 06h00. Thus, for fluctuating temperature conditions, temperature increased from the lowest to the highest temperature over 7 hours at a rate of ~0.7°C/30min, and decreased from the highest to the lowest temperature over 17 hours at a rate of ~0.3°C/30min. All temperature manipulations were undertaken in controlled temperature incubators (MIR-154, SANYO, Japan, for constant temperature conditions, and KB 115 (E3.1) Binder cooling incubator, Tuttlingen, Germany for fluctuating temperature conditions) and were monitored using Thermochron iButtons™ (model:DS1920G, Maxim Integrated, San Jose, CA, USA), under a 12h light:12h dark photoperiod.

2.3 Measuring springtail development and survival

To assess variation in development rate and survival, the number of days that springtails took to develop, along with the number of individuals that survived, were measured over different developmental stages. Springtails have three stages of development: egg, juvenile, and adult (Hopkin 1997). Thus, the effect of fluctuating and constant temperatures on development was measured between egg to juvenile and juvenile to adult stages, but also for the entire developmental period from egg to adult. F2 eggs, less than 24 hours old, were collected using a paintbrush and placed into 70 ml vials containing a moist plaster-of-Paris:charcoal (9:1) substrate. For each species and temperature treatment, six replicates were established, each with 30-40 eggs (i.e. the development was examined for 180-240 eggs per temperature treatment per species). Springtails were provided a standard diet of plane tree bark (*Plantanus* sp.) *ad libitum* after hatching and the plaster-of-Paris substrate was kept moist as required.

2.3 Statistical analysis

Generalised linear models (Gaussian, identity link) (GLM) were used to assess the effect of development temperature and treatment (constant or fluctuating temperatures) on development rate (calculated as the inverse of the number of days taken to develop). The response of traits to temperature, or the performance curve (Angilletta 2009), has an optimum and a decline to the right-hand side of the optimum. Here, data to the right-hand side of the optimum were not included for the calculation of temperature effects on development (see Sørensen et al. 2018). In addition, development over the lower, non-linear part of the curve was not measured because of complexities in the analysis of this asymptotic area of the decline in development rate (Fig. 1) (Worner 1992). GLM analyses were conducted using all available replicates for each species and temperature treatment.

The sum of effective temperatures (SET) and the lower development threshold (LDT) were calculated for each species and developmental stage following the method described in Honěk (1996). LDT was calculated using the equation,

$$LDT = -b/a \quad (1)$$

and SET was calculated using the equation,

$$SET = 1/a \quad (2)$$

where a = slope and b = intercept of a linear regression of development rate and temperature.

Phylogenetic linear mixed models (PLMM) were used to assess the overall effect of fluctuations on LDT and SET in springtails over the three developmental stages, whilst accounting for variation introduced by species relatedness. In the PLMMs, treatment was a fixed effect and phylogeny was a random effect. The phylogenetic tree for the PLMM models (Fig. C1) was developed using previous work on the phylogenetic relationships of major springtail taxa (D'Haese 2002; Malcicka et al. 2017) and recent work on Australian springtail species (Janion-Scheepers et al. 2018). Branch lengths for the final tree were assigned using Grafen's method (Grafen 1989). The PLMMs was run using the package ASReml-R v 3.0 (Gilmour et al. 1995).

Logistic regression (Quasibinomial distribution, logit link), was used to analyse how developmental temperature and treatment affected survival in springtails. Even though husbandry for all species was the same, high mortality in *I. loftyensis* and *M. caeca* at the lower temperature treatments during juvenile development meant that these species were not included in the analysis of survival for juvenile to adult and egg to adult development. Due to short-term changes in species abundances in JMR, it was not possible to repeat the experiment for these replicates. This also meant that development rate, LDT and SET for these two species were not assessed in the juvenile to adult and egg to adult stages, as three or more developmental temperatures are required to estimate the parameters of a linear regression. Mean data are

provided for all species for development rate (Fig. 1; Table C1) and survival (Table C2), regardless of use in analyses.

All analyses were implemented in R v. 3.5.2 (R Core Team 2018) using the RStudio platform v1.1.463 (RStudio Team 2016).

3. Results

3.1 Egg to juvenile development and survival

Temperature significantly affected egg to juvenile development rate in all species, with development rate increasing as development temperature increased (Table 1, Table C3). By contrast, temperature affected survival in only half the species, with survival decreasing as temperature increased in these species (Table 2, Table C4). In two out of the ten species studied (i.e. *Cryptopygus* sp. and *D. trispinata*), fluctuating temperatures affected development rate differently to constant temperatures, and development rate was significantly faster at low temperatures and slower at high temperatures, compared with those species' responses to constant temperature conditions. Likewise, fluctuating temperatures affected survival in the same two species (*Cryptopygus* sp. and *D. trispinata*), with higher survival under fluctuating temperatures compared to survival under constant temperatures. Overall, fluctuating temperatures significantly lowered LDT (Wald $\chi^2_{(1)} = 10.71$, $p = 0.01$) and increased SET (Wald $\chi^2_{(1)} = 7.345$, $p = 0.024$), (Fig. 2A, Table C5) in comparison to constant temperatures, although this effect was inconsistent across species, with fluctuating temperatures having the opposite effect on two species (*M. caeca* and *D. trispinata*).

3.2 Juvenile to adult development and survival

Juvenile to adult development rate was significantly affected by development temperature, increasing as temperature increased for all species (Table 1, Table C3). Overall, survival was

not affected by temperature, with only *Cryptopygus* sp. showing a negative effect of increasing temperatures at this stage of development (Table 2, Table C4). Likewise, fluctuating temperature conditions had no effect on development rate and survival for the majority of species. *Sinella* sp. was the only species for which fluctuations affected development rate, with development rate being slower over all fluctuating temperatures in comparison to constant temperatures. Fluctuating temperatures affected survival differently to constant temperatures in two species (*D. trispinata* and *Lepidocyrtus* sp.) however their directions of response were inconsistent. Fluctuations reduced survival in *D. trispinata*, whilst increasing survival in *Lepidocyrtus* sp.. For this developmental stage, fluctuating temperatures did not significantly affect LDT (Wald $\chi^2_{(1)} = 0.741$, p = 0.412) or SET (Wald $\chi^2_{(1)} = 1.45$, p = 0.267) in comparison to constant temperatures, although the overall trend was for fluctuating temperatures to decrease LDT and increase SET (Fig. 2B, Table C5).

3.3 Egg to adult development and survival

Development rate from egg to adult was positively affected by temperature in all species (Table 1, Table C3). Survival was negatively affected by temperature in five out of the eight springtail species analysed. The fluctuating temperature treatment had almost no effect on development rate and survival compared to constant temperatures. Fluctuating temperatures affected development rate significantly in one species (*O.* cf. *folsomi*), with development rate faster at low temperatures, and slower at high temperatures, in comparison to constant temperatures. Fluctuating temperatures had no effect on survival in comparison to constant temperatures. There was also no effect of fluctuating temperatures on LDT (Wald $\chi^2_{(1)} = 1.033$, p = 0.338) or SET (Wald $\chi^2_{(1)} = 1.615$, p = 0.245) for this developmental stage. Fluctuating temperatures did tend to decrease LDT and increase SET overall; however, the direction of response was different in half of the species.

4. Discussion

Overall, the response of development rate over springtail ontogeny was similar, with development rate increasing as developmental temperature increased. This is consistent with what has previously been found for development rate in ectothermic organisms, including springtails (e.g. Birkemoe and Leinaas 2000; Liefting and Ellers 2008; Sengupta et al. 2016, 2017), with rates following a typical thermal performance curve (Huey and Stevenson 1979), increasing as temperature increases until a threshold is reached. Although not explicitly tested for, the threshold for most species in this study was between 25 and 30°C, regardless of developmental stage.

In contrast, the response of survival to developmental temperature was mixed. Survival typically follows a U-shaped curve, remaining the same regardless of temperature until upper and lower thresholds are reached (e.g. Angilletta et al. 2004). This was the case here for the high end of the developmental temperatures, with survival remaining the same between 10 and 20°C, and only affected during development at the higher temperatures (i.e. 25 and 30°C). Because very low temperatures were not assessed, a lower-end effect was not detected. Overall, developmental temperature had a negative effect on survival for 7 out of the 10 species during at least one stage of development, and throughout ontogeny. The most-affected stages were egg to juvenile and egg to adult development. This may be attributed to variation in thermal tolerance amongst the species, as the three species whose survival was unaffected (*D. trispinata, H. thermophila* and *Sinella sp.*) are known to have a higher thermal tolerance than those species for which survival was affected by temperature (see Chapter 2 results). Thus, the unaffected species may not have responded negatively to increasing developmental temperature because the higher threshold where survival is reduced was not or only just reached.

In terms of deviation from constant temperatures, fluctuating temperatures had varying effects on development rate, affecting 6 species over at least one developmental stage, but with no one species affected consistently over all stages. The difference in development rate between temperature treatments was most apparent at the extremes of the temperature range used (i.e. the 10 and 30°C developmental temperatures). Even though most differences were not significant, low developmental temperatures in general resulted in a slight increase in development rate, whilst for higher developmental temperatures, fluctuations resulted in a decrease in development rate. This result is in line with previous research on development rate in ectotherms, demonstrating that fluctuations at high temperatures are typically detrimental whilst fluctuations at lower temperatures either have no effect or are beneficial (Tanigoshi et al. 1976; Kersting et al. 1999; Kingsolver et al. 2009; Garcia-Ruiz et al. 2011; Fischer et al. 2011; Kaersgaard et al. 2013; De Majo et al. 2019; Spurgeon and Brent 2019). Likewise, for most species, survival was unaffected by fluctuations across all stages, and for the three species whose survival was significantly affected by fluctuations, the direction of response was inconsistent. These results typify what is expected of the effects of fluctuating and constant temperatures, with previous research showing that fluctuations can be beneficial, detrimental, or have no effect, when compared to constant temperatures (Ragland and Kingsolver 2008; De Majo et al. 2019).

What is noteworthy about the effect of fluctuations on development and survival is that, although there were significant effects of fluctuating temperatures on these traits, such effects were generally observed in the egg to juvenile or juvenile to adult stages (especially for survival), but fluctuations were found to have no effect on the traits over the full springtail lifecycle (i.e. egg to adult). These outcomes reflect growing evidence that effects of plasticity at one stage may not be reflected at another, or indeed over the full life cycle of a given ectotherm species. Similar results, showing that the effects of fluctuating temperatures differ

between developmental stages, have been found in other invertebrates (e.g. Fischer et al. 2003; Terblanche and Chown 2006; Arias et al. 2011; Slotsbo et al. 2016), and may mean that studies that fail to explicitly investigate the effects of fluctuations on all developmental stages (e.g. Fischer et al. 2011) could miss effects associated with fluctuating temperatures, with implications for using trait data in a predictive context.

These differing effects of fluctuating temperatures between developmental stages were also apparent in LDT and SET, where fluctuating temperatures in the egg to juvenile stage reduced LDT and increased SET, whilst the other two stages were not significantly affected. Regardless of significance, and the general trend for fluctuations to lower LDT and increase SET, the direction of effect was not consistent across species or from one stage to the other. For example, fluctuations in *P. notabilis* and *O.* cf. *folsomi* lowered LDT in the egg to juvenile stage, but increased LDT in the juvenile to adult and egg to adult stages. Additionally, the responses of LDT and SET to fluctuating temperatures in juvenile to adult and egg to adult stages in half of the species were opposite to the other half. This indicates that even though the general result of fluctuations was to reduce LDT and increased SET in springtails, the large interspecific and development stage variations are likely to result in different outcomes compared to constant temperatures should this data be used for predictive purposes.

The response to fluctuating temperatures in this study could be a result of the type of fluctuations used, even though the methodology here reflects the natural thermal habitat of springtails (i.e. the study used a standardised low thermal sinusoidal amplitude of ±5°C), it did not consider either short large spikes in temperature or wider sinusoidal amplitudes (i.e. ±10°C). Thus, it may be that using a thermal fluctuation with a low amplitude was not enough to elicit a consistently-robust effect in the traits that were measured, and that the most pronounced effects on development rate and survival come from larger variations in thermal amplitude (e.g. Folguera et al. 2011; Xing et al. 2019), such as extreme heat events (e.g.

Kingsolver and Buckley 2017; Zhu et al. 2019). As such, it may be that more extreme exposure to stressful conditions might yield different results to what was found in this study.

Additionally, it is possible that because the linear portion of development rate was assessed in this study (due to methodological restrictions), and thermal performance is a non-linear function, effects of fluctuations on development rate may have been missed, especially for the higher temperatures in relation to the upper threshold of development. For example, constant temperatures during egg development often allowed development rate to continue increasing at the higher developmental temperatures, whereas fluctuating temperatures either reduced development rate or precluded development altogether (i.e. fluctuating temperatures lowered the upper thermal threshold for development). This effect was less apparent in juvenile development, and in development overall, but indicates that the point at which trait values measured under fluctuating temperature conditions deviate from those under constant temperatures is likely to be at or near the thermal threshold — something that was not explicitly investigated in this study, but has important implications for using this type of trait data in models.

The results in this study demonstrate that the response of springtails to fluctuating temperatures is highly variable. Whilst most species were unaffected over the full developmental period from egg to adult, effects were more apparent within egg to juvenile and juvenile to adult stages, and may mean that effects associated with fluctuating temperatures could be missed if these stages are ignored. The effects of fluctuations were most apparent at high temperature, where constant temperatures often enabled springtails to continue to develop when fluctuating temperatures did not, most likely due to high temperature thresholds being reached sooner in the fluctuating temperature conditions. The effect of fluctuations on LDT and SET also showed a strong response to fluctuations, with fluctuating temperatures overall decreasing LDT (especially during egg development) and increasing in SET. These results

indicate that care should be taken when attempting to use similar trait data in models, not only in terms of variation between developmental stages and between species, but also when the temperature range being tested is close to or exceeds thermal thresholds. In such circumstances, it is likely that trait values measured under constant temperatures will misestimate performance at those temperatures.

