GEOGRAPHIC RANGE AND THE MOUNTAIN NICHE

ECOLOGY, ADAPTATION AND ENVIRONMENTAL CHANGE

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------ PRODUCED ON ARCHIVAL QUALITY PAPER ------

What has roots as nobody sees,
Is taller than trees,
Up, up, up it goes,
And yet never grows?

-- *J.R.R Tolkien, The Hobbit*

Abstract

The geographic range is one of the most fundamental traits of a species. For this reason, understanding the ecological and evolutionary drivers of the size, position, and structure of the range is a key research challenge. The geographic range also has an overriding influence on the environments to which a species is exposed and their spatial and temporal variation. This study addresses four questions relevant to understanding interactions between the environment and individuals, populations, species and communities, with a focus on mountain regions where environmental variation is particularly pronounced.

First, using meta-analysis and a single-genus case study, I explore the relationship between geographic range size and characteristics of the ecological niche. Range size can vary by several orders of magnitude among closely related species, but is strongly and consistently associated with both niche breadth and niche position: the most widespread species tend to be those with a broader niche and/or those that utilise resources that are common across the landscape.

Second, I investigate the relationship between niche and range limits by testing variation in physiological tolerance across environmental gradients in two mountain systems: beetles (Carabidae: *Nebria* Latreille) from the North American Cascade Range and grasshoppers (Acrididae: *Kosciuscola* Sjösted) from the Australian Alps. Whereas the *Nebria*, distributed across a 2000 m elevational gradient, showed almost no variation in thermal tolerance, the *Kosciuscola* showed significant interspecific variation in cold tolerance, consistent with the decrease in average temperature with elevation. I suggest that cold tolerance limits might constrain the upper range edge of at least one species.

Third, I explore how past climate cycles and Australia's dissected mountain landscape have influenced the population structure of an alpine-endemic grasshopper (*Kosciuscola tristis*) using a combination of genetic methods. Despite continuity of alpine habitat during Pleistocene glacial cycles and, by global standards, small-scale disjunctions in the present distribution of these environments, *K. tristis* showed deep lineage divergence associated with geographic breaks in alpine conditions. Fine-scale structure in the absence of clear geographic barriers suggests that habitat heterogeneity might structure populations at a regional scale.

The last component of this work tests the response of alpine invertebrate species and communities to reduced winter snow cover. This is a likely future scenario in the Australian high country, where the winter snowpack is already marginal. I show that Australia has a diverse subnivean arthropod fauna, characterised by the high relative abundance of springtails (Collembola), mites (Acari), spiders (Araneae) and beetles (Coleoptera). Experimental reduction of the winter snowpack caused shifts in community composition, driven by a small number of abundant arthropod taxa. These effects were apparent at a small spatial and temporal scale, with rapid recovery from experimental perturbation in spring.

Mountain ecosystems are threatened by climate change as they are already rare at a landscape scale, are typically fragmented and have limited scope for climate tracking. The work presented here highlights effects of small-scale environmental variation on species traits, genetic structure and communities, which could act to either buffer or exacerbate landscape-scale climatic changes.

Declaration

This is to certify that:

the thesis comprises only my original work towards the PhD except where indicated in the Preface,

due acknowledgement has been made in the text to all other material used,

the thesis is fewer than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Signed: ________________________________

Date: ________________________________

Preface

This thesis contains three studies published in peer-reviewed journals and one study currently under review. These studies appear in the thesis with minor modifications from their published/submitted format. The two additional results chapters are unpublished. Contributions to each chapter are outlined below.

For Chapter 2, the study was designed in collaboration with Jason P Sexton (JPS) and Megan Hirst (MH). The data were collected and analysed by myself, with advice from JPS, Michael D Jennions and Brian S Mautz. The initial draft of the manuscript was written by RAS, and both JPS and MH contributed to subsequent editing and preparation of the manuscript for publication. Of the total work, 60% was performed by RAS and this chapter is published in Ecology Letters (2013), 16, 1104-1114.

Chapter 3 was designed, analysed and written by RAS. Field data were collected by RAS, Sean D Schoville (SDS), and Roman Dudko (RD). SDS, Ary A Hoffmann (AAH) and JPS provided comments on the draft.

For Chapter 4, the study was designed in collaboration with SDS. Field collections were made by RAS, SDS and RD. Physiological data were collected by RAS, molecular data were collected by SDS, and equipment was provided by SDS and Warren Porter. SDS supervised the data analysis and writing of the manuscript. 80% of the work for this chapter was performed by RAS.

In Chapter 5, Michael A Nash (MAN) and AAH assisted in planning the experiments and provided equipment for the experiments. All data were collected and analysed by RAS. MAN and AAH supervised the analysis and writing of the manuscript as a publication. Editing in response to reviewer comments on the initial manuscript was performed by RAS, with comments and advice from MAN and AAH. 90% of the work for this chapter was performed by RAS and this work is currently in press in Ecography.

AAH and MAN assisted in the design, analysis and editing of Chapter 6. Most field collections were made by RAS, with some samples collected by MAN, Yoshinori Endo (YE) and Adam Miller (AM). YE did 25% of the molecular work, which contributed to his Master's thesis. The remaining 75% of the molecular work was done by RAS. The data analysis was done by RAS, with advice from YE, Phillippa Griffin, AAH, AM and

Kate DL Umbers (KDLU). YE, AAH, AM, MAN and KDLU provided input for the writing up of this chapter as a paper. Editing and re-analysis in response to reviewer comments on the initial manuscript were performed by RAS, with comments and advice from AAH and KDLU. RAS performed at least 70% of the work on this chapter, which is published in BMC Evolutionary Biology (2014), 14: 204.

The work in Chapter 7 was designed by RAS with significant input from AAH and MAN. Snow clearing fieldwork was carried out by RAS in 2013 and 2015 and by Anthony J Slatyer in 2014. Pitfall trap catches were sorted and counted by RAS. Family-level identification of invertebrates was done by RAS, with assistance from Penelope Greenslade and MAN. Data analysis and writing were performed by RAS, with advice and comments from AAH and MAN. The compilation of studies included in Appendix F was completed in collaboration with Veronica Briceño. 90% of the work for this chapter was performed by RAS. This chapter will be prepared for publication during the examination of the thesis.

Acknowledgements

A huge number of people have helped out with this project, by catching grasshoppers and beetles, shovelling snow, caring for baby grasshoppers, and providing advice, guidance, emotional support, laughter, and constructive criticism when and where it was most needed. Some parts of the project would simply not have been possible without them, while others would have been much the poorer.

First, I would like to thank my supervisors, Ary Hoffmann and Michael Nash, for their patience and guidance over the last 3½ years, and for giving me the freedom to pursue many different areas of interest. I am also thankful for their support when I disappeared for a week or two (or four) to go sailing. I would like to thank Sean Schoville, who welcomed me into his lab and provided me with an opportunity to experience "real" mountains in the Pacific Northwest. I am most grateful for the opportunity I had to become acquainted with a whole new part of the alpine world – nocturnal snowfield specialists – and many good times were had hiking up mountains, sliding down them, eating cookies, playing cards and camping in some incredible places. Sean has been a fantastic mentor over the last year and half, and two chapters of this thesis would not have existed without his support. I also thank my advisory committee of Mick Keough, Laura Parry and Ben Phillips for their guidance and encouragement along the way.

Kate Umbers, Nik Tatarnic, and Jason Sexton have been invaluable sources of advice and encouragement since I started this PhD, and I'm not sure I would have finished without them. We also got to enjoy many fun mountain field trips together – in the snow and rain and sunshine, searching for grasshoppers, planting seeds, and discussing grand plans for future research.

Throughout my fieldwork, I was assisted by people who cheerfully caught grasshoppers, shovelled snow and traipsed through the mountains with me. So, to Polly Adams, Eileen Baker, Nick Barnard, Leo Barnard, Milly Brent, Ron Brent, Shane Denecke, Julian Drummond, John Giacon, Maggie Haines, Sam Hoare, Martina Hoffmann, Hanna Kokko, Andrew Laurie, Suzi Macbeth, Alex Mraz, Pierre-Do Putallez, Tom Shafron, Harry Slatyer, Tony Slatyer, Robyn Slatyer, Evan Slatyer, Nick Ward and Mira Wawn – thank you! Thanks

also to Roman Dudko for patiently helping me identify beetles, providing great company in the field, and teaching me the awesome Russian card game appropriately titled "Fool". This last field season has been made considerably less strenuous and infinitely more fun by David McCoombe, Anthony Bock and Vincent Antony, who gave me tips on the best places to ski and many lifts up the mountain.

Thanks to the Hoffmann lab, my office buddies and my fellow PhD students, particularly Ian Smith, Rebecca Jordan, Ginger Yeh, Heng Yeap, Yoshinori Endo and Perran Ross with whom many a dessert was shared. Emily Thomson has provided invaluable assistance with logistics and paperwork navigation for which I am most grateful. This project would have been a lot more difficult without her help. To Warren Porter, Mehdi Kabbage, Aurelie Rakotondrafara, the Schoville lab, and others in the UW Entomology Department – thanks for being so welcoming to this vagrant Australian. A big thank you also to Hojun Song and his team, whose combined passion for all things Orthopteran is truly inspiring. I think I learnt more about grasshoppers in a week in Florida than I did in the rest of my PhD.

No acknowledgements would be complete without thanking my family. The Australian mountains have been a part of my life for as long as I can remember. They hold a special place in my heart and I have my parents to thank for that. I'm sure they didn't realise that, when giving me the task of clearing snow from the ski lodge balcony as a child, they were training me for my PhD! Since I started this project, my family have been not only a pillar of support through the many ups and downs, but also provided invaluable practical assistance by helping out in the field, designing and building pieces of equipment for me, and proof-reading my thesis.

Last, but by no means least, I would like to acknowledge the funding and in-kind support provided by a great many organisations: the Holsworth Wildlife Research Endowment, the Ecological Society of Australia, the Orthopterists' Society, the Victorian Environmental Assessment Council, the Norman Wettenhall Foundation, the Georgina Sweet Award, the National Geographic Society, the Mountain Adventure Centre Jindabyne, Mt Buller Resort, Thredbo Sports, the Bio21 Institute, the Melbourne Sustainable Societies Institute, and the University of Melbourne. I would also like to acknowledge the Traditional Owners of the lands within Australian alpine region, where much of this project was undertaken: the Bidawal, Monero-Ngarigo, Gunaikurnai, Jaithmathang, Taungurung, Mitambuta, Ngarigu-Currawong, Dhudhuroa, Waywurru, Wurundjeri, Walgal, and Ngunnawal Peoples.

Dedicated to the memory of my grandfather Ralph Slatyer and my grandmother Marjorie Lucas

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Chapter 1

Introduction

Every species occupies a defined geographic space and variation in the distribution of species at local, regional and global scales is a puzzle central to the study of ecology and evolution. This thesis is an exploration of the ecological factors contributing to the current distributions of species and the implications of a particular geographic distribution for adaptation and exposure to past and future environmental change. The work focuses on mountain regions, where the interplay between organisms and their environment is thrown into stark relief.

1.1 Geographic range

Likened to "a grand game of chess with the world for a board" by Charles Darwin (Darwin 1975, p528), the geographic range describes where a species occurs and, by extension, where it does not. In turn, the structure of the range describes the size, shape (including the degree of continuity) and position of this area in geographic space (Brown *et al.* 1996; Gaston 2003; Sexton *et al.* 2009).

The geographic range can be considered from two broad perspectives. First, the geographic range can be taken as a species trait – a product of micro- and macroevolutionary processes, ecological constraints and historical contingency. From this perspective, research has focused on determining what factors control the range size and position of individual species, and the large scale patterns of biodiversity, abundance and distribution that reflect the overlaid range structures of individual taxa (MacArthur 1972; Brown 1984; Brown & Maurer 1987; Gaston 2003).

Second, the geographic range can be viewed as one of the key factors determining the abiotic conditions to which a species is currently exposed and how these conditions have changed (and will change) over time. In this context, where a species occurs and the size and continuity of its distribution are likely to influence the selective forces shaping adaptation, the spatial arrangement of genetic lineages and patterns of contemporary population structure (Avise *et al.* 1987; Lenormand 2002; Kawecki & Ebert 2004; Yeaman & Otto 2011); range position also has an overriding influence on the exposure of species to natural and anthropogenic stressors (Williams *et al.* 2008).

