

The effect of temperature on the physiological and behavioural responses of Collembola

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Abstract

Climate change is one of society's greatest challenges and understanding its impacts one of biology's most pressing questions. The work reported in this thesis concerns that question. Specifically, it addresses the complexities of estimating those impacts and the ways in which soil organisms are likely to respond to extreme climatic events. Critical thermal limits, upper (CT_{max}) and lower (CT_{min}) estimates of thermal tolerance, show substantial variation associated with the experimental rate of temperature change, but the full range of variation has not been investigated in a broader comprehensive analysis. The estimates of thermal tolerance are frequently used in the models predicting species survival, range, and distribution in the face of climate change, thus it is essential to establish the importance of incorporating the rate effects into such models. Plasticity in the upper thermal limits of ectotherms is limited and many species will rely on behavioural thermoregulation to avoid climate extremes. The interaction between physiology and behaviour is another necessary element that needs to be incorporated into forecast models for climate change impacts, and is poorly known in soil arthropods such as Collembola (springtails). Furthermore, small soil arthropods, including Collembola, will be exposed to more frequent and intense droughts in the warming climate. Polar species of Collembola have been found to express phenotypic plasticity in drought resistance due to thermal acclimation, but whether the same is true for the temperate and tropical species remains unknown. Furthermore, this thesis explores how well current theoretical models explain the effects of experimental ramping rate on the critical thermal limits of revealing four different patterns of intraspecific variation in rate effects with phylogenetic signal present in CT_{max} , but not in CT_{min} . Failure rate model approach better explained the rate effects on CT_{max} than the thermal tolerance landscape approach, while neither approach could explain the majority of the empirical rate variation in CT_{min} . Four species of Collembola from the temperate and the sub-Antarctic regions of Australia were used to explore the links between environmental stress, upper thermal tolerance, body size and behaviour. Findings revealed that the species with the lowest thermal tolerance prefer deeper soil layers. There was a limited evolutionary capacity for improving upper thermal tolerance followed by a reduction in body size due to the higher developmental temperatures. Temperate species exhibited immediate behavioural response to extreme weather event simulations, especially to the heat wave treatments. Analysis indicated that vertical movement in the soil depends on the fine-scale structure of the soil matrix, especially soil pore distribution, where more elongated body is preferred in avoiding heat stress and small body width is beneficial during drought. Both temperate and tropical species lack developmental plasticity in desiccation resistance when reared under temperatures simulating future projections of their respective microclimate. Finally, this work proposed a hypothetical framework for further exploration of how failure rate and recovery rate of an organism may interact to determine thermal tolerance. Moreover, the results highlighted the need for incorporating thermoregulatory behaviour in the predictive models of soil-dwelling species' responses to climate change.

Publications during enrolment

- Kovacevic, A., Latombe, G., Chown, S.L., 2019. Rate dynamics of ectotherm responses to thermal stress. Proceedings of the Royal Society B: Biological Sciences 286, 20190174.
- Harrison, J.F., Waters, J.S., Biddulph, T.A., Kovacevic, A., Klok, C.J., Socha, J.J., 2018. Developmental plasticity and stability in the tracheal networks supplying *Drosophila* flight muscle in response to rearing oxygen level. Journal of Insect Physiology 106, 189–198.

Thesis including published works General 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 one original paper published in a peer-reviewed journal and two unpublished manuscripts. The core theme of the thesis is the effect of temperature on ectotherms and soil arthropods using Collembola as a model organism. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the School of Biological Sciences under the supervision of Professor Steven L. Chown and co-supervision of Professor Craig R. White.

(The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.)

Thesis Chapter	Publication Title	Publication Status	Nature and extent (%) of student contribution	Co-author name Nature and extent (%) of co-author's contribution
2	Rate dynamics of ectotherm responses to thermal stress	Published	Came up with the ideas to test models against each other, gathered all the data, did the meta-analyses and completed most of the modeling. Wrote the drafts. Handled the publication process. 80%	Guillaume Latombe Data modeling, manuscript editing 15% Steven L. Chown Manuscript editing 5%
3	The link between physiology and behaviour in the face of climate change	Not submitted	Conceptualized the work, tested the ideas, built the equipment, did the work, ran the statistical analyses and wrote the chapter. 95%	Steven L. Chown Manuscript editing 5%
4	The effect of developmental temperature on desiccation resistance of temperate and tropical Collembola	Not submitted	Conceptualized the work, tested the ideas, built the equipment, did the work, ran the statistical analyses and wrote the chapter. 95%	Steven L. Chown Manuscript editing 5%

In the case of Chapters 2, 3, and 4, my contribution to the work involved the following:

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature:

Date: 3/06/2019

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 signature:

Date: 3/06/2019

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Chapter 1

Introduction

"Man will endeavor to preserve the equilibrium, which exists, in the meteorological forces and vital conditions of countries... and thus save from extinction the myriad beauteous forms of life, which have shared with him the inheritance of this wonderful Earth."

Mary Somerville, 1870

1.1 Climate change

Climate change is characterised by the rise of average annual temperatures and altered global weather patterns, which are marked by an increase in the frequency, magnitude, and duration of extreme weather events and by an increase in daily temperature extremes (Easterling et al., 2000; Rahmstorf & Coumou, 2011; Hansen et al., 2012; IPCC, 2014). Extreme weather events are generally considered to be intense and to occur at low frequency, but climate change is altering these patterns, where extreme weather events such as heat waves, cold waves, droughts, large storms, bushfires, record rainfalls and floods have become increasingly common over the last decade (Coumou & Rahmstorf, 2012; IPCC, 2014; Perkins-Kirkpatrick & Gibson, 2017). Drought frequency is expected to increase not only in temperate, but also in tropical regions (IPCC, 2014; Fu, 2015). As global mean surface temperature increases, there is an increase in warmer-than-average days and hot days, and a decrease in colder-than-average days on seasonal timescales resulting in milder winters (IPCC, 2014). The rise in the minimum temperature occurs at the faster rate than the rise in the maximum temperature resulting in a decrease in the diurnal temperature range (Walther et al., 2002).

Australia, which encompasses many climate zones (Figure 1), is no exception to this global trend. The Australian southeast temperate region (Victoria) experienced an increase in the temperature extremes on both ends for 1.0 °C on average since 1960 (Ashcroft et al., 2012) with drying trend particularly evident in the past two decades (Hughes & Steffen, 2013). February 2017 was the heat record-breaking month with peak land surface temperatures (NASA Earth Observatory, 2017). Tropical regions of Australia can expect increased temperatures of between 1.0 °C and 2.2 °C by 2050 with potential rainfall decline by up to seven per cent on average depending on the emissions scenario (Williams & Crimp, 2012; Hughes & Steffen, 2013). The Wet Tropics World Heritage region, the centre of biodiversity, will experience an increasing number of hot days while projections of annual rainfall do not show a consistent tendency towards an increase or decrease (Hilbert et al., 2014).

When it comes to the Australian sub-Antarctic region, Macquarie Island, earlier studies documented increased precipitation (Adams, 2009), whereas later ones provide evidence of a decrease in precipitation frequency accompanied by an increase in the precipitation intensity (Wang et al., 2015). Furthermore, combining the past and forecast weather station data suggests that there will be a 0.8 °C increase in the surface air temperatures over 118-year time frame (1912-2030), primarily due to an increase in maximum temperatures and little or no change in minimum temperatures (Tweedie & Bergstrom, 2000).

The biological impact of climate change depends on the physiological sensitivity of organisms to changes in water availability and temperature. Findings suggest that tropical forest species are the most vulnerable and have the greatest extinction risk from

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global warming because these species are currently living very close to their optimal temperature (Williams et al., 2003; Deutsch et al., 2008; Tewksbury et al., 2008; Huey et al., 2009; Diamond et al., 2012; Huey et al., 2012). Although the impact on terrestrial organisms is predicted to increase with latitude following the greater rate of warming with increasing latitude, warming in the tropics, despite its smaller magnitude, appears to have the most deleterious consequences (Deutsch et al., 2008). Extreme climatic events can have large impacts on biodiversity (Thibault & Brown, 2008; Ryan et al., 2015; Sanz-Lázaro, 2016; Harris et al., 2018). Species vulnerability to climate change generally depends on their exposure and sensitivity, but also their resilience and potential to adapt (Williams et al., 2008). Abiotic factors such as temperature and humidity have direct and the most obvious effect on fitness and performance of animals, especially ectotherms, whose body temperature closely trails the environmental temperature. Body temperature has an influence on both physiology and behaviour of ectotherms and, as a consequence, affects their ecology.



Figure 1 Climate map of Australia based on a modified Köppen classification system. Classification derived from 0.025×0.025 degree resolution mean rainfall, mean maximum temperature and mean minimum temperature gridded data. All means are based on a standard 30-year climatology (1961-1990) (The Australian Bureau of Meteorology; www.bom.gov.au). Licensed under Creative Commons Attribution 3.0 licence.

1.2 Thermal performance curve (TPC)

Thermal performance curves (TPCs), frequently used for assessments of climate change risk, describe the relationship between temperature and performance of an organism (Huey & Stevenson, 1979). This concept is especially relevant for ectotherms, whose physiological processes and regulation of body temperature typically depend on the temperature of their surroundings. TPCs have become a central topic in thermal biology (Angilletta, 2009; Rezende et al., 2014) and its various applications (e.g. Deutsch et al., 2008; Sinclair et al., 2016), many of which are focused on predicting species distributions and responses to climate change. Common performance measurements are locomotion, growth, development, fecundity, and survivorship (Angilletta, 2009). The general characteristics or parameters of a TPC curve are the thermal optimum where performance is at its highest, the thermal (performance) breadth, the thermal limits (CT_{max} and CT_{min}) and the tolerance range (Huey & Stevenson, 1979; Angilletta, 2009) (Figure 2). These measures differ in ecological significance such that thermal performance breadth is relevant to the concept of niche width, while endpoints set absolute limits on survival (Huey & Stevenson, 1979). The endpoints of a typical thermal performance curve are the upper and lower critical thermal limits (CTLs), where performance declines to zero. These endpoints are often defined behaviourally as the temperatures that result in loss of the righting response, coordination, or equilibrium, or the onset of stereotypical behaviour or thermal spasms, all depending on the performance measurement and the organism being measured (Lutterschmidt & Hutchinson, 1997; Chown & Nicolson, 2004). CTLs are commonly measured using a dynamic (ramping) method where temperature is gradually increased or lowered at a constant rate of temperature change until endpoint is observed. Empirical studies have shown that both upper and lower critical thermal limits can be altered according to the experimental rate of temperature change (Figure 2). Although the CTLs of many ectotherm species are found to increase with faster rates of temperature change, the converse response has also been demonstrated (Mora & Maya, 2006; Terblanche et al., 2007; Vinagre et al., 2015). Rate effects have been explored in several studies, frameworks and theoretical models, which propose to explain why critical thermal limits vary with the rate of temperature change (Kilgour & McCauley, 1986; Terblanche et al., 2007; Cooper et al., 2008; Rezende et al., 2014; Kingsolver & Umbanhowar, 2018).



Body Temperature (°C)

Figure 2 Varying rates of temperature change affect the ends of thermal performance curve, CT_{max} (red circle, rate effect marked by red dashed line) and CT_{min} (blue circle, rate effect marked by blue dashed line), by moving them up or down on the thermal scale, therefore changing the performance breadth (P_{breadth}) and shifting the overall position of the performance curve (solid blue line) on the thermal scale. Thermal performance curve shift is illustrated by the grey dotted lines.

The extent to which the evolution of thermal performance curves is influenced by non-thermal variables and by a fluctuating environment is also not fully understood. Upper thermal limits show less geographical variation than lower thermal limits, supporting the climatic variability hypothesis (Addo-Bediako et al., 2000). Thermal tolerance ranges increase with latitude in frogs (Snyder & Weathers, 1975), insects (Addo-Bediako et al., 2000), and lizards (Clusella-Trullas et al., 2011) and this increase is mostly due to shift in CT_{min} rather than in CT_{max} (Addo-Bediako et al., 2000; Huey et al., 2009; Sunday et al., 2010; Clusella-Trullas et al., 2011). Critical thermal maximum and thermal optimum in ectotherms, such as lizards and snakes, are more closely related to temperature variation and precipitation than they are affected by mean annual temperature. Critical thermal minimum, on the other hand, appears to be affected by mean annual temperature more than temperature variation and precipitation. Together, these observations suggest that responses to climate change will be complex, and dictated by the interactive effects of multiple climate variables (Clusella-Trullas et al., 2011). Considering that non-thermal variables such as relative humidity and moisture availability are directly affected by temperature, understanding how they influence the evolution of thermal performance curves is therefore important because of predictions that not only temperature but also other variables will change with changing climate.

1.3 Time-temperature relationships

Time-temperature relationships were originally explored in the food processing industry to determine how long it takes to kill a specific bacterium at a set temperature (Bigelow, 1921; Tang et al., 2007). Studies of thermal death time curves (TDTs) showed that there is a linear relationship between the logarithms of the thermal death time and the temperature (Bigelow, 1921). In the case of TDTs, temperature is kept constant throughout the experiment (static method), which leads to a single underlying relationship between temperature, exposure time, and mortality. This relationship follows a typical dose-response curve. The higher the temperature is, the shorter the tolerance time will be. Fry (1971) and Cossins & Bowler (1987) introduced the concept of 'incipient lethal temperature' (ILT) that separates the zone of tolerance and the zone of resistance on a thermal scale when mortality of an organism goes from being independent

of the exposure time to being dependent on the exposure time at a certain temperature (Figure 3).



Figure 3 Incipient lethal temperature (ILT), which separates the zone of tolerance and the zones of resistance, is the lowest lethal temperature at the longest time of exposure at which 50% of the population dies of exposure (Fry, 1971). In the zone of tolerance, 50% mortality will not occur, even for extended exposure periods. The maximal range over which lethal temperature (LT₅₀, LT₁₀₀ – temperature at which 50% and 100% of the population dies, respectively) may be observed is usually less than 5 °C (Cossins & Bowler, 1987).

1.4 Theoretical models describing the effect of rate of temperature change on critical thermal limits

One of the most recent proposals describing the relationship between critical thermal limits and the rate of temperature change associating it with the exposure time to stressful temperature is the thermal tolerance landscape (Rezende et al., 2014). This framework is solely focused on explaining the most commonly observed rate effect where critical thermal limits increase with the faster rate of temperature change, while it overlooks the converse response. The first empirical prediction of the thermal tolerance landscape is that upper critical thermal limits and upper lethal temperatures share the same inverse linear relationship between the logarithm of the thermal death time and temperature typical of a dose-response curve. It is assumed that the same relationship is shared between the lower critical thermal limits and lower lethal temperatures. Another prediction is that critical thermal limits are inadequate descriptors of thermal tolerance on conceptual grounds because they are highly dependent on the methodological context and make a comparison of results across studies difficult. This led to redefining critical thermal limits as a knockdown (death) temperature at 1 min of exposure. Finally, z-value, a measure of thermal sensitivity originating from TDTs, is assumed to be highly correlated with redefined critical thermal limits because the same has been found for lethal temperatures and z-value (Rezende et al., 2014). On the other hand, a related, but more flexible and inclusive modelling approach assumes that failure rate (min⁻¹) of an organism increases with the rising temperature following a non-linear trend (Kingsolver & Umbanhowar, 2018). Failure rate models claim to separate statistical effects accounted for by the model, and biological effects of an organism such as acclimation. This approach attributes any residual variation not explained by the failure rate model to the biological effects, thus considering and incorporating the converse response along with the most commonly observed response to the rate of temperature change (Kingsolver & Umbanhowar, 2018).

How well the thermal tolerance landscape and failure rate models describe and fit the empirical data on critical thermal limits across the broad range of ectotherm species remains unknown. More recently, an interspecific approach to predict thermal acclimation capacity of ectotherms across many species of different body sizes and from different habitats has also been adopted (Rohr et al., 2018). Ramping rate was included as an explanatory variable, but since the approach was interspecific it confounded intraspecific and inter-specific variation assuming that rate effect always takes the same form, although the empirical evidence shows otherwise (Mora & Maya, 2006; Terblanche et al., 2007; Vinagre et al., 2015; Allen et al., 2016).

1.5 Behavioural response to the environmental stress

Most terrestrial ectotherms are not tolerant to extreme heat and must rely on thermoregulatory behaviour in heterogeneous microclimates for survival (Kearney et al., 2009; Huey et al., 2012; Sunday et al., 2014; Duffy et al., 2015). Behaviour is often the first line of response when animals are faced with unfavourable conditions or any changes in their environment and can determine their immediate success or failure in the new modified environment (Sih et al., 2011; Candolin & Wong, 2012; Wong & Candolin, 2014). In estimating thermal tolerance, changes in species distributions, and species responses to climate change, especially when considering small soil ectotherms, microclimates and thermoregulatory behaviour displayed across soil layers of different depth, including horizontal variability in microclimate due to differences in vegetation structure, play a key role since they can alter climate change impact projections (Bonebrake et al., 2014; Sunday et al., 2014; Duffy et al., 2015). There is an expectation

that immediate, short-term behavioural responses will be able to mitigate poor thermal tolerance, while over long term, plasticity may affect this interaction (Blackburn et al., 2014; Sgrò et al., 2016). However, upper thermal limits show much less variation with latitude than the lower thermal limits (Addo-Bediako et al., 2000) indicating that plasticity in thermal tolerance may be evolutionary constrained (Gunderson & Stillman, 2015, Janion-Scheepers et al., 2018). Therefore, it might be up to behavioural thermoregulation and microclimates that act as a refuge to protect the organisms from the environmental extremes and play a role in the evolution of thermal plasticity (Bogert, 1949; Huey et al., 1991; Huey et al., 2003). Dowd et al. (2015) note that numerous studies showed how variation in body temperature is much greater between microclimates in close proximity than over much larger spatial scales. The role microclimate plays in behavioural physiology is clearly important (Scheffers et al., 2013; Kaspari et al., 2014; Pincebourde & Casas, 2019) indicating that incorporating behavioural thermoregulation into models for predicting climate change impacts on organisms is essential for achieving more precise and realistic results (Kearney et al., 2009).

1.6 Desiccation resistance

Climate projections of more frequent and intense droughts in temperate and tropical regions suggest that implications of climate change on small ectotherms largely depend upon their desiccation resistance (Easterling et al., 2000; IPCC, 2014; Fu, 2015). Along with thermal tolerance, the ability to withstand exposure to dry conditions with relative humidity lower than optimal is one of the key factors influencing the population

dynamics and geographic range of ectotherms. For example, insects use desiccation resistance and desiccation tolerance to deal with desiccation stress (Edney, 1977). While desiccation tolerance is the ability to tolerate body water loss, desiccation resistance concerns the different mechanisms to avoid dehydration such as an increase in body water content or reduction in the rate of water loss (Edney, 1977; Gibbs & Matzkin, 2001; Chown & Nicolson, 2004). Desiccation resistance, or the ability of an organism to resist and reduce water loss, is essential during drought and of particular importance for small ectotherms like insects and soil arthropods (Edney, 1977). Considering that drought frequency and intensity are expected to increase in temperate and tropical regions along with an increase in the average global temperatures (IPCC, 2014, Fu; 2015), desiccation resistance becomes even more important.

Desiccation resistance of small ectotherms in response to varying humidity conditions has been explored in many studies (Holmstrup et al., 2001; Sjursen et al., 2001; Kaersgaard et al., 2004; Terblanche et al., 2005; Elnitsky et al., 2008) and findings suggest that pre-exposure to mild drought conditions increases subsequent drought tolerance. On the other hand, studies exploring the effect of temperature on desiccation resistance are less numerous, largely confined to flying insects and yield contrasting results (Hoffmann & Watson, 1993; Hoffmann et al., 2003; Hoffman et al., 2005; Parkash et al., 2005; Terblanche et al., 2005; Fischer & Kirste, 2018). Selection studies have shown that desiccation resistance can evolve and increase in *Drosophila melanogaster* (Rose et al., 1992; Blows & Hoffmann, 1993). Furthermore, summer acclimation has been found to improve desiccation resistance in *D. melanogaster* relative to both winter and constant temperature conditions (Hoffmann et al., 2005). However, contrasting

results have been found in the selection studies for the most desiccation resistant population of *Drosophila birchii* from the Australian tropical rainforest, suggesting that drier conditions may limit some species distributions and contribute to their extinction risk (Hoffmann et al., 2003). Short-term acclimation also affects water loss rates, where lower acclimation temperature has been shown to cause higher water loss rates when an organism was exposed to desiccation at the higher temperature than the one it was acclimated at, suggesting that higher acclimation temperatures may signal a need for an increased protection against desiccation (Leinaas et al., 2009). How developmental temperatures may affect desiccation resistance is generally not widely understood.

1.7 Thesis aims

Given the fact that thermal resistance traits are key factors limiting species distributions (Addo-Bediako et al., 2000; Chown & Nicolson, 2004; Diamond et al., 2012; Araújo et al., 2013; Overgaard et al., 2014; García-Robledo et al., 2016; Bennett et al., 2016), my first data chapter (Chapter 2) will address the effects of experimental rate variation on critical thermal limits and investigate how the two recently proposed theoretical models apply to the empirical data. Many empirical studies have shown that the experimental protocol using slower and ecologically relevant warming rates lowers critical thermal limits. On the other hand, a significant number of studies has revealed the opposite trend or found no effect of the experimental ramping rate on critical thermal limits of ectotherms (Mora & Maya, 2006; Terblanche et al., 2007; Vinagre et al., 2015; Allen et al., 2016). The thermal tolerance landscape framework focuses on explaining the most commonly observed response where slower rates lower critical thermal limits, redefines

critical thermal limits and promotes using static assays at different temperatures whenever possible (Rezende et al., 2014). On the other hand, the more flexible set of the failure rate models acknowledges both the other empirical outcomes besides the most commonly observed response and separates statistical and biological effects, which are assigned to any residual variation of the best-fitting failure rate model (Kingsolver & Umbanhowar, 2018). Applying these two theoretical approaches to the empirical critical thermal limit data was necessary to distinguish the prevalence of different species' responses to the rate of temperature change and to either confirm or invalidate the claims and assumptions of these models before proceeding to the further empirical measurements of thermal tolerance traits using dynamic or ramping method. To establish what is happening both within and among species of ectotherms when using slower (ecologically relevant) and faster ramping rates, I conduct a comprehensive empirical analysis to investigate the full range of responses of critical thermal limits to the variation in the rate of temperature change (Chapter 2).

Chapter 3 of my thesis simultaneously examines behaviour and physiological traits tied to that specific behaviour under lab simulations of different extreme weather events on populations that have been reared under temperature regimes mimicking both present and projected future (2050) climates. This work investigates the impact of 2 °C increase in the global average temperature relative to the present climate and the effect of extreme weather events such as drought, heat wave, and the combination of the two since multiple environmental stressors may interact in driving behavioural and physiological responses to climate change (Darling & Côté, 2008; Gunderson et al., 2016). The goal here is to identify key physiological traits, climate change drivers (temperature and

extreme weather events) and presence/absence of behavioural thermoregulation in the populations reared under different conditions. Furthermore, I explore how these traits adapt under different rearing conditions, information necessary to improve mechanistic models predicting how a species will respond to climate change (Kellermann & van Heerwaarden, 2019).

Chapter 4 is focused on exploring the effect of developmental temperature on desiccation resistance, which can affect population dynamics and distribution of ectotherms. Climate projections predicting changing rainfall patterns and more dry seasons, which have been found to reduce insect abundances (Wolda, 1978; Tauber et al., 1998; Branson, 2008), suggest that implications of climate change on small ectotherms also depend upon their desiccation resistance (Easterling et al., 2000; IPCC, 2014; Fu, 2015). However, ectotherms will be exposed to the desiccation stress in the new warmer climate and the effect of temperature is also important. Temperature effects on desiccation resistance, whether acclimation or developmental temperature, are still generally largely unexplored with only several studies available, largely focused on Diptera (Hoffman, 1990; Hoffman & Watson, 1993; Hoffman et al., 2005; Terblanche et al., 2005; Chown et al., 2007; Leinaas et al., 2009; Terblanche et al., 2010; Fischer & Kirste, 2018).

Climate change has a large impact on both terrestrial and aquatic ecosystems (Parmesan et al., 2006; Pinsky et al., 2019), but soil systems and soil organisms are equally subjected to the impacts from changing climate. Recent estimates based on general data from global insect species and soil temperature modelling for Australian systems, suggest that by 2100 physiological heat tolerances may be exceeded in many

species in these systems (Duffy et al., 2015). To explore questions involving behaviour and physiological traits I will be using Collembola (springtails) as a model organism since they are the key components of soil ecosystems and soil food web occupying almost all terrestrial habitats (Petersen & Luxton, 1982; Hopkin, 1997; Rusek, 1998). As excellent bioindicators of an environmental change due to their sensitivity to both desiccation and temperature changes, they are an ideal soil organism for studying how behaviour and physiological traits may be altered with changing temperature and moisture availability. The overarching goal of my thesis is to gain insight into how climate change affects behaviour and physiology, both developmentally and under extreme conditions, in order to improve accuracy and precision in forecasting the impacts of changing climate on key populations of soil system invertebrates in Australia.