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Tables

Table 1

Summary outcome of generalised linear models (Gaussian distribution, identity link) comparing the effects of constant (CT) and fluctuating (FT) temperature treatments on development rate over three life stages in 10 springtail species. The table includes results for development rate variation in egg to juvenile, juvenile to adult and egg to adult stages. Two species (*I. loftyensis* and *M. ceaca*) were not assessed for juvenile to adult or egg to adult development rate due to low sample size. See Table C3 for full outcome. Boldface indicates significant results.

Egg to Juvenile

Species	Developmental temperature		Treatment (FT)		Interaction (temperature x treatment)	
	Estimate	**P-value**	**Estimate**	**P-value**	**Estimate**	**P-value**
Cryptopygus sp.	0.0106	**<0.00001**	0.0147	**0.0205**	-0.0009	**0.0086**
Desoria trispinata	0.0107	**<0.00001**	-0.0054	0.6420	0.0005	0.3880
Folsomia sp.	0.0065	**<0.00001**	0.0094	0.2910	-0.0007	0.1381
Hemisotoma thermophila	0.0145	**<0.00001**	0.0340	**0.0275**	-0.0015	0.0522
Isotopenola loftyensis	0.0061	**<0.00001**	0.0062	0.2320	-0.0003	0.2240
Lepidocyrtus sp.	0.0095	**<0.00001**	0.0213	0.1194	-0.0014	0.0605
Mucrosomia caeca	0.0049	**<0.00001**	-0.0065	0.1870	0.0003	0.2920
Orthonychiurus cf. *folsomi*	0.0050	**<0.00001**	0.0119	**0.0351**	-0.0008	**0.0066**
Parisotoma notabilis	0.0078	**<0.00001**	0.0137	0.0513	-0.0007	0.0527
Sinella sp.	0.0093	**<0.00001**	0.0290	**0.0132**	-0.0020	**0.0005**

Juvenile to adult

Species	Developmental temperature		Treatment (FT)		Interaction (temperature x treatment)	
	Estimate	**P-value**	**Estimate**	**P-value**	**Estimate**	**P-value**
Cryptopygus sp.	0.0043	**<0.00001**	0.0082	0.3181	-0.0005	0.2882

Desoria trispinata	0.0048	**<0.00001**	-0.0041	0.7638	0.0002	0.7676
Folsomia sp.	0.0021	**<0.00001**	0.0094	**0.0429**	-0.0007	**0.0130**
Hemisotoma thermophila	0.0063	**<0.00001**	-0.0057	0.5870	0.0002	0.6700
Isotopenola loftyensis						
Lepidocyrtus sp.	0.0022	**<0.00001**	0.0127	0.0989	-0.0010	**0.0484**
Mucrosomia caeca						
Orthonychiurus cf. *folsomi*	0.0018	**<0.00001**	0.0080	0.0759	-0.0006	**0.0156**
Parisotoma notabilis	0.0038	**<0.00001**	-0.0204	**0.0270**	0.0012	**0.0334**
Sinella sp.	0.0015	**<0.00001**	-0.0077	**0.3300**	0.0005	0.1890

Egg to adult

Species	**Developmental temperature**		**Treatment (FT)**		**Interaction (temperature x treatment)**	
	Estimate	**P-value**	**Estimate**	**P-value**	**Estimate**	**P-value**
Cryptopygus sp.	0.0030	**<0.00001**	0.0054	0.1730	-0.0003	0.1370
Desoria trispinata	0.0033	**<0.00001**	-0.0016	0.8120	0.0001	0.8040
Folsomia sp.	0.0016	**<0.00001**	0.0058	**0.0363**	-0.0005	**0.0095**
Hemisotoma thermophila	0.0044	**<0.00001**	0.0012	0.8150	0.0000	0.8780
Isotopenola loftyensis						
Lepidocyrtus sp.	0.0018	**<0.00001**	0.0096	0.0723	-0.0007	**0.0339**
Mucrosomia caeca						
Orthonychiurus cf. *folsomi*	0.0013	**<0.00001**	0.0052	**0.0383**	-0.0004	**0.0054**
Parisotoma notabilis	0.0026	**<0.00001**	-0.0071	0.0711	0.0004	0.0813
Sinella sp.	0.0013	**<0.00001**	-0.0051	0.3060	0.0004	0.1620

Table 2

Summary outcome of logistic regression (Quasibinomial, logit link) comparing the effects of constant (CT) and fluctuating (FT) temperature treatments on survival over three developmental stages in 10 springtail species. Full outcomes provided in Table C4. The species *I. loftyensis* and *M. ceaca* were not assessed for juvenile to adult or egg to adult development rate due to low sample size. Boldface indicates significant results.

Egg to hatching

Species	**Developmental temperature**		**Treatment (FT)**		**Interaction (temperature x treatment)**	
	Estimate	**P-value**	**Estimate**	**P-value**	**Estimate**	**P-value**
Cryptopygus sp.	-0.0531	0.1846	3.0759	**0.0416**	-0.1711	**0.0078**
Desoria trispinata	-0.0099	0.7493	2.0966	**0.0435**	-0.1241	**0.0085**
Folsomia sp.	-0.1387	**0.0003**	0.7982	0.5149	-0.0613	0.2726
Hemisotoma thermophila	-0.0384	0.1750	-0.8016	0.3430	0.0335	0.3850
Isotopenola loftyensis	-0.1248	**0.0013**	-1.4592	0.1710	0.0551	0.2645
Lepidocyrtus sp.	-0.2219	**0.0000**	-1.4809	0.2510	0.0874	0.1520
Mucrosomia caeca	-0.0573	0.1190	1.0491	0.3520	-0.0726	0.2080
Orthonychiurus cf. *folsomi*	-0.1852	**0.0002**	-2.4038	0.0801	0.0882	0.1430
Parisotoma notabilis	-0.1741	**0.0002**	-0.5863	0.6689	0.0226	0.7078
Sinella sp.	0.0137	0.6643	-0.0447	0.9577	-0.0422	0.2964

Hatching to adult

Species	**Developmental temperature**		**Treatment (FT)**		**Interaction (temperature x treatment)**	
	Estimate	**P-value**	**Estimate**	**P-value**	**Estimate**	**P-value**
Cryptopygus sp.	-0.0689	**0.0038**	-1.2946	0.0667	0.0823	**0.0263**
Desoria trispinata	-0.0435	0.1346	-2.4579	**0.0130**	0.1299	**0.0096**
Folsomia sp.	-0.0228	0.5220	1.2578	0.2030	-0.0595	0.2840

Hemisotoma thermophila	0.0082	0.7210	-0.3502	0.6130	-0.0015	0.9630
Isotopenola loftyensis						
Lepidocyrtus sp.	-0.0188	0.6521	2.9837	**0.0080**	-0.2156	**0.0020**
Mucrosomia caeca						
Orthonychiurus cf. *folsomi*	0.0388	0.1790	-0.0553	0.9440	0.0050	0.9100
Parisotoma notabilis	0.0199	0.4500	-0.4901	0.4700	0.0064	0.8630
Sinella sp.	-0.0072	0.8310	-0.7602	0.4860	0.0491	0.3520

Egg to adult

Species	**Developmental temperature**		**Treatment (FT)**		**Interaction (temperature x treatment)**	
	Estimate	**P-value**	**Estimate**	**P-value**	**Estimate**	**P-value**
Cryptopygus sp.	-0.0723	**0.0053**	-0.0795	0.9113	0.0022	0.9512
Desoria trispinata	-0.0374	0.1930	-0.5839	0.4950	0.0168	0.6810
Folsomia sp.	-0.0772	**0.0098**	1.5560	0.0795	-0.0928	0.0554
Hemisotoma thermophila	0.0016	0.9370	-0.4446	0.4850	0.0032	0.9160
Isotopenola loftyensis						
Lepidocyrtus sp.	-0.1326	**0.0002**	0.3944	0.6581	-0.0490	0.3544
Mucrosomia caeca						
Orthonychiurus cf. *folsomi*	-0.0892	**0.0028**	-0.5558	0.5174	0.0176	0.6675
Parisotoma notabilis	-0.0728	**0.0052**	-0.6329	0.3812	0.0162	0.6534
Sinella sp.	-0.0030	0.9300	-0.4734	0.6410	0.0172	0.7200

Figures

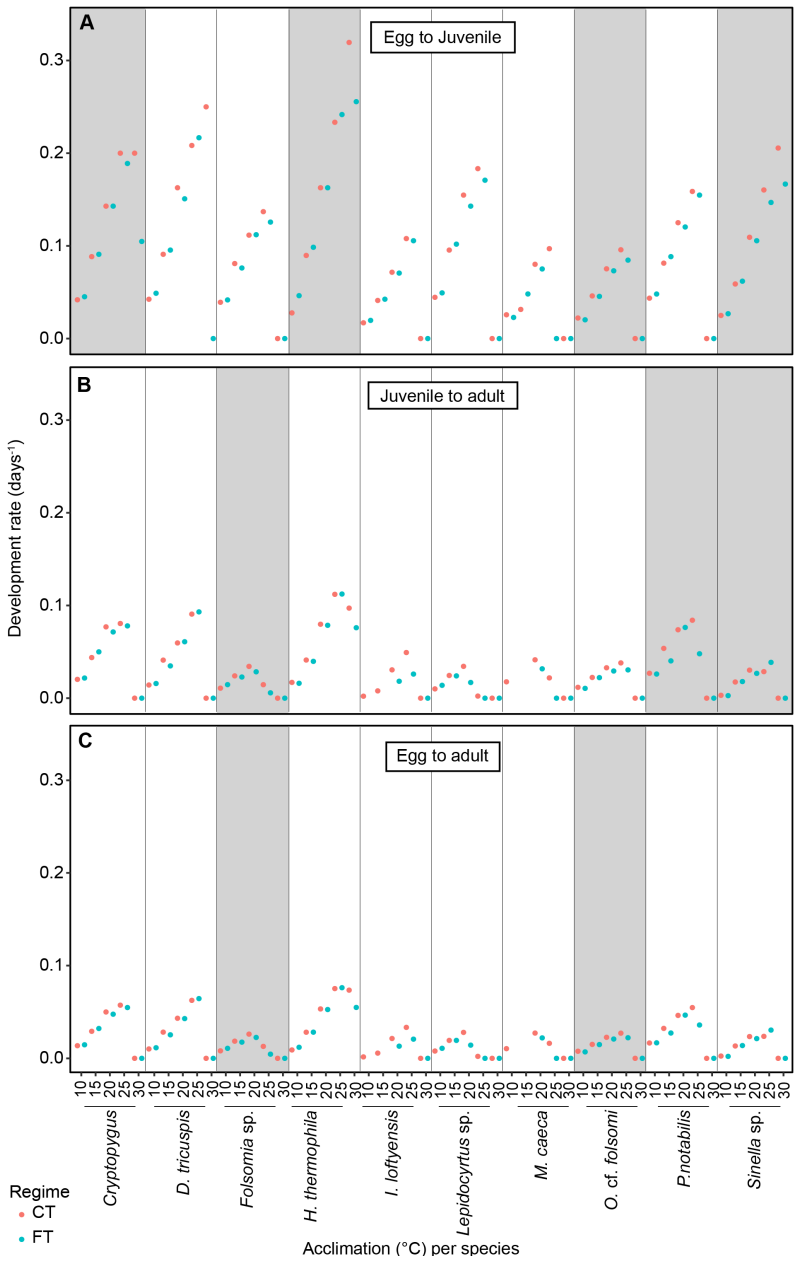

Figure 1

Mean response of development rate to constant (red) and fluctuating (blue) temperature treatments ranging from 10 to 30°C across different developmental stages for 10 springtail species. **A** shows the response of egg to juvenile development rate, **B** shows the response of juvenile to adult development rate, and **C** shows the response of egg to adult development rate. Shaded columns show species for which the response to the fluctuating temperature treatment is different to the constant temperature treatment. See Table C1 for mean, standard deviation and sample size and Tables 1, C3 for results of GLM comparing fluctuating and constant temperature treatments.