This thesis considers the geographic range from both of these perspectives: as it relates to macroecological patterns, adaptive variation, population structure and exposure to environmental change.

1.2 The ecological niche

The geographic range of a given species is shaped by both ecological and evolutionary processes, which define the places where it is able to maintain viable populations and those where conditions are unfavourable (Macarthur 1972, Brown 1996). Thus studies of distributions are intrinsically entwined with the concept of the ecological niche. The distribution is first constrained by the environmental resources required for population persistence (Grinnell 1917; Hutchinson 1957). These resources define the *n*-dimensional fundamental niche (Fig. 1.1; Hutchinson 1957).

At its most basic level, the fundamental niche comprises the physiological requirements of a species (Hutchinson 1957). Physiological tolerances will determine where a species can or cannot occur via interactions with the environment; geographic distributions are thus ultimately shaped by the availability of appropriate environmental conditions (Grinnell 1917; Hutchinson 1957; Jackson & Overpeck 2000; Schulte *et al.* 2011). Among these environmental conditions, temperature is considered to play a particularly important role through its cascading effects on individual fitness and population dynamics (Merriam 1894; Grinnell 1917; Hutchins 1947; Jeffree & Jeffree 1994; Bozinovic *et al.* 2011). For this reason, much of the work that follows focuses on the thermal environment and its effects on individuals and communities.

Hutchinson's original formulation of the *n*-dimensional niche concept implicitly allowed for all possible combinations of environments (along different axes) being potentially available (Hutchinson 1957; Jackson & Overpeck 2000; Fig. 1.1). In the real world, however, this is not the case as many niche variables co-vary (Jackson & Overpeck 2000). Jackson and Overpeck (2000) therefore defined a second constraint on the distribution of species that reflects the availability of required resources across the landscape (Fig. 1.1). This is the potential niche (Jackson & Overpeck 2000). A final constraint is placed on where species occur by biotic interactions and this subset – what we observe as a species' geographic range – represents the realised niche (Hutchinson 1957). Notably, the potential and realised niche limits are most likely to coincide in conditions of high abiotic stress and low productivity, and these conditions prevail in high-elevation mountain environments (Dobzhansky 1950; MacArthur 1972; Brown *et al.* 1996; Callaway *et al.* 2002; Maestre *et al.* 2009; Normand *et al.* 2009; Hargreaves *et al.* 2014).

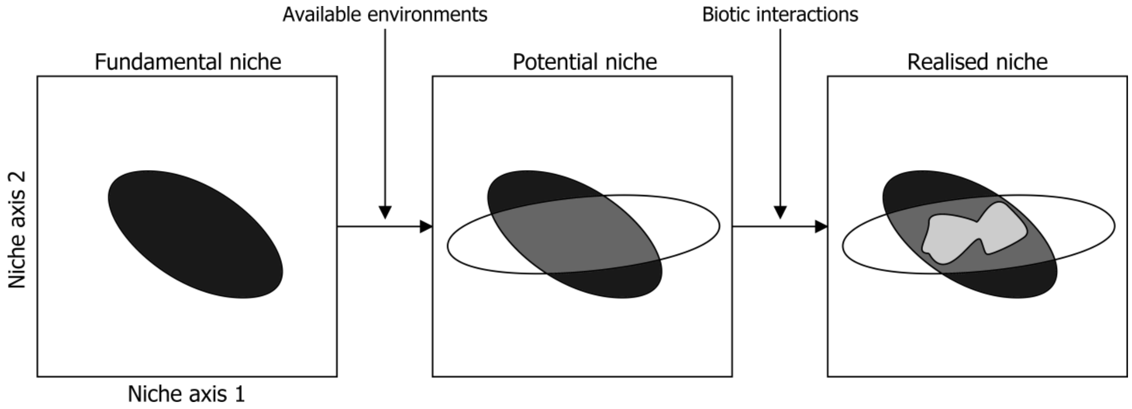

Figure 1.1. The ecological niche along two axes. Only some particular combinations of the two variables are present at any point in time, constraining the fundamental niche to a subset of its theoretical space (the potential niche). Biotic interactions (e.g. competition) place further constraints on the areas in which a species can persist, and this represents the realised niche. Figure modified from Jackson and Overpeck (2000).

1.3 The mountain niche

High-mountain environments cover roughly 5% of the Earth's terrestrial land area outside of Antarctica and are the only terrestrial biogeographic unit with a global distribution (Körner 2003, 2011). For their area, mountains also host a disproportionately high biodiversity and endemic species richness (Mani 1968; Myers *et al.* 2000; Crisp *et al.* 2001; Körner & Spehn 2002; Körner & Paulsen 2004). Mountains represent a dynamic physical and climatic landscape over evolutionary and ecological timescales and sustain a diverse array of environments within a small geographic area. These features mark mountain regions as ideal natural laboratories for studying the interaction between species and their environment. In the following sections, I highlight three research areas in which mountain ecosystems provide particularly compelling case studies, and which will be the focus of this thesis.

First, environmental clines on mountains parallel latitudinal clines but over much smaller spatial scales and without the confounding effects of changing seasonality and day length that are associated with the latter (Hopkins 1920). Across the relatively small spatial scale of mountain environmental gradients, ecological traits are the most likely determinants of the position of populations within the gradient, with factors such as historical contingency, stochasticity, and dispersal ability likely to play a much smaller role (Cahill *et al.* 2014; Hargreaves *et al.* 2014). The small spatial scale of environmental variation on mountains is also important when considering the ecological scale at which macroecological patterns can be tested. Often, the appropriate ecological scale at which a pattern should apply and be tested – intra- or inter-specific – is unclear, and the extent to which patterns can be extrapolated across scales has rarely been tested (Chown 2001; Gaston *et al.* 2009). To test intra-specific patterns across latitudes requires species with large latitudinal ranges, a problem articulated by Chown and Gaston (2010). These species are a minority (Gaston & Chown 1999b; Gaston & Blackburn 2000). Studies of latitudinal variation thus, by necessity, limit either (a) the scope of the question (inter-specific comparisons), or (b) the scope of the taxa ("common" species with large geographic distributions). On mountains, although most species still tend to occupy a narrow elevational range (Mani 1968), populations and species are distributed across more comparable spatial scales, enabling simultaneous studies of inter- and intra-specific variation.

Second, a combination of a dynamic climatic history and complex contemporary topography have made mountain regions rich contexts for studies of the genetic consequences of environmental change and ecological barriers to dispersal. Much of this research has focused on population dynamics during Pleistocene glacial cycles, where alpine environments were repeatedly isolated and re-connected (e.g. Hewitt 1999, 2000; Knowles 2000; Knowles 2001; Schoville & Roderick 2009; Schoville *et al.* 2012). Whereas low-elevation taxa are likely to have become isolated during glacial (cold) periods, alpine taxa might have expanded their ranges during these times with populations becoming more contiguous (DeChaine & Martin 2004). During interglacial (warm) periods, alpine taxa would have retreated to higher elevations, becoming more isolated and promoting divergence and speciation (DeChaine & Martin 2004). As we now live in an interglacial period, from this perspective, alpine taxa currently occupy their climatic refugia.

A third area which has, and continues to be, a critical focus of mountain research is the response of these ecosystems to anthropogenic climate change. Over the last 50 years, the global average surface temperatures have increased by 0.7°C a phenomenon known broadly as "global warming" (Stocker *et al.* 2013). The impacts of global warming are likely to be unevenly distributed across ecosystems, due to geographic variation in the rate and magnitude of change as well as intrinsic characteristics of species and ecosystems (Deutsch *et al.* 2008; Sheldon *et al.* 2011; Gunderson & Leal 2012; Huey *et al.* 2012). Mountain ecosystems are particularly at risk: warming is occurring more rapidly at high elevations than in lowland areas (Beniston *et al.* 1997; Diaz & Bradley 1997; Kotlarski *et al.* 2012; Kotlarski *et al.* 2015), changes in temperature and precipitation regimes are expected to be discordant (McCain & Colewell 2011) and habitat area loss is predicted to be disproportionately high (Dirnböck et al. 2011). Alpine regions typically contain a high proportion of endemic, cold-adapted species whose capacity to respond to climatic change is largely unknown (Bale and Hayward 2010). However, "thermophilisation" of high-mountain vegetation in Europe, where cold-adapted species in mountain-top communities are being replaced by warm-adapted species from lower-elevation areas, indicates that erosion of specialist alpine communities is already occurring (Gottfried *et al.* 2012).

1.3.1 Mountain life zones

The mountain life zone concept was first formalised by Alexander von Humboldt, in his 1805 "Essay on the Geography of Plants" (von Humboldt 1805). Here, von Humboldt described transitions from lowland forest to high-elevation grassland, devoid of trees. This concept has proven invaluable for comparative studies of mountain biota, as it allows for the lowering of isotherms towards higher latitudes. Leslie Holdridge later developed a classification system based on temperature, precipitation, and elevation (Holdridge 1967), thus defining life zones based solely on climate. Holdridge's classification adopted the terms of "montane", "sub-alpine", "alpine" and "nival". Most recently, Körner (2011) defined seven zones based on thermal characteristics of the growing season, the presence or absence of frost, and the position of the high-elevation climatic treeline – the limit of tall, upright tree growth (Wardle 1974).

In the work that follows, I have adopted the life zone terminology used by Holdridge (Holdridge 1967) and, where applicable, the definitions of Costin (1957) (Fig. 1.2). Costin (1957) described ecological transitions in Australia's high country using both abiotic and biotic features to delineate successive life zones. According to this classification, the montane zone receives sporadic snow that persists for less than one month per year and grades gradually into lowland vegetation. The winter snowline, above which there is continuous snow cover for more than one month per year, defines the transition from the montane to the sub-alpine zone. Above this, the treeline marks the beginning of the alpine zone. In most parts of the world, the treeline corresponds to the 6 – 7°C isotherm of the mean growing season temperature (Körner & Paulsen 2004). At the highest elevations, the nival zone is defined by continuous (year-round) snow cover. A nival zone is not present in Australia, but covers a large proportion of the mountain area in the Pacific Northwest, USA, described below. Based on these definitions, snow forms a critical component of the ecology of the sub-alpine, alpine and nival zones, which collectively can be termed "alpine regions". Although classification of life zones is problematic (Mani 1968), it is useful for the purposes of the studies that follow in describing the general structure of the environment (e.g. the presence/absence of trees or snow).

1.3.2 Mountain environments

Mountain environments are perhaps best characterised by their variability. Steep environmental gradients, correlated with elevation, include changes in temperature, solar radiation, humidity, and snow cover (Whiteman 2000; Körner 2003). Environmental variation is also manifest at much smaller scales – metres to millimetres – which can negate, or even reverse, clines in atmospheric conditions (Jennings 1979; Geiger *et al.* 1995; Scherrer & Körner 2010; Wundram *et al.* 2010). Further, de-coupling of atmospheric and near-ground climatic conditions tends to increase with elevation (Körner *et al.* 1983; Körner 2003) and during the day, when the effects of solar radiation can overwhelm atmospheric temperature gradients (Scherrer & Körner 2010). Mountain environments also show pronounced temporal variation. Tropical mountains, for example, are known for remarkable diurnal temperature fluctuations, which can be 10 times greater than seasonal variation (Mani 1968; Sarmiento 1986). In contrast, temperate mountain environments are characterised by strong seasonality, the effects of which are mediated (for near-ground microclimates) by winter snow cover (Mani 1968; Geiger *et al.* 1995).