1.8 Collembola (springtails)

Collembola (springtails) are small soil microarthropods (body length: 0.12-17 mm, most commonly < 2 mm) forming the largest of the three lineages of modern hexapods that are no longer considered insects. They have very wide global distribution with *ca.* 9,000 described species worldwide and many new species still being discovered (Bellinger et al., 1996-2019). Collembola inhabit terrestrial habitats ranging from Antarctica and the Arctic to the tropics, and represent one of the most widespread and abundant groups in the soil fauna (Petersen & Luxton, 1982; Hopkin, 1997; Rusek, 1998). Common morphological features for the majority of species are a furca, a forked, tail-like appendage on the 4th abdominal segment, giving them ability to jump (Figure 4). Species inhabiting deeper soil layers either have reduced furca or they completely lost it.

Collembola also have a ventral tube, the collophore, a sticky appendage used for attaching to surfaces, maintaining water balance, and for cleaning (Hopkin, 1997).



Figure 4 Parisotoma notabilis showing furca and collophore (arrows)

Collembola are key components of soil ecosystems and soil food webs, and as decomposers are involved in nutrient recycling and control of soil microbial communities (Petersen & Luxton, 1982; Hopkin, 1997; Rusek, 1998). The Collembola diet mostly consists of decaying plant material, soil microorganisms and fungal hyphae, which in turn affects the growth of fungi, and the health of the plant root system (Hopkin, 1997). Their small body size makes them very susceptible to desiccation, which is magnified by the fact that most Collembola species have cutaneous respiration and no tracheal system (Sminthuridae or globular springtails are an exception) (Verhoef & Witteveen, 1980). Based on their ecology, Collembola are classified into the following life forms: atmobiotic, epedaphic, hemiedaphic, and euedaphic (Gisin, 1943; Potapov et al., 2016). Atmobiotic species are large and inhabit vegetation – grasses and bushes, while epedaphic species inhabit litter surface (Potapov et al., 2016). Hemiedaphic springtails are leaf-litter dwellers and live under tree bark, while euedaphic life forms live in the soil or deeper litter where the environmental conditions are more stable (Gisin, 1943; Potapov et al., 2016).

Collembola reproduce both sexually via spermatophore deposition that is later picked up by a female and asexually through parthenogenesis, which is the case for many species living in the deeper soil layers (Thibaud, 2012). Mating behaviour has been observed only in the order Symphypleona. Eggs are laid in small batches, sometimes individually, depending on the species, and the embryonic development depends on the environmental conditions, primarily temperature, as is the case for other ectotherms (Verhoef, 1981).

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Chapter 2

Rate dynamics of ectotherm responses to thermal stress

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Chapter 2

Rate dynamics of ectotherm responses to thermal stress

"Nature never breaks her own laws."

Leonardo da Vinci

2.1 Abstract

Critical thermal limits (CTLs) show much variation associated with the experimental rate of temperature change used in their estimation. Understanding the full range of variation in rate effects on critical thermal limits and their underlying basis is thus essential if methodological noise is not to overwhelm or bias the ecological signal. We consider the effects of rate variation from multiple intraspecific assessments and provide a comprehensive empirical analysis of the rate effects on both the critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}) for 47 species of ectotherms, exploring which of the available theoretical models best explains this variation. We find substantial interspecific variation in rate effects, which takes four different forms (increase, decline, no change, mixed), with phylogenetic signal in effects on CT_{max} , but not CT_{min} . Exponential and zero exponential failure rate models best explain the rate effects on CT_{max} . The majority of the empirical rate variation in CT_{min} could not be explained by the failure rate models. Our work demonstrates that rate effects cannot be ignored in comparative analyses, and suggests that incorporation of the failure rate models into such analyses is a useful further avenue for exploration of the fundamental basis and implications of such variation.

2.2 Introduction

Thermal performance curves describe the effects of temperature on physiological processes. The endpoints of a typical thermal performance curve (TPC) are the upper and lower critical thermal (CT) limits (CTLs), where performance declines to zero (Deutsch et al., 2008; Angilletta, 2009; Rezende et al., 2014; Sinclair et al., 2016). Practicably, these endpoints are often defined behaviourally as the temperatures that result in loss of the righting response, coordination, or equilibrium, or the onset of stereotypical behaviour or thermal spasms (Lutterschmidt & Hutchinson, 1997; Chown & Nicolson, 2004; Owen & Dillon, 2018). CTLs are usually measured using a dynamic (ramping) method where temperature is gradually increased or lowered at a constant rate of temperature change until an endpoint is observed. Typically, CTLs of ectotherms increase with faster rates of temperature change, but the converse has also been found (Mora & Maya, 2006; Terblanche et al., 2007; Vinagre et al., 2014). Irrespective, the effects of varying rates of temperature change can be large (Mora & Maya, 2006; Terblanche et al., 2007), and can also interact unpredictably with acclimation effects (Allen et al., 2016). Given the use of CTLs to explore fundamental ecological questions, such as the functional basis of community structure (Hamblin et al., 2017) and the influence of trait variation on niche modelling (Kearney et al., 2014; Valladares et al., 2014; Herrando-Pérez et al., 2018), and to estimate important environmental change impacts (Diamond et al., 2011; Overgaard et al., 2014; García-Robledo et al., 2016), understanding the full range of variation in rate effects on CTLs is essential.

Several proposals have now been made for the ways in which rate effects (which are related to changes in exposure time to stressful temperature (Kilgour & McCauley, 1986; Cooper et al., 2008; Rezende et al., 2014) influence CTLs and their

estimation. The most comprehensive of these is the idea of thermal tolerance landscapes (Rezende et al., 2014). The approach is based on the premise that a single underlying relationship exists between temperature, exposure time, and mortality (see also (Tang et al., 2007)), which follows a typical dose-response curve. Here, critical thermal maximum (CT_{max}) is defined as a knockdown (death) temperature at 1 min of exposure (Rezende et al., 2014) (not 0 min of exposure (Cooper et al., 2008)). A similar argument is applied to critical thermal minimum (CT_{min}). In both cases, the theoretical expectation is that CTLs should improve (higher CT_{max} , lower CT_{min}) with increasing rates of experimental temperature change (or ramping rate). Several studies have now considered the thermal tolerance landscape approach, the most comprehensive of which is a recent analysis of 11 *Drosophila* species focussing especially on knockdown time (Jørgensen et al., 2019).

A related approach models CT_{max} based on the assumption that failure rate increases with rising temperature (Kingsolver & Umbanhowar, 2018). The statistical failure rate model predicts that CT_{max} should increase, typically in a non-linear fashion, with both experimental ramping rate and experimental starting temperature, and is capable of explaining more than half of the variation in CT_{max} values associated with changing ramping rates and starting temperatures. This model is thought to be likewise applicable to CT_{min} estimates (Kingsolver & Umbanhowar, 2018). Notably, the failure rate model is considered a first order model to distinguish statistical effects, which are expected to lead to the positive relationship between an increasing ramping rate and critical thermal limits, from biological effects that might lead either to no effect or to a negative relationship. Here, statistical effects represent variance in CTLs explained by the failure rate model showing an improvement in CTLs (higher CT_{max} , lower CT_{min}), where failure rate (M=1/time) is expected to be higher at the higher (or at the lower for CT_{min}) temperature of failure. Residual variance not explained by the failure rate model is attributed to the biological effects.

A recent work, particularly focused on predicting the thermal acclimation capacity of ectotherms across many different species, body sizes, latitudes, traits, and habitats, also includes the effect of ramping rate as one of the variables, but only at the interspecific level and at the high temperature end (Rohr et al., 2018). However, the study uses an interspecific approach, which confounds intraspecific with interspecific variation, assuming, in contrast to existing empirical data (Mora & Maya, 2006; Terblanche et al., 2007; Vinagre et al., 2015; Allen et al., 2016), that rate effects at the two levels have similar magnitudes and take the same form, and leaving the effect of ramping rate on the extent of variation of both CT_{max} and CT_{min} at the intraspecific level unexplored.

Given the growing significance of CTL estimates in both basic and applied ecological research (Diamond et al., 2011; Araújo et al., 2013; Overgaard et al., 2014; García-Robledo et al., 2016; Bennett et al., 2018), methodological variation in their estimates, and the existence of various theoretical frameworks for the expected nature of its impacts, here, we examine CTL variation with time of exposure to stressful temperatures within species, considering also how exposure time effects on CTLs vary among species. To investigate what the form of the intraspecific response is, across multiple taxa, we quantify how much overall variance can be explained by time alone, how much by species identity, and how much by higher level phylogenetic effects. In doing so we consider closely Rezende et al.'s (2014) variables CT_{max} and z (CT_{min} and z'). The variable z is defined as a constant that characterises the sensitivity to temperature change, and is a key component of the thermal tolerance landscape models (Rezende et al., 2014). We do so to determine whether z (z') conform to the original theoretical estimates, and whether CT_{max} and CT_{min} estimated using this approach provide values in keeping with what has been measured. Next, we examine the failure rate model approach (Kingsolver & Umbanhowar, 2018), which provides more flexibility than the log-linear model of the thermal tolerance landscape approach (Rezende et al., 2014), to determine whether each species follows the response expected by the set of failure rate models originally proposed. We provide the best fitting model to the data at the species level and determine the variation attributed to the statistical effects of failure rate and to residual biological variation (Kingsolver & Umbanhowar, 2018). Furthermore, we extend the set of failure rate models to analyse CT_{min} responses.

2.3 Methods

2.3.1 Data collection

We used a systematic review approach (Pullin & Stewart, 2006) to find studies that measure upper and/or lower CTLs of ectotherms using different rates of temperature change. Search databases included Web of Science, Google Scholar, and Research Gate using the keywords ('critical thermal limits' OR 'thermal tolerance') AND ('rate of temperature change' OR 'ramping rate' OR 'heating rate' OR 'cooling rate') AND ('critical thermal maxima' OR 'CTmax' OR 'CTmin' OR 'critical thermal minima'). In addition, we examined studies listed in the references of the articles found through the database search. Upper and/or lower CT limits were extracted from tables, figures and the main text. Data extraction from figures was undertaken using Plot Digitizer software (Huwaldt & Steinhorst, 2015).

Because the effect of ramping rate (i.e. time of exposure) on CTLs of ectotherms at the intraspecific level is of central interest, inclusion criteria for studies comprised: (i) at least two different rates of temperature change per species to establish a time-temperature pattern of response, (ii) the same life stage, and (iii) a consistent acclimation temperature within single species across multiple ramping rates. If the study contained more than one acclimation temperature, while simultaneously testing for the effect of ramping rate, we chose the acclimation temperature that was the closest to the recorded environmental temperature at the time of the species collection reported in the study. Based on these criteria the CT_{max} and CT_{min} datasets included 41 (184 data points) and 23 (77 data points) species of ectotherms, respectively. Each data point represents an arithmetic mean of a CTL corresponding to a particular rate of temperature change. Thus, these datasets differ from those used in a recent synthesis which focuses on thermal tolerance and acclimation capacities of ectotherms at different acclimation temperatures that use a single ramping rate per species in all of the 254 cases investigated (Rohr et al., 2018).

2.3.2 Analytical approach

Time of exposure for each CTL with its corresponding rate of temperature change was calculated according to:

$$t = \frac{CT_{max} - ST}{r} \tag{1}$$

$$t' = \frac{ST - CT_{min}}{r} \tag{2}$$

where *ST* is the starting experimental temperature, r is the rate of temperature change, t is the time of exposure (t'is the equivalent of t for decreasing temperatures), CT_{max} is the upper critical limit, and CT_{min} is the lower critical limit. To examine the CTL variation with time of exposure to stressful temperature and quantify how much overall variance can be explained by time alone, time and species, and by phylogenetic signal, we undertook two sets of analyses. First, we used a linear model on a semi-logarithmic scale to determine how much of the overall variance in CTLs (i.e. separately for CT_{max} and CT_{min}) is explained by the time of exposure across the entire dataset. In essence, the analyses suggest how much variance might be accounted for by a simple failure rate type model (Kingsolver & Umbanhowar, 2018). Then, we included species as an additional term to determine how much variance is attributable to species-specific responses. Because we found a strong species-specific response of the pattern variation in the CTLs to the time of exposure, we also undertook a phylogenetic analysis in each case using the phylogenetic generalised least squares (PGLS) method with the 'caper' package in R (Orme, 2013). Phylogenetic trees were generated using the 'rotl' package (Michonneau et al., 2016) from the comprehensive tree of life (Hinchliff et al., 2015). Tree branch lengths were estimated using Grafen's arbitrary branch lengths transformation where branch length is set to a length equal to the number of descendant tips minus one (Grafen, 1989). All analyses were performed in R v.3.5.1 (R Core Team, 2014).

Following the approach recommended by Rezende et al. (2014), we tested the expectation that CTLs should improve (higher CT_{max} , lower CT_{min}) with increasing rates of experimental temperature change following the proposed log-linear model (Rezende et al., 2014). We used a linear model of CTLs against a logarithm of time for each species to calculate the intercept and slope of these lines (Table S1 and Table S2, Supplementary Information), which, according to the thermal tolerance landscape framework, correspond to parameters CT_{max} and z (and CT_{min} and z' for low temperatures), respectively (Rezende et al., 2014). We also calculated goodness-of-fit of the line for each species (as the coefficient of determination). In a few instances where we had only two ramping rates per species, we used the CTL data only to

establish the time-temperature pattern of response, but did not include the goodnessof-fit. Two species, *Cryptolestes ferrugineus* and *Glossina pallidipes*, along with *Thaumatotibia leucotreta* (larval stage only), were removed from examination of the association between CT_{min} and z' parameters because the CT_{min} estimates were major outliers. We tested whether parameters z (z') and CT_{max} (CT_{min}) were as strongly correlated as proposed in the thermal tolerance landscape framework (Rezende et al., 2014) and also perform a phylogenetic analysis on these traits.

To test the failure rate model approach and determine whether each species follows the response expected by the set of proposed failure rate models (that assume that the relationship between the failure rate and temperature can follow different parametric forms, such as exponential and power-law forms), we modified and expanded the R code provided by the authors (Kingsolver & Umbanhowar, 2018). Because the authors provide the full code for the best fitting model to their species' dataset only, we expanded the code to provide the probability density function (pdf), expected time to failure, and CTL estimates for the remaining seven models (https://doi.org/10.26180/5c467981f3158). We selected the best fitting failure rate model for each species in our datasets using the corrected Akaike Information Criterion (AICc) (Burnham & Anderson, 2004) because mean CTL values were used within species, and we reported the variance attributed to the statistical effects of failure rate previously described above (Table S3, Supplementary Information). Five species showing a converse response from the one expected by the set of the failure rate models (i.e. a CT_{max} decreasing with increasing rate) were excluded from the CT_{max} analysis, because the statistical effects described by the failure rate models cannot account for a converse response (Kingsolver & Umbanhowar, 2018). Nevertheless, we applied these models to species exhibiting no effect (i.e. no change

in CTLs) or a mixed response (i.e. an increase in CTL followed by a decrease at faster ramping rates) to evaluate if statistical effects account, at least partially, for any CTL variation of these types of responses. Likewise, we extend this code to analyse the response patterns of CT_{min} and exclude nine species owing to their converse response not described by the failure rate models (Table S4, Supplementary Information and https://doi.org/10.26180/5c467981f3158). Both of these scripts can be easily applied to any species dataset investigating the effect of ramping rate and starting temperature on either heat or cold tolerance at the level of species. It is important to highlight that we tested these models using the reported mean values of CTLs for each ramping rate and a single experimental starting temperature per species, with the analyses focusing on the effect of ramping rate at the intraspecific level. All analyses were performed in R v.3.5.1 (Grafen, 1989) using 'bbmle' package v.1.0.20 applying the Nelder-Mead algorithm within function *mle2* (Bolker, 2011).

2.4 Results

In the overall time-temperature relationship, 43% of the overall variance in CT_{max} can be explained by the time of exposure to heat stress ($r^2 = 0.426$, p < 0.0001, Figure 1a). If the analyses are undertaken using rate data only (i.e. excluding starting times) the outcomes are similar (Figure S2, Supplementary Information). On the other hand, there was no relationship between CT_{min} and time of exposure to cold stress ($r^2 =$ 0.0028, p > 0.60, Figure 1c). Adding species identity as an additional factor revealed a strong species-specific response incorporating four different time-temperature patterns of response. Most (94.4%) of variance in CT_{max} can be explained by the time of exposure to heat stress and species identity ($r^2 = 0.944$, p < 0.0001, Figure 1b). Similarly, 94.7% of variance in CT_{min} is explained by the time of exposure to cold stress and species identity ($r^2 = 0.947$, p < 0.0001, Figure 1d). Variation among species in the relationships was not a consequence of body size variation among them as no interaction between a body size measure and rate was found when body size was included in an examination of the relationship between time and CTL (Figure S1 and Tables S6 and S7, Supplementary Information). Of the 40 species examined in the *CT_{max}* analysis (*T. leucotreta* was excluded from percentage analysis since adults and larvae have different responses for both *CT_{max}* and *CT_{min}*), more than half showed a decline in *CT_{max}* with exposure time (Fig. 2). Fifteen percent of species showed no effect, 12% an increase in thermal tolerance with exposure time, and 5% had a mixed response (Figure 2). In the case of *CT_{min}*, response patterns were more evenly distributed among categories (Figure 3). Forty-one percent of the 22 species showed an increase in *CT_{min}* with exposure, which is the contrary of what is typically predicted (Rezende et al., 2014), 27% showed a decrease, 5% had a mixed response, and cold tolerance of 27% of the species remained unchanged regardless of the time of exposure (i.e. ramping rate) (Figure 3).

Following the thermal tolerance landscape approach (Rezende et al., 2014), we calculated the intercept and slope of the lines for log_{10} -transformed time of exposure on CT_{max} values for each species that correspond to the CT_{max} and z. We also calculated variance explained by the time of exposure for each species excluding species that had only two ramping rates. Regressing log_{10} -transformed time of exposure on CT_{max} values of 39 species resulted in relationships with lower goodnessof-fit (median $r^2 = 0.872$, 95% CI between 0.656 and 0.976) (Figure 2 and Table S1, Supplementary Information) than those derived from upper lethal limit approaches reported in the original study seeking to integrate the two approaches (median $r^2 =$ 0.985, 95% CI between 0.876 and 0.999) (Rezende et al., 2014). Analyses show that z

accounts for 70% of the variation in CT_{max} as estimated by the coefficient of determination (slope = 2.90, p < 0.0001, $r^2 = 0.702$, Table 1a and Figure 4a). Within insects and crustaceans, the variance explained by this model corresponds to 76% and 67%, respectively (slopes = 2.52 and 1.88, p < 0.0001 and p = 0.0069), while within fishes and springtails this model cannot explain the variance in CT_{max} (slopes = -0.12 and 4.21, p = 0.9145 and p = 0.0694). Parameters CT_{max} and z vary across species, with CT_{max} ranging between 27.8 °C and 64.9 °C and z ranging between 0.04 and 8.9. Likewise, we performed the same analysis on cold tolerance (Figure 3 and Table S2, Supplementary Information). CT_{min} curves of 21 species of ectotherms resulted in relationships with lower goodness-of-fit (median $r^2 = 0.858$, 95% CI between 0.694 and 0.956) (Figure 3) than those reported using lower lethal limit values in the original study seeking to integrate the two approaches (median $r^2 = 0.955$, 95% CI between 0.876 and 0.993) (Rezende et al., 2014). After excluding three outliers from the analysis, we found that the association between parameters CT_{min} and z' was not significant (slope = -0.68, p = 0.5167, r² = 0.022, Table 1b and Figure 4b), suggesting that z' does not explain the variation in CT_{min} in 21 species of ectotherms, which is in contrast with the high correlation found in studies using lower lethal limits and the general thermal tolerance landscape expectation (slope = -4.99, p < 0.0001, $r^2 = 0.965$ (Rezende et al., 2014)). The range of CT_{min} variation was lower than for CT_{max} , with CT_{min} ranging between -7.30 °C and 9.61 °C, and z' between 0 and 4.03, which is not in keeping with the results of the thermal landscape approach (Rezende et al., 2014).

Estimates of a phylogenetic signal in z, a constant characterising the sensitivity to temperature change, were moderate. Pagel's λ in z alone was 0.43, while lower phylogenetic signals were detected in the effects of response ($\lambda = 0.26$), habitat ($\lambda =$ 0.05), and climate ($\lambda = 0.40$) on z. On the other hand, no phylogenetic signal was detected in z' alone, nor in the effects of response, habitat, and climate on z' (Table S5, Supplementary Information).

The most common failure rate models across species in both CT_{max} and CT_{min} analyses were exponential and zero exponential models, followed by a zero-power threshold, zero exponential threshold and exponential threshold models. Five species had a converse trend to which we could not apply statistical effects described by the failure rate models as recognised by the authors, thus, we assigned the response to the biological effects only (Figure 2 and Table S3, Supplementary Information). Outcomes of the CT_{min} analysis showed that the most common cold tolerance response was a converse response, which could not be accounted for by the statistical effects of the failure rate models. Therefore, we similarly assigned the variance to the biological effects (Figure 3 and Table S4, Supplementary Information). The zeropower threshold model was the most common best-fit model closely followed by the exponential and zero exponential models, including exponential threshold and zero exponential threshold models. Using mean CT_{max} values for each ramping rate we found that the best-fitting model for G. pallidipes are exponential and zero exponential models, giving a different outcome from the zero-power threshold model, which was the best-fitting model for the full G. pallidipes dataset containing CT_{max} values for each individual tested.

2.5 Discussion

Our results demonstrate that the commonly expected direction of response characterised by an improvement of CTLs (higher CT_{max} , lower CT_{min}) with the increasing rate of temperature change is not universally observed pattern among the ectotherms. While time of exposure generally has a negative effect on CT_{max} , such a

generalization cannot be made for CT_{min} . Similarly, ontogeny, body size, and sex, along with nutritional status and the extent of desiccation stress have varying effects on heat and cold tolerance (Terblanche et al., 2011; Overgaard et al., 2012; Madeira et al., 2012; Klockmann et al., 2016; Terblanche et al., 2017).

A strong species-specific response for both CT_{max} and CT_{min} , independent of body size (unlike the situation found in interspecific analyses (Rohr et al., 2018)), reveals four different thermal tolerance response patterns to the increasing exposure time (i.e. slower ramping rate). The proposal that static and dynamic experimental methods share similar relationships with exposure time in a thermal tolerance landscape framework (Rezende et al., 2014) thus appears to be an oversimplification of empirical observations. Species showing an increase in thermal tolerance with the exposure time might fit the log-linear time-temperature trend (Rezende et al., 2014) well, but in the opposite direction from the one expected. This model generally produces a poor fit for species with no effect and mixed trends in their CTLs. Indeed, assessment of the method used by Rezende et al. (2014) revealed that the parameters z(z), a constant characterising the sensitivity to the temperature change, and CT_{max} (CT_{min}) , redefined as a knockdown temperature at 1 min of exposure, are not always highly correlated as originally proposed. These outcomes are in agreement with a recent comprehensive assessment of the thermal landscapes approach, which demonstrated that heat tolerance parameters, z and CT_{max} at 1 min of exposure in 11 Drosophila species are not correlated (Jørgensen et al., 2019). The lower correlation of z and CT_{max} than the one found in the study by Rezende and the colleagues (Rezende et al., 2014) may have several explanations. One of these may be variation of species' CT_{max} response patterns to the ramping rate, as two thirds of the species show the decline in thermal tolerance with the longer exposure time (i.e. slower

ramping rate), while the rest of the species yield different responses. The other explanation, supported by evidence from modelling analyses, is that experimental noise (or small sample size), autocorrelation and unwarranted extrapolation, are responsible for the initial finding of a strong relationship between z and CT_{max} (Jørgensen et al., 2019). Perhaps unsurprisingly, therefore, we also found no correlation between z' and CT_{min} , a result while different to the original thermal landscapes idea (Rezende et al., 2014), is in keeping with the growing body of literature testing it (Jørgensen et al., 2019). The variation of CT_{min} with the dynamic temperature change is much more pronounced, where the most dominant pattern among the species tested is an increase in cold tolerance with the increasing exposure time. Parameter z also appears to be more phylogenetically constrained ($\lambda = 0.43$) than parameter z' where no phylogenetic signal was detected ($\lambda = 0$), which is in agreement with the previous studies detecting a phylogenetic constraint in upper thermal limits (Hoffmann et al., 2012; Kellermann et al., 2012).