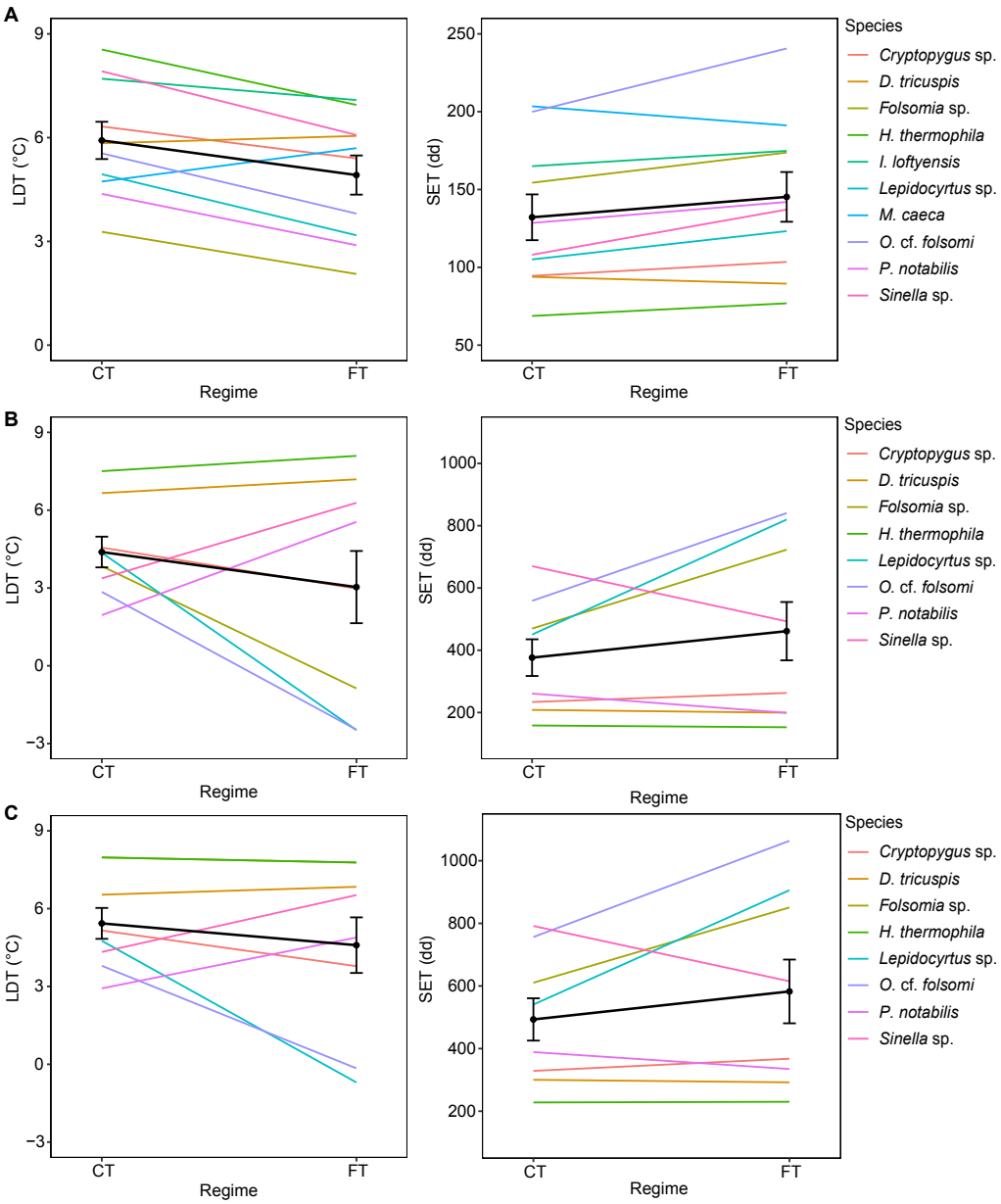

Figure 2

The difference between lower developmental threshold (LDT) and sum of effective temperatures (SET) for springtails exposed to constant (CT) and fluctuating temperature treatments (FT), across three stages of development. Development rate measured at 3 to 5 developmental temperatures between 10 and 30°C was used to calculate LDT and SET following the method in Honek (1996). LDT and SET are provided for three stages of development; egg to juvenile **A**, juvenile to adult **B,** and egg to adult **C**. Data are provided for 10 springtail species for the egg to juvenile stage, and for 8 species for the other stages (2 species were excluded due to a lack of data across more than 3 developmental temperatures). Mean and standard error for LDT and SET are shown in black. See Table C5 for LDT and SET values per species.

Appendix C: Supplementary data

Table C1

Mean development rate plus standard deviation, and number of replicates for 10 springtail species acclimated to constant (CT) and fluctuating (FT) temperatures increasing from 10 to 30°C in 5°C increments. Values are given for the developmental stages: egg to juvenile; juvenile to adult; and egg to adult. FT treatments vary by ±5°C around the constant temperature values.

Species	Developmental temperature	Egg to Juvenile				Juvenile to Adult				Egg to Adult			
		CT treatment		FT treatment		CT treatment		FT treatment		CT treatment		FT treatment	
		mean (s.d.)	n	mean (s.d.)	n	mean (s.d.)	n	mean (s.d.)	n	mean (s.d.)	n	mean (s.d.)	n
Cryptopygus sp.	10	0.042 (0.003)	6	0.045 (0.001)	6	0.02 (0.001)	6	0.022 (0.003)	6	0.014 (0)	6	0.015 (0.001)	6
	15	0.088 (0.004)	6	0.091 (0)	6	0.044 (0.007)	6	0.05 (0.005)	6	0.029 (0.003)	6	0.032 (0.002)	6
	20	0.143 (0)	6	0.143 (0)	6	0.077 (0)	6	0.071 (0)	6	0.05 (0)	6	0.048 (0)	6
	25	0.2 (0)	6	0.189 (0.017)	6	0.081 (0.007)	6	0.078 (0.013)	6	0.057 (0.004)	6	0.055 (0.007)	6
	30	0.2 (0)	6	0.157 (0.029)	4								
Desoria trispinata	10	0.042 (0.003)	6	0.049 (0.003)	6	0.017 (0.001)	5	0.019 (0.001)	5	0.012 (0.001)	5	0.014 (0)	5
	15	0.091 (0)	6	0.095 (0.005)	6	0.041 (0.003)	6	0.035 (0.005)	6	0.028 (0.002)	6	0.025 (0.002)	6
	20	0.163 (0.01)	6	0.151 (0.012)	6	0.06 (0.014)	6	0.061 (0.013)	6	0.043 (0.007)	6	0.043 (0.006)	6
	25	0.208 (0.02)	6	0.217 (0.026)	6	0.091 (0.018)	6	0.093 (0.026)	6	0.063 (0.009)	6	0.064 (0.014)	6
	30	0.25 (0)	6										
Folsomia sp.	10	0.039 (0.003)	6	0.042 (0.001)	6	0.013 (0.001)	5	0.015 (0.002)	6	0.01 (0.001)	5	0.011 (0.001)	6
	15	0.081 (0.01)	6	0.076 (0.004)	6	0.024 (0.004)	6	0.023 (0.003)	6	0.018 (0.003)	6	0.017 (0.002)	6
	20	0.112 (0.008)	6	0.112 (0.011)	6	0.034 (0.004)	6	0.028 (0.005)	6	0.026 (0.002)	6	0.023 (0.003)	6
	25	0.137 (0.009)	6	0.126 (0.01)	6	0.017 (0.002)	5			0.015 (0.001)	5		

	30												
Hemisotoma thermophila	10	0.028 (0.01)	6	0.046 (0.003)	6	0.02 (0.007)	5	0.016 (0.001)	6	0.011 (0.001)	5	0.012 (0.001)	6
	15	0.09 (0.003)	6	0.098 (0.004)	6	0.041 (0.004)	6	0.04 (0.005)	6	0.028 (0.002)	6	0.028 (0.003)	6
	20	0.163 (0.01)	6	0.163 (0.01)	6	0.08 (0.009)	6	0.079 (0.014)	6	0.053 (0.003)	6	0.053 (0.007)	6
	25	0.233 (0.026)	6	0.242 (0.02)	6	0.112 (0.011)	6	0.112 (0.019)	6	0.075 (0.005)	6	0.076 (0.01)	6
	30	0.319 (0.034)	6	0.256 (0.043)	6	0.097 (0.029)	6	0.091 (0.02)	5	0.074 (0.019)	6	0.066 (0.009)	5
Isotopenola loftyensis	10	0.017 (0)	6	0.02 (0)	6	0.006 (0)	2			0.005 (0)	2		
	15	0.041 (0.001)	6	0.042 (0.003)	6	0.016 (0.004)	3			0.011 (0.002)	3		
	20	0.072 (0.003)	6	0.071 (0.002)	6	0.031 (0.002)	6	0.027 (0.002)	4	0.021 (0.001)	6	0.02 (0.001)	4
	25	0.108 (0.01)	6	0.106 (0.006)	6	0.049 (0.012)	6	0.026 (0.006)	6	0.033 (0.005)	6	0.021 (0.004)	6
	30												
Lepidocyrtus sp.	10	0.044 (0.002)	6	0.049 (0.002)	6	0.012 (0.001)	5	0.014 (0.001)	6	0.009 (0)	5	0.011 (0)	6
	15	0.095 (0.005)	6	0.102 (0.005)	6	0.024 (0.005)	6	0.024 (0.005)	6	0.019 (0.003)	6	0.019 (0.003)	6
	20	0.155 (0.013)	6	0.143 (0)	6	0.034 (0.008)	6	0.025 (0.008)	4	0.028 (0.006)	6	0.021 (0.006)	4
	25	0.183 (0.018)	6	0.171 (0.028)	6								
	30												
Mucrosomia caeca	10	0.026 (0.001)	6	0.023 (0.006)	6	0.018 (0.001)	6			0.01 (0)	6		
	15	0.047 (0.003)	4	0.048 (0.003)	6								
	20	0.08 (0.004)	6	0.075 (0.003)	6	0.041 (0.004)	6	0.32 (0.007)	6	0.027 (0.002)	6	0.022 (0.004)	6
	25	0.097 (0.005)	6			0.022 (0.018)	6			0.016 (0.013)	6		
	30												
Orthonychiurus cf. *folsomi*	10	0.022 (0.001)	6	0.024 (0.001)	5	0.012 (0.001)	6	0.013 (0.001)	5	0.008 (0)	6	0.008 (0)	5
	15	0.046 (0.003)	6	0.045 (0.001)	6	0.022 (0.001)	6	0.022 (0.003)	6	0.015 (0.001)	6	0.015 (0.001)	6

	20	0.075 (0.005)	6	0.073 (0.009)	6	0.033 (0.005)	6	0.029 (0.006)	6	0.023 (0.003)	6	0.021 (0.003)	6
	25	0.096 (0.008)	6	0.085 (0.003)	6	0.038 (0.004)	6	0.03 (0.007)	6	0.027 (0.002)	6	0.022 (0.004)	6
	30												
Parisotoma notabilis	10	0.044 (0.002)	6	0.048 (0.002)	6	0.027 (0.003)	6	0.026 (0.005)	6	0.017 (0.001)	6	0.017 (0.002)	6
	15	0.081 (0.005)	6	0.088 (0.004)	6	0.054 (0.003)	6	0.04 (0.009)	6	0.032 (0.001)	6	0.027 (0.004)	6
	20	0.125 (0)	6	0.12 (0.007)	6	0.074 (0.008)	6	0.076 (0.006)	6	0.046 (0.003)	6	0.047 (0.002)	6
	25	0.159 (0.012)	6	0.155 (0.013)	6	0.084 (0.008)	6	0.048 (0.017)	6	0.055 (0.004)	6	0.036 (0.01)	6
	30												
Sinella sp.	10	0.025 (0.001)	6	0.027 (0.001)	6	0.006 (0)	3	0.008 (0)	2	0.005 (0)	3	0.006 (0)	2
	15	0.059 (0.002)	6	0.062 (0.002)	6	0.017 (0.004)	6	0.018 (0.004)	6	0.013 (0.003)	6	0.014 (0.002)	6
	20	0.109 (0.005)	6	0.106 (0.006)	6	0.03 (0.007)	6	0.027 (0.002)	6	0.023 (0.004)	6	0.021 (0.001)	6
	25	0.16 (0.023)	6	0.147 (0.01)	6	0.03 (0.011)	6	0.039 (0.006)	6	0.024 (0.007)	6	0.03 (0.003)	6
	30	0.206 (0.038)	6	0.167 (0)	6								

Table C2

Mean survival (standard deviation) (%) across 6 replicates for 10 springtail species reared at constant (CT) and fluctuating (FT) temperatures ranging from 10 to 30°C. Values are given for the developmental stages: egg to juvenile; juvenile to adult; and egg to adult. FT treatments vary by ±5°C around the constant temperature values.

Species	Developmental temperature	Egg to Juvenile		Juvenile to Adult		Egg to Adult	
		CT treatment	FT treatment	CT treatment	FT treatment	CT treatment	FT treatment
		mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)
Cryptopygus sp.	10	83.6 (18.7)	87.7 (9.1)	41.7 (16.6)	40 (13.5)	32.9 (9)	35.4 (13.9)
	15	94.7 (9.3)	92.7 (9.3)	59.5 (15.4)	59.5 (17.9)	55.6 (11.3)	54.7 (16.3)
	20	90.1 (8.5)	82.1 (19)	59.4 (11.7)	56 (9.9)	53.1 (9.1)	44.7 (7.4)
	25	75.1 (20.9)	90.4 (15.6)	55.1 (17.8)	49.7 (10)	41.2 (15.5)	45.8 (13.7)
	30	76.3 (4.3)	3.4 (3.1)	0	0	0	0
Desoria trispinata	10	77.7 (13.6)	74.2 (10.5)	31.3 (19.6)	25.9 (16.1)	22.9 (14.7)	19.1 (11.9)
	15	72.3 (7.4)	78.7 (8.5)	75.2 (10)	46.3 (14.7)	54.5 (9.6)	36.6 (13.1)
	20	75.8 (14.1)	81.2 (13.3)	67.3 (15.2)	65.9 (21)	50.3 (12.6)	51.6 (9.7)
	25	91.1 (5.5)	91.4 (7.8)	68.5 (16.7)	56.6 (12.6)	62.1 (14.8)	52.1 (14.1)
	30	62.9 (24.4)	0	0	0	0	0
Folsomia sp.	10	67.9 (11.4)	77.4 (16.3)	32.4 (18.7)	56 (12.9)	21.9 (13.2)	44.2 (17.3)
	15	84.4 (6.5)	83.1 (16.1)	60.5 (17.9)	68.4 (19)	51.1 (14.7)	54.5 (6.2)
	20	85.4 (18.7)	70.1 (28)	60.5 (13.9)	55.7 (18.5)	53.1 (20.2)	38.7 (21.9)
	25	77 (14)	39.3 (14.1)	24.1 (16.8)	16.7 (40.8)	18.8 (14.7)	0
	30	0	0	0	0	0	0
Hemisotoma thermophila	10	89 (12.5)	81.3 (12.1)	34.2 (25.1)	23 (15.8)	30.7 (24.9)	17.5 (10)