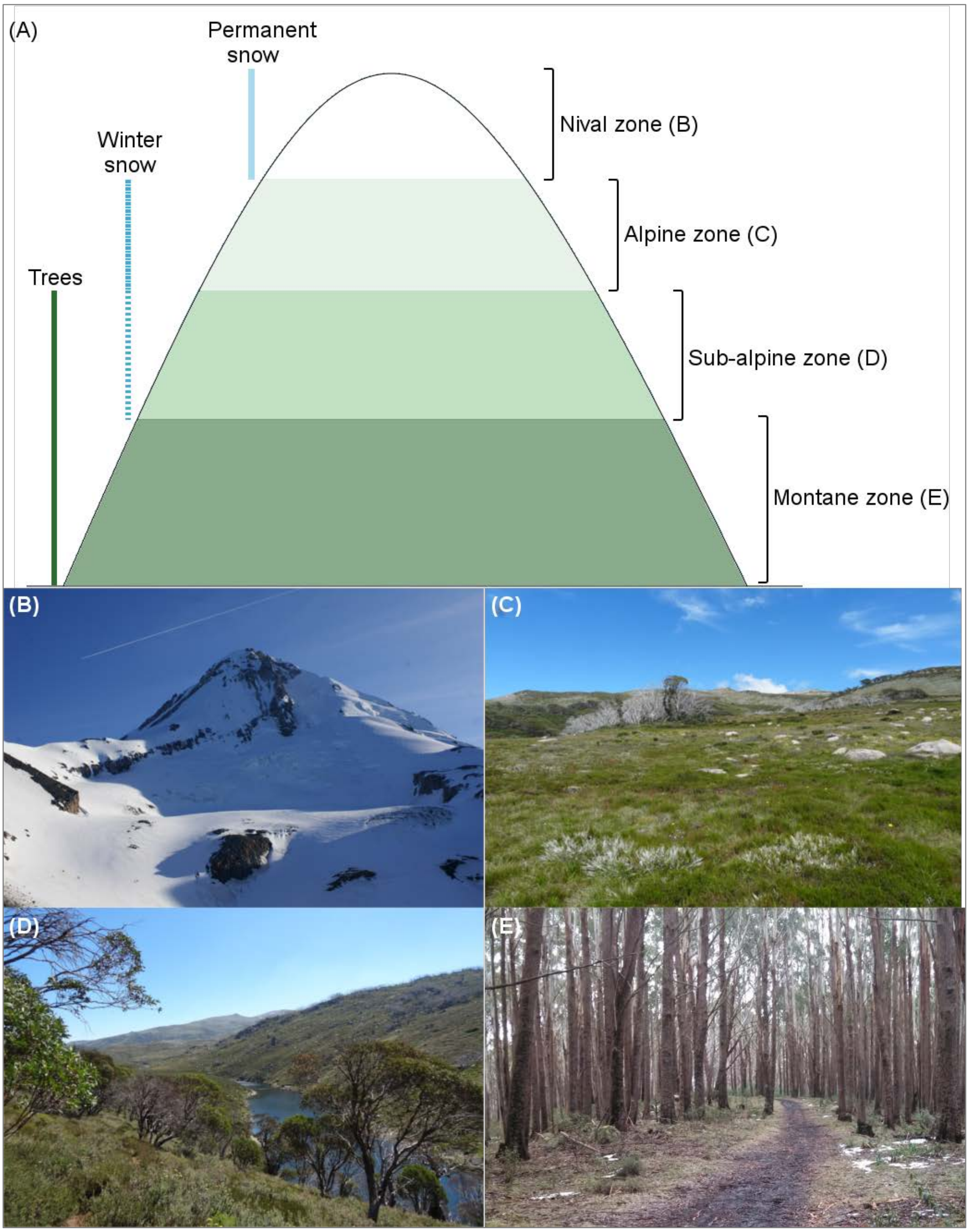

Figure 1.2. Mountain life zones. (A) Life zones are defined by the presence/absence of trees and the presence/persistence of snow. The nival zone (B) has permanent snow cover; below this, the alpine zone (C) is characterised by seasonal snow cover and an absence of trees; the sub-alpine zone (D) extends from the snow line to the high-elevation treeline; montane forest (E) occurs below the winter snowline. Images are representative of the respective zones in the Australian Alps, with the exception of (B) which is from the Cascade Range (Australia has no nival zone).

1.3.2.1 Temperature and radiation

Globally, the dry air temperature lapse rate (the decrease in temperature with elevation) averages 6°Ckm^{-1} (Dillon *et al.* 2006). On steep mountain sides, this means that locations just a few hundred metres apart can experience several degrees difference in temperature. Lapse rates also vary geographically. In the Snowy Mountains of Australia, for example, Slatyer (1978) estimated mean minimum and maximum temperature lapse rates of 2.1 and 9.7°Ckm^{-1}, respectively. On Mt Rainier, USA, the corresponding lapse rates are 3.9°Ckm^{-1} (minimum temperature) and 5.6°Ckm^{-1} (maximum temperature) (Minder *et al.* 2010).

Macroclimatic trends are mediated by features such as topography, aspect and circulation patterns. These produce regional and local deviations from global macroclimatic patterns and considerable climatic variation over scales of a few kilometres down to a few millimetres (Mani 1968; Davidson & Reid 1985; Geiger *et al.* 1995; Körner 2003; Wundram *et al.* 2010; Scherrer & Körner 2011). This small-scale variation often has noticeable impacts on vegetation communities. For example, the temperature at a given site within a mountain depends on both its elevation and its exposure to solar radiation (Whiteman 2000). As a consequence, the elevation of the treeline commonly varies with aspect, being higher on warmer, north-facing slopes in the southern hemisphere and south-facing slopes in the northern hemisphere (Körner 2003). Similarly, downslope flow of cool air into valleys can produce temperature inversions, or "frost hollows"; associated inversions in the treeline result in treeless valleys with "alpine" vegetation, surrounded by wooded slopes (Costin 1954; Moore & Williams 1976; Jennings 1979; Davidson & Reid 1985; Whiteman 2000). When mountain chains run perpendicular to prevailing winds, cooling of the air as it is uplifted over the mountain produces condensation on the windward side of the mountain and a rain shadow on the leeward side (Whiteman 2000). As a result, vegetation communities on the windward and leeward sides of a mountain can also differ markedly (Franklin *et al.* 1988; Costin *et al.* 2000).

Clear sky solar radiation increases with elevation, as there is less depletion through atmospheric absorption and scattering (Whiteman 2000). As a result, microclimatic temperature variability also tends to increase with elevation, producing large temperature differences between the air and the ground (e.g. up to 50°C), between areas in the sun and those in the shade (e.g. see Chapter 5), and rapid temperature fluctuations due to changes in cloud cover (e.g. 10°C in a few seconds) (Mani 1968; Geiger *et al.* 1995; Scherrer & Körner 2010). An organism's exposure to (and ability to utilise) such variation will depend on its size, habitat use and behaviour (Huey 1974; Stevenson 1985; Kearney *et al.* 2009;

Sunday *et al.* 2014; Schoville *et al.* 2015). Diurnal insects, for example, can elevate their body temperature to much higher than ambient temperature by moving to warm microhabitats – an option unavailable to nocturnal species (Kearney *et al.* 2009; Buckley *et al.* 2013).

1.3.2.2 Snow

Arguably the most significant feature in the ecology of mountain ecosystems is snow (Billings & Mooney 1968; Mani 1968). Mani (1968) considered that snow was "absolutely indispensable for the high altitude insects", because of its ameliorating effects on atmospheric temperature fluctuations and aridity, its contribution to soil moisture, and its use as a foraging ground for nival arthropods. Snow has a low thermal conductivity and Pruitt (1970) estimated that a depth of approximately 20 cm was needed to effectively insulate the space beneath the snow pack – the "subnivean space" – from diel fluctuations of ambient temperature. The snow layer is thus an ecotone between the warm, humid and thermally constant subnivean space and the cold, dry and fluctuating ambient air (Aitchison 2001).

For plants and small animals, the presence of winter snow means a change from the high diurnal and microclimate variability of the snow-free months to a stable and homogenous winter climate (Mani 1968, Dillon et al. 2006). As a consequence, many alpine mammals rely on the snowpack for overwinter survival (Halpin & Bissonette 1988; Gese *et al.* 1996; Korslund & Steen 2006), late-lying snow produces distinct vegetation communities (Bliss 1956; Billings & Bliss 1959; Williams & Ashton 1987; Edmonds *et al.* 2006; Björk & Molau 2007; Williams *et al.* 2015) and many small invertebrates utilise the subnivean space or the snow layer itself for winter activity (Mani 1968; Leinaas 1981; Hågvar 2010).

1.3.3 Mountain landscapes

The topographic complexity of mountain landscapes, where peaks are separated by lower-elevation regions, means that the elevational range of a species has a direct impact on the geographic connectivity of its habitat: the distance between equal-elevation points generally increases with elevation. For high-elevation species, valleys that extend below their lower range edge can act as dispersal barriers because fitness is lower in these environments (due to unfavourable abiotic conditions or biotic interactions) (MacArthur 1972; Bech *et al.* 2009). Similarly, mountain peaks can limit dispersal for lower-elevation species (e.g. Liggins *et al.* 2008). Alpine regions have thus frequently been described as "sky islands". These islands, geographically separated by low-elevation "seas", have a more dynamic history than oceanic islands, with past glacial and inter-glacial periods causing repeated division, reconnection and re-colonisation of mountain tops (Dillon *et al.* 2006). Global warming is expected to drive upslope shifts (to track climates through space) in the distribution of species and increasing isolation of populations on different mountain peaks (Parmesan & Yohe 2003; Beckage *et al.* 2008; Rubidge *et al.* 2012; Bässler *et al.* 2013). These distribution shifts can quickly fragment suitable habitat, with populations becoming restricted to shrinking habitat islands (Roland *et al.* 2000; Rubidge *et al.* 2012).

1.3.4 Mountains in a changing climate

Species can respond to environmental change over short to long time-scales via behaviour, phenotypic plasticity, adaptive evolution or distribution shifts. Behavioural thermoregulation can allow individuals to avoid short-term stress in heterogeneous habitats (Huey *et al.* 1977; Grant & Dunham 1988; Kearney *et al.* 2009; Sunday *et al.* 2014). Mountain environments are characterised by high microclimatic heterogeneity. Scherrer and Körner (2010) note that this heterogeneity is such that temperature variation at the scale of metres is considerably greater than predicted increases in atmospheric temperature due to global warming (i.e. 1.5 – 2°C by 2080 – 2100, relative to 1900)(Collins *et al.* 2013). These temperature mosaics, which are driven by microtopographic variation, might thus provide thermal refugia for small organisms such as insects (Scherrer & Körner 2010).

Changing environmental conditions can induce plastic and/or genetic shifts in species traits. Individual phenotypes are determined by the interaction between genotypes and the environment, and phenotypic plasticity allows a single genotype to express multiple phenotypes under different environmental conditions (Bradshaw 1965; West-Eberhard 2003). Plasticity can be reversible (e.g. acclimation: Chown & Terblanche 2007) or non-reversible (e.g. developmental plasticity: Smith-Gill 1983) but, as it is non-genetic, occurs within a single generation and can thus facilitate tolerance to rapidly changing environmental conditions (Spicer & Gaston 1999; Jump & Peñuelas 2005). Genetic (evolutionary) adaptation, by contrast, arises from natural selection on genetic traits. It thus requires multiple generations and will therefore occur more slowly than plastic responses (Hoffmann & Sgrò 2011). Nevertheless, adaptive responses to rapid environmental change have been documented (Franks *et al.* 2007; Hoffmann & Sgrò 2011; Karell *et al.* 2011; Juha 2012), and existing local adaptation in populations at range margins means that genotypes able to cope with changing climatic conditions might already exist (Moritz *et al.* 2012).

A final option available to species – worthy of particular consideration in the context of mountains – is a distribution shift. Under global warming, species with sufficient dispersal ability and geographic opportunity are expected to track their climatic niche towards high latitudes and elevations (i.e. cooler conditions) (La Sorte & Jetz 2010), and there is already substantial evidence for such shifts (e.g. Walther *et al.* 2002; Parmesan & Yohe 2003; Le Roux & McGeoch 2008; Chen *et al.* 2009; Chen *et al.* 2011; Bässler *et al.* 2013; Menéndez *et al.* 2014). High-elevation species are, however, physically limited in their opportunity to track their climatic niche through space – their distribution cannot extend above the elevation of the mountain (McCain & Colewell 2011). Movement between mountains (i.e. to track climate geographically) is also likely to be hindered by limited dispersal ability (see below) and the island-like nature of many mountain systems.