In addition, significant disparities exist when comparing CT_{min} estimates from the empirical studies using a dynamic method and CT_{min} values predicted by the thermal tolerance landscape. For example, Jian et al. (2015) found that the overall limits to activity (CT_{min}) for three species of beetles in the adult life stage, *Cryptolestes ferrugineus*, *Tribolium castaneum*, and *Sitophilus oryzae*, were 2.0 °C, 6.0 °C, and 6.5 °C respectively (Jian et al., 2015). Empirical results from this study differ substantially from CT_{min} values predicted by Rezende et al. (2014), where CT_{min} limits for *C. ferrugineus*, *T. castaneum*, and *S. oryzae* were estimated to be -100.96 °C, -85.17 °C, and -38.98 °C respectively (Rezende et al., 2014). This discrepancy arises from redefining CT_{min} as death or knockdown temperature at 1 min of exposure (Rezende et al., 2014) as opposed to the lowest limit to activity, as used across multitude of cold tolerance studies (Huey et al., 1992; Kelty & Lee, 1999; Powell & Bale, 2006). The lowest ever reported CT_{min} of -16 °C for *Diamesa* Meigen, a Himalayan glacier species, remains much higher than these three estimates (Kohshima, 1984). Because the thermal tolerance landscape framework seems to overestimate empirical CT_{min} values, the approach requires more exploration.

The set of failure rate models proposed by Kingsolver & Umbanhowar (2018), where failure rate is higher at the higher (or at the lower for CT_{min}) temperature of failure, generally corresponds to a decline in thermal tolerance with exposure time, a similar expectation to the thermal tolerance landscape framework (Rezende et al., 2014). However, failure rate models are more flexible than the log-linear models in a sense that they allow curves to take different shapes and include the presence or absence of a threshold temperature. In addition, other patterns of thermal tolerance response to the increasing ramping rate, which cannot be attributed to the statistical effects of failure rate, can also be incorporated generally (Kingsolver & Umbanhowar, 2018). Contribution of the statistical effects to the mixed and no effect responses is generally small, likely because the variation is owing to the biological effects. The models are not applicable to the converse response currently including 12% (for CT_{max}) and 41% (for CT_{min}) species, which can be also attributed to the biological effects. It is important to note that we obtained a different best-fitting model for G. pallidipes than the authors (Kingsolver & Umbanhowar, 2018) because we used mean CT_{max} values per ramping rate as opposed to CT_{max} values for each individual tested, which demonstrates how the outcome of the analysis may change when an individual variation is incorporated into or excluded from the intraspecific study. The most common best-fit models in CT_{max} analysis suggest that failure rate increases exponentially from the experimental starting temperature until CT_{max} is reached.

Results of other species support the presence of temperature threshold after which failure rate follows an exponential or a power increase before reaching CT_{max} . CT_{min} analysis supports the presence of a temperature threshold, but there are also species showing an exponential increase in failure rate from the experimental starting temperature until CT_{min} is reached.

Based on the four response patterns found, we propose a set of hypothetical relationships between failure rate (Kingsolver & Umbanhowar, 2018), recovery rate, and critical thermal limits that may explain the variation in species' responses (Figure 5). If recovery rate cannot catch up with the failure rate during prolonged time of exposure (Figure 5a), thermal tolerance declines with exposure time. On the other hand, if recovery rate improves with time owing to a rapid physiological response, thermal tolerance increases with exposure time (Figure 5b). In the case of the no effect response, time of exposure (i.e. ramping rate) might have no effect on thermal tolerance (Figure 5c). Alternatively, failure rate and recovery rate could be matched due to some form of beneficial physiological response by an organism. Finally, a mixed response could reveal an inflection point (Mora & Maya, 2006), with a pattern of declining thermal tolerance from an intermediate ramping rate towards faster and slower rates (Figure 5d). This response potentially reveals an optimal ramping rate at which maximum tolerance gain is achieved due to acclimation to the rapid change in temperature, while an organism simultaneously becomes exposed to the deleterious effects of temperature extremes.

What these four relationships provide is a framework for further exploration of the way in which differing damage accumulation rates and organismal-level physiological and biochemical response rates interact to determine thermal tolerance. Clearly, time of exposure (given different ramping rates) is an important component

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thereof, especially given the high Q₁₀ of the processes leading to heat stress-related physiological failure (Jørgensen et al., 2019). However, as an early study showed (Terblanche et al., 2007), so too is the starting temperature of the process, since this may determine the extent to which an organism is already outside the zone of tolerance (Cossins & Bowler, 1987), which precedes the onset of damage. Just what the effect is of starting temperature on experimental outcomes is not yet well resolved. The proposed framework suggests that future work should focus on three main areas. First, determining whether starting temperature has as large effect as ramping rate on outcomes, as a single study suggests it might (Terblanche et al., 2007) and whether a threshold effect, indicating that differences in starting temperatures inside or outside the organism's zone of tolerance (i.e. on either side of the incipient lethal temperature (Cossins & Bowler, 1987)) are important. Second, further considering the outcomes of the failure rate approach in the context of the framework proposed here to determine the extent to which simple failure rate models may afford the null expectation for thermal limits in the absence of biological responses (Kingsolver & Umbanhowar, 2018). The failure rate models provide good fits to the available data and are readily interpretable both in a statistical and physiological context. Finally, investigation of whether differential rates of damage and repair really are responsible for variation in the rate-thermal limit response. Very high rates of Q₁₀ for thermal limits (Jørgensen et al., 2019) suggest that, at least at the highest temperatures, any form of repair will be rapidly overwhelmed given generally lower thermal sensitivities of routine physiological functions including, for example, protein synthesis (Elias et al., 2014). Of course, even in the absence of investigation of these questions, it is clear that rate variation cannot be ignored in compiled comparative

studies, either of critical thermal limits or of their implications for environmental change.

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2.8 Figures



Figure 1

Time-temperature relationship for upper and lower critical thermal limits of ectotherms. a) General relationship between time of exposure and CT_{max} of 41 species of crustaceans, fishes, insects and springtails. b) The association between time of exposure and CT_{max} at the intraspecific level reveals four different patterns of species-specific responses to the heat stress for 41 species of ectotherms. c) General relationship between time of exposure and CT_{min} of 23 species of crustaceans, insects, and springtails. d) The association between time of exposure and CT_{min} at the intraspecific level reveals four different patterns to the intraspecific level reveals for 41 species of crustaceans, insects, and springtails. d) The association between time of exposure and CT_{min} at the intraspecific level reveals four different patterns of species-specific responses to the cold stress for 23 species of ectotherms.



Phylogeny of 41 species of ectotherms used in the CT_{max} analysis and their response patterns describing the change in heat tolerance with the increasing exposure time (i.e. slower ramping rates). Variance explained by the statistical (dark blue) and biological (light blue) effects using a failure rate model (Kingsolver & Umbanhowar, 2018) and goodness-of-fit of the thermal landscape model (Rezende et al., 2014).



Phylogeny of 41 species of ectotherms used in the CT_{max} analysis and their response patterns describing the change in heat tolerance with the increasing exposure time (i.e. slower ramping rates). Variance explained by the statistical (dark blue) and biological (light blue) effects using a failure rate model (Kingsolver & Umbanhowar, 2018) and goodness-of-fit of the thermal landscape model (Rezende et al., 2014).



a) The association between parameters CT_{max} and z for 41 species of ectotherms b) The association between parameters CT_{min} and z' for 21 species of ectotherms. CT_{max} (CT_{min}) and z (z') correspond to intercept and slope of log-linear model of CTLs against a logarithm of time for each species (Rezende et al., 2014).



Hypothetical relationship between failure rate and recovery rate and their effect on thermal tolerance. a) Thermal tolerance declines with exposure time if recovery rate cannot catch up with the failure rate; b) Thermal tolerance increases with exposure time if recovery rate improves over time and overcomes failure rate; c) Thermal tolerance is not affected by exposure time when failure rate and recovery rate are closely matched; d) Thermal tolerance initially shows an increase (or a decrease) in thermal tolerance, reaches a peak high (or low) temperature at a certain ramping rate (i.e. time of exposure), followed by a decrease (or an increase) in thermal tolerance. This response potentially reveals an inflection point or ramping rate, which either decreases or improves recovery rate relative to the failure rate.

2.9 Tables

Table 1

Outcome of the linear models examining the relationship between parameters CT_{max} and z and CT_{min} and z' among 41 and 23 species of ectotherms, respectively.

a) CT_{max}	Estimate	s.e.	t	Р				
CT_{max} (Intercept)	34.696	1.147	30.240	< 0.0001				
Ζ	2.905	0.299	9.703	< 0.0001				
$F_{(1,40)} = 94.14, p < 0.0001, R^2 = 0.7018$								
b) <i>CT_{min}</i>	Estimate	s.e.	t	Р				
CT_{min} (Intercept)	3.413	1.780	1.918	0.0703				
<i>z'</i>	-0.681	1.031	-0.661	0.5167				
$F_{(1,19)} = 0.44, p=0.5167, R^2 = 0.022$								

2.10 Supplementary Information

Table S1. Heat Tolerance - Thermal death time parameters according to Rezende et al. 2014

Species	Class	Life stage	Habitat	CT_{max}	Z	r ²	Response	Reference
Acanthemblemaria hancocki	Actinopterygii	adult	aquatic	36.63	0.39	0.335	Mixed	Mora & Maya 2006
Atta sexdens rubropulosa	Insecta	adult	terrestrial	53.29	2.17	0.417	Decrease	Ribeiro et al. 2012
Bathygobius soporator	Actinopterygii	adult	aquatic	39.27	0.08	0.056	No effect	Vinagre et al. 2015
Ceratitis capitata	Insecta	adult	terrestrial	42.05	0.36	0.801	Increase	Nyamunkondiwa & Terblanche 2010
Ceratitis rosa	Insecta	adult	terrestrial	40.26	0.78	0.976	Increase	Nyamunkondiwa & Terblanche 2010
Clupea harengus	Actinopterygii	larvae	aquatic	31.82	3.28	0.885	Decrease	Moyano et al. 2017
Cydia pomonella	Insecta	adult	terrestrial	38.41	2.30	0.808	Increase	Chidawanyika & Terblanche 2011
Cyrtobagous salviniae	Insecta	adult	aquatic	57.79	6.34	0.861	Decrease	Allen et al. 2012
Deuteraphorura one	Collembola	adult	terrestrial	43.35	3.35	0.852	Decrease	Allen et al. 2016
Deuteraphorura two	Collembola	adult	terrestrial	46.62	3.81	0.942	Decrease	Allen et al. 2016
Dicentrarchus labrax	Actinopterygii	larvae	aquatic	32.14	0.04	0.128	No effect	Moyano et al. 2017
Drosophila melanogaster	Insecta	adult	terrestrial	42.67	1.75	0.984	Decrease	Chown et al. 2009
Drosophila subobscura	Insecta	adult	terrestrial	42.17	3.13	na	Decrease	Castañeda et al 2015
Eurypanopeus abbreviatus	Malacostraca	adult	aquatic	37.42	0.81	1.000	Increase	Vinagre et al. 2015
Exosphaeroma antikraussi	Malacostraca	adult	aquatic	40.60	1.32	na	No effect	Faulkner et al. 2014
Exosphaeroma gigas	Malacostraca	adult	aquatic	34.95	2.12	0.819	Decrease	Faulkner et al. 2014
Exosphaeroma laeviusculum	Malacostraca	adult	aquatic	49.26	5.04	0.802	Decrease	Faulkner et al. 2014
Folsomia candida	Collembola	adult	terrestrial	45.37	3.49	0.852	Decrease	Allen et al. 2016
Glossina pallidipes	Insecta	adult	terrestrial	56.41	7.49	0.716	Decrease	Terblanche et al. 2007, 2008
Hippolyte obliquimanus	Malacostraca	adult	aquatic	34.66	0.06	0.980	No effect	Vinagre et al. 2015
Hyale grandicornis	Malacostraca	adult	aquatic	52.68	8.58	0.976	Decrease	Faulkner et al. 2014
Hyale hirtipalma	Malacostraca	adult	aquatic	34.90	3.05	0.977	Decrease	Faulkner et al. 2014
Hypogastrura assimilis	Collembola	adult	terrestrial	51.75	3.81	0.942	Decrease	Allen et al. 2016
Hypogastrura viatica	Collembola	adult	terrestrial	52.10	5.20	0.993	Decrease	Allen et al. 2016
Lepomis gibbosus	Actinopterygii	juvenile	aquatic	36.25	0.43	0.216	Decrease	Becker & Genoway 1979
Linepithema humile	Insecta	adult	terrestrial	54.58	5.90	0.981	Decrease	Jumbam et al. 2008; Chown et al. 2009
Menippe nodifrons	Malacostraca	adult	aquatic	38.71	0.32	0.629	Increase	Vinagre et al. 2015

Nylanderia fulva	Insecta	adult	terrestrial	56.04	7.82	1.000	Decrease	Bentley et al. 2016
Oncorhynchus kitsutch	Actinopterygii	juvenile	aquatic	30.75	1.13	0.939	Decrease	Becker & Genoway 1979
Oncorhynchus mykiss	Actinopterygii	juvenile	aquatic	27.84	0.24	0.478	No effect	Galbreath et al. 2004
Palaemon northropi	Malacostraca	adult	aquatic	40.10	0.33	0.363	Decrease	Vinagre et al. 2015
Parablennius marmoreus	Actinopterygii	adult	aquatic	37.10	0.56	0.997	Decrease	Vinagre et al. 2015
Pycnopsyche guttifer	Insecta	larvae	aquatic	38.25	3.26	0.669	Decrease	Houghton et al. 2014
Rutilus rutilus	Actinopterygii	adult	aquatic	31.58	0.17	0.026	No effect	Cocking 1956
Salmo salar	Actinopterygii	juvenile	aquatic	37.49	2.03	0.883	Decrease	Elliott & Elliott 1995
Salmo trutta	Actinopterygii	juvenile	aquatic	34.54	1.90	0.665	Decrease	Elliott & Elliott 1995; Galbreath et al. 2004
Salvelinus fontinalis	Actinopterygii	juvenile	aquatic	31.07	0.75	0.944	Decrease	Galbreath et al. 2004
Solenopsis invicta	Insecta	adult	terrestrial	62.57	8.66	0.996	Decrease	Bentley et al. 2016
Tenebrio molitor	Insecta	adult	terrestrial	42.54	0.19	0.031	Mixed	Allen et al. 2012
Thaumatotibia leucotreta	Insecta	adult	terrestrial	45.78	1.42	0.971	No effect	Terblanche et al. 2017
Thaumatotibia leucotreta	Insecta	larvae	terrestrial	64.87	8.86	0.908	Decrease	Terblanche et al. 2017
Xenylla humicola	Collembola	adult	terrestrial	50.62	4.32	0.981	Decrease	Allen et al. 2016

 CT_{max} - intercept of the curve

z - slope of the curve

 r^2 - Variance explained by the linear semi-logarithmic model (na = have only two rates of temperature change, variance not calculated)

Response - Change in heat tolerance as time of exposure increases

Table S2. Cold Tolerance - Thermal death time parameters according to Rezende et al. 2014

Species	Class	Life stage	Habitat	CT_{min}	z′	\mathbf{r}^2	Response	Reference
Ceratitis capitata	Insecta	adult	terrestrial	8.39	1.15	0.995	Increase	Nyamunkondiwa & Terblanche 2010
Ceratitis rosa	Insecta	adult	terrestrial	8.06	0.93	0.974	Increase	Nyamunkondiwa & Terblanche 2010
Cryptolestes ferrugineus	Insecta	adult	terrestrial	57.45	12.36	na	Increase	Jian et al. 2015
Cydia pomonella	Insecta	adult	terrestrial	2.44	0.70	1.000	No effect	Chidawanyika & Terblanche 2011
Cyrtobagous salviniae	Insecta	adult	aquatic	3.17	3.60	0.785	Decrease	Allen et al. 2012
Deuteraphorura one	Collembola	adult	terrestrial	-2.83	0.27	0.084	No effect	Allen et al. 2016
Deuteraphorura two	Collembola	adult	terrestrial	-2.29	0.92	0.956	Decrease	Allen et al. 2016
Drosophila melanogaster	Insecta	adult	terrestrial	7.58	0.56	0.754	Increase	Chown et al. 2009
Folsomia candida	Collembola	adult	terrestrial	-4.87	1.45	0.421	Decrease	Allen et al. 2016
Glossina pallidipes	Insecta	adult	terrestrial	-232.51	143.52	0.837	Decrease	Terblanche et al. 2007, 2008
Hypogastrura assimilis	Collembola	adult	terrestrial	0.79	1.31	0.993	Increase	Allen et al. 2016
Hypogastrura viatica	Collembola	adult	terrestrial	-0.90	2.46	0.912	Increase	Allen et al. 2016
Linepithema humile	Insecta	adult	terrestrial	-2.34	0.99	0.943	Decrease	Jumbam et al. 2008; Chown et al. 2009
Litopenaeus vannamei	Malacostraca	post larvae	aquatic	9.61	0.23	na	No effect	Kumlu et al. 2010
Locusta migratoria	Insecta	adult	terrestrial	-0.25	0.10	0.009	Mixed	Findsen et al. 2014
Nylanderia fulva	Insecta	adult	terrestrial	6.76	0.22	0.289	No effect	Bentley et al. 2016
Penaeus semisulcatus	Malacostraca	juvenile	aquatic	-3.79	4.03	0.973	Decrease	Kir & Kumlu 2008
Sitobion avenae	Insecta	adult	terrestrial	4.34	2.69	0.933	Increase	Powell and Bale 2006
Solenopsis invicta	Insecta	adult	terrestrial	2.91	0.56	0.814	No effect	Bentley et al. 2016
Tenebrio molitor	Insecta	adult	terrestrial	9.40	1.85	0.858	Increase	Allen et al. 2012
Thaumatotibia leucotreta	Insecta	adult	terrestrial	7.95	2.49	0.944	Increase	Terblanche et al. 2017
Thaumatotibia leucotreta	Insecta	larvae	terrestrial	-11.86	11.30	0.694	Decrease	Terblanche et al. 2017
Tribolium castaneum	Insecta	adult	terrestrial	6.00	0.00	na	No effect	Jian et al. 2015
Xenylla humicola	Collembola	adult	terrestrial	-7.30	1.13	0.380	Increase	Allen et al. 2016

CTmin - intercept of the

curve z'- slope of the curve

 r^2 - Variance explained by the linear semi-logarithmic model (na = have only two rates of temperature change, variance not calculated) Response - Change in cold tolerance as time of exposure increases

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Table S3. CT _{max} models	- Best models and the	variance accounting for t	he statistical effect	s of the failure rate	model according to	o Kingsolver &	Umbanhowar, 2018

Species	Class	Life stage	Habitat	Best Failure Rate Model	Variance	Response	Reference	Comments
Acanthemblemaria hancocki	Actinopterygii	adult	aquatic	Zero Exponential Threshold	0.436	Mixed	Mora & Maya 2006	
Atta sexdens rubropulosa	Insecta	adult	terrestrial	Exponential/Zero Exponential	0.460	Increase	Ribeiro et al. 2012	
Bathygobius soporator	Actinopterygii	adult	aquatic	Zero Power Threshold	0.053	No effect	Vinagre et al. 2015	Other good model fit: Exponential Threshold and Zero Exponential Threshold
Ceratitis capitata	Insecta	adult	terrestrial	Converse trend	na	Decrease	Nyamunkondiwa & Terblanche 2010	
Ceratitis rosa	Insecta	adult	terrestrial	Converse trend	na	Decrease	Nyamunkondiwa & Terblanche 2010	
Clupea harengus	Actinopterygii	larvae	aquatic	Exponential/Zero Exponential	0.312	Increase	Moyano et al. 2017	
Cydia pomonella	Insecta	adult	terrestrial	Converse trend	na	Decrease	Chidawanyika & Terblanche 2011	
Cyrtobagous salviniae	Insecta	adult	aquatic	Exponential/Zero Exponential	0.893	Increase	Allen et al. 2012	
Deuteraphorura one	Collembola	adult	terrestrial	Exponential/Zero Exponential	0.869	Increase	Allen et al. 2016	
Deuteraphorura two	Collembola	adult	terrestrial	Exponential/Zero Exponential	0.952	Increase	Allen et al. 2016	
Dicentrarchus labrax	Actinopterygii	larvae	aquatic	Exponential/Zero Exponential	0.125	No effect	Moyano et al. 2017	
Drosophila melanogaster	Insecta	adult	terrestrial	Zero Power Threshold	0.986	Increase	Chown et al. 2009	Other good model fit: Exponential Threshold and Zero Exponential Threshold
Drosophila subobscura	Insecta	adult	terrestrial	Only two rates of temp. change	na	Increase	Castañeda et al 2015	
Eurypanopeus abbreviatus	Malacostraca	adult	aquatic	Converse trend	na	Decrease	Vinagre et al. 2015	
Exosphaeroma antikraussi	Malacostraca	adult	aquatic	Only two rates of temp. change	na	No effect	Faulkner et al. 2014	
Exosphaeroma gigas	Malacostraca	adult	aquatic	Exponential/Zero Exponential	0.745	Increase	Faulkner et al. 2014	
Exosphaeroma laeviusculum	Malacostraca	adult	aquatic	Exponential/Zero Exponential	0.744	Increase	Faulkner et al. 2014	
Folsomia candida	Collembola	adult	terrestrial	Exponential/Zero Exponential	0.869	Increase	Allen et al. 2016	
Glossina pallidipes	Insecta	adult	terrestrial	Exponential Threshold	0.864	Increase	Terblanche et al. 2007, 2008	
Hippolyte obliquimanus	Malacostraca	adult	aquatic	Zero Power Threshold	0.981	No effect	Vinagre et al. 2015	Other good model fit: Exponential Threshold and Zero Exponential Threshold
Hyale grandicornis	Malacostraca	adult	aquatic	Zero Exponential	0.952	Increase	Faulkner et al. 2014	
Hyale hirtipalma	Malacostraca	adult	aquatic	Exponential/Zero Exponential	0.893	Increase	Faulkner et al. 2014	
Hypogastrura assimilis	Collembola	adult	terrestrial	Exponential/Zero Exponential	0.949	Increase	Allen et al. 2016	
Hypogastrura viatica	Collembola	adult	terrestrial	Exponential/Zero Exponential	0.994	Increase	Allen et al. 2016	
Lepomis gibbosus	Actinopterygii	juvenile	aquatic	Exponential/Zero Exponential	0.234	Increase	Becker & Genoway 1979	
Linepithema humile	Insecta	adult	terrestrial	Exponential/Zero Exponential	0.985	Increase	Jumbam et al. 2008; Chown et al. 2009	
Menippe nodifrons	Malacostraca	adult	aquatic	Converse trend	na	Decrease	Vinagre et al. 2015	
Nylanderia fulva	Insecta	adult	terrestrial	Exponential/Zero Exponential	0.999	Increase	Bentley et al. 2016	
Oncorhynchus kitsutch	Actinopterygii	juvenile	aquatic	Exponential/Zero Exponential	0.942	Increase	Becker & Genoway 1979	
Oncorhynchus mykiss	Actinopterygii	juvenile	aquatic	Exponential/Zero Exponential	0.762	No effect	Galbreath et al. 2004	
Palaemon northropi	Malacostraca	adult	aquatic	Exponential Threshold/Zero Exponential Threshold	0.381	Increase	Vinagre et al. 2015	Other good model fit: Zero Power Threshold
Parablennius marmoreus	Actinopterygii	adult	aquatic	Zero Power Threshold	0.997	Increase	Vinagre et al. 2015	Other good model fit: Exponential Threshold and Zero Exponential Threshold
Pycnopsyche guttifer	Insecta	larvae	aquatic	Exponential/Zero Exponential	0.723	Increase	Houghton et al. 2014	
Rutilus rutilus	Actinopterygii	adult	aquatic	Exponential/Zero Exponential	0.000	No effect	Cocking 1956	
Salmo salar	Actinopterygii	juvenile	aquatic	Exponential/Zero Exponential	0.628	Increase	Elliott & Elliott 1995	
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Salmo trutta	Actinopterygii	juvenile	aquatic	Exponential/Zero Exponential	0.414	Increase	Elliott & Elliott 1995; Galbreath et al. 2004	
Salvelinus fontinalis	Actinopterygii	juvenile	aquatic	Exponential/Zero Exponential	0.626	Increase	Galbreath et al. 2004	
Solenopsis invicta	Insecta	adult	terrestrial	Exponential Threshold	0.999	Increase	Bentley et al. 2016	Other good model fit: Zero Exponential Threshold
Tenebrio molitor	Insecta	adult	terrestrial	Exponential/Zero Exponential	0.023	Mixed	Allen et al. 2012	
Thaumatotibia leucotreta	Insecta	adult	terrestrial	Zero Power Threshold	0.974	No effect	Terblanche et al. 2017	Other good model fit: Exponential Threshold and Zero Exponential Threshold
Thaumatotibia leucotreta	Insecta	larvae	terrestrial	Zero Exponential Threshold	0.938	Increase	Terblanche et al. 2017	Other good model fit: Exponential Threshold and Zero Power Threshold
Xenylla humicola	Collembola	adult	terrestrial	Exponential/Zero Exponential	0.983	Increase	Allen et al. 2016	