	15	87.5 (8.3)	89 (9.3)	56.1 (19.9)	57.5 (8.2)	49.6 (19.7)	50.8 (6.7)
	20	88.5 (6.4)	89.7 (7.1)	72.6 (14.1)	51.6 (14.1)	64.9 (15.7)	46 (11.6)
	25	88.7 (7.4)	79.5 (14.3)	48.3 (15.8)	51.1 (20.4)	42.9 (14.4)	40.2 (15.9)
	30	82.2 (17)	84.6 (17)	46 (27.3)	26.6 (16.9)	35.4 (17)	24.2 (15.5)
Isotopenola loftyensis	10	68.4 (23.1)	53.9 (15.9)	21.7 (40.2)	0	3.4 (8.4)	0
	15	67.3 (12.3)	49.1 (19.6)	22.9 (31.2)	0	16.1 (22.8)	0
	20	84.3 (9.8)	81.9 (16.4)	43.9 (13.9)	37.3 (32)	37.6 (15.5)	26.8 (22.4)
	25	67.6 (21.1)	67.9 (11.4)	58.3 (9.7)	61.1 (35.7)	39 (11.6)	19.2 (19.1)
	30	0	0	0	0	0	0
Lepidocyrtus sp.	10	77.1 (14.7)	56.9 (11.6)	41.3 (22.8)	54.4 (15.7)	30.2 (17.8)	31 (12.1)
	15	74.5 (14.2)	82.6 (12.9)	65.3 (20.5)	60.1 (16.6)	47.4 (11.6)	49.3 (15.9)
	20	93.8 (4.6)	91.7 (6.6)	45.8 (12.6)	14.6 (18.2)	43.2 (13.5)	13.7 (16.7)
	25	12.2 (5.8)	44.5 (19.3)	16.7 (40.8)	0	0	0
	30	0	0	0	0	0	0
Mucrosomia caeca	10	53 (17.7)	46.9 (14.7)	38.9 (17.4)	0	18.7 (7)	0
	15	20.2 (22.9)	28.4 (30.5)	0	0	0	0
	20	57.6 (10.3)	77.6 (8.6)	55.3 (27.1)	20.8 (13.2)	31.5 (15)	16 (10.3)
	25	57.9 (34.2)	0	22.4 (29.2)	0	12.1 (11.6)	0
	30	0	0	0	0	0	0
Orthonychiurus cf. *folsomi*	10	84.1 (8.8)	51.7 (34.9)	66.1 (9.7)	62 (39.1)	55.5 (10.1)	33.3 (30.8)
	15	82.7 (8.3)	85.6 (12.4)	73.1 (19.8)	67 (23.5)	60 (16.6)	55.8 (16.9)
	20	82.9 (10.8)	79.6 (9.2)	78.8 (10.5)	84 (11.3)	64.8 (8.7)	66.3 (7.7)
	25	82.1 (14.1)	75 (7.6)	76.7 (5.1)	76.9 (11.5)	62.6 (9.4)	57.5 (8.7)
	30	0	0	0	0	0	0

Parisotoma notabilis	10	80.9 (20.3)	77.3 (19.5)	44.4 (12)	32.5 (16.5)	35.9 (11.1)	25.3 (13.3)
	15	77.8 (11)	75.1 (13.6)	63.7 (12.9)	41.6 (14.4)	49.3 (11.9)	31.4 (12.8)
	20	90.5 (8.5)	83.6 (17.4)	53 (11.5)	67.3 (9.8)	47.8 (11)	55.9 (12.6)
	25	72.8 (16.6)	77.8 (9.5)	57.7 (17.1)	34.6 (20.8)	40.3 (8.7)	26.6 (15)
	30	0	0	0	0	0	0
Sinella sp.	10	79.5 (7.9)	58.5 (23.8)	10.4 (11.7)	9.1 (15.2)	8.7 (10.1)	4.7 (7.5)
	15	79 (22.6)	84 (9.2)	64.2 (18.1)	45 (18.3)	48 (10.8)	38.9 (19.5)
	20	96.6 (4.3)	76 (19.3)	64.5 (16.6)	80.9 (14.5)	62 (15.5)	61.5 (19.8)
	25	87.4 (8.6)	86.7 (16)	65.9 (10)	68.5 (15.6)	57.7 (11)	58.6 (14.7)
	30	80.1 (8.8)	41.7 (8.2)	0	0	0	0

Table C3

Full outcome of generalised linear models (Gaussian distribution, identity link) comparing the effects of constant (CT) and fluctuating (FT) temperature treatments on development rate (days^{-1}) over three stages of development in 10 springtail species. Table includes results for development rate variation in egg to juvenile, juvenile to adult and egg to adult stages. Note that *I. loftyensis* and *M. ceaca* were not assessed for variation in development rate in the juvenile to adult or egg to adult stages due to low sample size. Boldface indicates significant results.

Egg to Juvenile

Species	**Developmental temperature**				**Treatment (FT)**				**Interaction (temperature x treatment)**			
	Estimate	**Std. Error**	**t value**	**P-value**	**Estimate**	**Std. Error**	**t value**	**P-value**	**Estimate**	**Std. Error**	**t value**	**P-value**
Cryptopygus sp.	0.0105799	0.00024	44.989	**<0.00001**	0.0146937	0.0061099	2.405	**0.02045**	-0.0009152	0.0003326	-2.752	**0.00858**
Desoria trispinata	0.0106517	0.0003458	30.805	**<0.00001**	-0.0054241	0.0115976	-0.468	0.642	0.0005218	0.0005989	0.871	0.388
Folsomia sp.	0.0064782	0.0003382	19.16	**<0.00001**	0.0093915	0.0087862	1.069	0.29095	-0.0007224	0.0004783	-1.51	0.13806
Hemisotoma thermophila	0.0145438	0.000446	32.61	**<0.00001**	0.0339575	0.0149579	2.27	**0.0275**	-0.001536	0.0007724	-1.989	0.0522
Isotopenola loftyensis	0.0060611	0.0001953	31.034	**<0.00001**	0.0061503	0.0050742	1.212	0.232	-0.000341	0.0002762	-1.235	0.224
Lepidocyrtus sp.	0.0095165	0.0005152	18.472	**<0.00001**	0.0212563	0.013385	1.588	0.1194	-0.0014035	0.0007286	-1.926	0.0605
Mucrosomia caeca	0.0049148	0.0001588	30.948	**<0.00001**	-0.006545	0.0048713	-1.344	0.187	0.0003157	0.0002952	1.07	0.292
Orthonychiurus cf. *folsomi*	0.0050023	0.0002051	24.384	**<0.00001**	0.0119362	0.005485	2.176	**0.03508**	-0.0008463	0.0002962	-2.857	**0.00656**
Parisotoma notabilis	0.007783	0.0002631	29.586	**<0.00001**	0.0136920	0.0068345	2.003	0.0513	-0.0007406	0.000372	-1.991	0.0527
Sinella sp.	0.0092536	0.0003773	24.529	**<0.00001**	0.0289624	0.0113176	2.559	**0.013225**	-0.0019603	0.0005335	-3.674	**0.000535**

Juvenile to adult

Species	**Developmental temperature**				**Treatment (FT)**				**Interaction (temperature x treatment)**			
	Estimate	**Std. Error**	**t value**	**P-value**	**Estimate**	**Std. Error**	**t value**	**P-value**	**Estimate**	**Std. Error**	**t value**	**P-value**
Cryptopygus sp.	0.0042794	0.0003115	13.737	**<0.00001**	0.0081736	0.01	1.01	0.31808	-0.0004737	0.0004406	-1.075	0.28817
Desoria trispinata	0.0048	0.0005148	9.324	**<0.00001**	-0.0041073	0.0135782	-0.302	0.76377	0.0002166	0.000728	0.298	0.76755
Folsomia sp.	0.0021295	0.000205	10.389	**<0.00001**	0.0093856	0.0044456	2.111	**0.0429**	-0.0007466	0.0002834	-2.634	**0.013**

Hemisotoma thermophila	0.0063163	0.0004033	15.66	**<0.00001**	-0.005661	0.0103519	-0.547	0.587	0.00024	0.0005591	0.429	0.67
Isotopenola loftyensis												
Lepidocyrtus sp.	0.0022208	0.0003335	6.659	**<0.00001**	0.0127034	0.00745	1.705	0.0989	-0.0010012	0.0004859	-2.061	**0.0484**
Mucrosomia caeca												
Orthonychiurus cf. *folsomi*	0.00179	0.0001652	10.837	**<0.00001**	0.0080333	0.004416	1.819	0.0759	-0.0006003	0.0002385	-2.517	**0.0156**
Parisotoma notabilis	0.0038341	0.000289	13.268	**<0.00001**	-0.0204056	0.0088674	-2.301	**0.027**	0.0011933	0.0005406	2.207	**0.0334**
Sinella sp.	0.0014924	0.0002733	5.461	**<0.00001**	-0.0077193	0.0078169	-0.988	**0.33**	0.0005369	0.0004017	1.337	0.189

Egg to adult

Species	**Developmental temperature**				**Treatment (FT)**				**Interaction (temperature x treatment)**			
	Estimate	**Std. Error**	**t value**	**P-value**	**Estimate**	**Std. Error**	**t value**	**P-value**	**Estimate**	**Std. Error**	**t value**	**P-value**
Cryptopygus sp.	0.003041	0.000150	20.274	**<0.00001**	0.0053931	0.0038968	1.384	0.173	-0.0003212	0.0002121	-1.514	0.137
Desoria trispinata	0.00333	0.000262	12.733	**<0.00001**	-0.0016480	0.0068970	-0.239	0.812	0.00009248	0.0003698	0.25	0.804
Folsomia sp.	0.0016385	0.0001213	13.513	**<0.00001**	0.0057553	0.0026297	2.189	**0.03628**	-0.0004633	0.0001677	-2.763	**0.00954**
Hemisotoma thermophila	0.004387	0.000195	22.495	**<0.00001**	0.00118	0.0050060	0.236	0.815	-0.00004171	0.0002703	-0.154	0.878
Isotopenola loftyensis												
Lepidocyrtus sp.	0.0018493	0.0002298	8.048	**<0.00001**	0.0095729	0.0051332	1.865	0.0723	-0.0007456	0.0003348	-2.227	0.0339
Mucrosomia caeca												
Orthonychiurus cf. *folsomi*	0.0013225	0.0000904	14.628	**<0.00001**	0.00517	0.0024171	2.137	**0.03832**	-0.0003823	0.0001305	-2.929	**0.00542**
Parisotoma notabilis	0.0025714	0.0001242	20.705	**<0.00001**	-0.0070753	0.003811	-1.857	0.07114	0.0004161	0.0002323	1.791	0.0813
Sinella sp.	0.0012632	0.000173	7.303	**<0.00001**	-0.0051361	0.0049481	-1.038	0.306	0.0003625	0.0002543	1.426	0.162

Table C4

Full outcome of logistic regression (Quasibinomial, logit) comparing the effects of constant (CT) and fluctuating (FT) temperature treatments on survival over three developmental stages in 10 springtail species. Table includes results for survival in egg to juvenile, juvenile to adult and egg to adult stages. Note that *I. loftyensis* and *M. ceaca* were not assessed for variation in survival for the juvenile to adult or egg to adult development stage due to low sample size. Boldface indicates significant results.

Egg to hatching

Species	**Developmental temperature**				**Treatment (FT)**				**Interaction (temperature x treatment)**			
	Estimate	**Std. Error**	**t value**	**P-value**	**Estimate**	**Std. Error**	**t value**	**P-value**	**Estimate**	**Std. Error**	**t value**	**P-value**
Cryptopygus sp.	-0.05308	0.03951	-1.343	0.18457	3.0759	1.47517	2.085	**0.042**	-0.17107	0.06202	-2.758	**0.00783**
Desoria trispinata	-0.00994	0.031	-0.321	0.74932	2.09659	1.014822	2.066	**0.04347**	-0.1241	0.045483	-2.728	**0.00849**
Folsomia sp.	-0.13865	0.03636	-3.814	**0.000343**	0.79819	1.21785	0.655	0.514887	-0.06134	0.05536	-1.108	0.272564
Hemisotoma thermophila	-0.03837	0.02792	-1.374	0.18	-0.80164	0.83768	-0.957	0.343	0.03354	0.03829	0.876	0.385
Isotopenola loftyensis	-0.12482	0.03674	-3.398	**0.001257**	-1.45923	1.05215	-1.387	0.170969	0.05507	0.04886	1.127	0.264454
Lepidocyrtus sp.	-0.2219	0.0470	-4.719	**0.000016**	-1.4809	1.2781	-1.159	0.251	0.08743	0.06025	1.451	0.152
Mucrosomia caeca	-0.05734	0.0362	-1.584	0.119	1.04907	1.11733	0.939	0.352	-0.07261	0.05696	-1.275	0.208
Orthonychiurus cf. *folsomi*	-0.18518	0.04717	-3.926	**0.000239**	-2.40384	1.34854	-1.783	0.08008	0.08822	0.05939	1.485	0.143033
Parisotoma notabilis	-0.17407	0.04404	-3.953	**0.000219**	-0.58625	1.36339	-0.43	0.668851	0.02256	0.05987	0.377	0.707785
Sinella sp.	0.01371	0.031	0.436	0.6643	-0.04468	0.83961	-0.053	0.9577	-0.04215	0.03999	-1.054	0.2964

Hatching to adult

Species	**Developmental temperature**				**Treatment (FT)**				**Interaction (temperature x treatment)**			
	Estimate	**Std. Error**	**t value**	**P-value**	**Estimate**	**Std. Error**	**t value**	**P-value**	**Estimate**	**Std. Error**	**t value**	**P-value**
Cryptopygus sp.	-0.06889	0.02274	-3.029	**0.00376**	-1.29455	0.69174	-1.871	0.0667	0.08232	0.03603	2.284	**0.0263**
Desoria trispinata	-0.04346	0.02857	-1.521	0.1346	-2.45787	0.95448	-2.575	**0.013**	0.12993	0.04824	2.693	**0.0096**
Folsomia sp.	-0.0228	0.03528	-0.646	0.522	1.25781	0.97377	1.292	0.203	-0.05948	0.05486	-1.084	0.284

Hemisotoma thermophila	0.008234	0.022927	0.359	0.721	-0.35016	0.688471	-0.509	0.613	-0.00151	0.032765	-0.046	0.963
Isotopenola loftyensis												
Lepidocyrtus sp.	-0.01881	0.04144	-0.454	0.65209	2.98371	1.0737	2.779	**0.00799**	-0.21555	0.06545	-3.294	**0.00196**
Mucrosomia caeca												
Orthonychiurus cf. *folsomi*	0.038836	0.028395	1.368	0.179	-0.055339	0.787191	-0.07	0.944	0.004977	0.043968	0.113	0.91
Parisotoma notabilis	0.01989	0.02609	0.762	0.45	-0.49014	0.6732	-0.728	0.47	0.0064	0.03688	0.174	0.863
Sinella sp.	-0.0072	0.033506	-0.215	0.831	-0.760215	1.082941	-0.702	0.486	0.049054	0.052243	0.939	0.352