1.3.5 Mountain arthropods

As far as currently known, the upper limit of arthropod life extends to an elevation of 6700 m – a record held by the Salticid spider *Euophrys omnisupersetes* (Wanless 1975). Collembola (springtails), Acari (mites) and Araneae (spiders) are ubiquitous in mountain regions globally. Insects are also common, particularly the Plecoptera (stoneflies), Coleoptera (beetles), Lepidoptera (moths & butterflies), and Diptera (flies). Between the lower edges of the montane forests and high-elevation alpine and nival habitats, clines in abiotic conditions, as discussed above, are associated with variation in life history traits, morphology, physiology, and behaviour that are observed both among and within species.

1.3.5.1 Life history

Insects typically have long life cycles in cold climates. For example, species in the cold-specialist order Grylloblattodea (ice crawlers) are estimated to live for 5 – 10 years (Walker 1937; Nagashima *et al.* 1982; Visscher *et al.* 1982). Hodkinson (2005) reviewed life history adaptations in insects (and Collembola) along elevational gradients and noted six distinct strategies that could be adopted in response to colder temperatures and a shorter growing season towards higher elevations: (a) fewer instars (Alexander & Hilliard 1964; Berner & Blanckenhorn 2006); (b) fewer generations (Randall *et al.* 1981); (c) a multi-year life cycle (Green 1983); (d) lowered temperature thresholds for development (Hill & Hodkinson 1995); (e) accelerated development and/or growth (Dingle & Mousseau 1994; Berner *et al.* 2004; Berner & Blanckenhorn 2006; Karl & Fischer 2009; Minards *et al.* 2014); and (f) shorter diapause at lower temperatures (Hodkinson 2005). Among grasshoppers, examples of simultaneous shifts in the number of eggs within a clutch (more at higher elevations) and the number of clutches (fewer at higher elevations) also point to constraining effects of season length on life history traits (Dearn 1977).

1.3.5.2 Morphology

Patterns of geographic variation in body size were first described by Carl Bergmann some 150 years ago (Bergmann 1847, in Blackburn et al. 1999). "Bergmann's rule" was formulated (and recently re-defined) for endotherms and describes a pattern of larger body size (mass) with higher latitude (Blackburn *et al.* 1999). This rule also appears applicable to ectotherms across latitudinal gradients (Blanckenhorn & Dermont 2004; Chown & Gaston 2010). Among mountain insects, there is considerable variation in the direction of the body size cline with elevation (Shelomi 2012). Increases in body size with elevation are thought to result from a negative relationship between developmental temperature and body size, and are particularly prevalent among the Orthoptera and Coleoptera; the opposite pattern – with smaller individuals at higher elevations – might result from resource limitation (Mayr 1956; James 1970; Chown & Klok 2003; Shelomi 2012).

Many mountain insects show some degree of wing reduction (brachyptery) (Mani 1968; Hodkinson 2005). In the Northwest Himalaya, for example, nearly 50% of the high-elevation insects are wingless (Mani 1962). Within species, wing reduction is observed as an increasing proportion of wingless individuals (e.g. Dearn 1978). Wing reduction can also be observed as a community effect, with an increasing proportion of brachypterous species towards higher elevations (Darlington 1943; Kavanaugh 1985). The most commonly evoked driver of brachyptery is habitat stability, while habitat age, energetic constraints and costs of dispersal have also been suggested (Roff 1990; Zera & Denno 1997). One consequence of brachyptery is that the effects of habitat patchiness (across the mountain landscape) might be enhanced (Bohonak 1999).

An increase in melanism in higher elevation populations is a commonly-observed phenomenon, particularly among the Lepidoptera (Mani 1968; Watt 1968; Kingsolver 1983; Guppy 1986; Sømme 1989; Ellers & Boggs 2002). The ecological importance of increasing melanism is much-debated with suggested adaptive significance for desiccation resistance (darker individuals have lower desiccation rates) (Parkash *et al.* 2008a; Parkash *et al.* 2008b; Harris *et al.* 2013; King & Sinclair 2015), protection from damage induced by ultra-violet radiation (Mani 1968; Bestide *et al.* 2014), immune function (melanin pigments are important cuticle components) (Dubovskiy *et al.* 2013), and thermal biology (dark individuals warm up more quickly and achieve higher body temperatures) (Clusella-Trullas *et al.* 2007).

1.3.5.3 Thermal physiology

Temperature is critical for the ecology of ectotherms, as their limited ability to regulate body temperature means that fitness is intrinsically linked to the thermal environment (Huey & Stevenson 1979; Chown & Nicolson 2004; Angilletta 2009). Hence, the overwhelming majority of studies on physiological trait variation in mountain environments concern thermal limits to activity. It should be noted, however, that this number still falls far short of the studies that have tested for comparable latitudinal patterns (Spicer & Gaston 1999; Addo-Bediako *et al.* 2000; Sunday *et al.* 2011; Hoffmann *et al.* 2013). These latitudinal studies have detected idiosyncratic results for different species and taxa. Although some studies have found strong clinal variation in physiological limits and preferences (Gaston & Chown 1999a; Buse *et al.* 2001; Karl *et al.* 2008), others, on different taxa, have found weak, absent or even reversed clines in physiological traits (Ashby 1998; Buckley *et al.* 2013; Buckley *et al.* 2014; Schoville *et al.* 2015). Physiological variation will be discussed in greater depth in later chapters.

1.4 An introduction to two mountain systems

The work that follows in this thesis focuses on two mountain systems: the Cascade Range of North America, and the Australian Alps. These ranges present contrasting geological histories, contemporary climates and faunal assemblages.

1.4.1 The Cascade Range

1.4.1.1 History and environment

The "Cascades" provide the backdrop for many classical studies in mountain ecology and biogeography (e.g. Merriam 1899; Edwards 1986, 1987). Stretching 1100 km along the northwest coast of North America, the range comprises a series of isolated volcanic peaks at its southern end, progressing to continuous mountains in the north (Fig. 1.3). At their southern extremity, the Cascades are roughly 50 km wide and most peaks are relatively low (1200 – 1500 m a.s.l); the range expands both in width and height towards the north, where many peaks stand over 2400 m high (Mackin & Cary 1965). Mt Rainier, at 4392 m, is the highest mountain in the range. The Cascades are part of the vast North American Cordillera which extends some 6900 km from the northwest corner of Alaska to the Isthmus of Panama (Fenneman 1931).

By geological standards, the Cascades are young mountains. Major uplift of the range did not occur until 5 – 4 million years ago (Ma) (Mackin & Cary 1965; Cheney & Hayman

2007; Mustoe & Leopold 2014). This uplift produced what is now one of the strongest rain shadows in the world, with the range acting as a barrier to the prevailing westerly winds coming off the Pacific Ocean (Siler *et al.* 2013). As a consequence of this rain shadow, the western slopes of the Cascades are wet and montane/sub-alpine forests are dominated by firs, while the eastern slopes support dry pine forests and grassland (Franklin *et al.* 1988). As both a physical and climatic barrier, the Cascades mark a major phylogeographic boundary in the North American northwest (reviewed: Shafer *et al.* 2010).

High-elevation areas of the Cascades were heavily glaciated during the Pleistocene, with the last glacial advance occurring roughly 12500 years ago (Porter & Swanson 2008; Osborn *et al.* 2012). However, the dynamic geologic history of the region continues. In the last 10000 years, the Cascades have experienced both renewed glacial expansion ("neoglaciation") (Davies 1988; Alt & Hyndman 1995) and considerable volcanic activity (Harris 1988). Today, the Cascades region contains roughly 80% of the glaciated land area in the contiguous USA, though many glaciers are retreating rapidly (Jackson & Fountain 2007; Fountain *et al.* 2014; Marcinkowski & Peterson 2015). At the same time, many of the volcanoes are still active (Alt & Hyndman 1995) – the Mt St Helens eruption of 1980 is a notable example.

The Cascades mountains have a maritime climate with wet, mild winters and cool, relatively dry summers (Franklin & Dyrness 1988). The snowpack is characteristically deep – St John and Warren (1937) report an annual snowfall of 20 m in the winter of 1932-33 in the Paradise Valley on Mt Rainier! The snowpack is also highly variable, tied closely to regional climate cycles more so than to global climate trends (Stoelinga *et al.* 2010; Abatzoglou 2011; Johnstone & Mantua 2014; Trujillo & Molotch 2014). The Cascades have a notably narrow alpine zone with permanent snow and ice extending to relatively low elevations (e.g. ~ 2000 m on Mt Rainier) (Franklin & Dyrness 1988).

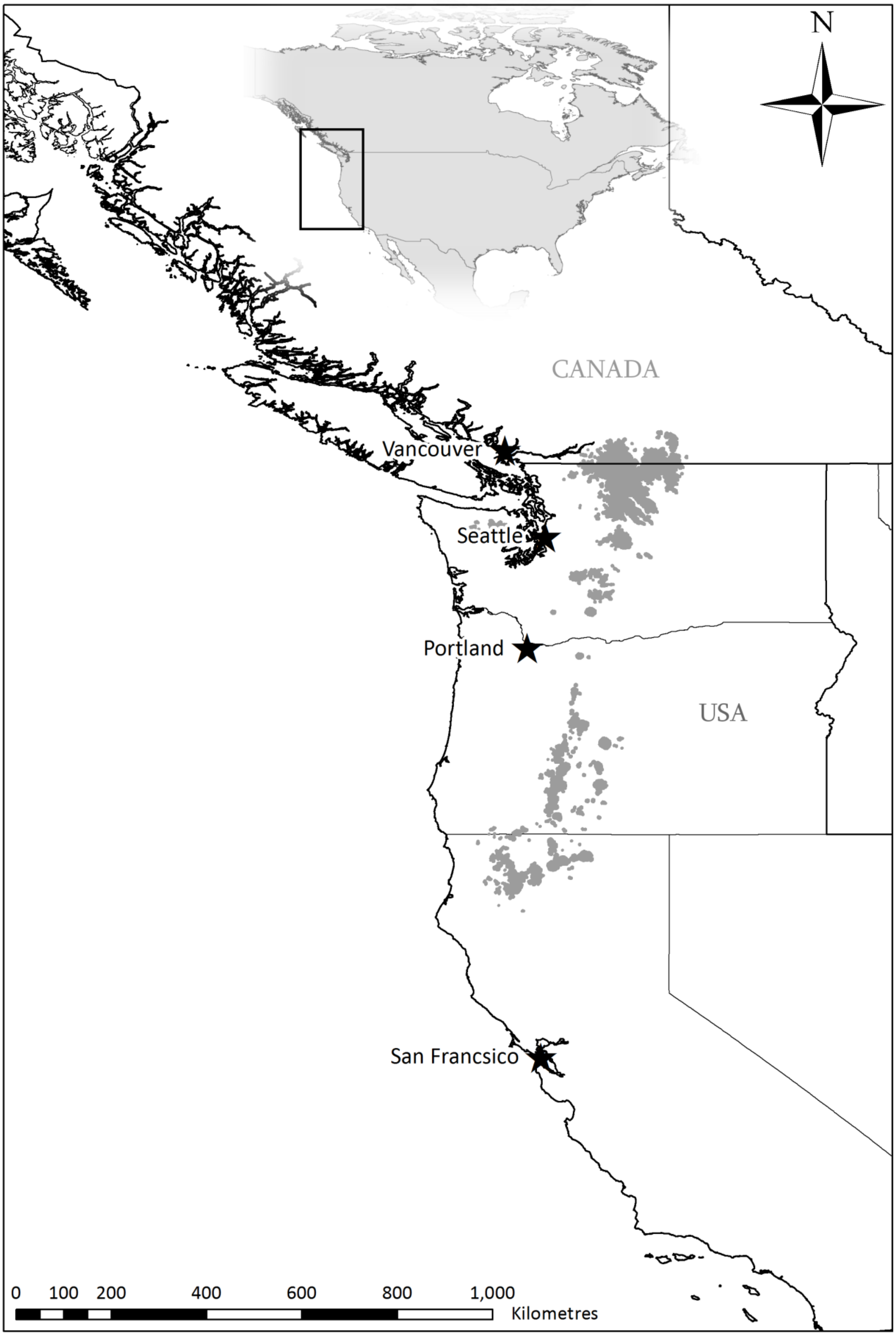

Figure 1.3. Sketch of the Cascade Range of North America. Mountains of the Cascades are shown in grey.