Best Failure Rate Model - model with the lowest AICc (na = converse trend from the one expected by the model OR have only two rates of temperature change, variance not calculated)

Variance - Variance accounting for the statistical effects of the failure rate model (na = only two rates of temperature change, variance not calculated)

Response - Change in heat tolerance as ramping rate increases

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Species	Class	Life stage	Habitat	Best Failure Rate Model	Variance	Response	Reference	Comments
Ceratitis capitata	Insecta	adult	terrestrial	Converse trend	na	Decrease	Nyamunkondiwa & Terblanche 2010	
Ceratitis rosa	Insecta	adult	terrestrial	Converse trend	na	Decrease	Nyamunkondiwa & Terblanche 2010	
Cryptolestes ferrugineus	Insecta	adult	terrestrial	Only two rates of temp. change	na	Decrease	Jian et al. 2015	
Cydia pomonella	Insecta	adult	terrestrial	Zero Power Threshold	0.999	No effect	Chidawanyika & Terblanche 2011	Other good model fit: Exponential Threshold and Zero Exponential Threshold
Cyrtobagous salviniae	Insecta	adult	aquatic	Exponential/Zero Exponential	0.823	Increase	Allen et al. 2012	
Deuteraphorura one	Collembola	adult	terrestrial	Exponential Threshold/Zero Exponential Threshold	0.096	No effect	Allen et al. 2016	Other good model fit: Zero Power Threshold
Deuteraphorura two	Collembola	adult	terrestrial	Zero Power Threshold	0.958	Increase	Allen et al. 2016	Other good model fit: Exponential Threshold and Zero Exponential Threshold
Drosophila melanogaster	Insecta	adult	terrestrial	Converse trend	na	Decrease	Chown et al. 2009	
Folsomia candida	Collembola	adult	terrestrial	Zero Power Threshold	0.469	Increase	Allen et al. 2016	Other good model fit: Exponential Threshold and Zero Exponential Threshold
Glossina pallidipes	Insecta	adult	terrestrial	Exponential Threshold	0.996	Increase	Terblanche et al. 2007, 2008	
Hypogastrura assimilis	Collembola	adult	terrestrial	Converse trend	na	Decrease	Allen et al. 2016	
Hypogastrura viatica	Collembola	adult	terrestrial	Converse trend	na	Decrease	Allen et al. 2016	
Linepithema humile	Insecta	adult	terrestrial	Exponential/Zero Exponential	0.945	Increase	Jumbam et al. 2008; Chown et al. 200	19
Litopenaeus vannamei	Malacostraca	post larvae	aquatic	Only two rates of temp. change	na	No effect	Kumlu et al. 2010	
Locusta migratoria	Insecta	adult	terrestrial	Exponential/Zero Exponential	0.014	Mixed	Findsen et al. 2014	
Nylanderia fulva	Insecta	adult	terrestrial	Exponential Threshold/Zero Exponential Threshold	0.296	No effect	Bentley et al. 2016	
Penaeus semisulcatus	Malacostraca	juvenile	aquatic	Zero Power Threshold	0.871	Increase	Kir & Kumlu 2008	Other good model fit: Exponential Threshold and Zero Exponential Threshold
Sitobion avenae	Insecta	adult	terrestrial	Converse trend	na	Decrease	Powell and Bale 2006	
Solenopsis invicta	Insecta	adult	terrestrial	Exponential Threshold	0.818	No effect	Bentley et al. 2016	Other good model fit: Zero Power Threshold
Tenebrio molitor	Insecta	adult	terrestrial	Converse trend	na	Decrease	Allen et al. 2012	
Thaumatotibia leucotreta	Insecta	adult	terrestrial	Converse trend	na	Increase	Terblanche et al. 2017	
Thaumatotibia leucotreta	Insecta	larvae	terrestrial	Exponential Threshold	0.917	Decrease	Terblanche et al. 2017	Other good model fit: Zero Exponential Threshold and Zero Power Threshold
Tribolium castaneum	Insecta	adult	terrestrial	Only two rates of temp. change	na	No effect	Jian et al. 2015	
Xenylla humicola	Collembola	adult	terrestrial	Zero Power Threshold	0.424	Increase	Allen et al. 2016	Other good model fit: Exponential Threshold and Zero Exponential Threshold

Best Failure Rate Model - model with the lowest AICc (na = converse trend from the one expected by the model OR have only two rates of temperature change, variance not calculated)

Variance - Variance accounting for the statistical effects of the failure rate model (na = only two rates of temperature change, variance not calculated)

Response - Change in cold tolerance as ramping rate increases

Table S5

Outcome of Phylogenetic Generalised Least Squares (PGLS) analyses showing change in the sensitivity to temperature change (z and z') with the type of response observed to the increased time of exposure to high (z) and low (z') stressful temperatures, habitat and climate.

Response (z)	Estimate	s.e.	t	Р					
Decrease	3.369	0.725	4.654	<0.0001					
Increase	-3.038	0.971	-3.128	0.003					
Mixed	-3.013	1.403	-2.148	0.038					
No Effect	-2.435	0.900	-2.706	0.010					
$F_{(3,36)} = 5.905, p = 0.002194, R^2 = 0.33, ML\lambda = 0.26$									
Response (z')	Estimate	s.e.	t	Р					
Decrease	2.198	0.407	5.398	<0.0001					
Increase	-0.688	0.519	-1.326	0.204					
Mixed	-2.098	0.997	-2.104	0.052					
No Effect	-1.868	0.551	-3.389	0.004					
$F_{(3,16)} = 4.60, p = 0.01667, R^2 = 0.46, {}_{ML}\lambda = 0.00$									
Habitat (z)	Estimate	s.e.	t	Р					
Aquatic	1.913	0.577	3.315	0.002					
Terrestrial	1.671	0.849	1.968	0.056					
$F_{(1,38)} = 3.873, p = 0.05639, R^2 = 0.09, ML\lambda = 0.05$									
Habitat (z')	Estimate	s.e.	t	Р					
Aquatic	2.620	0.580	4.520	0.0003					
Terrestrial	-1.603	0.629	-2.550	0.020					
$F_{(1,18)} = 6.5, p = 0.02012, R^2 = 0.22, ML\lambda = 0.00$									
Climate (<i>z</i>)	Estimate	s.e.	t	Р					
Polar	2.896	1.383	2.094	0.043					

Sub-tropical	-0.357	1.889	-0.189	0.851					
Temperate	-0.645	1.124	-0.574	0.569					
Tropical	-0.367	1.253	-0.293	0.771					
$F_{(3,36)} = 0.118, p = 0.949, R^2 = 0.0098, ML\lambda = 0.40$									
Climate (z')	Climate (z') Estimate s.e. t P								
Polar 1.795 0.839 2.140 0.048									
Sub-tropical -0.680 1.186 -0.573 0.574									
Temperate	-0.470	0.897	-0.524	0.607					
Tropical	-1.405	1.186	-1.185	0.253					
$F_{(3,16)} = 0.518$, p = 0.6757, R ² = 0.0886, _{ML} $\lambda = 0.00$									

Table S6

Result of GLM testing the effects of rate of time to failure, body length and interaction on CT_{max} of juvenile and adult fish: $CT_{max} \sim \log_{10}(\text{FailTime}) * \log_{10}(\text{Body Length})$

CT_{max}	Estimate	s.e.	t	Р
CT _{max} (Intercept)	65.019	10.219	6.362	< 0.0001
log ₁₀ (Time to failure)	-6.488	3.413	-1.901	0.063
log ₁₀ (Body Length)	-14.604	5.360	-2.724	0.009
log ₁₀ (Time to failure) * log ₁₀ (Body Length)	2.451	1.763	1.390	0.170

Table S7

Result of GLM testing the effects of rate of time to failure, body length and interaction on CT_{max} of crustaceans, insects, and springtails combined: $CT_{max} \sim \log_{10}$ (FailTime) * $\log_{10}(\text{Body Length})$

CT _{max}	Estimate	s.e.	t	Р
CT_{max} (Intercept)	60.086	4.825	12.452	< 0.0001
log ₁₀ (Time to failure)	-8.355	2.219	-3.766	0.0003
log10(Body Length)	-10.331	4.669	-2.213	0.029
log ₁₀ (Time to failure) * log ₁₀ (Body Length)	2.916	2.083	1.400	0.165

GLM models of time and body size interaction were run separately for fish and all the other taxa to avoid sampling bias in our analysis, since fish was the only taxonomic group in our dataset with body length above 31 mm (Figure S3). CT_{max} variation among fish species was not a consequence of interaction between time and body length, even after excluding fish larvae from the dataset (Table S6). Likewise, CT_{max} variation among species from other taxonomic groups combined was not a consequence of interaction between time and body length (Table S



Figure S1

Relationship between body length (mm) and CT_{max} of 38 species of ectotherms



Figure S2

Left panel shows the overall time-temperature relationship. Right panel shows the overall rate-temperature relationship. The outcomes of the study are similar.

References

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Chapter 3

The link between physiology and behaviour in the face of climate change

"... I cannot conceive of the time when knowledge of soils will be complete. Our expectation is that our successors will build on what has been done, as we are building on the work of our

predecessors."

R.S. Smith, Director of the Illinois Soil Survey, 1928

3.1 Abstract

The interaction between physiology and behaviour is a necessary element that needs to be incorporated into forecast models for climate change impacts. Plasticity in the upper thermal limits of ectotherms is limited. Many species will rely on behavioural thermoregulation to avoid climate extremes such as heat waves, droughts, and the combination of the two. While the link between physiology and behaviour has been established for some frog, lizard, ant, and leaf arthropod species, it is poorly known for soil arthropods such as Collembola. Future climate projections suggest that soil organisms in the Australian temperate zone will be exposed to 2 °C warmer temperatures in 2050 as a consequence of climate change, along with more frequent heat waves and droughts. The Australian sub-Antarctic region (Macquarie Island) will also experience higher temperatures and more frequent dry periods. In this chapter, four species of springtails are used to explore the links between environmental stress, upper thermal tolerance, body size and behaviour. The temperate Collembola species exhibit immediate behavioural responses to extreme weather event simulations, especially heat wave

treatments, have limited evolutionary capacity to improve their upper thermal tolerance traits (cessation of voluntary movement and critical thermal maximum), and exhibit a reduction in body size (length and width) in response to the higher developmental temperatures. Furthermore, developmental temperature has the greatest impact on the thermal tolerance of species living closer to the soil surface, while in species inhabiting deeper soil layers the reduction of body size is the most prominent change at higher developmental temperatures. Cessation of voluntary movement and critical thermal maximum are found to be highly correlated across all four species tested. At the intraspecific level, this correlation is higher in species with higher thermal tolerance. Findings show that vertical movement in the soil depends on the fine-scale structure of the soil matrix, especially soil pore distribution. Longer body length becomes an important factor in avoiding heat stress, while smaller body width appears to be beneficial during drought.

3.2 Introduction

Forecasting the impacts of global climate change and extreme weather events on species distribution, biodiversity, and risk of extinction remains one of the central questions in current biology (Williams et al., 2008; Foden et al., 2013). Global trends in the rise of average annual temperatures are accompanied by an increase in the frequency, intensity, and duration of extreme weather events (Easterling et al., 2000; Rahmstorf & Coumou, 2011; Hansen et al., 2012; IPCC, 2014). Droughts are expected to increase not only in temperate, but also in tropical regions (IPCC, 2014; Fu, 2015). Australia is no exception and is expected to follow this global trend (Steffen et al., 2013). Indeed, February 2017

was a record-breaking month, with the highest land surface temperatures recorded in Australia (NASA Earth Observatory, 2017). Australia encompasses many climate zones including the sub-Antarctic region, Macquarie Island. While the overall precipitation in this area may indeed increase over time (Adams, 2009), a more recent study predicts less frequent, but more intense rainfall, suggesting prolonged dry periods (Wang et al., 2015) accompanied by an increase in maximum temperatures and little or no change in minimum temperatures (Tweedie & Bergstrom, 2000).

Most terrestrial ectotherms are not tolerant to extreme heat and rely on thermoregulation to survive (Kearney et al., 2009; Huey et al., 2012; Sunday et al., 2014; Duffy et al., 2015). Recent estimates based on general data from global insect species and soil temperature modelling for Australian systems suggest that soil ecosystems and particularly small ectotherm inhabitants like insects, may be under heat threat (Duffy et al., 2015). Future projected temperatures are likely to exceed upper thermal tolerance in many of these species by 2100 (Duffy et al., 2015). Therefore, interaction between shortterm behavioural responses and physiological tolerance traits over the long term is going to play a key role in determining the survival of individuals and species under extreme environmental conditions (Abram et al., 2016). While the interaction between behaviour and physiological traits for terrestrial ectotherms is known for some frog and lizard species (Scheffers et al., 2013), ants (Kaspari et al., 2014; Baudier et al., 2015), and arthropods inhabiting the phyllosphere (Pincebourde & Casas, 2019), the nature of this relationship is poorly known for soil arthropods such as Collembola, which are the key components of soil ecosystems and the soil food web (Petersen & Luxton, 1982; Hopkin, 1997; Rusek, 1998).

Collembola, more commonly known as springtails, are amongst the most widespread and abundant organisms in the soil fauna, spanning all continents and climate zones, including Antarctica and the sub-Antarctic islands (Hopkin, 1997). These soil microarthropods show a distinct vertical stratification in soil, usually with different life forms (Gisin, 1943; Hopkin, 1997; Berg et al., 1998). Collembola are highly sensitive to desiccation and environmental changes, especially changes in temperature (Petersen & Luxton, 1982; Hopkin, 1997; Rusek, 1998). These traits make them excellent bioindicators and an ideal model organism for gaining general understanding how soildwelling organisms, critical for stable soil structure and its productivity, will cope with rapidly changing climates. For Collembola as a taxonomic group, three trait diversity indices (richness, evenness and divergence) are highly correlated with vertical stratification in soil, where soil-dwelling species show the highest trait richness (Ellers et al., 2018). Variability in the habitat has been shown to shape the responses to temperature extremes and, thus, surface-dwelling species of springtails have a greater ability to maintain increased heat tolerance across acclimation temperature and show an increased extreme heat tolerance compared to soil-dwelling species exposed to a relatively stable thermal environment (van Dooremalen et al., 2012). A recent study, spanning the tropics to the sub-Antarctic, found that alien species of springtails have the broadest thermal tolerance ranges and the highest critical thermal maxima (the temperature at which activity ends), which will benefit them in the warming world compared to the indigenous species (Janion-Scheepers et al., 2018). However, both alien and indigenous groups show little potential for evolutionary change in tolerance to warm temperature (Janion-Scheepers et al., 2018).

Predictions of how soil organisms will respond to environmental temperatures need to consider the underground microclimate and soil as a thermal buffer that can represent a refuge from the temperature extremes and unsuitable conditions encountered at the soil surface (Scheffers et al., 2013; Sunday et al., 2014; Duffy et al., 2015). Several studies have found that the relationship between physiology and behaviour can be variable and primarily depends on the type of the environmental stressor, so it is crucial to establish how this relationship changes with different aspects of climate change and how individuals and populations may respond (Wikelski & Cooke, 2006; Killen et al., 2013). Stressful abiotic conditions can either amplify or mask behavioural and physiological differences among individuals (Killen et al., 2013). In turn, these effects may increase or decrease phenotypic variation resulting in physiological and behavioural traits that may be under pressure of natural selection depending on the specific environmental conditions. A review by Abram et al. (2016) on the behavioural effects of temperature on ectothermic animals points out the rarity of hypothesis-based research concerning temperature-induced behavioural adjustment. This is surprising considering that thermoregulatory behaviour may successfully mitigate the effects of harmful temperatures on ectotherms in heterogeneous microclimates (Kearney et al., 2009; Sunday et al., 2014; Duffy et al., 2015). Thermoregulatory behaviour can reduce natural selection (Bogert, 1949; Huey et al., 2003; Abram et al., 2016), ultimately limiting physiological adaptation (Buckley et al., 2015), and, more practically, altering climate change impact projections (Bonebrake et al., 2014). Behavioural adjustment is usually the first response to altered conditions (Wong & Candolin, 2014). Yet, so far only a few studies investigated the behavioural response of Collembola to temperature. Turning

subarctic peat cores upside down in the field revealed that the vertical stratification of Collembola depends both on substrate quality and abiotic conditions with relative importance of these factors being species specific (Krab et al., 2010). A single Collembola species *Folsomia candida* was exposed to above-freezing cooling temperatures since it was thought that avoidance behaviour is key survival strategy for springtails (Hopkin, 1997; Boiteau & MacKinley, 2013). Contrary to expectations, the vertical soil distribution of *F. candida* at 5, 10, and 15 °C was similar to that observed at constant 20 °C, suggesting that *F. candida* remained in the upper soil layers to acclimate to cold temperatures (Boiteau & MacKinley, 2013). In both of these studies, the individual movement of springtails was not distinguished from abundance and differences in birth and death rates at different soil depths. The extent of behavioural responses of Collembola under abiotic stress, along with the physiological traits that might be connected to presence or lack of this behaviour, remains unknown.

Properties of soil, especially the space between the soil particles, affect the movement of the soil organisms. Ant species living in habitats with a high soil compaction are found to have a smaller mesosoma (middle part of the body) length than ant species living in habitats with a low soil compaction (Schmidt et al., 2016). By contrast, in two species of tephritid flies, *Rhagoletis juglandis* and *Rhagoletis suavis*, large individuals were found deeper in the sand than the small individuals (Davis et al., 2015). Body size has also been found to influence vertical distribution of Collembola in the soil matrix where smaller individuals generally showed preference for deeper soil layers at the interspecific and intraspecific levels (Detsis, 2000). This may be due to smaller individuals being less desiccation tolerant, or because the dimension of soil pores

affected the springtails' vertical distribution within the soil matrix (Heisler & Kaiser, 1995; Salmon et al., 2005). Alternatively, it has been proposed that the eggs were perhaps laid in deeper soil layers so the proportion of juveniles there was larger (Detsis, 2000). Body size often correlates positively with higher upper thermal tolerance in arthropods (Kingsolver & Huey, 2008; Terblanche et al., 2011; Bokhorst et al., 2012), although the opposite has also been found (Brans et al., 2017), likely depending on the specific physiological circumstances.

Higher developmental temperatures are generally expected to reduce body size of individuals (Atkinson, 1994), which is also an ecological response to climate change (Sheridan & Bickford, 2011). In Collembola, smaller individuals were generally found to prefer deeper soil layers (Detsis, 2000). It has not been established how extreme weather events affect this distribution of body size of Collembola across soil layers and whether projected warmer developmental temperatures may alter this pattern. Moreover, it is unknown whether the observed preference of smaller individuals for deeper soil layers (Detsis, 2000) is related to their physiological tolerances, and if the difference in thermal tolerance could be the driving factor of the vertical distribution of Collembola in the soil matrix.

Given the evidence of little capability for evolution of increases in thermal tolerances, and indications that tolerances will be exceeded into the future in soil organisms, exploring the potential for behavioural responses to thermal conditions is critical (Pinsky et al., 2019), yet under-investigated in soil organisms. Here, I address these questions by simultaneously investigating behaviour and physiological traits in four temperate and sub-Antarctic species of Collembola reared under temperatures simulating

present-day conditions of their respective microclimates. To assess the effect of higher developmental temperatures on both behaviour and physiology I rear two temperate species under future projections of their microclimate and compare a set of physiological traits and the behavioural response for both populations. Future projections simulate the 2 °C average global warming predicted to occur in 2050 under the RCP8.5 climate scenario (Moss et al., 2010; Peters et al., 2012). Although RCP8.5 is considered the "worst-case" future climate scenario, current emissions seem to track this pathway (Peters et al., 2012; Brown & Caldeira, 2017.).

Multiple environmental stressors interact to affect behavioural and physiological responses to climate change (Darling & Côté, 2008; Gunderson et al., 2016). To simultaneously investigate behaviour and physiological traits under extreme weather conditions, I expose different species and rearing populations of Collembola to lab simulations of drought, heat wave, and the combined effect of the two stressors. Here, I am looking at a general response to increasing temperatures and drying conditions, and whether Collembola avoid heat or drought stress before reaching any limits. Specific responses to thermal limits were not examined, but rather behavioural responses to shortterm high-temperature and/or drought conditions. Then, I extract live individuals and investigate whether the choice of soil layer across all species is associated with the thermal tolerance traits of individuals such as cessation of voluntary movement (CVM) and critical thermal maximum (CT_{max}) . Furthermore, I investigate the relationship between CVM, a measure of voluntary thermal maximum that might be more reliable since it reflects an animal's own perception of thermal stress (Camacho et al., 2018), and CT_{max} . To test whether smaller individuals tend to move to deeper soil layers under heat

stress, as suggested by prior studies, and to investigate how this trend might change with different types of abiotic stress caused by extreme weather, I measure body size (length and width) of individuals and investigate body size distribution across the soil layers for all species. Identifying key physiological traits and climate change drivers for the presence or absence of behavioural thermoregulation and exploring how these traits adapt under different rearing conditions is essential to improve mechanistic models that predict species responses to climate change (Kellerman & van Heerwaarden, 2019). These findings establish behavioural and physiological patterns in Collembola and reveal the relationship between behaviour and physiology in the face of climate change.

3.3 Materials and methods

3.3.1 Collection sites, species identification, and status

Temperate species, *Orthonychiurus* sp. and *Sinella* sp. were collected during 2016 and 2017 from the Jock Marshall Reserve (37°54'S, 145°8'E) located in Clayton, Victoria, Australia (Figure S1, Supplementary Information). Jock Marshall Reserve is an ecological sanctuary located within an urban area and an environmental research facility with indigenous vegetation consisting of open woodlands and diverse ground layer of grasses and herbs. The sub-Antarctic species, *Ceratophysella denticulata* and *Mucrosomia caeca*, were collected from Macquarie Island (54°30'S, 158°57'E) in 2017 (Figure S1, Supplementary Information). Macquarie Island is a UNESCO World Heritage Site located in the Southern Ocean. This sub-Antarctic island is 34 km long and 5 km wide, around half-way between Australia and Antarctica. The climate is cool, oceanic, cloudy, wet, and windy with vegetation that includes grasslands (mainly tussocks), herb

fields, and the cushion plant Azorella macquariensis (Selkirk et al., 1990; Selkirk-Bell & Selkirk, 2013). Collembola represents around 30 % of the terrestrial arthropod species on Macquarie Island (Greenslade, 2002). All species were live-extracted from the soil and leaf litter using Berlese-Tullgren funnel method (Southwood & Henderson, 2000). An experienced taxonomist identified the two sub-Antarctic species, while DNA barcoding (Ratnasingham & Hebert, 2007) was used to identify two temperate species. Sequences of 10 specimens of two temperate species were compared with more than 75,000 springtail sequences available through the Barcode of Life Data Systems (BOLD) (www.barcodinglife.org; Table S1, Supplementary Information). Sequences are available on BOLD (www.boldsystems.org) as part of Project COLMU (Collembola of Monash University). Sinella sp. and M. caeca were identified as indigenous to Australia and to the sub-Antarctic islands, respectively. Species were considered indigenous if they could not be identified using available keys, if they were not previously represented in BOLD, or if they were represented and already collected across Australia, New Zealand, or south of the Wallace line (following Janion-Scheepers et al., 2018). Undescribed species that had sequences present in BOLD from other regions were considered alien species. Orthonychiurus sp. and C. denticulata were classified as alien species. Orthonychiurus sp. and *M. caeca* are euedaphic (soil-dwelling), while *Sinella* sp. and *C. denticulata* are hemiedaphic (litter-dwelling) life forms (Janion et al., 2010).