Egg to adult

Species	**Developmental temperature**				**Treatment (FT)**				**Interaction (temperature x treatment)**			
	Estimate	**Std. Error**	**t value**	**P-value**	**Estimate**	**Std. Error**	**t value**	**P-value**	**Estimate**	**Std. Error**	**t value**	**P-value**
Cryptopygus sp.	-0.07232	0.02493	-2.901	**0.00531**	-0.07947	0.71046	-0.112	0.91134	0.00216	0.03515	0.061	0.9512
Desoria trispinata	-0.03736	0.02832	-1.319	0.193	-0.58391	0.85094	-0.686	0.495	0.01678	0.04064	0.413	0.681
Folsomia sp.	-0.07724	0.02888	-2.674	**0.00984**	1.55601	0.87076	1.787	0.07946	-0.09281	0.04742	-1.957	0.05539
Hemisotoma thermophila	0.001643	0.020841	0.079	0.937	-0.444596	0.632623	-0.703	0.485	0.003165	0.029927	0.106	0.916
Lepidocyrtus sp.	-0.13256	0.03254	-4.074	**0.00015**	0.3944	0.88642	0.445	0.65811	-0.04903	0.0525	-0.934	0.3544
Orthonychiurus cf. *folsomi*	-0.08918	0.02852	-3.126	**0.00283**	-0.5558	0.85298	-0.652	0.51738	0.01763	0.04081	0.432	0.66745
Parisotoma notabilis	-0.07283	0.02502	-2.91	**0.00517**	-0.6329	0.71707	-0.883	0.38122	0.01623	0.03595	0.451	0.65339
Sinella sp.	-0.00296	0.033353	-0.089	0.93	-0.473374	1.010763	-0.468	0.641	0.017191	0.047778	0.36	0.72

Table C5

Lower developmental threshold (LDT) and sum of effective temperatures (SET) for 10 springtail species measured across three stages of development. For both fluctuating and constant temperature treatments, development rate measured at 3 to 5 developmental temperatures between 10 and 30°C was used to calculate LDT and SET following the method in Honek (1996). The amplitude of fluctuations was ±5°C around the constant temperatures. LDT and SET are provided for three stages of development; egg to juvenile, juvenile to adult and egg to adult. Data are provided for 10 springtail species for the egg to juvenile stage, and for 8 species for the other stages (2 species were excluded due to a lack of data across more than 3 developmental temperatures).

Species	**Treatment**	**Developmental stage**					
		Egg to Hatching		**Hatching to Adult**		**Egg to Adult**	
		LDT (°C)	**SET (dd)**	**LDT (°C)**	**SET (dd)**	**LDT (°C)**	**SET (dd)**
Cryptopygus sp.	CT	6.32	94.52	4.56	233.68	5.16	328.86
	FT	5.40	103.47	2.98	262.76	3.78	367.65
Desoria trispinata	CT	5.84	93.88	6.66	208.33	6.54	300.33
	FT	6.05	89.50	7.19	199.34	6.84	292.21
Folsomia sp.	CT	3.28	154.36	3.84	469.59	3.94	610.31
	FT	2.06	173.74	-0.87	723.12	0.60	850.85
Hemisotoma thermophila	CT	8.55	68.76	7.50	158.32	7.97	227.95
	FT	6.95	76.88	8.09	152.53	7.78	230.14
Isotopenola loftyensis	CT	7.70	164.99				
	FT	7.09	174.82				
Lepidocyrtus sp.	CT	4.94	105.08	4.36	450.29	4.76	540.75
	FT	3.18	123.26	-2.48	819.94	-0.70	906.04
Mucrosomia caeca	CT	4.73	203.47				
	FT	5.70	191.19				
Orthonychiurus cf. *folsomi*	CT	5.55	199.91	2.85	558.66	3.80	756.43
	FT	3.80	240.62	-2.46	840.55	-0.16	1063.72
Parisotoma notabilis	CT	4.37	128.49	1.95	260.82	2.93	388.89
	FT	2.89	142.00	5.55	198.91	4.89	334.73
Sinella sp.	CT	7.92	108.07	3.37	670.06	4.33	791.64
	FT	6.08	137.11	6.28	492.78	6.52	615.12

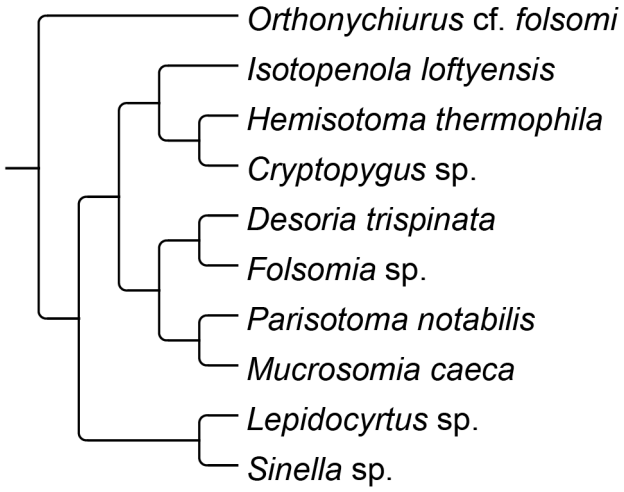

Figure C1

Phylogeny of 10 springtail species used in this study. The phylogenetic tree was developed using previous work on the phylogenetic relationships of major springtail taxa (D'Haese 2002; Malcicka et al. 2017) and recent work on Australian springtail species (Janion-Scheepers et al. 2018).

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Chapter 4

Thermal tolerance, disturbance and soil invasion

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Abstract

Biological invasions constitute a significant threat to biodiversity and are increasing globally. As a consequence, species' traits are increasingly used to improve predictions of the likelihood and course of invasions. Recently, it was proposed that alien species may have an advantage over indigenous species because alien species typically come from disturbed areas that experience high temperature extremes. However, as native environments of indigenous species may experience similar extremes to environments of alien species, any advantage that alien species have may be limited by geographic location. To explore this idea, we examined the critical thermal maxima (CT_{max}) of 24 indigenous and 21 alien springtail species originating from tropical, temperate and sub-polar latitudes. We expected that CT_{max} would be higher for alien species than indigenous species from the tropical and sub-polar locations and equal for species from the temperate location, because temperate areas typically experience a larger number of temperature extremes. We found the following results. **1.** Irrespective of location, the CT_{max} of alien species was 1.37°C higher than that of indigenous species **2.** The CT_{max} of tropical and sub-polar alien species was higher (by 1.56ºC and 4.86ºC, respectively) than those of their indigenous counterparts. **3.** The CT_{max} of temperate alien species was 1.28ºC lower than that of temperate indigenous species. These results show that the effects of alien species on indigenous biodiversity under climate change may be location-dependent. Thus, alien species origin, and the thermal environment of origin in relation to that of the area being invaded, should be considered when predicting the outcomes of species introductions and assessing risks to biodiversity.

1. Introduction

Biological invasions constitute a significant threat to biodiversity. The introduction of species by humans to new areas, and the subsequent establishment and spread of introduced species,

can result in large-scale transformation of ecosystems (Simberloff et al. 2013; Gallardo et al. 2016) and extinction of native species (Blackburn et al. 2004; Clavero and García-Berthou 2005; Maxwell et al. 2016). Introductions of species that go on to become invasive are increasing around the globe (Seebens et al. 2017). In consequence, much interest exists in determining whether the outcomes of introductions can be explained by environmental characteristics, species traits, phylogenetic relatedness, introduction effort, or some combination thereof (Richardson and Pyšek 2006; Pyšek et al. 2012; Allen et al. 2017; Redding et al. 2019). A better understanding of how invasions proceed is essential for forecasting the outcomes of existing introductions (van Kleunen et al. 2010a), and for risk assessments to improve biosecurity (McGeoch and Jetz 2019; Nunez-Mir et al. 2019).

Trait-based approaches have long been a feature of investigations into the factors contributing to the success of introduced species (Baker 1974; Leibhold et al. 1995; Kolar and Lodge 2001). They have risen to prominence recently, however, given the increasing availability of trait data (e.g. Jones et al. 2009; Kattge et al. 2011; Parr et al. 2017), the need to investigate how traits influence success along invasion pathways (van Kleunen et al. 2010b), and the demonstrated value of trait-based approaches for understanding both plant and vertebrate invasions (Whitney and Gabler 2008; van Kleunen et al. 2010a; Sahlin et al. 2011; Capellini et al. 2015; Allen et al. 2017; Nunez-Mir et al. 2019; Redding et al. 2019).

Less attention has been paid to understanding the role of trait differences along invasion pathways for invertebrates, with most focus currently given to aquatic groups (e.g. Roy et al. 2002; Stachowicz et al. 2002; Grabowksi et al. 2007; Bates et al. 2013). In the context of soil invasions, most of the focus on invertebrates and their traits centres on ants (Holway et al. 2002; Bertelsmeier et al. 2017), with little information available on other groups (Pey et al. 2014). Yet concerns are growing about the extent and impacts of invasive alien species in soil systems (Cicconardi et al. 2017; Coyle et al. 2017; Ricciardi et al. 2017; Ferlian et al. 2018).

Springtails (Collembola) are one group for which understanding of trait differences among indigenous and non-indigenous species is developing. A diversifying range of studies has shown that invasive alien species generally have wider thermal limits, higher development rates, greater egg hatching success at high temperatures, greater desiccation resistance, and lower development thresholds, relative to their indigenous counterparts (Chown et al. 2007; Janion et al. 2010; Treasure et al. 2019; Philipps et al. 2020).

A consistent finding of these comparisons is that invasive alien springtail species have higher critical thermal maximum temperatures (CT_{max} – a maximum threshold temperature for activity), lower critical thermal minimum temperatures (CT_{min} – a minimum threshold temperature for activity), and broader thermal tolerance ranges (CT_{range}), than their common indigenous counterparts across a wide span of latitudes from tropical to sub-polar locations (e.g. Janion-Scheepers et al. 2018; Phillips et al. 2020). The fact that lower critical thermal limits, and thermal tolerance ranges, vary among species from different environments is unsurprising and in keeping with findings for terrestrial invertebrates (and indeed for other organisms) globally (Addo-Bediako et al. 2000; Sunday et al. 2011; Holmstrup 2018). By contrast, the consistent differences in CT_{max} (of about 3.0°C on average) between invasive alien springtail species and their common indigenous counterparts (Janion-Scheepers et al. 2018; Phillips et al. 2020) is remarkable. Critical thermal maxima generally show limited variation among terrestrial species and populations, and limited variation over time in both invertebrates and vertebrates (e.g. Addo-Bediako et al. 2000; Huey et al. 2009; Diamond et al. 2012; Araújo et al. 2013; Pinsky et al. 2019; Sunday et al. 2019). These maxima have also been demonstrated to show limited evolutionary potential and phenotypic plasticity (Mitchell and Hoffmann 2010; Hoffmann et al. 2013; Blackburn et al. 2014; Gunderson and Stillman 2015; Diamond et al. 2017; Janion-Scheepers et al. 2018; Castañeda et al. 2019).

To explain these differences in critical thermal limits between indigenous and invasive alien springtail species, the hypothesis was proposed that variation in extreme temperatures and disturbance among the native ranges of indigenous and invasive alien species might be responsible (Bertelsmeier et al. 2017; Coyle et al. 2017). Three major assumptions are implicit in this hypothesis. **Assumption 1.** Globally, alien springtail species tend to be introduced from locations, or simply from habitats in any location, that regularly experience disturbance. **Assumption 2.** Disturbed areas tend to experience higher extreme temperatures than undisturbed areas. **Assumption 3.** These two interacting effects result in higher CT_{max} values for introduced species than their indigenous counterparts, except in areas where indigenous species experience conditions similar to those of introduced species in their native ranges (Fig. 1).

Since it is widely known that most invasive alien springtail species are widespread European species from disturbed habitats (King et al. 1985; Greenslade 2002; Greenslade and Convey 2011; Janion et al. 2011; Porco et al. 2012; Cicconardi et al. 2017; Baird et al. 2019), Assumption 1 has some support. Thus, many invasive alien springtail species originate from a small area of the globe known to have a long history of both natural and anthropogenic disturbance since the last glacial maximum (Fyfe et al. 2015; Kaplan et al. 2016; Marquer et al. 2017). For this reason, we set out to examine explicitly Assumptions 2 and 3, which to date have not been carefully considered. We focus especially on Assumption 3, testing the hypothesis that higher CT_{max} values in invasive alien species than in their indigenous counterparts should be found in tropical and sub-polar assemblages that are less regularly exposed to extreme temperatures, as opposed to mid-latitude assemblages that routinely encounter extreme temperatures (Addo-Bediako et al. 2000; Hoffmann 2010) — especially in the southern hemisphere. Nonetheless, we also examine Assumption 2 by comparing outcomes from a range of studies on the thermal environments of disturbed areas.

2. Materials and methods

2.1 Springtail collection and maintenance

We measured the critical thermal maxima (CT_{max}) of 45 springtail species (21 alien, 24 indigenous) (Fig. 2A) from 11 families, originating from tropical, temperate, and sub-polar locations within Australia (see Table D1 for species). The families included Entomobryidae (10), Isotomidae (13), Hypogastruridae (6), Neanuridae (4), Onychiuridae (3), Brachystomellidae (3), Katiannidae (2), Neelidae (1), Sminthurididae (1), Dicyrtomidae (1), Orchesellidae (1). We measured field-caught springtails and did not maintain springtails until the F2 generation before trait assessment, as has been done previously (e.g. Janion-Scheepers et al. 2018). We did this to ensure comparability among data and because, where differences in CT_{max} among field-caught and F2 generations have been sought, they have typically been found to be small and statistically non-significant (Phillips et al. 2020).