1.4.1.2 The nival arthropods: cryophily and the snow habitat

The great extent of permanent and long-lasting snow on the high mountains of the Cascades is associated with a distinct "nival" arthropod fauna. Nival arthropods are often cryophilic, with activity and survival bound within a narrow range of low temperatures (Mani 1962; Schoville *et al.* 2015). The capacity to function at temperatures near 0°C, rather than tolerance of extreme cold, is considered the primary determinant of life in these environments (Mani 1968; Edwards 1986, 1987; Schoville *et al.* 2015). Mani (1962) notes the ability to utilise cold as an adaptation to avoid the effects of desiccation, with water loss occurring more slowly at low temperatures; species can thus occupy high-elevation environments *because of* the cold (Mani 1962). Hygrophily is also common among nival insects, with individuals of many species concentrated at, or restricted to, snowfield edges, glacial margins and the edges of glacial lakes (Mani 1962).

Swan (1963) described snow-associated species assemblages as "aeolian communities", with a trophic food base provided by insect fallout and other organic debris, transported by the wind from lowland habitats: in effect, the energy in these systems is derived from external sources (Swan 1963). The aeolian arthropod community of Mt Rainier, the highest peak in the Cascades, is representative of other mountains in the range and comprises springtails, beetles (a staphylinid and five carabid species), a grylloblattid (Grylloblattida: *Grylloblatta* sp.), an annelid worm (Oligochaeta: *Mesenchytraeus solifugus raineirensis*) and a harvestmen (Opiliones: *Liomitopus* sp.) (Mann *et al.* 1980; Edwards 1987). With the exception of some of the springtails, these species are nocturnal or active in the late afternoon (Mann *et al.* 1980).

Carabid beetles (Coleoptera: Carabidae) in the genus *Nebria* (Latreille 1802) (Fig. 1.4) are ubiquitous in aeolian and lower montane communities throughout the northern hemisphere (Mann *et al.* 1980; Gereben 1995). Over 40 species occur in North America, 15 of which are found in the Cascades (Kavanaugh 1978). All the species are cool- or cold-adapted and largely restricted to riparian, tundra, talus or snowfield habitats (Kavanaugh 1978). Speciation in the group is thought to have occurred in the last 1.8 million years, considerably more recently than much of Australia's alpine fauna (see below) (Kavanaugh 1978). Many of the mountains in the Cascades contain multiple *Nebria* species with distinct elevational distributions, raising the question of what drives these patterns of zonation (Chapter 4).

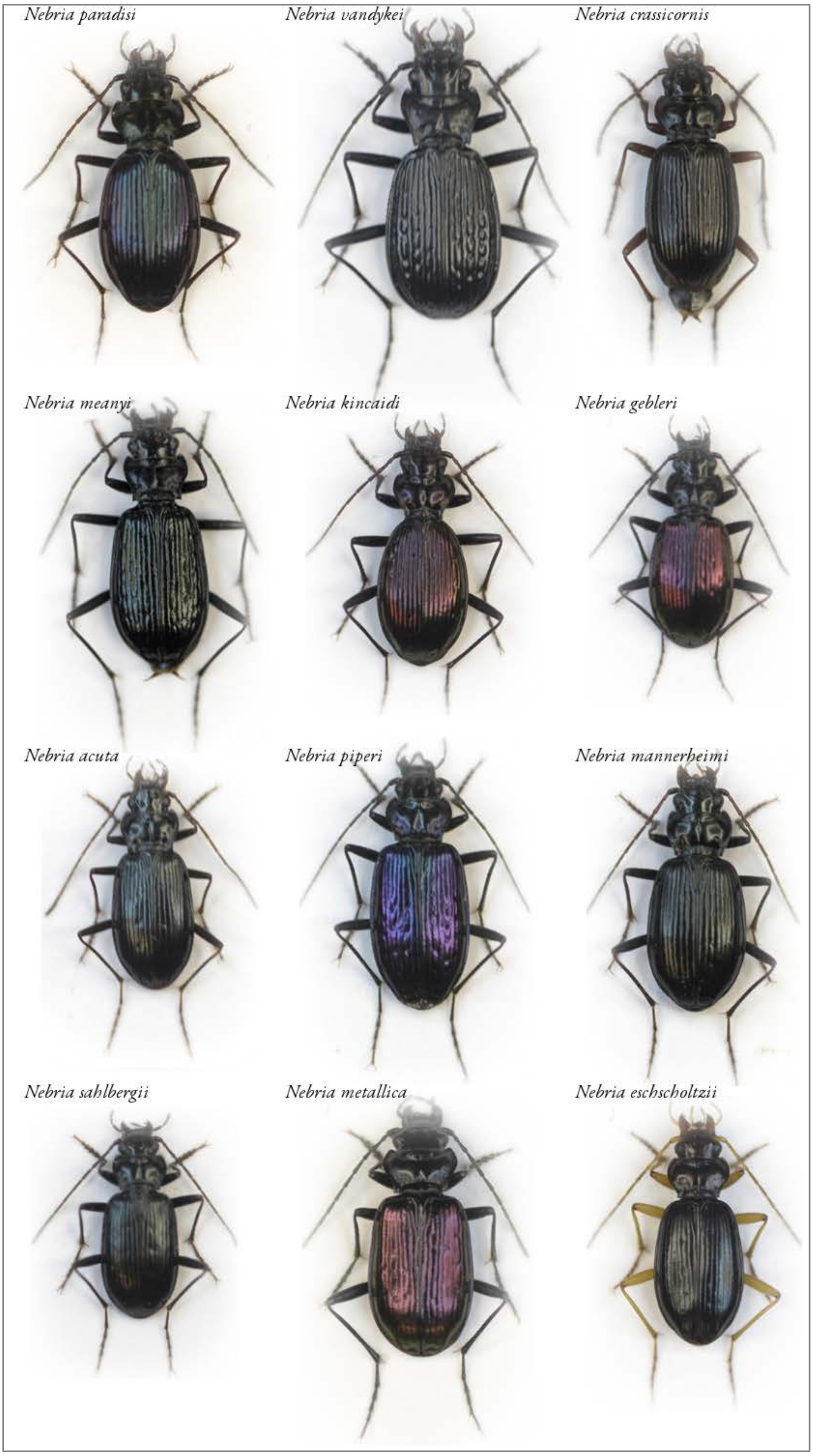

Figure 1.4. The 12 species of *Nebria* found on Mt Rainier, Washington, USA. Photos: SD Schoville.

1.4.2 *The Australian Alps*

1.4.2.1 History and environment

Australia's alpine region, comprising sub-alpine and alpine environments, covers just 0.15% (11500 km^2) of the country's land area (Costin 1957; Pickering & Armstrong 2003) (Fig. 1.5). The entirety of this area is within the southeastern states of New South Wales, Victoria and Tasmania, and the Australian Capital Territory and most is protected within a network of eight National Parks.

On the mainland, the alpine region marks the highest point along the Great Dividing Range – a series of uplands and escarpments covering 3500 km along the country's eastern seaboard from northern Queensland to western Victoria. The alpine region itself, however, has a linear extent of just 500 km (Costin 1989). This region is divided into five main sub-regions that are distinct geographically, geologically, and with respect to their component fauna (Hills 1975; O'Sullivan *et al.* 2000; Chapple *et al.* 2005; Koumoundouros *et al.* 2009; Endo *et al.* 2014; Slatyer *et al.* 2014). The highest peaks occur within the Snowy Mountains sub-region (New South Wales), where they form an extensive, undulating alpine ridge that includes Mt Kosciuszko, the highest mountain in Australia at 2228 m. A southern low pressure system that moves perpendicular to the main north-south axis of the mountains extends alpine conditions to lower elevations than would normally be expected, giving Australia a true alpine zone despite the low vertical range of the mountains (Costin 1989). Nevertheless, Australia's mountains are distinguished by lacking a nival zone – there is nowhere that snow persists year-round and thus a specialised snowfield fauna, so apparent in the high mountains of the Cascades, is absent (Green 1982).

In contrast to the young and geologically active Cascade Range, the mountains of southeastern Australia are thought to have experienced their most recent uplift 40 – 30 Ma (Holdgate *et al.* 2008). This event raised the mountains above their present height, with subsequent erosion shaping the undulating plateaus that characterise the area today (Holdgate *et al.* 2008). Despite the ancient origins of the landscape, however, mountain *climates* in Australia are relatively young. The onset of alpine conditions, as we know them today, occurred during the Pliocene (5 – 2.5 Ma), with the rapid cooling and drying of Australia's southeast (Gallagher *et al.* 2003). Cold-adapted alpine species are believed to have evolved during this period and persisted through subsequent climatic shifts (Galloway & Kemp 1984; Kershaw *et al.* 1986; Endo *et al.* 2014).

Unlike most continental areas, Australia experienced very little glaciation during the Pleistocene glacial cycles. On the mainland, glaciation occurred only around Mt Kosciuszko, though a broader area was affected by periglacial activity (Carr & Costin 1956; Galloway 1965; Colhoun *et al.* 1996; Barrows *et al.* 2002; Barrows *et al.* 2004). Depression of the snowline during colder glacial periods would have provided continuous sub-alpine to alpine conditions across the entire extent of the current mountains (Galloway 1965; Mark *et al.* 2005). However, as will be discussed in Chapter 6, faunal assemblages show high sub-region endemicity and deep genetic divergence, suggesting long periods of isolation.

At present, Australia's mountain climate is highly variable, on both an annual and diurnal time scale. Snow can fall in any month of the year and diurnal temperature fluctuations can exceed 40°C during the summer (Costin 1954; Carr & Turner 1959; Nash *et al.* 2012). Frosts are also frequent, with Charlotte Pass, at 1840 m and near Mt Kosciuszko, experiencing an average 215 frosts per year (Costin 1954). The highest air temperatures are in January and February, while July is the coldest month (BOM 2015). While atmospheric temperatures generally decrease with elevation (Slatyer 1978), frost hollows are a common feature of the Australian mountain landscape and create a complex mosaic of environments that do not always conform to classic climatic gradients (Costin 1989).

The Australian mountain vegetation comprises 12 major structural formations, including heathlands, grasslands, herbfields, fens, and bogs (Costin 1954; Kirkpatrick & Bridle 1999), and 56 distinct plant communities have been described in the sub-alpine and alpine zones (McDougall & Walsh). The species composition and distribution of these formations is controlled by elevation, climate, local topography, snow duration, wind, water availability, base rock type and soil characteristics (Carr & Turner 1959; Kirkpatrick & Bridle 1999; McDougall & Walsh 2007; Pickering & Green 2009). McDougall and Walsh (2007) estimate that roughly 30% of plants found in the Australian alpine region are endemic and Crisp *et al.* (2001) identified the Australian alpine region as one of 12 centres of plant endemism in Australia.

The Australian alpine region is considered to be particularly vulnerable to climate change, from a national and global perspective, due to its small vertical range, limited extent and the relative isolation of high-elevation habitats (Hennessy *et al.* 2007; Steffen *et al.* 2009; La Sorte & Jetz 2010; Laurance *et al.* 2011; Reisinger *et al.* 2014). Australia's high-mountain species face severe geographic constraints to distribution shifts: the maximum vertical range of the alpine zone is just 400 m, while combined sub-alpine and alpine environments span less than 1000 m of elevation. In addition, distances between sub-alpine/alpine habitats on isolated mountains are often in the order of 50 – 100 km. Thus, the only options available to Australia's alpine species are to tolerate or adapt to changing environmental conditions, or persist in refugia with suitable microclimates.

1.4.2.2 Precipitation and snow

Mountain precipitation in Australia is enormously important from both an ecological and economic perspective. The Australian alpine region contains the headwaters to many of the river systems running into the Murray Darling Basin, which sits west of the mountain range and drains nearly 14% of the Australian continent. The Basin contains more than 70% of Australia's irrigated land area and the value of irrigated agricultural production is estimated at over AU$6 billion (ABS 2014). In addition, water from the Snowy and Kiewa rivers – both originating in the high country – is used for hydroelectric power generation in New South Wales and Victoria, respectively.