3.3.2 Rearing temperatures

According to the Australian Bureau of Meteorology weather station data (www.bom.gov.au), the highest mean maximum temperature over the last 30 years (1981-2010) was during February, in both Jock Marshall Reserve (JMR) and on Macquarie Island. For determining rearing temperatures, the present fluctuating temperatures from the JMR site were based on the NicheMapR hourly microclimate model (Kearney et al., 2014), while future predictive climate models for 2050 were downloaded from the NASA Earth Exchange Global Daily Downscaled Projections (NEX-GDDP; Thrasher et al., 2012) dataset under the RCP8.5 future climate scenario (Peters et al., 2012). The NicheMapR microclimate model dataset was used to extract the average hourly soil temperature data for the month of February at 3 cm depth under 50% and 75% shade levels, which were averaged to obtain the final soil temperatures (Kearney et al., 2014). These parameters of soil depth and shading most closely represent JMR sampling sites. The highest mean maximum air temperature in JMR during February is 25.9 °C, while the highest mean minimum air temperature is 14.2 °C (www.bom.gov.au). The average maximum temperature at 3 cm soil depth is 27 °C, while the average minimum temperature is 16.3 °C (Kearney et al., 2014). Using a similar air-soil temperature relationship under the RCP8.5 future climate scenario, the temperature increase will be 2 °C in 2050 at 3 cm soil depth. Based on these estimates, two rearing temperatures over a 24-hr period simulating the natural daily fluctuations were determined: 1) present rearing temperatures for Collembola from JMR were set to fluctuate between 16.3 °C and 27 °C, and 2) future rearing temperatures were set to fluctuate between 18.3 °C and 29 °C (Table S2, Supplementary Information). For the sub-Antarctic species from Macquarie Island, I used only existing populations from 10 °C constant rearing temperature regimen due to limited Collembola numbers. Summary of surface air temperature statistics for the base meteorological observatory on Macquarie Island (1948-1998) showed that the mean maximum surface air temperature in February is about 10 °C, while soil temperature on Macquarie Island in February between 5 and 10 cm depth is roughly around 8-9 °C (Tweedie & Bergstrom, 2000). Prior lab rearing of sub-Antarctic Collembola has shown that they favour stable and low (but not too low) constant temperature in order to reach sufficiently large population numbers required for this experiment (Janion-Scheepers et al., 2018).

3.3.3 Colony maintenance

Species were reared at their respective temperature regimens described above in controlled-temperature incubators (MIR-154-PE, Panasonic, Japan) on a 12-h light: 12-h dark cycle. Incubator temperatures were monitored with Hygrochron iButtons (DS1923; Maxim Integrated, CA, USA), indicating the realized temperatures did not fluctuate with more than \pm 0.5 °C. Individuals (200-400) from the F0 generation of each species were randomly assigned to small (50 individuals) or large (up to 200 individuals) plastic lid vials lined with moistened Plaster-of-Paris:charcoal powder (9:1) substrates (Janion-Scheepers et al., 2018). *Platanus* sp. tree bark with algae was replaced and de-ionized water was added twice a week to provide adequate nutrition and maintain the high humidity in the vials. To minimize any parental and environmental effects, only egglaying adults of the F2 generation were used in the experiments (Janion-Scheepers et al., 2018).

3.3.4 Artificial soil preparation

Artificial soil was prepared according to the modified OECD Guidelines (OECD, 1984; OECD, 2016). The artificial soil mixture contained 10% finely ground peat moss, 20% colloidal kaolin clay, 69% river sand, and 1% of Calcium carbonate (BioReagent, suitable for insect cell culture) for a total of 150 grams of soil (dry weight) per column. The artificial soil mixture was sterilized at 100 °C for 2 hours to prevent foraging behaviour and movement between soil layers due to presence of bacteria and microorganisms as a source of food (Chauvat et al., 2014). A soil sample from JMR was collected the day after rainfall in order to determine the moisture fraction of the natural soil from the collection site and its water-holding capacity. The protocol described in Greene et al. (1988) was followed for determining moisture content and water-holding capacity of the soil. Moisture content of the artificial soil was adjusted according to the natural soil moisture content and further optimized to attain loose porous soil structure allowing freedom of the movement of Collembola throughout different soil layers. 29% moisture fraction (MF) in the artificial soil was used throughout soil layers, with the exception of 10% moisture fraction (MF) in the first soil layer (L0) to simulate drought conditions.

3.3.5 Simulations of extreme weather events

The experimental design consists of soil columns that simulate control, drought, heat wave, and a combination heat wave and drought treatments. Each soil column has five 4 cm x 4 cm x 4 cm compartments: the top heated air chamber with flow-through air and four soil-filled chambers representing four different soil layers (Figure S2, Supplementary Information). The majority of Collembola species are negatively

phototactic moving away from the light source (Salmon & Ponge, 1998; Fox et al., 2007). To avoid the influence of light on the Collembola movement, soil columns were constructed with a non-transparent PVC material (Monash Instrumentation Facility, Clayton Campus, Australia). Simulations of extreme weather conditions were achieved by manipulating air temperature of the top air chamber and soil moisture content of the first soil layer (L0, 0-4 cm depth) creating a thermal gradient down the soil column. The control was set up by adding de-ionized water corresponding to 29% moisture fraction (MF) of dry soil mass across all four soil layers while maintaining the air chamber temperature at 25 °C for the temperate and 10 °C for the sub-Antarctic species. To simulate drought, the moisture fraction of the first soil layer was changed to 10% MF, while the other three layers retained the MF of 29% as in the control treatment. The heat wave treatment started with 29% MF in all soil layers, as in the control, and manipulated only the air chamber temperature, ramping it up to 40 °C for the temperate and 25 °C for the sub-Antarctic species. Heat wave temperature was maintained for three and a half hours. Finally, a combination of heat wave and drought involved ramping the temperature up to 40 °C (temperate) and 25 °C (sub-Antarctic) starting with 10% MF in the first soil layer. The experimental set-up consisted of having an air pump connected to a flow meter (Flowbar-8, Sable Systems, Las Vegas, NV), scrubber column (silica to remove H₂O from the air), and a channel split into four streams to produce a rate of 30 ml min⁻¹ per soil column. Each soil column was connected to the thermal controller (TC-720, TE Technology, Inc., Traverse City, MI) run by the using the supplied software and monitored with the iButtons. The total duration of each soil column treatment experiment

was 378 minutes. A short experimental duration was chosen to prevent loss of individuals due to starvation or other temperature-unrelated causes.

Orthonychiurus sp., *Sinella* sp., *M. caeca*, and *C. denticulata* each have eight, five, ten, and five replicates per treatment, per rearing temperature, respectively, to evaluate behavioural response, with each number of replicates per species determined to be sufficient by power analysis (Table S3, Supplementary Information). Each soil column replicate used 40 adults of the F2 generation for a total of 6,160 individuals tested across all four species and a total of 154 soil column replicates. After each replicate run, the soil column was quickly separated by sliding steel plate inserts instead of the hollow ring inserts between different soil layers and springtails were extracted alive. Springtails found on the surface of the first soil layer (L0) were aspirated and remaining springtails were extracted from soil layers by sieving and flotation. Next, individuals were counted by soil layer and placed in pre-labelled plastic vials with moistened Plaster-of-Paris:charcoal mixture substrate and tree bark. All vials were placed in the incubators with the species' respective original rearing temperature. Any individuals that were not recovered during the extractions were assigned to as NR (not recovered).

3.3.6 Cessation of voluntary movement (CVM), critical thermal maximum (CT_{max}), and body size measurements

Collembola are in constant motion. However, this movement gradually slows down at high stressful temperature and completely ceases when the temperature approaches the critical thermal maximum temperature. An individual becomes static, but nevertheless retains the upright position. Therefore, cessation of voluntary movement (CVM) is the

temperature at which movement stops and an individual remains in one spot in the upright position (Mellanby, 1939). Critical thermal maximum (CT_{max}) is the temperature at which the loss of righting response of an individual is reached when tipped over with a brush (Chown et al., 2009). The rate of temperature change (heating rate) was set to 0.05 °C/min for all species. This rate of temperature increase is within the range of those rates of temperature change recorded for both temperate and sub-polar microhabitats (Allen et al., 2016). Experimental starting temperature for temperate and sub-Antarctic species was set to 20 °C and 10 °C, respectively. Custom-built Peltier plate set-ups for measuring CVM and CT_{max} included aluminium vials with clear lids, with thermocouples touching the bottom of the vials lined with the moistened Plaster-of-Paris:charcoal mixture substrate that contained Collembola individuals (Monash Instrumentation Facility, Clayton Campus, Australia; Figure S3, Supplementary Information). Moistened Plasterof-Paris:charcoal mixture substrate was used to prevent desiccation of individuals during trials. Sample sizes varied depending on the number of individuals extracted from each soil layer and up to 20 individuals were tested within each aluminium vial. A single observer (myself) undertook all of the experimental work between 9:00 and 18:00 hours. After CVM and CT_{max} limits were recorded, specimens were preserved in ethanol and stored in 0.5 ml Eppendorf tubes.

Scaled photos of individuals preserved in ethanol were taken with a Leica microscope (M205 C, Leica Microsystems, Wetzlar, Germany) and body size (body length, mm) of all individuals was measured using ImageJ v1.52k (Rasband, 1997-2018; https://imagej.nih.gov/ij/). Body width was extrapolated for each species based on the body length:width relationship of 20 individuals of each species spanning different body

sizes with a goodness-of-fit (r^2) between 0.56 and 0.91. The schematic of the rearing and the experimental design used for this study is presented in Figure S4, Supplementary Information.

3.3.7 Soil porosity measurement

Photographs of both wet (29% moisture fraction) and dry soil (10% moisture fraction) surface at the same scale were taken with a Leica microscope (M205 C, Leica Microsystems, Wetzlar, Germany). Both images were converted to 8-bit binary images (size: 17.53x12.47mm) in ImageJ v1.52k (Rasband, 1997-2018; https://imagej.nih.gov/ij/) and thresholds have been adjusted so that the soil pore space is represented in black (Figure 7). Using command "Analyze Particles" in ImageJ, surface area of each pore space (mm²) was calculated. The summed area of pore space divided by total area of the image gave the areal porosity over that plane (Nimmo, 2004).

3.3.8 Statistical analysis

The behavioural response of Collembola across soil layers was quantified in the form of individual counts per soil layer. To investigate the effect of extreme weather simulation treatments on behavioural response relative to the control by soil layer and species, I subsetted the data according to species, developmental temperature and soil layer, and used Generalised Linear Models (GLMs) corrected for overdispersion and Negative Binomial Models. P-values were corrected for false discovery rates (FDR) to reduce the likelihood of falsely rejecting the null hypothesis (Benjamini & Hochberg, 1995) with the *p.adjust* function in R v.3.5.1 (R Core Team, 2014).

A linear model describing the relationship between CVM and CT_{max} across all species did not meet assumptions of normality (Shapiro Wilk's test, W= 0.951, p<0.001) or homogeneity of variances (Fligner-Killeen test, p<0.001). Visual inspection of the data did not meet assumptions of normality either despite the large sample size (Figure S5, Supplementary Information). Therefore, non-parametric Kendall's tau correlation coefficient was used to examine the relationship between CVM and CT_{max} at both interspecific and intraspecific levels.

To investigate how thermal tolerance traits, CVM and CT_{max} , vary with soil depth within each treatment, I used non-parametric Kruskal-Wallis tests and Dunn's post-hoc tests with Bonferroni corrections performed with 'FSA' v0.8.22 package (Ogle et al., 2018) because ANOVAs did not meet the assumptions of normality (Figure S6 and Figure S7, Supplementary Information).

To explore how body length varies with soil depth and within each treatment, I used Welch's one-way test and pairwise t-tests with Bonferroni corrections to find which layers differ between each other since the model met assumptions of normality (Shapiro Wilk's test, W= 0.999, p<0.083; Figure S8, Supplementary Information), but did not meet the assumptions of equal variances (Levene's test, F=25.129, df=4, p<0.0001).

Non-parametric Kruskal-Wallis tests and Dunn's post-hoc tests with Bonferroni corrections were used to analyse how body width varies with soil depth and within each treatment since ANOVA did not meet assumptions of normality (Shapiro Wilk's test, W= 0.982, p<0.0001) or homogeneity of variances (Levene's test, F=13.485, p<0.0001) (Figure S9, Supplementary Information).

To establish an overall relationship between CT_{max} and body length as a proxy of body size, I compared mean CT_{max} and mean body length values for all soil layers. The linear model did not meet the assumptions of normality (Shapiro Wilk's test, W= 0.987, p=0.00037; Figure S10, Supplementary Information). Therefore, Kendall's tau correlation coefficient was used to examine the relationship between CT_{max} and body length at both interspecific and intraspecific levels.

The effect of developmental temperature on physiological traits (CVM, CT_{max} , body length, and width) was investigated for two temperate species, *Orthonychiurus* sp. and *Sinella* sp. using either classic equal variances t-tests or Welch's t-tests allowing for unequal variances.

3.4 Results

3.4.1 Thermoregulatory behaviour

Behavioural response was investigated at the species level. Temperate species reared under present-day and future projected temperature scenarios (hereafter present and future, respectively), *Orthonychiurus* sp. and *Sinella* sp., had no individuals present on the soil surface (L0) in heat wave and heat wave & drought treatments (Figures 1 and 2). For *Orthonychiurus* sp. (present), the heat wave treatment caused a 67% decrease in number of individuals found in (L1), while they decreased by 37% under the future scenario. For *Orthonychiurus* sp. (present and future), the heat wave treatment caused an increase between 61% and 75% in the number of individuals in deeper soil layers (L2 through L4). Furthermore, the drought treatment caused a 72% decrease in the number of individuals on the soil surface for *Orthonychiurus* sp. (future) (Figure 1). For *Sinella* sp.

(present and future), the drought treatment resulted in a decrease in the number of individuals on the soil surface (L0), of 43% and 46%, respectively. Drought, heat wave, and heat wave & drought treatments increased the number of *Sinella* sp. (future) individuals in (L1) by 41, 84, and 82%, respectively. This was not the case for *Sinella* sp. (present) individuals. On the other hand, (L2) was only affected by the heat wave treatment in the present-day population, showing a 400% increase in the number of individuals on average (Figure 2).

Sub-Antarctic species, *M. caeca* and *C. denticulata*, both reared under present-day temperatures only, had no individuals on the soil surface in any of the treatments including the control. *Mucrosomia caeca* had 51% more individuals in (L1) in the drought treatment than in the control. By contrast, (L2) in drought, heat wave, and heat wave & drought treatments showed a decrease in number of individuals by 50, 62, and 45%, respectively (Figure 3). *Ceratophysella denticulata*, tested only for the heat wave treatment, showed no behavioural response. Outcomes of Generalised Linear Models and Negative Binomial Models are presented in Table S4, Supplementary Information.

3.4.2 Thermal tolerance traits: CVM and CT_{max}

Kendall's tau correlation coefficient (R=0.79, p<0.001) indicated an overall strong positive correlation between CVM and CT_{max} for all species combined (Figure 4a). At the intraspecific level, correlation coefficients ranged from 0.12 in *M. caeca*, the species with the lowest CVM and CT_{max} estimates, to 0.54 in *Sinella* sp., the species with the highest CVM and CT_{max} estimates (Figure 4b).

CVM differed between soil layers for all species combined (Kruskal-Wallis: $X^2 =$ 1619.2, df=4, p<0.001) and results of Dunn's post hoc test for multiple comparisons between soil layers revealed that CVM decreased with the increasing soil depth up to soil layer (L3), where CVM of individuals found in (L3) and (L4) was not significantly different (Figure 5A, left panel, Table 1). CVM decreased between 1 °C and 2.4 °C with each subsequent soil layer, on average, with the overall difference of 5.5 °C in CVM between individuals found in (L0) and (L4) (Table 2). This general pattern of CVM decline was clear within each treatment (Figure 5A, right panel). In the control and drought treatments, the decline of CVM with increasing soil depth stopped after (L2), while in the heat wave and heat wave & drought treatments decline occurred up to (L3) (Table 3). At the treatment level, CVM decreased between 1.1 and 3.6 °C with each subsequent layer with the overall difference varying between 3.9 and 7.0 °C in CVM between individuals found in (L0) and (L4) depending on the treatment. This difference was the most apparent in the drought treatment where the overall CVM difference between (L0) and (L4) individuals, was 7.0 °C on average. At the species level, CVM differed between soil layers within all species (Kruskal-Wallis: $X^2 > 8.34$, df = 3 and 4, p<0.040). However, the general trend of CVM decline with the increasing soil depth was either lost or less consistent at the intraspecific level (Table S5, Supplementary Information).

Similarly, this was the case for CT_{max} (Kruskal-Wallis: X² = 1731, df=4, p<0.001). Results of Dunn's post-hoc tests for multiple comparisons between soil layers revealed that CT_{max} followed a similar trend as CVM and decreased with the increasing soil depth up to soil layer (L3), where CT_{max} of individuals found in (L3) and (L4) was not

significantly different (Figure 5B, left panel, Table 1). CT_{max} decreased between 0.8 °C and 1.9 °C with each subsequent soil layer with the overall difference of 4.2 °C in CT_{max} between individuals found in (L0) and (L4) (Table 2). This general pattern has been maintained within each treatment (Figure 5B, right panel). In the control, the decline of CT_{max} with increasing soil depth ceased after (L2), while in the heat wave and heat wave & drought treatments the decline occurred up to (L3) (Table 3). CT_{max} appeared to be more sensitive to drought treatment than CVM, where the decline of CT_{max} stopped after (L2), but continued in soil layer (L4), which had lower CT_{max} compared to (L2). At the treatment level, CT_{max} decreased between 0.9 and 2.7 °C with each subsequent layer where the overall difference varied between 3.1 and 5.2 °C in CT_{max} between individuals found in (L0) and (L4). Again, this difference was the most apparent in the drought treatment where the overall CT_{max} difference between individuals found in (L0) and (L4) was 5.2 °C, on average. At the intraspecific level, CT_{max} differed between soil layers in Orthonychiurus sp. (present) (Kruskal-Wallis: $X^2 = 18.21$, df = 4, p=0.001), Orthonychiurus sp. (future) ($X^2 = 30.47$, df = 4, p<0.001), Sinella sp. (present) ($X^2 =$ 33.48, df = 4, p<0.001) and *M. caeca* ($X^2 = 9.53$, df = 3, p=0.023), but not in *Sinella* sp. (future) ($X^2 = 4.77$, df = 3, p=0.189) and C. denticulata ($X^2 = 5.39$, df = 3, p=0.145) (Table S5, Supplementary Information).

3.4.3 Body size

Body length generally increased with soil depth (Welch one-way test: F=109.06, df=4, p<0.0001), by 8 % between (L0) and (L4) for all species, which was contrary to the expectation that smaller individuals prefer deeper soil layers (Figure 5C, left panel). At

the treatment level, the common general trend was only retained in the heat wave treatment, while there was a significant overlap of body lengths between soil layers in all the other treatments (Figure 5C, right panel; Table 2). Nevertheless, body length in the deepest soil layer was greater than in the shallowest layer where animals were present. Body length of individuals in (L0) and (L4) increased by 9% and 3%, in control and drought treatment, respectively. In heat wave and heat wave & drought treatments, body length (L1) to (L4) increased with soil layer by 19% and 17%, respectively.

Although there was an overlap between soil layers, body width generally decreased with soil depth, by 10 % between (L0) and (L4) for all species (Kruskal-Wallis: $X^2 = 132.66$, df=4, p-value <0.001; Figure 6, left panel). At the treatment level, body widths of individuals in (L0) and (L4) were reduced by 10% and 18%, in control and drought treatment, respectively. The reduction of body widths of individuals in (L1) and (L4) was 5% and 6% in heat wave and heat wave & drought treatments, respectively (Figure 6, right panel; Table 2).

3.4.4 Soil porosity

The total area of each soil image plane was 218.60 mm². The summed area of pore space in the wet soil was 134.37 mm² giving the 61% areal porosity. Dry soil had the summed area of pore space of 64.50 mm², which divided by the total area gave the 30% areal porosity (Figure 7).

3.4.5 Body length and CT_{max} correlation

 CT_{max} was not strongly correlated with body length and Kendall's tau correlation indicated an overall weak negative correlation for all species combined (R = -0.21, p<0.001). At the species level, only *M. caeca* showed a significant weak negative correlation (R = -0.31, p<0.001), while the correlation between CT_{max} and body length was not significant for the other three species.

3.4.6 The effect of developmental temperature on physiological traits

Equal variance t-tests, conducted separately for each species, confirmed the higher CVM in the future reared populations of *Orthonychiurus* sp. (t=-3.3283, <0.001) and *Sinella* sp. (t=-3.3443, p<0.001). However, the effect sizes for both species were small, 0.07 and 0.09 °C, respectively. Welch's unequal variance t-tests confirmed the effect of developmental temperature on CT_{max} for both species (*Orthonychiurus* sp., t=4.0915, p<0.001; *Sinella* sp., t=-5.9523, p<0.001) with small effect sizes of 0.09 and 0.17 °C, respectively. Interestingly, *Orthonychiurus* sp. (future) had slightly lower CT_{max} than *Orthonychiurus* sp. (present), on average.

Equal variance t-test and Welch's t-test, conducted for *Orthonychiurus* sp. and *Sinella* sp., respectively, confirmed a reduction of body length in the future reared populations for both species (*Orthonychiurus* sp., t=12.507, p<0.001; *Sinella* sp., t=3.9667, p<0.001). While the effect size for *Sinella* sp. was only 3.5% reduction in the overall body length, the medium effect size of future rearing treatment caused a 6.7% reduction in body length of *Orthonychiurus* sp. Equal variance t-test and Welch's t-test, conducted for *Orthonychiurus* sp. and *Sinella* sp., respectively, confirmed the reduction

of body width in the future reared populations for both species (*Orthonychiurus* sp., t=12.5, p<0.001; *Sinella* sp., t=3.9508, p<0.001). The effect size for *Sinella* sp. was small showing a 3.4% reduction in body width, while a medium effect size of future rearing treatment caused 7.1% reduction in body width of *Orthonychiurus* sp.

3.5 Discussion

Behavioural responses of springtails to extreme weather treatments varied among species. On average, the largest behavioural response was to the heat wave treatment (40 °C) in temperate species (Orthonychiurus sp. and Sinella sp.), which not only caused complete absence of individuals on the soil surface, but also caused Orthonychiurus sp. (present and future) to move to deeper soil layers beyond the uppermost soil layer (L1). Similarly, in the heat wave & drought treatment there were no temperate species present on the soil surface, but this treatment did not generally affect other soil layers except in Sinella sp. (future) where it increased number of individuals in soil layer (L1). Drought treatment had the most impact on Orthonychiurus sp. (future) and Sinella sp. (present and future) by reducing their number on the soil surface. In the indigenous sub-Antarctic species M. *caeca* drought treatment appeared to slow down movement of individuals from the soil layer (L1) to deeper soil layers. On the other hand, all three treatments appeared to reduce the number of *M. caeca* in the soil layer (L2). *Ceratophysella denticulata* did not show a behavioural response to heat wave treatment, likely because this is alien species with a broad habitat where polar heat wave of 25 °C is not considered stressful. Heat wave simulations for temperate species (40 °C) exceeded their upper thermal tolerances indicating that when terrestrial ectotherms are exposed to extreme heat they must rely on thermoregulation to survive (Kearney et al., 2009; Huey et al., 2012; Scheffers et al., 2013; Sunday et al., 2014; Duffy et al., 2015). *Mucrosomia caeca* preferred the deepest soil layer regardless of the treatment and had no individuals on the soil surface, while majority of *C. denticulata* remained in the first soil layer, even during polar heat wave treatment. Among the four species tested, the vertical stratification in soil during extreme weather treatments matched their life form (euedaphic and hemiedaphic).

Findings in my study demonstrated that springtails responded behaviourally over the short-term to warming events and drought simulations focusing on the individual movement of springtails. For example, a removal of the insulating snow layer in the Australian alpine region appears to create a less suitable environment for some Collembola species because of an increase of daily temperature fluctuations (Vasseur et al., 2014), while other species appear to benefit from it and increase in number (Slatyer et al., 2017). This could potentially explain why the alien sub-Antarctic species C. *denticulata* did not show a behavioural response to the heat wave treatment and largely remained in the upper soil layer (L1). These behavioural responses also implied that the short-term effects of high temperatures, and possibly drought, could be mediated by behavioural change. Thermoregulatory behaviour observed here indicated that the impacts of climate change might not necessarily be as large as predicted, at least for Collembola, in keeping with suggestions for terrestrial ectotherms generally (Pinsky et al., 2019). However, understanding a full set of springtail behavioural responses across their different life forms is necessary to make complete predictions of responses for Collembola in general. While euclaphic life forms may be able to move into deeper soil layers more easily due to their morphology, as was the case for Orthonychiurus sp. and *M.caeca*, their hemiedaphic (*Sinella* sp. and *C. denticulata*) and epiedaphic counterparts may largely depend on the fine-scale structure of the soil matrix and soil pore distribution within. Vertical movement of microarthropods in the soil depends on pre-existing burrows and tunnels made by ecosystem engineers such as earthworms (Cameron et al., 2013). Moreover, a study found that none of the 53 species of springtails in the alluvial forest soils belonging to different life forms appear deeper than 20 cm (Kolesnikova et al., 2013). If soil temperatures at this depth exceed their physiological limits, even with behavioural thermoregulation, the outcomes will be negative.