Tropical springtails were collected in April 2019 from Kamerunga Regional Park in Cairns, Queensland, Australia (16.8733° S, 145.6831° E), by sifting leaf litter and by manual aspiration (following Janion-Scheepers et al. 2018). Eleven tropical species were identified, of which 5 were indigenous and 6 were alien (Fig. 1A; Table D1). Tropical springtails were sorted into morpho-species, and kept for ~24 hours at room temperature (verified as 22.5-26.5ºC via Thermochron iButtons™, model DS1920G, Maxim Integrated, San Jose, CA, USA) in 70ml pots containing a saturated plaster-of-Paris substrate and plane tree bark (*Plantanus* sp.) to feed on *ad libitum* (see Hoskins et al. 2015; Janion-Scheepers et al. 2018) before CT_{max} data were collected. On completion of data collection, springtails were preserved in vials of 100% ethanol for species identification and DNA barcoding.

Temperate springtails were collected between July 2017 and December 2018 from Jock Marshall Reserve (JMR), Monash University Clayton Campus, Victoria Australia (37.9096° S, 145.1400° E), via manual aspiration or extraction from leaf litter/soil samples using a

Berlese-Tullgren funnel system (Southwood and Henderson 2009). Eighteen temperate springtail species were identified, of which 10 were indigenous and 8 were alien (Fig. 1A; Table D1). Temperate species were maintained in 70 ml plastic vials with a plaster-of-Paris:charcoal substrate (9:1). Springtails were fed a standard diet of plane tree bark (*Plantanus* sp.) *ad libitum* (Hoskins et al. 2015; Janion-Scheepers et al. 2018), and their substrate kept saturated to prevent desiccation. Springtails were kept in a controlled temperature room at 20°C (verified as 20.3±0.4°C via Thermochron iButtons™, model DS1920G, Maxim Integrated, San Jose, CA, USA) on a 12h dark:12h light photoperiod for 7 to 14 days before CT_{max} data were collected.

Sub-polar springtails were collected on two occasions from Macquarie Island (54°30' S, 158°57' E) during the resupply missions of March/April 2016 and February/March 2017. Macquarie Island is a sub-Antarctic island considered a part of Australia. Springtails were collected via beating vegetation and manual aspiration in the field, and collecting turf samples (10x10x5cm). During transportation to Monash University (1-2 weeks), turf samples were stored at 5°C and monitored with Hygrochron iButtons (DS 1923-F5, Maxim Integrated, San Jose, CA, USA). Springtails were then extracted from the samples at room temperature (~20 to 22°C) using a Berlese-Tullgren funnel system (Southwood and Henderson 2009), and sorted into species. Sixteen sub-polar species were identified, of which 8 were indigenous and 8 were alien (Fig. 1A; Table D1). Sub-polar species were maintained as per the method described above, in a controlled temperature room at 10°C (verified as 10.14±0.2°C via Thermochron iButtons™, model DS1920G, Maxim Integrated, San Jose, CA, USA) on a 12h dark:12h light photoperiod for 7 to 14 days before CT_{max} data were collected.

2.2 Springtail identification

Springtail identification was conducted following the methods described in Janion-Scheepers et al. (2018). Springtails from Macquarie Island are well known, and were identified using the key developed for them (Greenslade and Van Klinken 2006). Tropical and temperate springtails were first identified to morpho-species, then later to Family and Genus (and species where possible), using keys for Australian and European springtail fauna (e.g. Fjellberg 1998; Hopkin 2007; Greenslade et al. 2014). All identifications were confirmed with the assistance of specialist springtail taxonomists and via sequencing of the mitochondrial cytochrome oxidase subunit 1 gene. Resulting sequences were compared with sequences available through the Barcode of Life Data Systems (BoLD) (www.barcodinglife.org) and via the basic local alignment search tool (BLAST) in GenBank (www.blast.ncbi.nlm.nih.gov). Mitochondrial DNA extraction and sequencing were undertaken by the Biodiversity Institute of Ontario, University of Guelph, Canada. Alien species were classed as those with samples in the BoLD or GenBank databases from locations other than Australia — in particular, the Northern Hemisphere. Where sequencing was unsuccessful (4 out of 45 species), species were identified using descriptions from several sources (Fjellberg 1998; Hopkin 2007; Greenslade et al. 2014).

2.3 Climate characteristics

Frequency of maximum daily temperature was determined for each collection location (Fig. 1) to illustrate how often each location experiences extreme temperatures. Maximum daily temperature was sourced from climate statistics available from the Australian Bureau of Meteorology (www.bom.gov.au) from weather stations located at or near collection locations, and encompass daily measurements from 1980 to 2019. A frequency histogram of maximum daily temperature was generated in R v. 3.6.3 (R Core Team 2018) using the RStudio v. 1.1.463 platform (RStudio Team 2016).

2.4 Critical thermal maximum

To measure the critical thermal maxima (CT_{max}) of each species, springtails were placed into a 40 ml plastic vial positioned in a hollow metal stage (custom built by Monash University Instrument Facility, Clayton Campus, Victoria, Australia). For sub-polar and temperate species, the stage was connected to a programmable Grant water bath (TFX200, Grant Instruments, Cambridge, UK). For tropical species, the stage was connected to a Peltier-thermoelectric cooler plate (CP-121HT plate with a TC-720 controller, TE Technology, Michigan, USA) with liquid heat exchange attachments (unbranded aluminium-alloy four-channel 120 mm water block). We determined CT_{max} by ramping temperature at a rate of 0.05°C per minute, and measuring the temperature at which springtails lost motor function (as in Janion-Scheepers et al. 2018). This was achieved by running heated liquid (50:50 water:propylene glycol mix for sub-polar and temperate species, and water using an Aquapro AP210 water pump for tropical species) through the metal stage. The ramping rate of 0.05°C per minute was used to correspond closely to the rate of temperature change observed in temperate and tropical microhabitats (Allen et al. 2016), and was also applied to sub-polar springtails to make data for species from different geographic locations comparable.

Ramping was initiated at 10°C for sub-polar springtails and 20°C for temperate and tropical springtails, after holding springtails at these temperatures for 15 minutes. Springtails were monitored every 30 minutes until cessation of movement was observed, then checked every 10 minutes (or every increase of 0.5°C) until motor function was lost. Loss of motor function was defined as the inability of a springtail to return to an upright position after being tipped onto their side or back with a paintbrush (Janion-Scheepers et al. 2018). Vial temperatures were measured with thermocouples (type K for sub-polar and temperate species, or type J for tropical species) and a temperature data logger (RDXL 12SD, Omega Engineering, USA). Typically, CT_{max} was measured for 30-50 springtails per species over 2-3 replicates.

Both methods used to examine CT_{max} (i.e. water bath and Peltier) were tested for accuracy using a temperature data logger (RDXL 12SD, Omega Engineering, USA) and thermocouple (type K), and were confirmed to have a mean ramping rate of 0.047°C per minute. Temperate and sub-polar CT_{max} experiments were undertaken at Monash University, Clayton Campus, Victoria, and tropical experiments were undertaken at the field site location in Cairns, Queensland.

2.5 Statistical analysis

A phylogenetic generalised least squares (PGLS) model was used to assess the difference in mean CT_{max} of indigenous and alien springtail species from tropical, temperate and sub-polar locations. The covariance matrix for the models assumed an evolutionary model of Brownian motion (BM), since type I error rates of Ornstein-Uhlenbeck (OU) models are high when phylogenies have less than 200 species (as was the case here). In this case, the OU model parameter is indistinguishable from that of the BM model (Cooper et al. 2016). The maximum-likelihood (ML) estimate of Pagel's lambda (λ) (Pagel 1999) was used to assess the effect of phylogeny on CT_{max}, where a lambda of 0 indicates no phylogenetic effect and a lambda of 1 indicates a strong phylogenetic effect. The analysis was repeated with ordinary least squares (OLS) using the 'lm' function in R (see justification in Janion-Scheepers et al. 2018) (Table D2). Analyses were implemented in the packages "ape' (v. 5.3; Paradis et al. 2004) and 'caper' (v. 1.0.1; Orme 2013).

The phylogenetic tree for the PGLS model (Fig. D1) was based on previous work that uses molecular-based methods to assess phylogenetic relationships of major springtail taxa (D'Haese 2002; Malcicka et al. 2017) and recent work on Australian and South African springtail species (Janion-Scheepers 2018; Liu et al. 2020). Branch lengths for the final tree were assigned using Grafen's method (Grafen 1989).

A frequency histogram of individual CT_{max} values for springtails was generated to illustrate how the instances of extreme temperature events in tropical, temperate and sub-polar locations might influence thermal tolerance in springtails. All analyses were conducted in R v. 3.6.3 (R Core Team 2018) using the RStudio v. 1.1.463 platform (RStudio Team 2016).

3. Results

Irrespective of geographic location, the CT_{max} of alien springtails was 1.37°C higher than that of indigenous springtails (Fig. 2; Table 1). On average across locations, alien springtails had a higher CT_{max} compared to indigenous springtails from sub-polar and tropical locations (by 1.56 and 4.86°C, respectively) and a lower CT_{max} compared to indigenous springtails from the temperate location (by 1.28°C) (Fig. 2; Table 1).

The PGLS model indicates that the mean CT_{max} of alien species does not differ among locations but the mean CT_{max} of indigenous species does, with the difference between alien and indigenous springtails being greatest for sub-polar species (Table 2). There was no significant difference in CT_{max} between indigenous and alien springtails from the temperate location (Table 2). Likewise, CT_{max} did not vary significantly among alien springtails across locations (Fig. 2). The difference between the CT_{max} of alien and indigenous species was significantly greater in species from polar environments than species from temperate environments; however, the effect of species status was not significantly different between species from tropical and temperate locations.

The frequencies of maximum daily temperature at all locations (Fig. 1) illustrate that the temperate location experiences more variation in maximum daily temperature (7.3 to 46.7 ºC) in comparison to the other two locations, and also experiences many days where the temperature exceeds the CT_{max} of the species from this location (species means: CT_{max} = 33.7 to 43.9 ºC) (Fig. 1; Table D1). However, maximum daily temperature at the sub-polar location

(range of daily maximum temperature = -4.6 to 14.4 ºC) never approaches the CT_{max} of the sub-polar species (species means: CT_{max} = 29.7 to 38.3 ºC). Likewise, temperature at the tropical location, even experiencing higher temperatures (range of daily maximum temperature: 16.7 to 39.8 ºC), rarely exceeds 37 ºC and the CT_{max} of the tropical species tested (species means: 37 to 41.5ºC). Thus, the temperate springtails are likely to experience more evolutionary pressure to develop higher thermal tolerance than either the tropical or sub-polar springtails.

4. Discussion

The outcomes presented here demonstrate that, although alien springtails have an overall advantage over indigenous springtails in terms of thermal tolerance (i.e. alien springtails generally have a higher CT_{max} than their indigenous counterparts), this advantage varies depending on geographic location. Thus, these outcomes lend support to Assumption 3, that alien springtail species have higher CT_{max} than their indigenous counterparts, except in temperate areas where springtails experience more extreme maximum temperatures that exceed their thermal tolerances. In other words, in those areas where indigenous species experience conditions similar to those of introduced species in their native range.

Assumption 2 suggests that locational variation in thermal tolerance may arise due to differences in the level of disturbance experienced by alien and indigenous species at different locations. Disturbance, whether natural (e.g. glaciation, fire) or anthropogenic (e.g. urbanisation), increases temperature, predominantly by reducing vegetation. For example, Liu et al. (2020) found that the reduction of vegetation by wildfires in fynbos shrublands in South Africa increased ground temperature by 7.5 °C. Furthermore, rocky areas recently exposed by retreating glaciers have been shown to have highly-variable and often extreme temperatures, compared to more vegetated areas (Mathews 1999; Fickert 2017). Similarly, disturbance in the form of urbanisation results in substantial heat islands with temperatures typically much higher

than in surrounding areas (e.g. Imhoff et al. 2010), affecting invertebrate physiology and especially thermal tolerances (Chown and Duffy 2015). For example, ants living in urban areas have been shown to develop higher thermal tolerances than those in rural settings (e.g. Angilletta et al. 2007; Diamond et al. 2017). Conversion of forest landscapes to agriculture has similarly profound effects on thermal landscapes (Bonan 1999). Of the locations included in this study, those with highest levels of disturbance and thermal variability (temperate and tropical locations) were also those that exhibited CT_{max} values closest to the values observed for the alien springtails in this study. It is difficult to determine the exact environment from which the alien species originate; however, as alien springtails in Australia typically originate from Europe — an area that has experienced high levels of natural and anthropogenic disturbance (Fyfe et al. 2015; Kaplan et al. 2016; Marquer et al. 2017) — it is probable that the indigenous and alien environments of the tropical and temperate locations in this study are similar enough to the invasive environment to elicit a comparable response in CT_{max}. However, in this case, the less extreme temperatures found in the tropical location seem to indicate that alien species will have an advantage, whilst in the temperate location the opposite is true.

Although the approach to measuring CT_{max} in this study attempts to mitigate any variation introduced by different methods of data collection, considerations should be made for differences in the temperature at which the experiments were initiated and ramping rate used. Despite the limited variation and adaptability of CT_{max} among species (Addo-Bediako et al. 2000; Janion-Scheepers et al. 2018; Castañeda et al. 2019; Sunday et al. 2019), differences in starting temperature can have a considerable impact on CT_{max}. In particular, lower starting temperatures are known to reduce thermal tolerance (e.g. Terblanche et al. 2007) as species spend more time under potentially stressful thermal conditions before reaching their maximum thermal threshold. The lower CT_{max} observed as a result is possibly due to accumulation of damage (e.g. Cossins and Bowler 1987), or desiccation and starvation (Terblanche et al. 2011)

— although the latter two are unlikely in this study, given the duration of the experiments and the methodology used. This could explain why alien species from the sub-polar location had lower CT_{max} values in comparison to species from other locations, although this could also be linked to a relaxation of selection pressure due to lower environmental temperatures at the sub-polar location (e.g. Oyen et al. 2016). The rate at which temperature increases has also been shown to affect CT_{max} values, with faster rates of increase generally yielding higher values (e.g. Terblanche et al. 2007; Chown et al. 2009; Allen et al. 2016). The rates used here closely reflect conditions observed in tropical and temperate locations, and thus may be less reflective of the slower rates of temperature increase typically observed in sub-polar areas (e.g. Allen et al. 2016). However, as previous work on springtails using a similar approach to the one here has yielded similar results (e.g. Allen et al. 2016; Janion-Scheepers et al. 2018), any effect of disparity between the ramping rate used and what the springtails experience naturally is unlikely to be substantial.