Precipitation in the Australian alpine region is highly variable on both an inter- and intra-annual timescale (Dai *et al.* 2014; Theobald *et al.* 2015). Between 1958 and 2012, annual precipitation over the Snowy Mountains ranged from 760 mm to 2800 mm, with an overall average of 1715 mm (Theobald *et al.* 2015). Precipitation is typically twice as high during the winter and early spring (June – September) as during the warmer months (Chubb *et al.* 2011). This is due to a northward shift in the position of the mid-latitude storm track which, in turn, allows cold fronts to move across the southeast of the continent (Larsen & Nicholls 2009; Theobald *et al.* 2015). These cold fronts are responsible for roughly 70% of wintertime precipitation in the high country, while the remaining 30% is associated with high-pressure systems (Dai *et al.* 2014). Accordingly, a decline in winter precipitation has been attributed to changes in the frequency and intensity of cold fronts (Nicholls 2010; Chubb *et al.* 2011).

An overall drying trend has been observed across the region in recent decades and during the "Millenium Drought" (1996 – 2000) alpine regions experienced the largest reductions in rainfall in southeastern Australia (Riseby *et al.* 2013). Climate models predict both drier and wetter periods for the Australian high country, but with drier conditions on average by 2030 (Post *et al.* 2012). Total snow accumulation in Australia is closely linked to precipitation (Whetton *et al.* 1996; Fiddes *et al.* 2015) and, since 1958, precipitation in the high country has become more intense, but with fewer events (Theobald *et al.* 2015). With this has come a decline in small snowfall events (Fiddes *et al.* 2015), a decline in snow depth (Nicholls 2005; Davis 2013; Fiddes *et al.* 2015) and a shortening of the snow season (Green

& Pickering 2009; Bhend *et al.* 2012; Sánchez-Bayo & Green 2013). Trends and predictions for snow cover in the Australian alpine region are discussed further in Chapter 7.

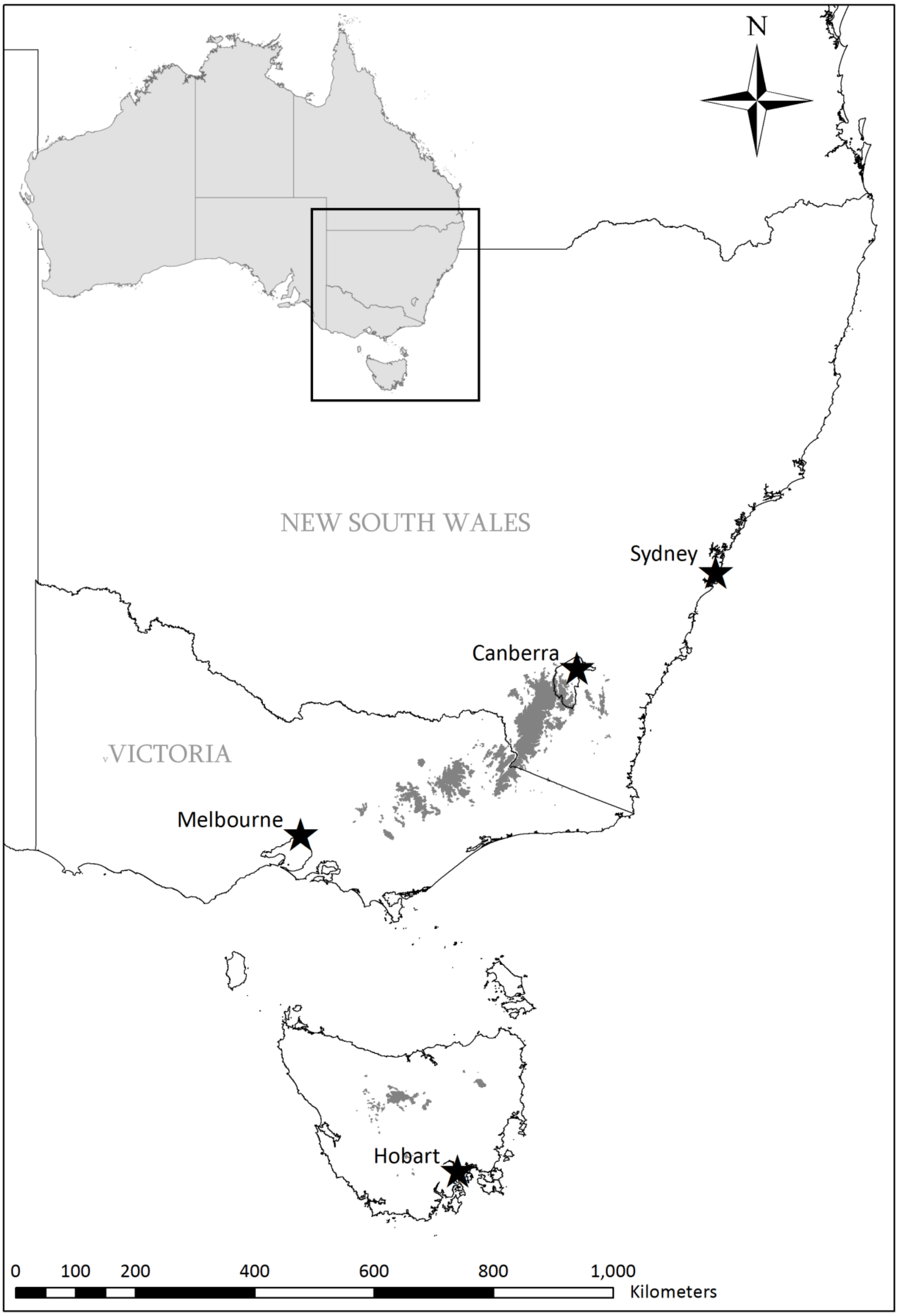

Figure 1.5. Outline of the Australian high country. Elevations above 1500 m are shaded in grey.

1.4.2.3 The mountain arthropods: thermophily in the alpine region

The Australian high country is unusual among global mountain systems in the rarity of mammalian herbivores above the snowline (Green & Osborne 2012). Indeed, the largest native mammals in Australia's alpine zone are the broad-toothed rat (*Mastacomys fuscus*) and the bush rat (*Rattus fuscipes*), at 100 – 150 grams (Green & Osborne 2012). There is, however, a rich arthropod fauna, with the Hymenoptera and Orthoptera distinctly abundant (Costin 1989; Williams *et al.* 2014). Over 1000 invertebrate species from 175 families are known from the alpine region; many of these are endemic to the high country, or even to a specific mountain (Green & Osborne 2012). The alpine stonefly (*Thaumatoperla alpina*), the largest of Australia's stoneflies (Order Plecoptera), for example, is known from only 12 sites, all on the Bogong High Plains in Victoria (TSSC 2011). Also in Victoria, the alpine silver xenica butterfly (*Oreixenica latialis theddora*) is endemic to Mt Buffalo, a small (310 km^2) isolated plateau to the west of the main axis of the Great Dividing Range (Field 2013). Although no winter specialist species are known, there has been only one survey of winter-active arthropods (Green 1997).

Grasshoppers (Orthoptera: Acrididae) are among the most abundant insects and are dominant herbivores in the sub-alpine and alpine zones (Green & Osborne 2012). Although considered a thermophilic group, grasshoppers are common in high mountain areas worldwide (Alexander 1951). Unlike the nival fauna of the Cascades, mountain grasshoppers are primarily diurnal and thermophilic. Large differences in temperature over small spatial scales allows small, mobile animals, such as grasshoppers, to achieve body temperatures considerably higher than ambient air and thus to persist in high-elevation environments despite the atmospheric cold (Mani 1968; Geiger *et al.* 1995; Buckley *et al.* 2013).

One of the most conspicuous elements of Australia's alpine insect fauna is the *Kosciuscola* (Sjösted 1934) grasshopper (Fig. 1.6). The five species in the genus are endemic to the Australian high country, with four species on the mainland mountains and one in Tasmania. They are unique among grasshoppers in their temperature-controlled colour change, which is most pronounced in the chameleon grasshopper, *K. tristis,* but might also occur in the other species of the genus (KDL Umbers, unpublished data). Although originally considered a mechanism for thermoregulation (Key & Day 1954), with individuals becoming lighter as their body temperature increases, subsequent work suggests that the effect of colour on body temperature is likely to be small (Umbers *et al.* 2013; Umbers *et al.* 2014). Nevertheless, field data indicate that grasshopper body temperatures can exceed 40°C when ambient temperature is barely 15°C (RA Slatyer, unpublished data). These grasshoppers are the focus of Chapters 5 and 6.

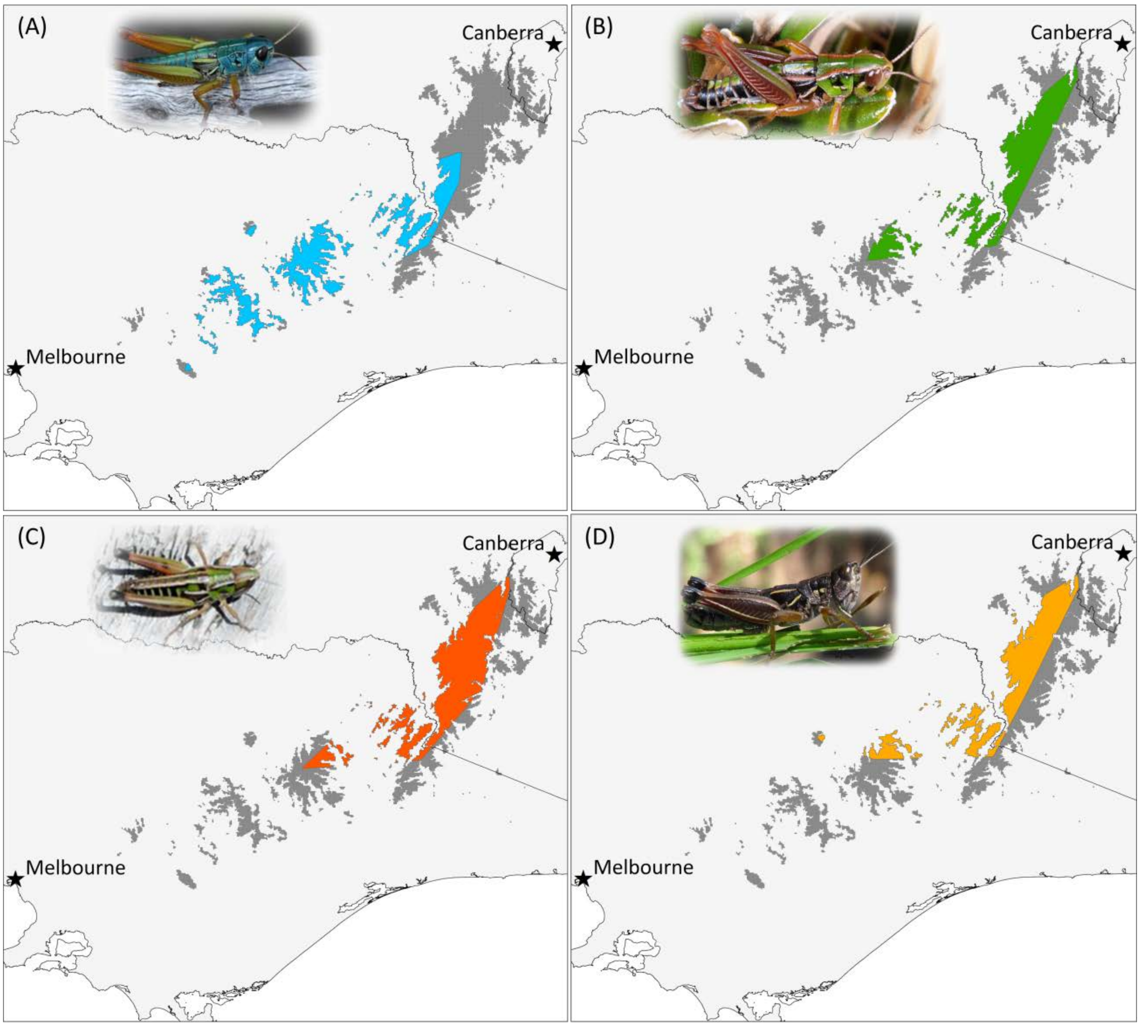

Figure 1.6. Approximate distributions of the mainland *Kosciuscola* within the high-country of southeastern Australia (shaded dark grey). (A) *Kosciuscola tristis,* (B) *Kosciuscola usitatus,* (C), *Kosciuscola cognatus,* and (D) *Kosciuscola cuneatus.* Distributions are estimated by a minimum convex polygon around occurrence data (*n* = 24 – 98) and should be taken only as a guide to the relative distributions of the four species. Photos: KDL Umbers (A, C, D) & MA Nash (B).