I found that across all four species investigated, both upper cessation of voluntary movement (CVM) and critical thermal maximum (CT_{max}) decreased with the increasing soil depth. Large effect size spanning several degrees (CVM: 5.5 °C and CT_{max}: 4.2 °C) between the thermal tolerance of individuals found on the soil surface and the individuals found in the deepest soil layer tested (L4, 12-16cm) and with 1-3 °C difference between individuals from each subsequent soil layer, on average, indicated that the upper thermal tolerance traits largely influenced vertical distribution of Collembola in the soil matrix. Covariation between thermal physiology and behaviour has been found in skinks Lampropholis delicata suggesting that thermal physiology should be included in the pace-of-life-syndrome (POLS) (Réale et al., 2010; Goulet et al., 2017). This relationship between thermal tolerance traits and thermoregulatory behaviour has not been previously found in Collembola and the findings emphasize the need for further understanding of the interactions between physiological and behavioural responses, especially in the warming climate, and how these vary among species. Moreover, drought appeared to be the treatment that affected vertical stratification of Collembola in the soil matrix the most due

to difference in their CVM and CT_{max} . The overall difference between individuals found in (L0) and (L4) was 7.0 and 5.2 °C for CVM and CT_{max} , respectively. Although the degree difference was smaller for CT_{max} , CT_{max} appeared to be more sensitive to drought because its pattern of decline continued up to the deepest soil layer. Species that moved deeper into the soil were more thermally sensitive. This result was similar to previous studies on ants from aboveground and belowground habitats where canopy ants had higher CT_{max} than understory and belowground species (Kaspari et al., 2014; Baudier et al., 2015). Similarly, there was a link between thermal tolerance limits and leaf temperatures in arthropods inhabiting the phyllosphere (Pincebourde & Casas, 2019), demonstrating that microclimate accounts for much of the variability of the critical thermal limits.

There was a strong positive interspecific correlation between CVM, a measure of voluntary thermal maximum, and CT_{max} (R = 0.79, p<0.001). At the intraspecific level, the positive correlation varied between 0.12 and 0.54 (p<0.001) and increased with the increasing thermal tolerance. This finding was in contrast with the previous study on lizards, which found no intraspecific correlation between CT_{max} and voluntary thermal maximum (Camacho et al., 2018). While Camacho et al. (2018) suggested that voluntary thermal maximum may evolve independently in lizards, findings here suggested that the relationship between CVM and CT_{max} in Collembola gets stronger with increasing thermal tolerance.

A study based on Collembola biomass across soil layers found that smaller individuals generally show a preference for deeper soil layers both at the interspecific and intraspecific levels under adverse summer conditions (Detsis, 2000). However, body length and body width were not taken into consideration. A study using body length as a proxy for body size found no significant relationship between average body size and CT_{max} value of the taxa in soil arthropod community (Franken et al., 2018). However, at the intraspecific level, the effect of body size on CT_{max} differed between taxonomic groups, with some having a significant negative relationship between CT_{max} and body size such as Isotoma riparia (Collembola) (Franken et al., 2018). Other arthropod taxa (not Collembola) showed either positive or, in the majority of cases, no intraspecific relationship between CT_{max} and body size (Franken et al., 2018). In my study, there was a weak negative correlation between body length and CT_{max} at the interspecific level, while at the intraspecific level only *M. caeca*, an indigenous sub-Antarctic species, showed a weak negative correlation. Across all species, body length of individuals in the deepest soil layer was about 8% longer. However, in both heat wave and heat wave & drought treatments, this gap widened and individuals found in the deepest soil layer were 17-19% longer than the ones found in the first soil layer (L1). By contrast, body width was generally less by 10% in the deepest soil layer relative to the first soil layer. Across treatments, drought seemed to have the biggest effect where soil layer (L4) had individuals with 18% smaller body width relative to the top soil layer (L1). Findings here suggested that generally, more elongated individuals with thinner body moved deeper into the soil. Body length became an important factor when Collembola were exposed to heat stress, while body width appeared to be an important factor during drought treatment. This likely happened due to the smaller total surface area of pore openings in dry than wet soil and could be comparable in certain respects to the ant study where ant species living in habitats with a high soil compaction had a smaller body width than ant
species living in habitats with a low soil compaction (Schmidt et al., 2017). In my study, both soils have low compaction, but dry soil had much smaller particles and thus, less air space between them.

The effect of developmental temperature on thermal tolerance was greater in *Sinella* sp. than in *Orthonychiurus* sp. For *Orthonychiurus* sp. (future) CVM was 0.07 °C higher, while CT_{max} was 0.09 °C lower. By contrast, for *Sinella* sp. (future) CVM was 0.09 °C higher and CT_{max} was also higher, for 0.17 °C. However, both species, one alien and one indigenous, showed little potential for evolutionary change in tolerance to warm temperatures (Janion-Scheepers et al., 2018) and the slight differences in thermal tolerance that were measured were not particularly ecologically significant.

Body size (length and width) was reduced in both species in the future reared populations as generally expected (Atkinson, 1994) and as an ecological response to climate change (Sheridan & Bickford, 2011). *Sinella* sp. (future) had 3.5% reduction in body length and 3.4% reduction in body width. For *Orthonychiurus* sp. (future), 2 °C higher developmental temperature reduced body length by 6.7% and body width 7.1%. These results indicated that 2 °C increase in the developmental temperatures had the greatest effect on thermal tolerance of *Sinella* sp. by slightly improving their CT_{max} (0.17 °C), and on body size of *Orthonychiurus* sp. by reducing their body size (both length and width ~7%). This was likely happening due to different physiological demands. *Sinella* sp. is hemiedaphic, covered in scales, and has a preference for warmer temperatures since it is generally found during warm summer season in JMR, while *Orthonychiurus* sp. can be found all throughout the year in high abundance, but as a euedaphic species without

scales, likely relies on thermoregulatory behaviour to avoid stressful environmental conditions.

The persistence of species and populations and species distribution and range largely depend on their resistance and response to multiple stressors or their simultaneous effect (Gunderson et al., 2016). Considering that extreme weather events can cause immediate physiological stress and even mortality (Easterling, 2000; Somero, 2010; Dowd et al., 2015), examining species' options to mediate or buffer exposure to weather extremes through acclimation to the new warmer climate and behavioural thermoregulation is required for predicting species' responses and the biological consequences to climate change (Huey et al., 2003; Baudier et al., 2015; Buckley & Huey, 2016; Baudier et al., 2018; Kellermann & van Heerwaarden, 2019). The study here provided useful data to address this question in the Collembola, indicating where future work is required to refine the novel findings reported here.

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3.8 Figures

Figure 1

Present and future reared *Orthonychiurus* sp. (alien) exhibited thermoregulatory behaviour by moving away entirely from the soil surface (L0) in the heat wave and heat wave & drought treatments. Heat wave treatment caused *Orthonychiurus* sp. from both rearing scenarios to move to deeper soil layers. The number of future reared *Orthonychiurus* sp. only, decreased in L0 during drought. Error bars indicate 95% CIs.



Present and future reared *Sinella* sp. (indigenous) exhibited similar thermoregulatory behaviour by moving away entirely from the soil surface (L0) in the heat wave and heat wave & drought treatments. Drought decreased the number of individuals on the soil surface (L0) in both populations. While present reared *Sinella* sp. did not show any difference in L1 count between control and the treatments, future reared *Sinella* sp. had more individuals present in L1 in all three treatments relative to the control. Error bars indicate 95% CIs.



Mucrosomia caeca (indigenous) and *Ceratophysella denticulata* (alien) have no individuals present on the soil surface (L0) in control or any of the treatments. Drought increases number of *M. caeca* present in (L1), while (L2) is negatively affected by all three treatments with reduced number of individuals present in this soil layer. *C. denticulata* shows no behavioural response. Error bars indicate 95% CIs.



a) Kendall's tau rank correlation coefficient (R = 0.79, p<0.001) indicates an overall strong positive general correlation between cessation of voluntary movement (CVM) and critical thermal maximum (CT_{max}) for all species combined. b) At the species level, correlation is lower with tau varying from 0.12 to 0.54 (p<0.001).

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A. Left panel: CVM decreases with the increasing soil depth for all species, rearing temperatures, and treatments combined (Kruskal-Wallis: $X^2 = 1619.2$, df=4, p-value <0.001). Right panel: CVM decreases with the increasing soil depth according to each treatment a) control, b) drought, c) heat wave, d) heat wave & drought.

B. Left panel: CT_{max} decreases with the increasing soil depth for all species, rearing temperatures, and treatments combined (Kruskal-Wallis: $X^2 = 1731$, df=4, p-value <0.001). Right panel: CT_{max} decreases with the increasing soil depth according to each treatment a) control, b) drought, c) heat wave, d) heat wave & drought.

C. Left panel: Body length increases with the increasing soil depth for all species, rearing temperatures, and treatments combined (Welch one-way test, F=109.06, df=4, p<0.001). Right panel: Trends of body length increase with the increasing soil depth show that body length of individuals from different soil layers overlap in many cases. Outcomes of Dunn's post-hoc tests (A and B) and pairwise t-tests (C) are illustrated by letters above the boxplots for a) control, b) drought, c) heat wave, and d) heat wave & drought. Similar letters denote no significant differences. Data points on the left panels represent species: *C. denticulata* (red), *M. caeca* (green), *Orthonychiurus* sp. (blue), *Sinella* sp. (purple).



Left panel: Body width decreases with the increasing soil depth for all species, rearing temperatures, and treatments combined (Kruskal-Wallis: $X^2 = 132.66$, df=4, p-value <0.001). Right panel: Outcomes of Dunn's post-hoc tests are illustrated by letters above the boxplots for a) control, b) drought, c) heat wave, and d) heat wave & drought. Similar letters denote no significant differences. Data points on the left panels represent species: *C. denticulata* (red), *M. caeca* (green), *Orthonychiurus* sp. (blue), *Sinella* sp. (purple).



Total areal soil porosity = 61 %

Total areal soil porosity = 30 %

Wet soil (29% moisture fraction) has larger total areal soil porosity (61%) than dry soil (10% moisture fraction), which has total areal soil porosity of 30%.

3.9 Tables

Table 1

Outcomes of Dunn's post-hoc tests show that CVM and CT_{max} decrease with the increasing soil depth up to soil layer (L3) for all species combined. Significant Bonferroni corrected p-values are in bold. Soil Layers are L0: Soil surface; L1: 0-4 cm; L2: 4-8 cm; L3: 8-12 cm; L4: 12-16 cm; NR: Not recovered.

Soil Louona	CV	M (°C)	<i>CT_{max}</i> (°C)		
Son Layers	Z	P-value	Z	P-value	
L0 - L1	5.06	<0.001	5.49	<0.001	
L0 - L2	14.32	<0.001	15.36	<0.001	
L1 - L2	14.62	<0.001	15.67	<0.001	
L0 - L3	20.88	<0.001	21.19	<0.001	
L1 - L3	24.16	<0.001	23.93	<0.001	
L2 - L3	7.27	<0.001	6.70	<0.001	
L0 - L4	24.35	<0.001	25.53	<0.001	
L1 - L4	33.19	<0.001	34.61	<0.001	
L2 - L4	9.62	<0.001	9.77	<0.001	
L3 - L4	1.28	1.00	1.93	0.533	

Table 2

Summary statistics showing means and standard errors (s.e.) for the thermal tolerance traits: cessation of voluntary movement (CVM) and critical thermal maximum (CT_{max}) for all species combined. Soil Layers are L0: Soil surface; L1: 0-4 cm; L2: 4-8 cm; L3: 8-12 cm; L4: 12-16 cm; NR: Not recovered.

	All trea	atments	Con	trol	Dro	ught	Heat	wave	Heat way	e & Drought
Soil	CVM	I (°C)	CVM	[(°C)	CVM	l (°C)	CVM	l (°C)	CV	M (°C)
Layer	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
LO	37.8	0.09	37.8	0.10	37.7	0.17	na	na	na	na
L1	36.8	0.05	36.7	0.08	36.4	0.10	37.2	0.11	37.0	0.09
L2	34.4	0.14	33.1	0.27	32.8	0.34	35.9	0.17	34.1	0.33
L3	32.6	0.14	32.4	0.30	31.0	0.32	33.8	0.22	31.8	0.26
L4	32.3	0.10	32.2	0.23	30.7	0.17	33.3	0.16	32.1	0.21
Soil	CTma	x (°C)	CTma	x (°C)	CTma	x (°C)	CTmax (°C)		CTmax (°C)	
Layer	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
LO	38.6	0.08	38.5	0.09	38.7	0.14	na	na	na	na
L1	37.8	0.04	37.6	0.06	37.5	0.08	38.1	0.08	38.1	0.08
L2	35.9	0.10	35.0	0.18	34.9	0.23	37.0	0.13	35.4	0.24
L3	34.7	0.10	34.5	0.21	34.0	0.22	35.4	0.17	34.2	0.18
L4	34.4	0.07	34.3	0.14	33.5	0.11	35.0	0.11	34.4	0.14
Soil	Body len	gth (mm)	Body len	gth (mm)	Body leng	gth (mm)	Body len	gth (mm)	Body le	ength (mm)
Layer	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
LO	1.395	0.013	1.387	0.017	1.410	0.020	na	na	na	na
L1	1.343	0.005	1.389	0.011	1.390	0.010	1.246	0.011	1.330	0.009
L2	1.449	0.012	1.409	0.023	1.470	0.027	1.422	0.021	1.551	0.021
L3	1.483	0.009	1.509	0.020	1.454	0.028	1.462	0.014	1.505	0.018
L4	1.502	0.006	1.514	0.017	1.436	0.015	1.483	0.010	1.492	0.013

Soil	Body wi	dth (mm)	Body wid	dth (mm)	Body wi	dth (mm)	Body wi	dth (mm)	Body w	vidth (mm)
Layer	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
LO	0.301	0.003	0.299	0.004	0.306	0.005	na	na	na	na
L1	0.290	0.001	0.304	0.002	0.297	0.002	0.271	0.002	0.284	0.002
L2	0.293	0.003	0.275	0.007	0.277	0.008	0.306	0.005	0.305	0.007
L3	0.274	0.003	0.279	0.006	0.250	0.008	0.283	0.005	0.266	0.006
L4	0.270	0.020	0.268	0.004	0.252	0.004	0.284	0.003	0.267	0.004

Table 3

Outcomes of Dunn's post-hoc tests show decreasing trends of CVM and CT_{max} with the increasing soil depth by treatment for all species combined. Significant Bonferroni corrected p-values are in bold. Soil Layers are L0: Soil surface; L1: 0-4 cm; L2: 4-8 cm; L3: 8-12 cm; L4: 12-16 cm; NR: Not recovered.

Tucctment	Soil Lavora	CVN	1 (°C)	CT _{max} (°C)		
Ireatment	Son Layers	Z	P-value	Z	P-value	
	L0 - L1	5.81	<0.001	5.25	<0.001	
	L0 - L2	13.51	<0.001	12.76	<0.001	
	L1 - L2	10.84	<0.001	10.43	<0.001	
Г	L0 - L3	14.4	<0.001	13.56	<0.001	
RO	L1 - L3	11.9	<0.001	11.37	<0.001	
LNC	L2 - L3	1.29	1.00	1.17	1.00	
Ŭ	L0 - L4	16.62	<0.001	15.77	<0.001	
	L1 - L4	14.44	<0.001	13.96	<0.001	
	L2 - L4	1.03	1.00	1.03	1.00	
	L3 - L4	-0.43	1.00	-0.3	1.00	
	L0 - L1	3.6	0.003	4.76	<0.001	
	L0 - L2	9.39	<0.001	9.14	<0.001	
	L1 - L2	8.49	<0.001	7.24	<0.001	
H	L0 - L3	11.27	<0.001	10.62	<0.001	
НЭ	L1 - L3	10.69	<0.001	9.03	<0.001	
SOL	L2 - L3	2.08	0.379	1.7	0.893	
DF	L0 - L4	15.13	<0.001	15.8	<0.001	
	L1 - L4	17.61	<0.001	17.15	<0.001	
	L2 - L4	2.64	0.082	3.5	0.005	
	L3 - L4	0.04	1.00	1.3	1.00	
	L1 - L2	6.41	<0.001	5.54	<0.001	
ΚE	L1 - L3	14.01	<0.001	13.63	<0.001	
MA	L2 - L3	6.49	<0.001	6.9	<0.001	
AT ,	L1 - L4	18.92	<0.001	18.38	<0.001	
HE	L2 - L4	9.02	<0.001	9.42	<0.001	
	L3 - L4	1.65	0.596	1.59	0.677	
	L1 - L2	7.04	<0.001	7.15	<0.001	
E	L1 - L3	13.77	<0.001	14.04	<0.001	
VAV JGH	L2 - L3	3.97	<0.001	4.08	<0.001	
T M SOL	L1 - L4	16.83	<0.001	16.36	<0.001	
DF	L2 - L4	4.18	<0.001	3.8	0.001	
Н	L3 - L4	-0.22	1.00	-0.78	1.00	



3.10 Supplementary Information

Figure S1

Two collection sites and four species of Collembola from the temperate and the sub-Antarctic Australian regions. *Orthonychiurus* sp. and *Sinella* sp. were collected from the Jock Marshall Reserve, Victoria and *Mucrosomia caeca* and *Ceratophysella denticulata* were collected from Macquarie Island.





Experimental setup: air is pumped through the gas flow meter and scrubbed of moisture before reaching the soil column. The Peltier-plate connected to the thermal controller heats up the air in the top air chamber simulating the natural warming of the soil by the sun and creating the thermal gradient down the soil column. The soil column is entirely made out of non-transparent PVC. I-Buttons record temperature and relative humidity of the soil matrix throughout the duration of the experiment.



© Photo by Ian Aitkenhead

Figure S3

Custom-built Peltier plate set-up for measuring cessation of voluntary movement (CVM) and critical thermal maximum (CT_{max}) uses aluminium vials lined with the moistened Plaster-of-Paris:charcoal mixture substrate. Temperature sensors and thermocouples are placed within each control vial (one control vial per stand).



Figure S4

Schematic of the rearing and experimental design used for this study.



Figure S5 Linear model assumptions $lm(CVM \sim CT_{max})$ for correlation between CVM and CT_{max} for all species were not met suggesting that the residuals are heavy-tailed on one end: a) Residuals versus fitted values, b) normal probability plot of residuals, and c) histogram of residuals



Figure S6 ANOVA (CVM~ Soil Layer + Species) assumptions plots for the effect of soil layer on CVM were not met suggesting that the residuals are heavy-tailed on both ends: a) Residuals versus fitted values, b) normal probability plot of residuals, and c) histogram of residuals



Figure S7 ANOVA (CT_{max} ~ Soil Layer + Species) assumptions plots for the effect of soil layer on CT_{max} were not met suggesting that the residuals are heavy-tailed: a) Residuals versus fitted values, b) normal probability plot of residuals, and c) histogram of residuals



Figure S8 ANOVA (Body Length~ Soil Layer) assumptions plots for the effect of soil layer on body length: a) Residuals versus fitted values, b) normal probability plot of residuals, and c) histogram of residuals



Figure S9 ANOVA (Body Width~ Soil Layer) assumptions plots for the effect of soil layer on body length: a) Residuals versus fitted values, b) normal probability plot of residuals, and c) histogram of residuals



Figure S10 Linear model assumptions $lm(CT_{max} \sim Body Length + Species)$ for correlation between CT_{max} and body length for all species were not met suggesting that the residuals have tails on both ends: a) Residuals versus fitted values, b) normal probability plot of residuals, and c) histogram of residuals

Table S1

List of barcoded species.

Species	Process ID	Sample ID/collection code	BIN	COI Seq. Length	Collection Site	Other collection sites outside of Australia (BOLD)	
Orthonychiurus sp.	30323F11_Ony_JMR_a	COLMU1591-18	BOLD:AAC3119	658	Victoria	New Zealand,	
	30323F12_Ony_JMR_b	COLMU1592-18	BOLD:AAC3118	658		Germany, South Africa Monaco	Africa, Monaco,
	30323G3_Ony_JMR_e	COLMU1595-18	BOLD:AAC3118	658		Canada	
<i>Sinella</i> sp.	30323F1_Sin_JMR_a	COLMU1581-18	BOLD:ADM8436	658	Victoria	None	
	30323F2_Sin_JMR_b	COLMU1582-18	BOLD:ADM8436	658			
	30323F3_Sin_JMR_c	COLMU1583-18	BOLD:ADM8436	658			
	30323F4_Sin_JMR_d	COLMU1584-18	BOLD:ADM8436	658			
	30323F5_Sin_JMR_e	COLMU1585-18	BOLD:ADM8436	658			

Table S2

Developmental temperature programs for temperate species simulating daily temperature fluctuations of the month with the highest mean maximum temperature (February). Cycle: 0=dark, 1=light

	Temperat	te (JMR)	Cycle
Hour	Present (°C)	Future (°C)	Light/ Dark
00:00	17.7	19.7	0
02:00	16.9	18.9	0
04:00	16.3	18.3	0
06:00	16.6	18.6	1
08:00	18.7	20.7	1
10:00	22.3	24.3	1
12:00	25.6	27.6	1
14:00	27.0	29.0	1
16:00	25.6	27.6	1
18:00	22.3	24.3	0
20:00	19.5	21.5	0
22:00	18.1	20.1	0

Table S3

Preliminary power analysis on species done after first five replicates of *Orthonychiurus* sp. and *M. caeca* indicated that with an effect size of 6-8 individuals and with standard deviation of 3.6-3.8 on average (power=0.8, p=0.05), replicate number should be 7 on average. With *Sinella* sp. this number was 3-4 replicates. In the case of *C. denticulata*, no effect was observed even after 5 replicates. Below are the power analyses done on the final observations.

Results of power analysis t-test approach used to determine number of replicates needed per species to detect difference of 4-17 individuals across different soil layers and treatments (power = 0.8, p = 0.05). It's important to note that for *Orthonychiurus* sp. Present (L0), there are no individuals observed on the soil surface relative to the control across all replicates of treatments compared.

Soil Layers are L0: Soil surface; L1: 0-4 cm; L2: 4-8 cm; L3: 8-12 cm; L4: 12-16 cm; NR: Not recovered.

Species	Soil Layer	Treatment compared to the control	effect size (delta)	pooled sd	sig. level	power	n	Avg. n	
.ds	LO	Heat wave	4.125	3.27	0.05	0.8	11		
<i>irus</i> : at	LO	Heat wave & Drought	4.125	3.27	0.05	0.8	11		
<i>ychiu</i> reser	L1	Heat wave	15.75	3.46	0.05	0.8	2		
P	L2	Heat wave	6	2.62	0.05	0.8	4		
Or	L4	Heat wave	8.375	2.37	0.05	0.8	3		
	LO	Drought	4.875	2.57	0.05	0.8	6	6.4	
ds <i>sr</i>	LO	Heat wave	6.75	2.96	0.05	0.8	4		
<i>hiur</i> r ure	LO	Heat wave & Drought	6.75	2.96	0.05	0.8	4		
o <i>nyc</i> . Fut	L1	Heat wave	8.375	4.3	0.05	0.8	5		
Orthu	L3	Heat wave	4.875	3.11	0.05	0.8	8		
	L4	Heat wave	5.375	4.57	0.05	0.8	12		
	LO	Drought	5	1.6	0.05	0.8	3	3 2 2	
<i>la</i> sp sent	LO	Heat wave	11.6	1.52	0.05	0.8	2		
Sinel Pre	LO	Heat wave & Drought	11.6	1.52	0.05	0.8	2		
-1	L2	Heat wave	5.6	4.79	0.05	0.8	13		
	LO	Drought	7.6	2.58	0.05	0.8	3	2.1	
iture	LO	Heat wave	16.6	3.36	0.05	0.8	2	5.4	
p. Fu	LO	Heat wave & Drought	16.6	3.36	0.05	0.8	2		
illa s	L1	Drought	7.6	2	0.05	0.8	3		
Sine	L1	Heat wave	15.6	2.29	0.05	0.8	2		
	L1	Heat wave & Drought	15.2	2.5	0.05	0.8	2	2	
	L1	Drought	3.9	3.54	0.05	0.8	14		
aeca	L2	Drought	4.1	3.61	0.05	0.8	13	12.5	
М. с	L2	Heat wave	5.1	3.47	0.05	0.8	8	12.3	
	L2	Heat wave & Drought	3.7	3.5	0.05	0.8	15		

Table S4

Outcomes of Generalised Linear Models (GLMs) and Negative Binomial Models investigating the effect of extreme weather treatments on the behavioural response (individual count) within each soil layer for four species of Collembola. Significant FDR corrected p-values are in bold indicating that the number of individuals found in the soil layer of the particular treatment differs from the control. Soil Layers are L0: Soil surface; L1: 0-4 cm; L2: 4-8 cm; L3: 8-12 cm; L4: 12-16 cm; NR: Not recovered.