Despite these considerations, the results of this study show that the interactions of alien species with indigenous biodiversity under climate change may be location-dependent, with a larger effect seen in those areas where the thermal tolerance of indigenous species is lower than that of their alien counterparts, as is the case in sub-polar and undisturbed locations. This is contrary to other work on springtails suggesting that alien species will have an advantage over indigenous species regardless of location, and also that under climate-change conditions, springtail communities are likely to be overtaken by invasive species (e.g. Janion-Scheepers et al. 2018). Thus, consideration of alien species origin, and the thermal environment of origin in relation to that of the area being invaded, should be considered when predicting the outcomes of species introductions and in improving assessments of risks to biodiversity. However, more research into thermal tolerance of other invertebrate taxa, including comparisons of disturbed and undisturbed areas at different geographic locations, are needed to confirm these results.

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Tables

Table 1

Mean, standard deviation and sample size of CT_{max} for indigenous and alien springtail species from three geographic locations in Australia.

Location	Indigenous		Alien	
	mean±s.e. (°C)	**n**	**mean±s.e. (°C)**	**n**
Tropical	37.861±0.921	5	39.425±0.781	6
Temperate	40.024±2.862	7	38.746±0.596	11
Sub-polar	31.797±1.831	8	36.657±0.726	8
All	36.741±4.234	24	38.112±0.464	21

Table 2

Outcome of PGLS showing the difference in critical thermal maximum (CT_{max}) between alien and indigenous springtails from tropical, temperate and sub-polar locations. ML^{λ} (maximum-likelihood estimate of Pagel's λ) indicates the phylogenetic effect, where 1 equals a strong effect and 0 equals no effect.

	Estimate	**Std. Error**	**t value**	**P-value**
Intercept (alien temperate)	38.71	0.712	54.37	<0.001
Status				
Indigenous temperate	1.425	0.966	1.475	0.148
Location				
Sub-polar (alien)	-1.974	0.996	-1.982	0.054
Tropical (alien)	0.719	1.155	0.622	0.537
Interaction (status x location)				
Indigenous x Sub-polar	-6.326	1.364	-4.638	<0.001
Indigenous x Tropical	-3.003	1.561	-1.924	0.062

$F(5,39) = 19.15$, $R^2 = 0.68$, $p < 0.001$, $ML^{\lambda} = 0$

Figures

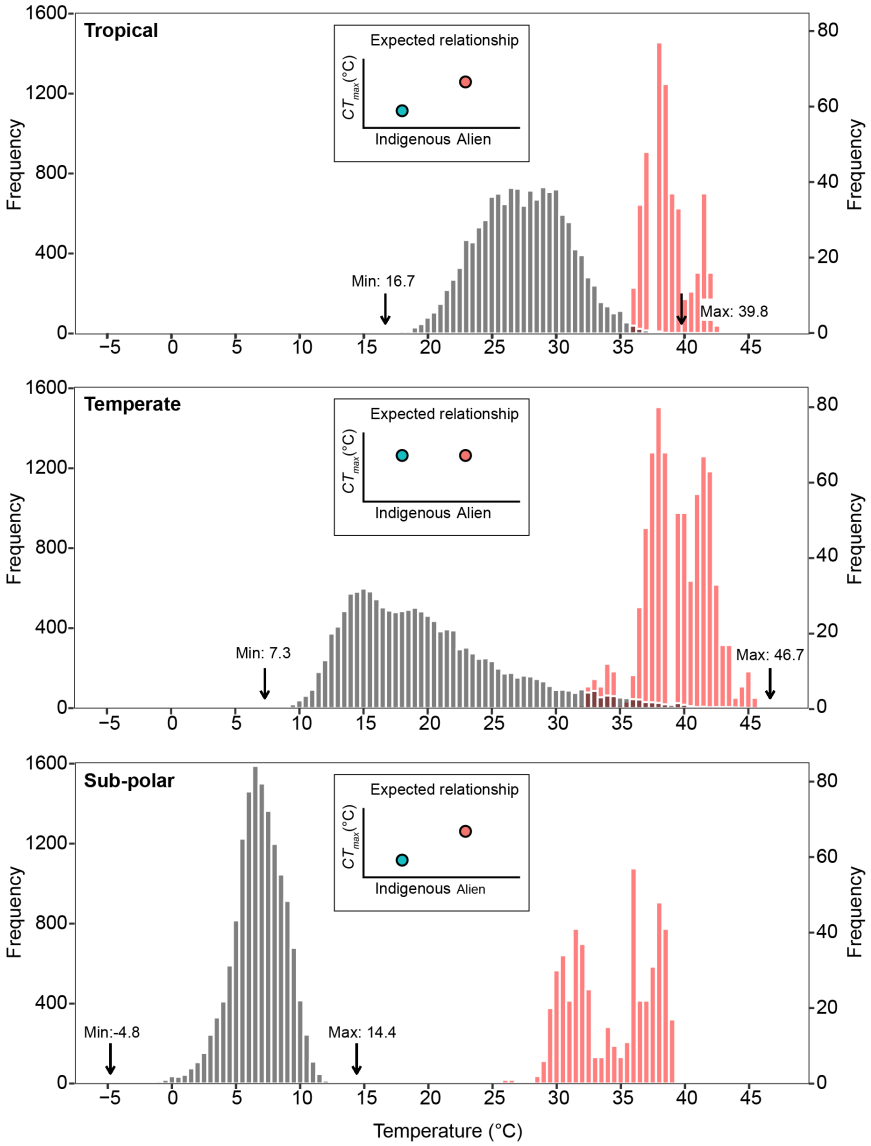

Figure 1

The main figure shows the frequency distribution of maximum daily temperature (°C) (black), along with the maximum and minimum temperatures, as well as the frequency distribution of CT_{max} values (red), at tropical, temperate, and sub-polar locations. Insets show the expected relationship between indigenous and alien CT_{max} at each location. The hypothesis is that CT_{max} values for alien species will be higher than their indigenous counterparts, except in those areas where indigenous species experience conditions similar to those of introduced species in their native range. In this case, the temperate climate is predicted to be closer to what is experienced by alien springtails in their native habitat. Maximum daily temperature was sourced from climate statistics available from the Australian Bureau of Meteorology (www.bom.gov.au), and encompass data from between 1980 and 2019.

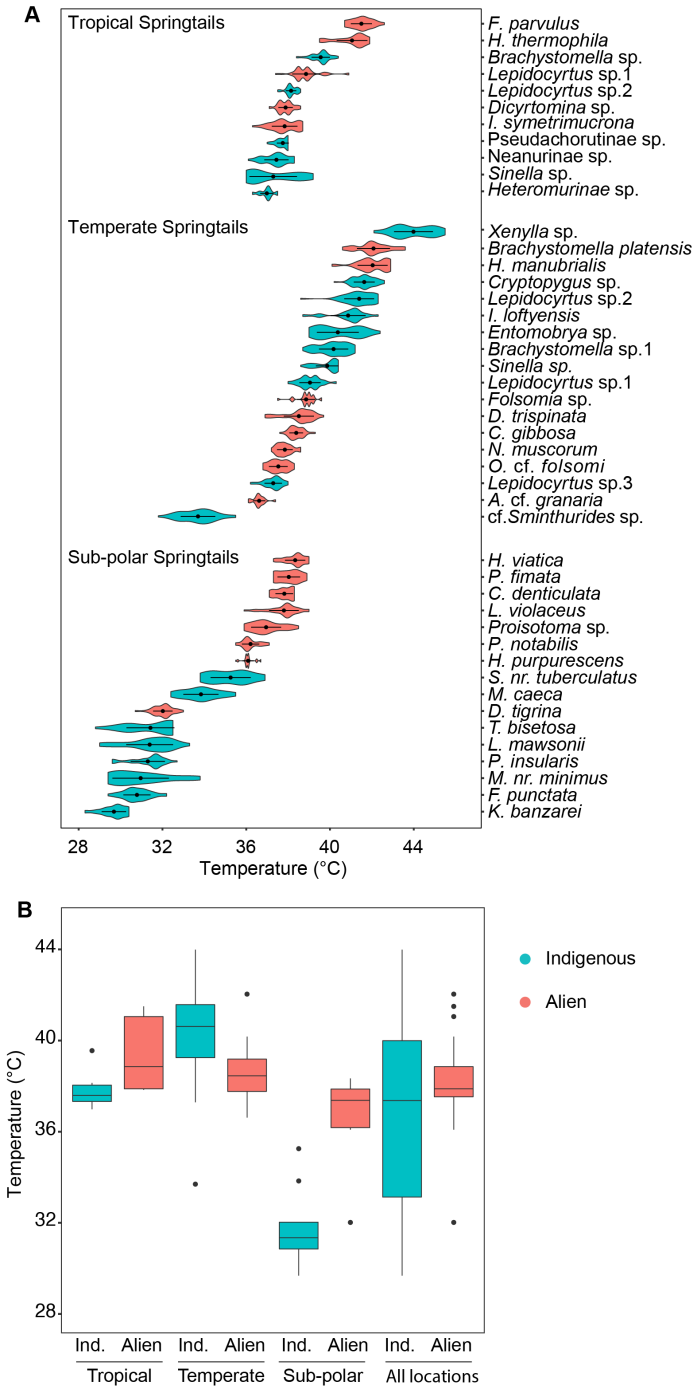

Figure 2

Critical thermal maxima (CT_{max}) of 45 springtail species **A**, and variation in CT_{max} between indigenous and alien springtails **B**, found at tropical, temperate and sub-polar locations. Plot **A** shows violin plots for each species, displaying the frequency density of individual CT_{max} data of indigenous (blue) and alien (red) springtail species. In each case, mean and standard deviation is also displayed in black (see Table D1 for values). Plot **B** shows boxplots of species means for indigenous and alien species for each location.

Appendix D: Supplementary data

Table D1

Mean, standard deviation, max, min and sample size of critical thermal maximum (CT_{max}) for the springtail species in this study. Species are separated by location and status and arranged in order of highest mean CT_{max} to lowest in each case. Higher taxonomic levels for each species are also provided.

Tropical

Species	Order	Family	mean (s.d.)	max	min	N
Alien						
Folsomides cf. parvulus	Entomobryomorpha	Isotomidae	41.5 (0.5)	42.6	40.7	41
Hemisotoma thermophila	Entomobryomorpha	Isotomidae	41.05 (0.73)	41.9	39.5	45
Lepidocyrtus sp.1	Entomobryomorpha	Entomobryidae	38.86 (0.66)	40.9	37.4	46
Dicyrtomina sp.	Symphypleona	Dicyrtomidae	37.88 (0.34)	38.6	37.1	42
Isotomiella symetrimucronata	Entomobryomorpha	Isotomidae	37.83 (0.61)	38.7	36.3	41
Indigenous						
Brachystomella sp.	Poduromorpha	Brachystomellidae	39.56 (0.43)	40.4	38.4	45
Lepidocyrtus sp. 2	Entomobryomorpha	Entomobryidae	38.14 (0.25)	38.6	37.5	47
Pseudachorutinae sp.	Poduromorpha	Neanuridae	37.75 (0.29)	38	37	40
Neanurinae sp.	Poduromorpha	Neanuridae	37.44 (0.58)	38.3	36.1	47
Sinella sp.	Entomobryomorpha	Entomobryidae	37.29 (1.14)	39.2	36	42
Heteromurinae sp.	Entomobryomorpha	Orchesellidae	36.98 (0.32)	37.5	36.3	50

Temperate

Species	Order	Family	mean (s.d.)	max	min	N
Alien						
Hypogastrura manubrialis	Poduromorpha	Hypogastruridae	42.04 (0.72)	42.9	40.1	52
Brachystomella platensis	Poduromorpha	Brachystomellidae	40.17 (0.7)	41.2	38.7	46
Folsomia sp.	Entomobryomorpha	Isotomidae	38.86 (0.43)	39.6	37.5	49
Desoria trispinata	Entomobryomorpha	Isotomidae	38.51 (0.72)	39.7	36.9	48
Ceratophysella gibbosa	Poduromorpha	Hypogastruridae	38.39 (0.33)	39.3	37.6	43
Neanura muscorum	Poduromorpha	Neanuridae	37.85 (0.37)	38.6	37.2	60
Orthonychiurus cf. *folsomi*	Poduromorpha	Onychiuridae	37.53 (0.45)	38.3	36.8	52
Anurida cf. granaria	Poduromorpha	Neanuridae	36.62 (0.3)	37.4	36.1	46
Indigenous						
Xenylla sp.	Poduromorpha	Hypogastruridae	43.99 (0.93)	45.5	42.1	44
Brachystomella sp.	Poduromorpha	Brachystomellidae	42.08 (0.79)	43.6	40.6	42
Cryptopygus sp.	Entomobryomorpha	Isotomidae	41.63 (0.5)	42.6	40.2	46
Lepidocyrtus sp. 2	Entomobryomorpha	Entomobryidae	41.4 (0.71)	42.3	38.6	45
Isotopenola loftyensis	Entomobryomorpha	Isotomidae	40.87 (0.84)	42.3	38.7	48
Entomobrya sp.	Entomobryomorpha	Entomobryidae	40.37 (1)	42.4	39	43

Sinella sp.	Entomobryomorpha	Entomobryidae	39.87 (0.54)	40.4	38.6	46
Lepidocyrtus sp.1	Entomobryomorpha	Entomobryidae	39.04 (0.51)	40.3	38	47
Lepidocyrtus sp.3	Entomobryomorpha	Entomobryidae	37.29 (0.42)	38	36.2	46
cf. *Sminthurides* sp.	Symphypleona	Sminthurididae	33.7 (0.83)	35.5	31.8	46