1.5 Structure of the thesis

This thesis is structured as a series of studies focused primarily on mountain environments. The studies explore the ecological underpinnings of geographic ranges and their limits, and the consequences of range position for past and present range structure and exposure to future environmental change.

Chapters 2 synthesises evidence for the relationship between niche breadth and geographic range size, drawing from previously published research to establish the generality of a niche breadth-range size relationship at a global scale using a meta-analytic approach.

The relationship between the ecological niche and range size is explored in more detail in Chapter 3, where I focus on a continental scale and ground beetles (Coleoptera: Carabidae) in the genus *Nebria* to test two alternative hypotheses for geographic range size variation.

At a smaller scale again, range structure in Chapter 4 is explored within a single mountain. Here, I particularly look at the relationship between physiology and elevational range limits. Again, this question is approached with *Nebria* beetles, in which 12 species are spread across a 2200 m elevational gradient on Mt Rainier, Washington, USA.

Physiological variation occurs both among and within species, and distinguishing between the two sources of variation is crucial for understanding the adaptive capacity of species and how they are likely to respond to environmental change. The relationship between inter- and intra-specific variation is explored in Chapter 5, with three species of Australian mountain-endemic grasshoppers (Orthoptera: Acrididae: *Kosciuscola*).

Mountain-endemic species occupy habitat islands that are dynamic over evolutionary time, while current mountain topography influences contemporary population structure. In Chapter 6, I focus on *Kosciuscola tristis*, the most elevationally-restricted but geographically widespread of Australia's mountain grasshoppers, to explore past and current patterns of population connectivity within the Australian alpine landscape.

Chapter 7 focuses on the impacts of climate change on Australia's alpine arthropod fauna. This work documents subnivean biodiversity and tests the effects of reduced snow cover on alpine arthropods in the Australian mountain environment, where winter snow faces an uncertain future.

Chapter 8 summarises and synthesises the results of the preceding chapters, and provides a discussion of future research directions.

Chapter 2 – 6 are written in the form of manuscripts, with Chapters 2, 4, 5, and 6 published or submitted to peer-reviewed academic journals. As such, there is some repetition within the introductions and discussions, descriptions of the study sites and organisms, and the reference lists.

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The geographical areas of distribution are the Chinese-lantern shadows produced by the different taxa on the continental screen: it is like measuring, weighing, and studying the behaviour of ghosts.

Eduardo H Rapoport, 1982, Areography
(transl. B Drausal)

Chapter 2

Niche breadth predicts geographic range size: a general ecological pattern

Abstract

The range of resources that a species uses (i.e., its niche breadth) might determine the geographic area it can occupy, but consensus on whether a niche breadth-range size relationship generally exists among species has been slow to emerge. The validity of this hypothesis is a key question in ecology in that it proposes a mechanism for commonness and rarity, and if true, may help predict species' vulnerability to extinction. We identified 64 studies that measured niche breadth and range size and we used a meta-analytic approach to test for the presence of a niche breadth-range size relationship. We found a significant positive relationship between range size and environmental tolerance breadth ($z = 0.49$), habitat breadth ($z = 0.45$), and diet breadth ($z = 0.28$). The overall positive effect persisted even when incorporating sampling effects. Despite significant variability in the strength of the relationship among studies, the general positive relationship suggests that specialist species might be disproportionately vulnerable to habitat loss and climate change due to synergistic effects of a narrow niche and small range size. An understanding of the ecological and evolutionary mechanisms that drive, and cause deviations from, this niche breadth-range size pattern is an important future research goal.

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2.1 Introduction

A species' niche breadth describes the suite of environments or resources, in the broadest sense, that it can inhabit or use (Gaston *et al.* 1997). Brown (1984) argued that, by utilising a greater array of resources, and maintaining viable populations within a wider variety of conditions, a species should become more widespread – this would lead to a positive correlation between niche breadth and geographic range size. This intuitively appealing hypothesis has been discussed, debated, and tested in numerous studies and books (e.g. Brown 1984; Gaston *et al.* 1997; Gaston & Blackburn 2000; Gaston *et al.* 2000; Arribas *et al.* 2012; Boulangeat *et al.* 2012), yet a general consensus on the presence or magnitude of such a relationship has been slow to emerge. Nevertheless, the validity of this niche breadth-range size hypothesis is a key question in ecology in that it proposes a mechanism for commonness and rarity, and if true, may help predict species' vulnerability to extinction under rapid environmental change (Harcourt *et al.* 2002; Boyles & Storm 2007; Heim & Peters 2011).

The hypothesised niche breadth-range size relationship is one of several suggested mechanisms explaining commonness and rarity. For example, it has been invoked to explain the common observation that local abundance is positively correlated to geographic range size (Brown 1984; reviewed in: Gaston & Blackburn 2000). It has also been applied to the evolution of resource utilization. For example, Janz and Nylin (2008) suggested that diversification among herbivorous insects is driven by alternating evolutionary periods of dietary generalisation (associated with range expansion) and specialisation (associated with local adaptation to new hosts).

Evolutionary mechanisms might also play a role in niche and range size determination. Among closely related species, variation in traits such as dispersal ability (e.g. Lester *et al.* 2007; Arribas *et al.* 2012), evolutionary age (e.g. Willis 1922; Webb & Gaston 2000) and niche position (the availability of resources within a species' niche) (Hanski 1993; Gregory & Gaston 2000) have each been used to explain differences in geographic range size. Increased dispersal ability and time since species inception should increase the likelihood of filling available niche space, and thus attaining a larger range size. However, recent reviews have failed to find evidence of consistent relationships between the above factors and range size (Lester *et al.* 2007) or have shown that predicted associations can arise purely through stochastic range size evolution (Pigot *et al.* 2012). For instance, if range size evolves randomly and if species with small ranges are more likely to go extinct, then a positive association between species age and range size could result because of loss of the small-ranged species, rather than because range size increases with species age (Pigot *et al.* 2012). Thus, variation in range size among species might be more strongly mediated by other factors such as niche characteristics (Laube *et al.* 2013).

In a comprehensive study on British birds, Gregory and Gaston (2000) found that niche position was closely associated with geographic range size. This supported earlier, more anecdotal reports, that widespread species were those that utilised widespread resources (reviewed in Gaston & Blackburn 2000) and Pither (2003) showed that tolerance of cold extremes (rather than the range of temperatures tolerated) was the most important factor determining the geographic ranges of trees and shrubs in North America. Yet, the niche breadth and niche position hypotheses for range size are not mutually exclusive; e.g. a species with a broader niche might be more likely to have widespread resources included within that niche.

Studies that have looked for evidence for a niche breadth-range size relationship have been limited by problems of sampling bias, spatial extent and niche definitions, making it difficult to discern the evidence for or against its generality. If, for example, resource use is determined for more individuals or at more locations for common and widespread species than for scarce and restricted species, then apparent niche breadth is likely to be greater in the former simply by chance (i.e. sampling effects; Burgman 1989). A positive relationship would therefore come about purely as a sampling artefact. The mechanisms determining range size might also vary with spatial scale. For example, Brändle *et al.* (2002a) found that as the spatial scale increased, the importance of dietary niche breadth in predicting range size decreased, while physiological tolerance became more important. Specialisation itself is also highly scale-dependent, such that a local specialist might be a generalist at a regional scale and vice versa (Gaston *et al.* 1997; Hughes 2000).

Finally, accurate niche measures (whether breadth or position) are obscured by the *n*-dimensional nature of the Hutchinsonian niche (Hutchinson 1957; Colwell & Futuyma 1971). Niche breadth can be measured in many ways (e.g. Table 2.1) and niche breadths along different axes are not necessarily correlated (e.g. Berkström *et al.* 2012), so the presence or absence of a statistical relationship between niche breadth and range size might be dependent on the niche axis measured (Harcourt *et al.* 2002). Inevitably, if enough niche axes are measured, some will be found that correlate positively with range size and others will be found that do not. In the absence of a clear rationale for choosing one component of the niche over another, most studies measure multiple niche axes. Habitat use, diet and environmental tolerance (here considered as a niche component and typically defined as thermal limits in animals and germination traits in plants) are the most frequently measured niche subsets. Despite these difficulties, a strong, positive relationship between niche breadth and range size among multiple, independent systems would provide general support for the niche breadth-range size hypothesis.

A lack of strong empirical evidence and studies that appropriately controlled for sampling effects led Gaston and Blackburn (2000) to conclude that there was little support for a general, positive relationship between niche breadth and range size. In the decade since their review, many more studies have investigated this relationship and a large proportion of these made efforts to account for the aforementioned confounding effects. Here we use a meta-analytic framework to test the relationship between niche breadth and range size. We consider three broad niche dimensions – habitat, diet and environmental tolerance – and test for differences in strength of the relationship between taxonomic groups and the spatial scale being considered. We find a consistent positive relationship between niche breadth and range size, which is strongest for environmental tolerance breadth. This relationship is present across all taxonomic groups examined and at different spatial scales and even holds when only studies that control for sampling effects are included.

2.2 Methods

2.2.1 Data compilation

We used four approaches to compile our dataset. First, we searched the literature using the ISI Web of Science database, with the search terms [niche breadth or niche width or tolerance breadth or diet breadth or habitat breadth or resource breadth] and [range size or geographic* range] in October 2012. Second, we identified original papers outlining commonly used metrics for niche breadth (Pielou 1974; Hurlbert 1978; Feinsinger *et al.* 1981; ter Braak 1986; Dolédec *et al.* 2000; Fridley *et al.* 2007; Warren *et al.* 2008) and used Web of Science to retrieve all publications citing each of these. Third, we identified two recent reviews of niche breadth (specifically thermal tolerance breadth) and geographic range (Bozinovic *et al.* 2011; Sunday *et al.* 2011) and scanned their reference list to locate additional, potentially relevant, papers. Finally, after identifying relevant studies (see *Inclusion criteria*) by the first three methods, we retrieved the publications cited in each of these. Combined, these methods yielded a total of 2,670 unique references. To reduce the risk of sampling bias, we did not include papers that were encountered by chance outside the search parameters. The titles and abstracts of all references were scanned and those that were clearly outside the scope of this meta-analysis were discarded, leaving a total of 180 references.

2.2.2 Inclusion criteria

We had three inclusion criteria: (1) does the study measure both niche breadth and range size? (2) Does the study use four or more species (see below)? (3) Does the paper include extractable data? For criterion 1, studies that reported niche breadth and/or range size as a categorical variable (e.g. wide versus narrow niche; large versus small range size) were included, in addition to those using other metrics for niche breadth and range size. We classified niche measures into three broad categories: habitat, diet and environmental tolerance. Range size measurements were divided into two categories, defined by whether estimates covered the entire geographic distribution of the species ("comprehensive") or only part of its distribution ("partial"). Where a study measured range size at both scales, we excluded the partial measure so that each study had only one effect size per niche breadth measure. We chose to do this because we were primarily interested in the relationship between niche breadth and geographic range size (i.e. "comprehensive" range size measure). For criterion 2, $n \geq 4$ is required for calculations of the effect size variance (v_z) (Borenstein *et al.* 2009). Criterion 3 meant that we sometimes excluded studies that measured both niche breadth and range size but where it was not possible to calculate the effect size. Exclusion was usually due to ambiguous information about the direction of the effect or data that were not reported in such a way that effect sizes could be extracted (Table 2.2).

Meta-analytic models assume statistical independence of effect sizes, and inclusion of multiple studies covering the same species would violate this assumption. If two or more studies covered the same species group, we included data from the study with the higher number of species. Two studies (Brändle *et al.* 2003b; Thompson & Ceriani 2003) measured the temporal germination niche breadth, which cannot readily be assigned to one of the three niche categories we examined. These studies were consequently excluded from the analysis, but the results are included in Table 2.2.