Species	Soil Layer	Treatment	Est.	SE	z value	p-value
		Control	1.63	0.17	9.57	<0.001
	τo	Drought	0.07	0.24	0.30	0.767
	LU	Heat wave	-1.63	0.40	-4.10	<0.001
		Heat wave & Drought	-1.63	0.40	-4.10	<0.001
	Soil Layer	Treatment	Est.	SE	t value	p-value
		Control	3.17	0.08	38.23	<0.001
	т 1	Drought	0.19	0.11	1.74	0.125
	LI	Heat wave	-1.08	0.16	-6.54	<0.001
		Heat wave & Drought	0.15	0.11	1.37	0.182
	L2	Control	1.25	0.30	4.15	0.001
		Drought	-1.14	0.61	-1.86	0.099
Orthorn of immediate		Heat wave	1.00	0.35	2.83	0.017
sp. PRESENT		Heat wave & Drought	-0.34	0.47	-0.72	0.477
		Control	0.86	0.35	2.50	0.055
	13	Drought	-1.56	0.83	-1.88	0.094
	LJ	Heat wave	0.95	0.41	2.33	0.055
		Heat wave & Drought	-0.64	0.59	-1.09	0.284
		Control	1.35	0.31	4.37	0.001
	T A	Drought	-1.35	0.69	-1.98	0.077
	L4	Heat wave	1.15	0.36	3.23	0.006
		Heat wave & Drought	-0.10	0.45	-0.23	0.823
		Control	0.81	0.27	3.00	0.023
	NIP	Drought	0.54	0.34	1.60	0.121
	INIX	Heat wave	0.58	0.34	1.70	0.121
		Heat wave & Drought	0.77	0.33	2.36	0.051

Species	Soil Layer	Treatment	Est.	SE	z value	p-value
		Control	2.05	0.13	16.12	<0.001
	τo	Drought	-0.99	0.24	-4.06	<0.001
	LU	Heat wave	-2.05	0.38	-5.45	<0.001
		Heat wave & Drought	-2.05	0.38	-5.45	<0.001
	Soil Layer	Treatment	Est.	SE	t value	p-value
		Control	3.11	0.09	34.32	<0.001
	т 1	Drought	0.12	0.12	0.97	0.457
	LI	Heat wave	-0.47	0.15	-3.19	0.007
		Heat wave & Drought	-0.07	0.13	-0.57	0.571
	L2	Control	0.97	0.35	2.76	0.040
		Drought	0.39	0.45	0.86	0.397
Outhomschiums		Heat wave	0.78	0.42	1.86	0.147
sp. FUTURE		Heat wave & Drought	0.51	0.44	1.16	0.343
		Control	0.56	0.36	1.55	0.177
		Drought	-0.07	0.52	-0.14	0.888
	LJ	Heat wave	1.33	0.41	3.27	0.011
		Heat wave & Drought	0.69	0.44	1.56	0.177
		Control	1.25	0.31	3.98	0.002
	ТЛ	Drought	-0.39	0.50	-0.78	0.440
	L4	Heat wave	0.93	0.37	2.50	0.037
		Heat wave & Drought	0.71	0.38	1.85	0.100
		Control	1.06	0.21	5.09	<0.001
	NR	Drought	0.53	0.26	2.02	0.107
	INIX	Heat wave	0.48	0.26	1.80	0.111
		Heat wave & Drought	0.23	0.28	0.83	0.412

Species	Soil Layer	Treatment	Est.	SE	z value	p-value
		Control	2.53	0.13	20.11	<0.001
	τo	Drought	-0.51	0.21	-2.46	0.014
	LU	Heat wave	-2.53	0.46	-5.45	<0.001
		Heat wave & Drought	-2.53	0.46	-5.45	<0.001
	Soil Layer	Treatment	Est.	SE	t value	p-value
		Control	3.07	0.14	21.76	<0.001
	т 1	Drought	0.30	0.19	1.62	0.167
	LI	Heat wave	0.14	0.19	0.72	0.485
		Heat wave & Drought	0.39	0.18	2.15	0.094
	Soil Layer	Treatment	Est.	SE	z value	p-value
	L2	Control	0.88	0.37	2.36	0.037
		Drought	-0.88	0.63	-1.40	0.217
C:		Heat wave	1.20	0.47	2.58	0.037
Sinella sp. PRESENT		Heat wave & Drought	-0.41	0.56	-0.72	0.472
		Control	0.34	0.43	0.79	0.804
		Drought	-0.34	0.65	-0.52	0.804
	LS	Heat wave	1.14	0.51	2.23	0.104
		Heat wave & Drought	0.13	0.59	0.23	0.820
		Control	0.00	0.50	0.00	1.000
	T 4	Drought	0.00	0.71	0.00	1.000
	L4	Heat wave	0.79	0.62	1.26	0.412
		Heat wave & Drought	0.88	0.62	1.42	0.412
	Soil Layer	Treatment	Est.	SE	t value	p-value
		Control	1.61	0.20	7.87	<0.001
	NR	Drought	-0.17	0.30	-0.58	0.763
		Heat wave	-0.33	0.32	-1.04	0.628
		Heat wave & Drought	0.08	0.28	0.27	0.790

Species	Soil Layer	Treatment	Est.	SE	z value	p-value
		Control	2.87	0.11	26.90	<0.001
	τo	Drought	-0.57	0.18	-3.19	0.001
	LU	Heat wave	-2.87	0.46	-6.24	<0.001
		Heat wave & Drought	-2.87	0.46	-6.24	<0.001
	Soil Layer	Treatment	Est.	SE	t value	p-value
		Control	2.92	0.05	60.76	<0.001
	т 1	Drought	0.34	0.06	5.45	<0.001
	LI	Heat wave	0.61	0.06	10.19	<0.001
		Heat wave & Drought	0.60	0.06	9.97	<0.001
	Soil Layer	Treatment	Est.	SE	z value	p-value
	L2	Control	0.00	0.45	0.00	1.00
		Drought	0.00	0.63	0.00	1.00
G: 11		Heat wave	0.69	0.55	1.27	0.824
Sinella sp. FUTURE		Heat wave & Drought	0.00	0.63	0.00	1.00
	1.2	Control	-	-	-	-
		Drought	-	-	-	-
	LS	Heat wave	-	-	-	-
		Heat wave & Drought	-	-	-	-
		Control	0.00	0.45	0.00	1.00
	I.4	Drought	0.00	0.63	0.00	1.00
	L4	Heat wave	0.00	0.63	0.00	1.00
		Heat wave & Drought	0.18	0.61	0.30	1.00
	Soil Layer	Treatment	Est.	SE	t value	p-value
		Control	1.57	0.23	6.96	<0.001
	ND	Drought	0.00	0.32	0.00	1.00
	INK	Heat wave	0.00	0.32	0.00	1.00
		Heat wave & Drought	0.22	0.30	0.74	0.942
Species	Soil Layer	Treatment	Est.	SE	t value	p-value
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	T 1	Control	1.34	0.20	6.60	<0.001
		Drought	0.71	0.25	2.86	0.012
	LI	Heat wave	-0.33	0.30	-1.09	0.575
		Heat wave & Drought	0.15	0.28	0.53	0.589
		Control	2.10	0.16	13.33	<0.001
	1.2	Drought	-0.69	0.27	-2.54	0.021
	L2	Heat wave	-0.97	0.30	-3.23	0.005
		Heat wave & Drought	-0.60	0.27	-2.26	0.030
	L3	Control	1.96	0.18	11.03	<0.001
Magaag		Drought	-0.33	0.27	-1.20	0.237
M. caeca		Heat wave	0.34	0.23	1.47	0.237
		Heat wave & Drought	0.29	0.23	1.24	0.237
	T A	Control	2.83	0.10	29.18	<0.001
		Drought	0.15	0.13	1.16	0.337
	L4	Heat wave	0.20	0.13	1.55	0.258
		Heat wave & Drought	-0.02	0.14	-0.13	0.897
		Control	1.39	0.16	8.59	<0.001
	ND	Drought	-0.16	0.24	-0.68	0.499
	INK	Heat wave	-0.22	0.24	-0.92	0.484
		Heat wave & Drought	0.22	0.22	1.03	0.484

Species	Soil Layer	Treatment	Est.	SE	t value	p-value
	т 1	Control	3.11	0.07	42.09	<0.001
	LI	Heat wave	-0.06	0.11	-0.52	0.617
	L2	Control	1.57	0.22	7.25	<0.001
C doutioulata		Heat wave	0.56	0.27	2.06	0.073
	L3	Control	0.96	0.18	5.27	0.002
C. ueniicululu		Heat wave	-0.17	0.27	-0.62	0.550
	L4	Control	1.61	0.20	7.99	<0.001
		Heat wave	-0.45	0.32	-1.38	0.204
	NR	Control	1.65	0.10	16.38	<0.001
		Heat wave	-0.04	0.14	-0.27	0.792

Table S5

Outcomes of Dunn's post-hoc tests show trends of CVM and CT_{max} with the increasing soil depth by treatment at the intraspecific level. Significant Bonferroni corrected p-values are in bold. Soil Layers are L0: Soil surface; L1: 0-4 cm; L2: 4-8 cm; L3: 8-12 cm; L4: 12-16 cm; NR: Not recovered.

TEMPERATE			PRESENT			FUTURE (2050)			
SPI	ECIES	CVM (°C)		CTn	nax (°C)	CV	'M (°C)	CTmax (°C)	
Species	Soil Layers	Z	P-value	Z	P-value	Z	P-value	Z	P-value
	L0 - L1	-3.39	0.007	-2.15	0.318	-3.68	0.002	-1.45	1.00
	L0 - L2	-1.38	1.00	-2.07	0.383	-1.30	1.00	-0.37	1.00
ė	L1 - L2	2.38	0.175	-0.37	1.00	2.83	0.046	1.35	1.00
s sn	L0 - L3	-0.21	1.00	0.77	1.00	1.20	1.00	0.53	1.00
hiur	L1 - L3	3.33	0.009	3.37	0.008	6.05	<0.001	2.48	0.132
onyc	L2 - L3	1.21	1.00	3.07	0.021	2.86	0.042	1.04	1.00
Drtha	L0 - L4	-1.64	1.00	-0.61	1.00	0.46	1.00	1.81	0.704
0	L1 - L4	2.21	0.274	2.08	0.376	6.24	<0.001	5.27	<0.001
	L2 - L4	-0.26	1.00	1.87	0.619	2.25	0.246	2.72	0.066
	L3 - L4	-1.47	1.00	-1.57	1.00	-0.98	1.00	1.39	1.00
	L0 - L1	2.86	0.043	-0.39	1.00	0.70	1.00	1.13	1.00
	L0 - L2	4.15	<0.001	3.15	0.016	2.78	0.032	1.98	0.288
	L1 - L2	2.75	0.060	3.99	0.001	2.68	0.044	1.80	0.430
ċ.	L0 - L3	-0.88	1.00	-0.94	1.00	-	-	-	-
la s]	L1 - L3	-2.46	0.140	-0.82	1.00	-	-	-	-
Sinel	L2 - L3	-3.71	0.002	-3.14	0.017	-	-	-	-
01	L0 - L4	1.81	0.706	3.72	0.002	-0.65	1.00	-0.24	1.00
	L1 - L4	0.66	1.00	4.21	<0.001	-0.72	1.00	-0.35	1.00
	L2 - L4	-0.87	1.00	1.33	1.00	-1.97	0.291	-1.21	1.00
	L3 - L4	2.13	0.331	3.77	0.002	-	-	-	-

SUB-ANTARCTIC SPECIES			PRESENT				
		CVM (°C)		СТи	nax (°C)		
Species	Soil Layers	Z P-value		Z	P-value		
	L1 - L2	-0.85	1.00	-2.98	0.017		
1	L1 - L3	0.97	1.00	-2.35	0.114		
aecc	L2 - L3	1.90	0.346	0.97	1.00		
М. с	L1 - L4	2.46	0.082	-2.21	0.162		
· · ·	L2 - L4	3.44	0.003	1.53	0.756		
	L3 - L4	1.68	0.553	0.52	1.00		
	L1 - L2	-3.42	0.004	0.88	1.00		
ata	L1 - L3	-2.05	0.242	0.35	1.00		
ticul	L2 - L3	0.13	1.00	-0.16	1.00		
deni	L1 - L4	-3.23	0.007	-1.94	0.315		
C.	L2 - L4	-0.19	1.00	-2.25	0.147		
	L3 - L4	-0.27	1.00	-1.52	0.767		

Chapter 4

The effect of developmental temperature on desiccation resistance of temperate and tropical Collembola

"The following facts I have noticed at Monte Video and frequently in this place: After a heavy thunder storm in a little pool in a courtyard which had only existed at most seven hours. I observed the surface strewed over with black specks: these were collected in groups, and precisely resembled pinches of gunpowder dropped in different parts on the surface of the puddle. These specks are Insects of a dark leaden colour; the younger ones being red. Viewed through a microscope, they were continually crawling over each other and the surface of the water; on the hand they possessed a slight jumping motion. The numbers on each pool were immense: and every puddle possessed some of the pinches . . . What are they? and how produced in such countless myriads? We have seen their birth is effected in a short time, and their life, from the drying of the puddles can not be of a much longer duration."

Charles Darwin, 1832, Insect Notes

4.1 Abstract

Phenotypic plasticity is necessary to mediate challenges many ectotherms face from changing climates, which now are characterized by more frequent and intense droughts and higher average global temperatures. This is especially true for small soil arthropods, such as Collembola, sensitive to desiccation and temperature changes. Unlike plasticity due to drought acclimation and mechanisms against desiccation stress, the effects of thermal acclimation and developmental temperature on desiccation resistance are seldom explored. Polar species of Collembola have been found to express phenotypic plasticity due to thermal acclimation, but whether the same is true for the temperate and tropical species remains unknown. Future climate projections suggest that soil organisms in the Australian tropics and the temperate zone will be exposed to 2 °C warmer temperatures in 2050 as a consequence of climate change. Findings in this study show that temperate and tropical species lack developmental plasticity in desiccation resistance when reared under temperatures simulating future projections of the warmest month of the year of their respective microclimate. As a result, present-day and future reared populations do not differ in the survival time to desiccation. In the absence of developmental plasticity, temperate and tropical species of Collembola will likely have to rely on behaviour to avoid desiccation stress in the new warming climates.

4.2 Introduction

Desiccation resistance, or the ability of an organism to resist and reduce water loss, is a trait of particular importance for survival of insects and small terrestrial arthropods susceptible to dehydration due to desiccation stress. While the effect of acclimation at a given humidity on desiccation resistance is well explored for small ectotherms, the effects of developmental temperature and thermal acclimation on desiccation resistance or desiccation rate are still generally rarely explored (Hoffman, 1990; Hoffman & Watson, 1993; Hoffman et al., 2005; Terblanche et al., 2005; Chown et al., 2007; Leinaas et al., 2009; Terblanche et al., 2010). Climate projections of more frequent and intense droughts in temperate and tropical regions suggest that implications of climate change on small ectotherms largely depend upon their desiccation resistance (Easterling et al., 2000; IPCC, 2014; Fu, 2015). Changing rainfall patterns and dry seasons have been found to reduce insect abundances (Wolda, 1978; Tauber et al., 1998; Branson, 2008). Furthermore, projected increase in the land surface temperatures will affect microhabitats within the soil matrix that harbour small ectotherms (Duffy et al., 2015) and generally increase temperatures under which they develop. A recent study on a long-term arthropod biomass data in Puerto Rico's Luquillo rainforest revealed that a 2 °C increase in forest temperature over the past 30 years was the major driver in reducing arthropod abundance causing synchronous decline in lizard, frog, and bird populations (Lister & Garcia, 2018). This substantial collapse of Luquillo rainforest's food web was tied to the arthropod biomass reduction, which decreased by 10 to 60 times (Lister & Garcia, 2018). Considering that droughts in both temperate and tropical regions are becoming more common (Easterling et al., 2000; IPCC, 2014; Fu, 2015), drought exposure coupled with development in the warming climate due to the rising average global temperatures may also have an effect on arthropod desiccation resistance. Acclimation or pre-exposure to one type of stress can create resistance to a different form of stress, in a response that is called cross-tolerance (Bubliy et al., 2011, 2013; Kalra et al., 2017; Gotcha et al., 2018).

Studies investigating the effects of developmental temperature and thermal acclimation on desiccation resistance, predominantly focused on different species and populations of *Drosophila*, found varying responses. Water loss rates in the desert fruit fly *Drosophila mojavensis* were unaffected by warmer static temperature acclimation, although adaptive changes in the cuticular lipids have been observed (Gibbs et al., 1998). *Drosophila birchii*, a tropical rainforest species found in north-eastern Australia, was not able to evolve further desiccation resistance, even after intense selection due to a lack of genetic variation in this trait (Hoffmann et al., 2003). Likewise, desiccation rate of a beetle *Chirodica chalcoptera* (Germar) did not significantly respond to thermal acclimation to three different constant acclimation temperatures (Terblanche et al., 2005). By contrast, acclimation to summer

fluctuating temperatures has been found to improve desiccation resistance in *Drosophila melanogaster*, relative to both winter and constant temperature conditions (Hoffmann et al., 2005). It is unclear at present if the apparent differences in thermal acclimation capacity among these species of *Drosophila* is genuine, or reflects different effects of constant and fluctuating summer conditions. This reinforces the question whether a treatment simulating fluctuating summer temperature of higher intensity might further improve desiccation resistance in some species of ectotherms. Studies on other taxa, such as *Oryzaephilus surinamensis* (grain beetle), *Centruroides sculpturatus* (scorpion), and *Drosophila pseudoobscura*, have found that higher static developmental temperatures cause a change in the cuticular lipid composition (Howard, 1993; Howard et al., 1995), thus reducing the rate of water loss (Toolson & Hadley, 1979; Toolson, 1982), which could consequently increase time to desiccation (Leinaas et al., 2009; but see Gibbs et al., 1998). Similarly, water loss rates were reduced in response to high temperature acclimation in *Scarabaeus spretus* (Terblanche et al., 2010).

Desiccation resistance is of particular importance for small soil ectotherms with cutaneous respiration, such as Collembola. Collembola (springtails) are one of the most abundant soil arthropods responsible for control of soil microbial communities and are very sensitive to desiccation (Petersen & Luxton, 1982; Hopkin, 1997; Rusek, 1998). Like in many terrestrial arthropods, water loss in Collembola under ambient temperatures primarily occurs through the cuticle (Edney, 1977; Hadley, 1994). Collembola use several strategies to reduce desiccation stress, including active regulation of internal osmotic pressure, low cuticular permeability, and water vapour absorption (Verhoef & Witteveen, 1980; Bayley & Holmstrup, 1999; Holmstrup et al., 2001; Holmstrup et al., 2002; Kaersgaard et al., 2004). Implementation of one or more of these strategies by Collembola generally has been shown to improve survival by extending time to desiccation and can be triggered by pre-acclimation at milder desiccation stress (Bayley & Holmstrup, 1999; Sjursen et al., 2001; Holmstrup et al., 2002; Elnitsky et al., 2008; Holmstrup & Bailey, 2013). Thermal acclimation, using static temperatures, has been found to have a large effect on desiccation resistance in six polar species of Collembola where the warmer static temperature improved the survival time of the invasive species (Chown et al., 2007). Short-term acclimation to the higher static temperature reduced the rate of water loss and increased the time to desiccation at either test temperature in *Pogonognathellus flavescens*, one of the invasive species tested (Leinaas et al., 2009). On the other hand, indigenous polar species generally benefited from the cold temperature acclimation (Chown et al., 2007). Thus far, it remains unknown how desiccation resistance of temperate and tropical species of Collembola will be affected by either acclimation or development under their respective ecologically relevant temperatures.

Experimental and large-scale studies have shown that insect species and populations living in environments with different water availability vary in their desiccation resistance and desiccation tolerance (Edney, 1977; Hadley, 1994; Addo-Bediako et al., 2001; Hoffmann et al., 2001; Chown, 2002; Marron et al., 2003; Parkash et al., 2005). Ectotherms living in arid environments are generally more resistant to desiccation compared to ectotherms living in the areas with greater water availability (Addo-Bediako et al., 2000; Gibbs & Matzkin, 2001; Tracy et al., 2010) suggesting that species of Collembola from the tropical regions should be more sensitive to desiccation stress compared to the temperate species. Resistance to desiccation varies between species of Collembola from different types of habitat and vegetation cover, such as lichens, humid forest litter, tussocks, etc. (Leinaas & Fjellberg, 1983; Leinaas & Somme, 1984). Indeed, while drought can be a limiting distribution factor for some Collembola species, in others it may have more effect on their abundance and patterns of demography and density (Hertzberg & Leinaas, 1998) and have no obvious correlation between vertical distribution in the natural environment and resistance to desiccation stress (Kaersgaard et al., 2004).

In the present study, I aim to explore whether there is an effect of ecologically relevant higher fluctuating developmental temperatures on a survival time to desiccation of temperate and tropical Collembola, at the level of species, by incorporating experimental rearing conditions pertinent to those predicted to occur due to global climate change. To do so, I investigate whether development under simulated future projected summer temperatures of the top soil layer, corresponding to a 2 °C increase in the average global temperature projected to occur in the year 2050, will have an impact on desiccation resistance of six species of Collembola from temperate and tropical regions of Australia relative to present-day conditions of their respective microclimate. The emphasis is placed on the individual mortality events and recording of the survival time for each individual. Furthermore, I assess how the mean survival time of desiccation varies with the climatic region.

4.3 Materials and Methods

4.3.1 Species and collection sites

Temperate species – *Orthonychiurus* sp., *Sinella* sp., and *Lepidocyrtus* sp.1 (Figure S1, Supplementary Information) - were collected from the Jock Marshall Reserve (37°54'S, 145°8'E), an environmental research facility with native vegetation consisting of open woodlands and diverse ground layer of grasses and herbs, located in Clayton, Victoria, Australia. The annual rainfall in this area averages 658.5 mm,

mean annual temperature is 14.8 °C, mean summer (Dec-Feb) maximum is 25.1 °C, and mean winter (Jun-Aug) minimum 6.5 °C (1981-2000, www.bom.gov.au). Tropical species – Thalassaphorura cryptopyga, Brachystomella sp., and Lepidocyrtus sp.2 (Figure S1, Supplementary Information) – were collected from Wooroonooran National Park (17° 26' S, 145°5'E) belonging to the Wet Tropics World Heritage area of Queensland, Australia. The annual rainfall in this tropical rainforest region averages 3321.8 mm, mean annual temperature is 24 °C, mean summer (Dec-Feb) maximum is 31.3 °C, and mean winter (Jun-Aug) minimum is 16 °C (1981-2000, www.bom.gov.au). In the field, Collembola were extracted from the leaf-litter samples by sieving and aspiration and placed into plastic vials lined with the moistened Plaster-of-Paris and charcoal mixture and native vegetation (Janion-Scheepers et al., 2018). Multiple leaf-litter samples were collected from each site, placed in resealable plastic bags and containers. Temperate collections were transported back to the lab within 1 hr and tropical collections within 5 days. Collembola were live-extracted using the Berlese-Tullgren funnel method (Southwood & Henderson, 2000) in a temperature controlled room maintained at 20 \pm 0.5 °C. Species were identified to the genus level with the help of an experienced taxonomist and samples were sent to the Barcode of Life Data Systems (BOLD) (www.barcodinglife.org) for DNA barcoding (Ratnasingham & Hebert, 2007) and further identification (Figure S2 and Table S1, Supplementary Information). Sequences are available on BOLD (www.boldsystems.org) as part of Project COLMU (Collembola of Monash University). Four out of six species (all except Orthonychiurus sp. and Thalassaphorura cryptopyga) in this study were identified as indigenous to Australia. This was the case if they were not previously represented in BOLD, or if they were represented and already collected across Australia, New Zealand, or south of the Wallace line (following Janion-Scheepers et al., 2018).