Polar

Species	**Order**	**Family**	**mean (s.d.)**	**max**	**min**	**N**
Alien						
Hypogastrura viatica	Poduromorpha	Hypogastruridae	38.34 (0.48)	39	37.3	42
Protaphorura fimata	Poduromorpha	Onychiuridae	38.03 (0.54)	38.9	37.3	33
Ceratophysella denticulata	Poduromorpha	Hypogastruridae	37.82 (0.42)	38.3	37.1	31
Lepidocyrtus violaceus	Entomobryomorpha	Entomobryidae	37.8 (0.71)	39	35.9	30
Proisotoma sp.	Entomobryomorpha	Isotomidae	36.95 (0.72)	38.5	35.9	32
Parisotoma notabilis	Entomobryomorpha	Isotomidae	36.21 (0.41)	37.1	35.5	31
Hypogastrura purpurescens	Poduromorpha	Hypogastruridae	36.09 (0.27)	36.7	35.5	31
Desoria tigrina	Entomobryomorpha	Isotomidae	32.02 (0.46)	33	30.7	34
Indigenous						
Sminthurinus nr. *tuberculatus*	Symphypleona	Katiannidae	35.25 (0.96)	36.9	33.8	31
Mucrosomia caeca	Entomobryomorpha	Isotomidae	33.84 (0.84)	35.5	32.4	31
Tullbergia bisetosa	Poduromorpha	Onychiuridae	31.43 (1.14)	32.5	28.8	31
Lepidocyrtus mawsonii	Entomobryomorpha	Entomobryidae	31.39 (1.12)	33.3	29	28
Parisotoma insularis	Entomobryomorpha	Isotomidae	31.3 (0.82)	32.7	29.6	33
Megalothorax nr. *minimus*	Neelipleona	Neelidae	30.96 (1.35)	33.8	29.4	29
Folsomotoma punctata	Entomobryomorpha	Isotomidae	30.53 (1.21)	32.2	26.2	35
Katianna banzarei	Symphypleona	Katiannidae	29.68 (0.58)	30.4	28.3	32

Table D2

Outcome of OLS showing the change in critical thermal maxima (CT_{max}) between alien and indigenous springtails across tropical, temperate and sub-polar locations in Australia.

	Estimate	**Std. Error**	**t value**	**P-value**
Intercept (alien temperate)	38.746	0.723	53.603	<0.001
Status				
Indigenous (alien)	1.278	0.970	1.318	0.195
Location				
Sub-polar (alien)	-2.0893	1.022	-2.044	0.0478
Tropical (alien)	0.679	1.165	0.582	0.564
Interaction (Status x Location)				
Indigenous x Sub-polar	-6.137	1.409	-4.355	<0.001
Indigenous x Tropical	-2.842	1.572	-1.807	0.078

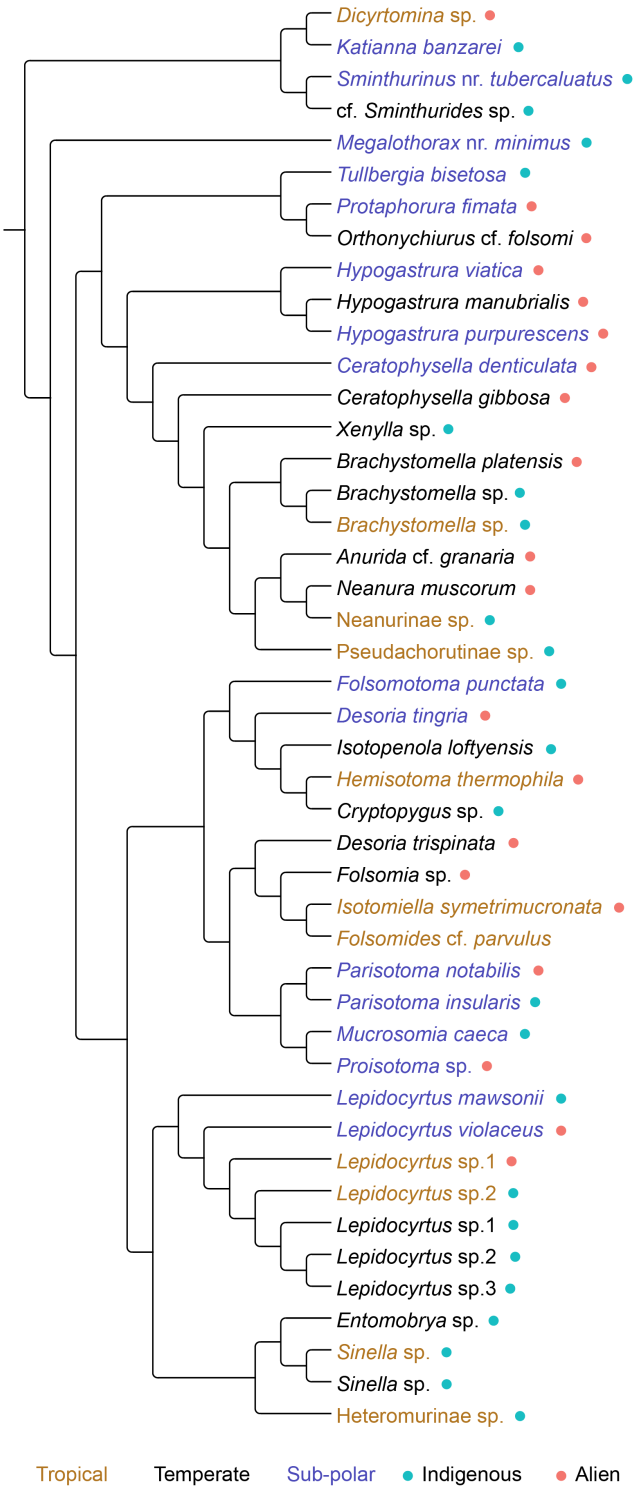

Figure D1

Phylogeny of the 45 springtails used in this study. The tree is based on previous work that uses molecular-based methods to assess phylogenetic relationships of major springtail taxa (D'Haese 2002; Malcicka et al. 2017) and recent work on Australian springtail species (Janion-Scheepers 2018; Liu et al. 2020).

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General Discussion

The recent increase in utilisation of trait data in models, brought about by attempts to better understand the effects of global temperature change on organisms, has resulted in a need to not only provide easy access to data for a wide variety of taxa and traits (e.g. Jones et al. 2009; Kattge et al. 2011; Gallagher et al. 2020), but to assess how variability in environmental and experimental temperature conditions will affect outcomes (e.g. 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). In order to address knowledge gaps in these areas in this thesis; I first synthesised scaling relationships from the literature (Chapter 1), second, examined the effect of fluctuating temperatures on springtails across thermal tolerance and developmental traits (Chapters 2 and 3, respectively) and finally examined the effect the native thermal environment of springtails has on conferring an advantage to invasive alien springtails in indigenous environments (Chapter 4).

Chapter 1

Chapter 1 provides the first comprehensive modern synthesis of scaling relationships for insects. The outcomes from this chapter revealed the data available for insect scaling relationships is not equal across traits, taxa or measurement levels. However, the large amount of work available on morphological and physiological traits indicates that there is a well-established fundamental basis for understanding how body size affects these type of traits. Nevertheless, without additional data, especially in regards to ontogenetic scaling (where almost all scaling relationships were on two species), it may be difficult to understand broader scaling patterns. The evidence of this was highlighted in how difficult it was to assess variation

in scaling values between interspecific, intraspecific and ontogenetic scaling for more than one trait.

Scaling relationships are of interest not only because they provide insights into the mechanisms underpinning biodiversity in organisms (Brown et al. 2004; Marquet et al. 2014; Nijhout and Callier 2015; White and Kearney 2014; Harrison 2017), but also because they offer a way for researchers to easily estimate trait values from body size (e.g. Benke et al. 1999). As such, the bias towards particular areas of research revealed in this study limits how this data might be used to predict biological or ecological outcomes. Cleary, a much broader range of species and traits would be useful to add to this dataset in the future. The focus of future research in insect scaling should not be to repeat the work that has already been done by, for example, re-examining aspects of metabolic scaling, but should instead be directed to filling in the gaps made apparent in this synthesis. This is especially true for those traits that are going to be valuable to address ongoing questions about the resilience of insects to climate change (e.g. Duffy et al. 2015).

In that regard, careful consideration should also be made for the way in which this type of data are collected. One of the difficulties I had in synthesising insect scaling data was the large variation in the methodologies and reporting of the results between the sources. This greatly reduced the number of entries in the dataset and is something that has been recognised as a general problem in synthesising trait data (e.g. Gallagher et al. 2020). Thus, it is important going forward to consider how researchers are able to maintain a high level of consistency to measuring traits so that data are not only more reliable, but are comparable across studies. With that in mind it is also important to consider how this information might be shared in the future. The dataset that I have developed will be available as a free to access online resource, however recent projects such as the Open Traits Network (Gallagher et al. 2020) are attempting to gather together a much larger trait database. Thus, researchers undertaking work on scaling in the

future, be it a single study or meta analyses of multiple studies should consider such options, that way the data won't become lost in the sea of literature.

Chapters 2 and 3

The outcomes of Chapters 2 and 3, highlight that the effects of fluctuating temperatures on traits are not straightforward. Evidence from previous works on a multitude of traits (e.g. Ragland and Kingsolver 2008; Bozinovic et al. 2011; Fischer et al. 2011; Kjaersgaard et al. 2013; Carrington et al. 2013) and mathematical theory (Ruel and Ayres 1999; Denny 2017) indicate that fluctuating temperature trait values will vary from constant temperature values. However, the outcome of the research in Chapter 2 contradicts this, with thermal tolerance traits unlikely to be significantly affected by fluctuating temperatures, at least in the Collembola under a relatively wide range of conditions. Thus, thermal tolerance trait data estimated under constant temperatures would accurately reflect trait values in natural systems and therefore use of constant temperature values in models would be acceptable. The findings in Chapter 3, however, showed the response of traits to temperature fluctuations was highly variable, with both developmental stage and species responding in different ways. As such it is likely that the use of developmental trait values estimated under constant temperatures would result in over or underestimation of performance, with a similar result occurring should constant values be used for predictive purposes.

These results indicate a need to formally test the effects of fluctuations on traits before using them in a predicative capacity. In this regard, testing not only the effect of fluctuations but also the effect of extreme temperature conditions should also be considered. This is one area that I did not test in this thesis, but that unquestionably requires examination, especially given evidence, from my work and others (e.g. Kingsolver and Buckley 2017; Zhu et al. 2019; Folguera et al. 2011; Xing et al. 2019), that temperatures closer to physiological thresholds are

likely to result in different outcomes for fluctuating versus constant temperatures. It may be that the response of traits to extreme temperature events will more consistently reflect the expected values than those I found during my research. Furthermore, as extreme temperature conditions are predicted to increase under climate change (IPCC 2018), research examining the response of traits to these types of events will become more necessary in forecasting the organismal and population level response to changing conditions (see also Valladares et al. 2014).

Chapter 4

The outcomes of Chapter 4 provide evidence for the hypothesis that the native thermal environments of invasive and indigenous species influence the advantage of invasive species in indigenous environments. This is an important finding as it not only has large implications for predicting the effects of invasive species on indigenous biodiversity, but also in the management of invasive species in invaded environments. For example, research has shown that invasive species tend to have an advantage in indigenous environments, because they have wider thermal limits, higher development rates, greater egg hatching success, greater desiccation resistance, and lower developmental thresholds, relative to their indigenous counterparts (Chown et al. 2007; Janion et al. 2010; Janion-Scheepers et al. 2018; Treasure et al. 2019; Phillips et al. 2020, for general theory see Enders et al. 2020). However, my results indicate that, for thermal tolerance at least, this advantage is likely to be restricted to indigenous thermal environments that are dissimilar to those in the native range of the invaders. Thus, the focus of invasive species management efforts could be concentrated on those environments where the impact is likely to be the greatest.

However, a problematic aspect of the research in this chapter was the absence of location-specific information on the origin of the invasive springtail species. One area of

emerging research, in this regard, is the utilisation of genetic data to determine where invasive species originate (e.g. Chown et al. 2015; Roe et al. 2019). Having this information would allow for improved understanding of the thermal environment of invasive species, and thus their traits in that environment, which will therefore enable much better predictions of the effects of invasive species in the invaded environment.

Furthermore, there is a general lack of investigation of community effects of invasive species. I think that I was more able to observe the effects in this study where others were not (e.g. Janion-Scheepers et al. 2018) because I focussed on thermal tolerance in communities of springtails. This lack of investigation of communities has been raised as a potential issue in regards to better understanding the effects of climate change (e.g. Araújo and Luoto 2007; Van der Putten et al. 2010; Gilman 2010; Chown and Gaston 2016; Donelson et al. 2019), but it has yet to be studied extensively. Although, this study only represents one example of how indigenous thermal environment might mitigate the effects of invasion, the results of this chapter are novel. Much more work in this area on communities in different environments and for multiple traits is required to determine if this is applicable across many species and systems. It does however, demonstrate that the species invasions that are predicted with future temperature conditions (e.g. Bellard et al. 2013; Hulme 2017) might not have such a dramatic effect on biodiversity in some locations.

Thesis conclusions

The synthesis of scaling relationships and findings in this thesis on the effect of experimental and environmental temperature on traits promotes understanding of how traits might be used in a predicative context. In particular, it provides easy access to insect scaling relationships that were previously scattered in the literature. Additionally, my work improves knowledge of how experimental temperatures, especially constant temperatures, might produce

trait values that are unacceptable to use in models. Furthermore, Chapter 4 provides evidence for a new and exciting hypothesis that suggests biodiversity in certain environments may be largely unaffected by species invasions at least from a climate change context of increasing temperature effects. This thesis greatly improves knowledge of how springtails are likely to respond to environmental change, something that remains of high importance due to the impact of changing environments on soil systems and the potential of this to affect all other terrestrial ecosystems.

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