4.3.2 Developmental temperatures and rearing

Temperate and tropical species were reared at temperature simulations of the month with the highest mean maximum temperature of the year over the span of the last 30 years (1981-2010) for both Australian regions (Bureau of Meteorology; http://www.bom.gov.au) (Table S2, Supplementary Information). Changing climate increases exposure to more extreme weather, therefore, the goal here was to push the rearing temperature to the upper limit that could still produce sufficient number of individuals needed for the experiment. Considering that I had species from different families, it was important to be conservative to an extent in choosing the rearing temperatures. Present-day soil temperatures were obtained from the NicheMapR hourly microclimate model by choosing the appropriate level of shading by vegetation that most closely represented each location (Kearney et al., 2014). Present rearing temperatures for Collembola from the Jock Marshall Reserve (JMR) were set to fluctuate between 16.3 °C and 27.0 °C, while for Collembola from Wooroonooran N.P. (WNP) rearing temperatures fluctuated between 23 °C and 27 °C over a 24-hr cycle. These temperatures corresponded to soil temperatures at 3 cm depth for each of the two sampling locations. Future predictive climate data for 2050 were downloaded from the NASA Earth Exchange Global Daily Downscaled Projections (NEX-GDDP; Thrasher et al., 2012) dataset under the RCP8.5 future climate scenario (Peters et al., 2012). The future projected air temperatures following the RCP8.5 climate scenario were then used to predict future soil temperatures for the year 2050. Prediction was based on maintaining the same relationship between the present air (Bureau of Meteorology; http://www.bom.gov.au) and the present soil temperatures (Kearney et al., 2014). Simulations of future projected temperatures were set to fluctuate between 18.3 °C and 29.0 °C for JMR and between 25 °C and 29 °C for WNP. All species were kept under 12-h light: 12-h dark light cycle in plastic vials lined with moist plaster-of-Paris mixture, fed with *Platanus sp.* tree bark, and watered with deionized water (following Janion-Scheepers, 2018). Egg laying adults of F2 generation were used in the experiments.

4.3.3 Desiccation resistance

Desiccation resistance was assessed at 90 % relative humidity (RH) and 25 °C. I chose this intermediate temperature, because it was roughly equal to the mean temperature of the temperate and tropical present and future rearing treatments. Pilot studies have shown that Sinella sp. lives more than 72 hours at 96% relative humidity, so I choose lower relative humidity (90 %) for all species to be able to track mortality of each individual caused by desiccation stress only and not by starvation. Animals were placed in 16 glass vials in total (diameter = 1 cm; height = 3 cm) that were arranged in a custom-made EVA-foam holder (Figure S3, Supplementary Information) and placed in the centre of a 420-ml air-tight plastic container with the clear lid and with 150 ml of 90% RH aqueous NaCl (181.2 g/l H₂O) (Holmstrup et al., 1997). The setup was held at 25 \pm 0.5 °C. The air inside the closed system equilibrated quickly following Raoult's law (Sjursen et al., 2001; Holmstrup et al., 2002). The RH stability was verified by a Hygrochron iButtons (DS 1923-F5; Maxim Integrated). Using this method, all individuals were exposed to the same relative humidity. Each of the eight glass vials (eight per rearing temperature) had five individuals that were observed in 10 to 30-min intervals, depending on the species. I

used 80 individuals per species (40 per rearing treatment) and recorded the time of death for each individual. The exception was *Thalassaphorura cryptopyga* from the tropics with 60 individuals total (30 per rearing treatment). Collembola were scored as dead if they lost the upright position and there was no antenna and leg movement.

4.3.4 Statistical analysis

Statistical analyses were conducted in Rv.3.5.1 (R Core Team, 2014). Survival was analysed using the Kaplan-Meier survival method (Kaplan & Meier, 1958) and log-rank test in 'survminer' package v.0.4.3 to compare the present and future survival curves of each species separately with each vial representing a single replicate. This approach was useful considering its emphasis on individual mortality events. Mean survival times of desiccation (LT_{50}) were calculated using *dose.p* function in the 'mass' package for each replicate within a species and developmental temperature. The effects of developmental temperatures on desiccation resistance (LT_{50}) of each species were examined with one-way analysis of variance (ANOVA). Kruskal-Wallis tests were used to analyse the effects of climate on LT_{50} . Finally, LT_{50} of each replicate (six replicates per treatment for *Thalassaphorura cryptopyga* and eight for all the others) were averaged to obtain a single LT_{50} value per species per treatment shown in Table 1.

4.4 Results

Overall, survival time at 90% RH at 25 °C ranged from 10 min for the shortest living individuals of *Thalassaphorura cryptopyga* from the tropics to more than 9.5 hours for the longest living individual of *Sinella* sp. from the temperate region (Figure 1). Temperate species, *Orthonychiurus* sp. ($X^2 = 0.233$, p=0.63), *Sinella sp.* ($X^2 = 0.658$,

p=0.42) and *Lepidocyrtus* sp. 1 ($X^2 = 0.21$, p=0.65) showed no change in survival time between the present and the future rearing treatments (Figure 1a). Tropical, *Brachystomella* sp. ($X^2 = 0.047$, p=0.83) and *Thalassaphorura cryptopyga* ($X^2 = 1.75$, p=0.19) also showed no change in survival time. Only *Lepidocyrtus* sp. 2 from the tropics showed an increase in survival time ($X^2 = 3.96$, p=0.047) for the future reared population (Figure 1b). However, the survival time of the future reared population was 80 min longer than the present and appeared to be driven by a single long-lasting individual. After the removal of this individual from the data analysis, there was no significant difference in the survival time to desiccation of the two rearing treatments ($X^2 = 3.08$, p=0.079, Figure 1b).

There was no effect of rearing temperature on LT_{50} for all species (*Orthonychiurus* sp.: F = 0.114, p=0.741; *Sinella* sp.: F = 0.303, p=0.591; *Lepidocyrtus* sp. 1: F = 1.273, p=0.278; *Thalassaphorura cryptopyga*: F = 2.11, p=0.177; *Brachystomella* sp.: F = 0.10, p=0.757; *Lepidocyrtus* sp. 2: F = 0.839, p=0.375; Figure 2). Mean (± SD) LT_{50} (min) for each species and the rearing treatment are presented in Table 1. There was an overall effect of climate on LT_{50} (X² = 57.26, p<0.0001) with the temperate species generally having higher desiccation resistance (i.e. longer time to desiccation) than the tropical species.

4.5 Discussion

Exposure to elevated developmental temperatures did not induce a plastic response in desiccation resistance of temperate and tropical species of Collembola, unlike the thermal acclimation in polar species of Collembola (Chown et al., 2007). Invasive and indigenous polar species from sub-Antarctic Marion Island did not differ in the extent of phenotypic plasticity or the absolute time to desiccation when acclimated at two

different static temperatures (5 and 15 °C) (Chown et al., 2007). Nevertheless, different forms of plasticity among the species suggested that warmer acclimation promotes survival of the invasive species while reducing survival time of desiccation in indigenous species (Chown et al., 2007). Further investigation revealed that the short-term acclimation to warmer conditions reduced the rate of water loss in the invasive species Pogonognathellus flavescens and, as a consequence, increased the time to desiccation (Leinaas et al., 2009). In addition, findings suggested that the effect of thermal acclimation diminished with increasing body size. This was interpreted as an indication that warmer acclimation induced plastic response to protect smaller animals from fast water loss (Leinaas et al., 2009), since smaller individuals are likely more vulnerable to desiccation due to larger body surface/volume ratio (Leinaas & Somme, 1984; Leinaas & Fjellberg, 1985; Hadley, 1994). Indeed, higher temperature generally reduces desiccation survival time due to increased saturation deficit at higher temperatures where rates of cuticular water loss increase with temperature (Chown & Nicolson, 2004). A combination of high humidity and lower temperature favours the survival of individuals, as expected (Chown & Nicolson, 2004; Lyons et al., 2014). Therefore, it has been suggested that higher acclimation temperature in the case of *P. flavescens* could be the signal for an increased protection against desiccation stress (Leinaas et al., 2009). By contrast, findings in my study suggest that developmental temperatures do not induce a plastic response in temperate and tropical species. Neither one of the six species tested, whether invasive or indigenous, coming from temperate or tropical location, displayed a change in the overall survival time of desiccation stress or in the mean LT₅₀ with increasing developmental temperature. One reason for the lack of noticeable developmental plastic response could be because of the magnitude of the

difference between treatments. The fluctuating developmental temperature treatments differed by 2 °C on the cold and warm ends, while the constant temperature acclimations of the polar Collembola differed by 10 °C. It is possible that a 2 °C difference was not large enough to induce a distinct plastic response. Alternatively, there could be a change in the cuticle composition and permeability or internal protective mechanisms, which responded to the new developmental conditions to maintain rather than enhance desiccation resistance. For instance, adaptive changes in the cuticular lipids have been observed in the desert fruit fly *Drosophila mojavensis* with higher constant acclimation temperature, but these changes did not result in reduced rates of water loss or improved desiccation resistance (Gibbs et al., 1998). Similarly, acclimation to different temperatures in two closely related species of ants did not have an effect on their drought survival, although the composition of cuticular hydrocarbons changed (Sprenger et al., 2018).

Along with temperature, water availability and precipitation are one of the most important drivers of ectotherm distribution and natural selection (Addo-Bediako et al., 2000; Bonebrake & Mastrandrea, 2010; Clusella-Trullas et al., 2011; Kellermann et al., 2012; Siepielski et al., 2017). The evolutionary response to desiccation in different *Drosophila* species appeared to be strongly inversely correlated to the average annual precipitation (Kellermann et al., 2012). However, limited evolutionary potential in *Drosophila* is suggested to lie within its phylogeny rather than only being limited to tropical species (Kellermann et al., 2012), but it also may depend on the type of thermal stress experienced (Blackburn et al., 2014). Results of my work revealed that climate had a strong effect on desiccation resistance where temperate species had significantly higher survival time to desiccation than species from the wet tropical region. No difference in desiccation resistance between

present-day and future reared populations indicates that the potential for developmental flexibility remains the same for both temperate and tropical species implying that species from both climate zones will face similar challenges with the ongoing climate change. A limited scope for adaptive desiccation resistance responses of Collembola to the future 2 °C warmer developmental temperatures suggest that the behavioural response will be the key strategy for ensuring persistence and survival under desiccation stress in the future warmer climate (Sunday et al., 2014).

Other desiccation resistance studies on Collembola specifically examining individual mortality events and time to desiccation have been undertaken on polar species (Leinaas & Somme, 1984; Hertzberg & Leinaas, 1998; Chown et al., 2007). In comparison, temperate and tropical species from this study generally exhibit similar or lower survival time to desiccation to polar species tested at the lower relative humidity (76 and 80% RH) and lower temperatures (5, 10, 15, 20 °C). At 25 °C, polar species such as *F. sexoculata* and *F. quadrioculata* had similar desiccation resistance to tropical species *Lepidocyrtus* sp. 2 and *Brachystomella* sp., respectively, although they were tested at more drying conditions (80% RH) (Hertzberg & Leinaas, 1998). This could indicate greater sensitivity to drought of tropical than polar species at similar warm conditions, but a broader comparative study, using identical techniques is needed to confirm this pattern.

Mild desiccation stress triggers different drought response mechanisms (Verhoef & Witteveen, 1980; Bayley & Holmstrup, 1999; Holmstrup et al., 2001; Holmstrup et al., 2002; Kaersgaard et al., 2004). Thermal acclimation to high versus low static temperatures in combination with drought acclimation has been found to improve desiccation resistance in the butterfly *Bicyclus anynana* (Fischer & Kirste, 2018). In *Glossina pallidipes*, plasticity in desiccation resistance, specifically thermal

acclimation responses of water loss rate were found to depend on the time-scale and magnitude of variation of the environmental conditions (Kleyhans et al., 2014). Therefore, the recommendation for the future investigations of desiccation resistance, for Collembola in particular, is the investigation of the known protective mechanisms induced by the mild desiccation stress (98.2% RH) mentioned above (Bayley & Holmstrup, 1999; Holmstrup et al., 2001; Elnitsky et al., 2008; Holmstrup & Bailey, 2013) under different ecologically relevant acclimation, developmental and test temperatures. Moreover, establishing the upper thermal limits for the activation and efficacy of these protective mechanisms under mild desiccation stress is an integrative approach necessary for advancing the knowledge of responses to desiccation stress in the warming world.

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4.8 Figures

Figure 1

Estimated survival by Kaplan-Meier analysis from observed survival times for a) temperate and b) tropical species of Collembola reared at present and future projected temperatures of their respective microclimate. Future* = Survival after the removal of a single *Lepidocyrtus* sp. 2 individual.



Figure 2

Mean LT_{50} (min) to desiccation of 50% of the individuals tested for each species ordered from the highest to the lowest. There was no significant difference between present and future developmental temperatures on LT_{50} . Error bars represent 95% CIs.

4.9 Tables

Table 1

Mean (\pm SD) LT₅₀ (min) for each species across replicates (n=8 per treatment, *n=6 per treatment; 5 individuals per replicate) for each of the rearing treatments at 90% RH and 25 °C.

Species	Climate	Present	Future
Orthonychiurus sp.	Temperate	117.44 ± 19.73	114.70 ± 11.77
<i>Sinella</i> sp.	Temperate	347.44 ± 31.71	356.01 ± 30.53
Lepidocyrtus sp. 1	Temperate	162.86 ± 30.81	177.12 ± 18.14
* Thalassaphorura cryptopyga	Tropical	9.89 ± 0.26	12.81 ± 4.93
Brachystomella sp.	Tropical	28.74 ± 13.50	26.78 ± 11.13
Lepidocyrtus sp. 2	Tropical	93.96 ± 19.66	110.24 ± 46.28

4.10 Supplementary Information



Figure S1

Temperate species collected from Jock Marshall Reserve: A) *Orthonychiurus* sp.; B) *Sinella* sp.; C) *Lepidocyrtus* sp. 1 and tropical species collected from Wooroonooran National Park: D) *Thalassaphorura cryptopyga*; E) *Brachystomella* sp.; F) *Lepidocyrtus* sp. 2

The effect of temperature on the physiological and behavioural responses of Collembola







Figure S3

The closed system used to control relative humidity: custom-made EVA-foam holder with 16 glass vials (diameter = 1 cm; height = 3 cm) placed in the centre of a larger air-tight container with the transparent lid immersed in the 90% RH aqueous NaCl solution. Each glass vial contains 5 individuals for a total of 40 individuals per rearing treatment and 80 individuals per species.

Table S1

List of barcoded species.

Species	Process ID	Sample ID/collection code	BIN	COI Seq. Length	Collection Site	Other collection sites outside of Australia (BOLD)
Orthonychiurus sp.	30323F11_Ony_JMR_a	COLMU1591-18	BOLD:AAC3119	658	Victoria	New Zealand,
	30323F12_Ony_JMR_b	COLMU1592-18	BOLD:AAC3118	658		Germany, South Africa Monaco
	30323G3_Ony_JMR_e	COLMU1595-18	BOLD:AAC3118	658		Canada
<i>Sinella</i> sp.	30323F1_Sin_JMR_a	COLMU1581-18	BOLD:ADM8436	658	Victoria	None
	30323F2_Sin_JMR_b	COLMU1582-18	BOLD:ADM8436	658		
	30323F3_Sin_JMR_c	COLMU1583-18	BOLD:ADM8436	658		
	30323F4_Sin_JMR_d	COLMU1584-18	BOLD:ADM8436	658		
	30323F5_Sin_JMR_e	COLMU1585-18	BOLD:ADM8436	658		
Lepidocyrtus sp. 1	30323F6_Lep_JMR_a	COLMU1586-18	BOLD:ADI8949	658	Victoria	None
	30323F7_Lep_JMR_b	COLMU1587-18	BOLD:ADI8949	658		
	30323F8_Lep_JMR_c	COLMU1588-18	BOLD:ADI8949	658		
	30323F9_Lep_JMR_d	COLMU1589-18	BOLD:ADI8949	658		
	30323F10_Lep_JMR_e	COLMU1590-18	BOLD:ADI8949	658		
Thalassaphorura	30323G9_Ony_QLD_a	COLMU1601-18	BOLD:AAA8183	658	Queensland	Indonesia,
cryptopyga	30323G10_Ony_QLD_b	COLMU1602-18	BOLD:AAA8183	658		China, Vietnam, Comoros.
	30323G11_Ony_QLD_c	COLMU1603-18	BOLD:AAA8183	658		Mayotte
	30323G12_Ony_QLD_d	COLMU1604-18	BOLD:AAA8183	658		
	30323H1_Ony_QLD_e	COLMU1605-18	BOLD:AAA8183	658		
	30323H2_Ony_QLD_f	COLMU1606-18	BOLD:AAA8183	658		
	30323H3_Ony_QLD_g	COLMU1607-18	BOLD:AAA8183	658		
	30323H4_Ony_QLD_h	COLMU1608-18	BOLD:AAA8183	658		
	30323H5_Ony_QLD_i	COLMU1609-18	BOLD:AAA8183	658		

Brachystomella sp.	30323E07_Bra_QLD_b	COLMU1575-18	No BIN allocated	242	Queensland	None
	30323E08_Bra_QLD_c	COLMU1576-18	No BIN allocated	243		
	30323E10_Bra_QLD_e	COLMU1578-18	No BIN allocated	242		
	30323E11_Bra_QLD_f	COLMU1579-18	No BIN allocated	243		
	30323E12_Bra_QLD_c	COLMU1580-18	No BIN allocated	242		
Lepidocyrtus sp. 2	30323G4_Lep_QLD_a	COLMU1596-18	BOLD:ADO7359	658	Queensland	None
	30323G5_Lep_QLD_b	COLMU1597-18	BOLD:ADO7359	632		
	30323G6_Lep_QLD_c	COLMU1598-18	BOLD:ADP1935	658		
	30323G7_Lep_QLD_d	COLMU1599-18	BOLD:ADP1935	639		
	30323G8_Lep_QLD_e	COLMU1600-18	BOLD:ADO7359	658		

Table S2

Developmental temperature programs for temperate and tropical species simulating daily temperature fluctuations of the month with the highest mean maximum temperature (February). Cycle: 0=dark, 1=light

	Temperate (JMR)		Tropical	Tropical (WNP)		
Hour	Present (°C)	Future (°C)	Present (°C)	Future (°C)	Light/ Dark	
00:00	17.7	19.7	24.0	26.0	0	
02:00	16.9	18.9	23.3	25.3	0	
04:00	16.3	18.3	23.0	25.0	0	
06:00	16.6	18.6	23.0	25.0	1	
08:00	18.7	20.7	23.8	25.8	1	
10:00	22.3	24.3	24.6	26.6	1	
12:00	25.6	27.6	26.2	28.2	1	
14:00	27.0	29.0	27.0	29.0	1	
16:00	25.6	27.6	26.4	28.4	1	
18:00	22.3	24.3	24.8	26.8	0	
20:00	19.5	21.5	24.2	26.2	0	
22:00	18.1	20.1	23.8	25.8	0	

Chapter 5

Conclusion

"The real voyage of discovery consists not in seeking new landscapes, but in having new eyes."

Marcel Proust

5.1 Summary of contributions

The world's new, warming climate poses numerous challenges and threats to biodiversity in both terrestrial and marine ecosystems. The increase in average global temperatures is accompanied by altered weather patterns, where long and intense heat waves, droughts, storms and floods are becoming more common (Easterling et al., 2000; Rahmstorf & Coumou, 2011; Hansen et al., 2012; IPCC, 2014). Species vulnerability to climate change generally depends on their exposure and sensitivity, but also their resilience and potential to adapt (Williams et al., 2008). Temperature and humidity have the most direct impact on fitness and performance of animals, especially ectotherms, whose life processes depend on the external heat sources. Thermal performance curves (TPCs), describing the relationship between temperature and performance of an organism, are often used in climate change risk assessments. The ends of TPCs, CT_{max} and CT_{min} , shift on the thermal scale within a species depending on ontogeny, body size, sex, acclimation, seasonality, experimental starting temperature, and the experimental rate of temperature change (Terblanche et al., 2011; Madeira et al., 2012; Overgaard et al., 2012; Klockmann et al., 2016; Terblanche et al., 2017; Agudelo-Cantero & Navas, 2019). Moreover, thermoregulatory behaviour is proposed as the major strategy for mitigating extreme weather conditions in small ectotherms, emphasizing the importance of its inclusion into models predicting species responses to climate change (Bonebrake et al., 2014; Sunday et al., 2014; Duffy et al., 2015).

In this thesis, I have focused on investigating the effects of the experimental rate of temperature change at the intraspecific level by applying and evaluating two different theoretical approaches – thermal tolerance landscape and failure rate model – to empirical data from a range of ectotherms including insects, arthropods, crustaceans and fishes. Meta-analysis of the effect of the experimental rate of temperature change on critical thermal limits demonstrates that the decoupled responses of CT_{max} and CT_{min} in ectotherms are highly species-specific. The results reveal that ectotherms have four different thermal tolerance response patterns to the increasing exposure time (i.e. slower ramping rate):

- Decrease: thermal tolerance declines with the increasing exposure time
- Increase: thermal tolerance improves with the increasing exposure time
- No effect: thermal tolerance is not affected by exposure time
- Mixed: thermal tolerance initially improves with the increasing exposure time, reaches a peak high (or low) temperature at a certain ramping rate (i.e. time of exposure), and then declines with even further increase of exposure time

A set of failure rate models allowing for curve flexibility and the concept of threshold temperature were found to be a good fit for the empirical data allowing for the separation of the statistical effects described by the model and biological effects due to the physiological response of an organism. Based on these findings, the hypothetical relationship between the failure rate and the recovery rate of an organism is proposed to describe how their interaction might affect thermal tolerance (Chapter 2). Future work should further explore the biotic and abiotic drivers of variation in these models. For example, to simultaneously investigate the effects of ontogeny, body size, sex, acclimation, seasonality, experimental starting temperature, and the experimental rate of temperature change on CT_{max} and CT_{min} , more data recording multiple trait measurements within a single study are needed to compare the interaction of multiple factors at the level of a single species and find what drives the specific pattern of response. I conclude that general comparative analyses using macrophysiological approaches of critical thermal limits could be in significant error without taking heating or cooling rates of experiments into consideration. Given that these rates vary widely among studies, and have significant effects on CT_{max} and CT_{min} , it may well be that current conclusions are only partially valid.

My research also aimed to identify the developmental effect of 2 °C increase in the average global temperatures on physiological traits and behaviour of soil arthropods using Collembola as a model organism, and establish the link between physiology and the observed behavioural response to the lab simulations of extreme weather events. Shortterm behavioural responses to extreme weather events were found to mitigate exposure to thermal extremes and poor thermal tolerance, while over the longer term, developmental plasticity showed that higher developmental temperature generally improves thermal tolerance, but the improvement was not sufficient enough to have a positive ecological impact. This indicates that behavioural thermoregulation will be the primary mode of dealing with unfavourable environmental conditions, at least in Collembola. This behavioural response appears to be limited by body size and morphology, and dependent on the fine-scale structure of the soil matrix. A more elongated body shape was more beneficial in avoiding heat, while species with smaller body width moved more easily through the dry soil with less pore space between the soil particles. These responses and traits beneficial for each response will likely depend on the type of soil, species morphology, and the specific physiological circumstances. Nevertheless, regardless of the soil type, the behavioural response has a limit and might not be sufficient if thermal limits are exceeded in deeper soil layers, which are used as a refuge from the surface temperatures (Chapter 3). Development under simulations of future predictions of the warmer microclimate temperatures demonstrated that the higher developmental temperature does not enhance desiccation resistance in either temperate or tropical Collembola, suggesting that springtails from both climatic regions have equally limited scope for adaptive desiccation resistance (Chapter 4).

Chapters 3 and 4 demonstrate that behavioural thermoregulation will be the primary strategy for survival and persistence of Collembola in the future warming climate since changes in the physiological traits will be either insufficient or non-existent to mediate an increase in the average environmental temperature or the exposure to the climate extremes. Furthermore, they provide novel findings regarding both the thermoregulatory behaviour under extremes in temperate and sub-Antarctic Collembola and the effect of temperature on desiccation resistance in temperate and tropical species. Overall they suggest that much further work needs to be undertaken on behavioural responses to thermal extremes and drought in springtails. While this work is often seen as difficult to do, my work has revealed that it is relatively straightforward to accomplish in a laboratory setting and can reveal remarkable insights. To further establish whether
desiccation resistance will remain unchanged in the future climate for both alien and indigenous species of temperate and tropical Collembola, a broader comparative study using similar methods is needed to confirm this, although the results here suggest that it might be the case.

Overall, the findings of this thesis have important implications for scientific models predicting species responses to climate change, since the choice of rates used in the experiments may underestimate species vulnerability to increasing global temperatures. Results here reinforce the need to choose the ecologically relevant rates of temperature change and daily fluctuations corresponding to the species' microclimate when estimating thermal tolerance. These rates are generally slower than many currently employed in the experimental studies, which may occur in some extreme circumstances and sudden changes in the weather front. In addition, the results here highlight the need for incorporating the behavioural response in the predictive models of species response and vulnerability to climate change.

5.2 References

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