2.2.3 Meta-analysis statistical methods

The common effect size we calculated was Fisher's *z*, which is the standard effect size measure used for correlational data (Borenstein et al. 2009). We preferentially calculated *z* using the correlation coefficient *r,* extracted from summary tables, the text or figures (using IMAGEJ v.1.46r: Schneider *et al.* 2012). When this approach was not possible, we converted test statistics (*t, F*) or *p*-values (Borenstein 2009). We then calculated the variance using the number of species as the sample size.

If there was more than one effect size per niche category (diet, habitat or tolerance) within a study, we calculated the study mean and its associated variance by following a hierarchical approach to combining data (see Slatyer *et al.* 2012). First, we calculated the mean effect if the same individuals contributed two or more effects to the same response (e.g. niche breadth calculated using two different indices). Second, if there was still more than one effect size per niche category (e.g. the study was carried out in multiple regions, with some species overlap), we calculated the weighted mean for the study for that category, using a standard fixed-effects model. For both steps, the variance of the mean of m effects (denoted X_i or X_j) each with a variance of V_i or V_j, and r_{ij} representing the correlation between X_i and X_j is:

$$V_{mean} = \left(\sum_{i=1}^{m} V_i + 2 \sum_{i,j} \left(r_{ij} \sqrt{V_i} \sqrt{V_j} \right) \right) \Big/ m^2$$

(modified from Borenstein *et al.* (2009), p228).

When the variance differed between effect-size estimates, we multiplied each V_i in the first sum by (W_i/W_{mean}) and each term in the second sum by $(W_i \times W_j)/(W_i \times W_j)_{mean}$, where $W_i = 1/V_i$ and $W_{mean} = (\sum W_i)/m$ (see Slatyer *et al.* 2012). r_{ij} was either provided in the publication or calculated as the proportion of species shared between datasets.

Finally, we ran separate random-effects models in the R package METAFOR (Viechtbauer 2010; R Core Team 2014), using study-level effects for each niche category (i.e., the effect sizes calculated after combining data if required, as described above). The null hypothesis for each analysis was that the mean effect size was zero and effect sizes were always calculated such that a positive value indicated that species with a broader niche occupied a larger geographic range. Heterogeneity was estimated using the I^2 statistic (Higgins &

Thompson 2002; Higgins *et al.* 2003), which is less sensitive to study number than the commonly quoted Cochrane's Q (Borenstein *et al.* 2009). To examine the influence of the scale of range measurement and taxonomic grouping on the relationship, we ran separate mixed-effects models for each niche category using the effect size as the response variable and scale of range measurement (comprehensive versus partial) and taxonomic group as explanatory factors. Finally, we used a mixed-effects model to test whether control of sampling effects influenced mean effect size by including sampling design (with or without some control for sampling effects) as an explanatory factor. We classified a study as having controlled for sampling effects if they: (a) used equal sampling to measure niche breadth for each species, (b) used a well-studied set of species for which sampling bias has been determined to not be influential (Cowley *et al.* 2001a) or (c) statistically adjusted for potential sampling bias.

2.2.4 Publication bias

Several methods are commonly used to test for publication bias and its potential impact on the results of the meta-analysis and we employed three methods. First, we tested for funnel plot asymmetry using a weighted regression and standard error as the predictor (Egger *et al.* 1997; Rothstein *et al.* 2005). A funnel plot, with study effect size on the x-axis and sample size (or standard error) on the y-axis, will tend to have a cluster of large studies around the mean effect whereas smaller studies are spread across the base of the graph (Borenstein *et al.* 2009). If there is no publication bias, studies are expected to be distributed symmetrically around the mean effect size. Asymmetry in the funnel plot is therefore an indicator of publication bias and most commonly involves the absence of studies from the lower left of the graph – i.e. small studies with nonsignificant or negative effect (Borenstein *et al.* 2009).

Our second approach was to use a "trim and fill" method, which suppresses the most extreme positive studies to generate a symmetric funnel plot and re-calculates the effect size without these studies (Duval & Tweedie 2000a, b). The method also provides an estimate of the number of hypothetically "missing" studies, based on asymmetry in the funnel plot (Duval & Tweedie 2000a, b). This method therefore estimates what impact potential publication bias might have on the results of the analysis.

Finally, we examined how robust our results are to the effects of publication bias using Rosenberg's fail-safe number. This is the number of new studies with a mean effect size of zero necessary to reduce the observed significance level to $\alpha = 0.05$ (Rosenberg 2005). Fail-safe numbers are often considered robust if they are greater than $5n + 10$, where n is the original number of studies (Rosenthal 1991). That is, when the fail-safe number is large relative to the number of studies, publication bias (if it exists) is unlikely to alter the conclusions of the analysis (Rosenberg 2005).

Table 2.1. Classification of niche breadth measures into habitat, diet and environmental tolerance categories.

Category	Detail	Examples
Habitat	Number of biomes occupied	Fernández and Vrba (2005)
	Categorical specialist/generalist	Kolb *et al.* (2006)
	Co-occurrence	Boulangeat *et al.* (2012)
	Environmental characteristics of occupied habitats	Lappalainen and Soininen (2006)
	Number of depth zones occupied	Harley *et al.* (2003)
	Number of different habitat types occupied	Harcourt *et al.* (2002)
	Number of habitat categories occupied	Carrascal *et al.* (2008)
	Number of host species used	Krasnov *et al.* (2005)
	Number of reef zones occupied	Berkström *et al.* (2012)
	Number of substrates occupied	Callaghan and Ashton (2008)
	Proportion of breeding habitats utilised	Brändle *et al.* (2002b)
	Proportion of different habitats utilised	Cowley *et al.* (2001a)
	Range of grain sizes in occupied habitats	Frost *et al.* (2004)
	Soil characteristics of occupied habitats	Burgman (1989)
	Vegetation characteristics of occupied habitats	Reif *et al.* (2006)
Diet	Categorical specialist/generalist	Rickart *et al.* (2011)
	Diversity of flowers used	Goulson *et al.* (2008)
	Number of different food types used	Eeley and Foley (1999)
	Number of host plant families used	Garcia-Barros and Benito (2010)
	Number of host plant genera used	Forister *et al.* (2011); Jahner *et al.* (2011)
	Number of host plant species used	Brändle *et al.* (2002a)
Tolerance	Annual temperature range in habitat	Pither (2003)
	Calcium requirements	Briers (2003)
	Elevational range	Brändle *et al.* (2002a); Essl *et al.* (2009)
	Germination temperatures	Luna and Moreno (2010); Luna *et al.* (2012)
	Thermal tolerance breadth	Calosi *et al.* (2008)

Table 2.2. Studies identified from the literature search that provided evidence for ("support") or against ("no support") the niche breadth-range size hypothesis but were unable to be included in the meta-analysis.

Study	Taxonomic group	Reason for exclusion	Support (+) No support (-)
Arribas *et al.* (2012)	Animal	< 4 species in study (*n* = 2).	-
Brändle *et al.* (2003b)	Plant	Germination niche breadth.	+
Calosi *et al.* (2008)	Animal	Same species as Calosi *et al.* (2010).	+
Debussche and Thompson (2003)	Plant	< 4 species in study (*n* = 2).	+/-
Krasnov *et al.* (2008)	Animal	Correlations between niche breadth and range size not provided and could not be calculated from available data.	+
Lambdon (2008)	Plant	Correlations between niche breadth and range size not provided and could not be calculated from available data.	+
Mühlenberg *et al.* (1977)	Animal	Correlations between niche breadth and range size not provided and could not be calculated from available data.	-
Shkedy and Safriel (1992)	Animal	< 4 species in study (*n* = 2).	+
Southward (1958)	Animal	Correlations between niche breadth and range size not provided and could not be calculated from available data.	-
Tales *et al.* (2004)	Animal	Correlations between niche breadth and range size not provided and could not be calculated from available data.	-
Thompson and Ceriani (2003)	Plant	Germination niche breadth.	-
Thompson *et al.* (1999)	Plant	Subset of the species used by Thompson *et al.* (1998).	-

2.3 Results

2.3.1 Random-effects meta-analysis

We extracted 75 effect sizes from 64 studies that met our inclusion criteria (see Table 2.3). Of these studies, 36 controlled for sampling bias or provided data to support the argument that their study was free from sampling bias. Studies covered animals (n = 45), plants (n = 17), algae (n = 2) and diatoms (n = 1). Habitat breadth was the most common measure of niche breadth (n = 44 studies; Fig. 2.1) followed by diet breadth (n = 20; Fig. 2.2) and environmental tolerance range (n = 11; Fig. 2.3) and several studies used two or more measures.

The mean effect size, 95% confidence intervals and results of tests for heterogeneity are given in Table 2.4. There was a significant positive relationship between niche breadth and geographic range size when niche breadth was measured as either habitat breadth ($z = 0.45$; Fig. 2.1) or environmental tolerance range ($z = 0.49$; Fig. 2.3). There was also a clear, but weaker, trend for positive relationship between diet breadth and geographic range size ($z = 0.28$; Fig. 2.2). There was high variability in effect sizes between studies, with 83% (diet) to 95% (habitat) of the total variation in estimated effect sizes due to heterogeneity between studies.

2.3.2 Mixed-effects meta-analysis: test of explanatory factors

We used two mixed-effects models to examine the influence of taxonomic group and measurement scale, respectively. Results from these analyses are shown in Tables 2.5 (taxonomic group) and 2.6 (measurement scale). Neither moderator had a statistically significant impact on the mean effect size, or accounted for a substantial amount of heterogeneity among studies (Tables 2.5, 2.6). Nevertheless, dietary breadth was significantly correlated with range size only in arthropods and the association between tolerance breadth and range size was marginally non-significant in studies on animals (Table 2.5). Sample size was small ($n = 5$) for the latter, however, and the effect size ($z = 0.49$) indicates a moderate to strong relationship.

Studies that controlled for sampling effects had lower average effect sizes than those that did not, although this difference was not statistically significant (Table 2.7). Whereas studies that did not control for sampling effects always had significant mean effects within each niche breadth measure (Table 2.7), of the controlled studies, only the habitat breadth-range size relationship had a mean effect significantly greater than zero. Although always positive, the mean effect size of studies that controlled for sampling effects was not statistically greater than zero when niche breadth was measured as diet ($z = 0.19$, $p = 0.125$, $n = 10$) or environmental tolerance ($z = 0.30$, $p = 0.570$, $n = 6$).

2.3.3 Publication bias

We did not find evidence for publication bias through regression tests for funnel plot asymmetry (Fig. 2.4) or the trim-and-fill analysis (Table 2.8). For the latter, there were no putatively missing studies for any niche breadth measure and "corrected" effect sizes (calculated after suppressing extreme positive studies) were identical to those from the initial random-effects model. For all random-effects models, the fail-safe number was high ($> 5n + 10$) relative to the number of studies in the analysis (Table 2.8), indicating that publication bias (if present) is unlikely to alter the conclusions of the analysis.

Table 2.3. Summary statistics and effect sizes for studies included in the meta-analysis; *n* is the number of species, *z* is the standard correlation effect size and *v(z)* is the variance of the effect size.

Study	**Niche category**	***n***	***z***	***v(z)***	**Scale**	**Group**	**Control for sampling effects**
Animals							
Bean *et al.* (2002)	Habitat	5	0.37	0.70	Partial	Chordata: Fishes	Y
Beck and Kitching (2007)	Diet	85	0.60	0.01	Partial	Arthropoda: Moths	Y
Berkström *et al.* (2012)	Diet	11	-0.39	0.13	Comprehensive	Chordata: Fishes	Y
	Habitat	11	-0.08	0.12	Comprehensive	Chordata: Fishes	N
Bonte *et al.* (2004)	Habitat	29	0.86	0.04	Partial	Arthropoda: Spiders	Y
Boyes and Perrin (2009)	Diet	5	0.69	0.50	Comprehensive	Chordata: Birds	N
Boyles and Storm (2007)	Diet	16	-0.19	0.08	Comprehensive	Chordata: Bats	Y
Brändle and Brandl (2001)	Habitat	51	0.06	0.02	Comprehensive	Chordata: Birds	N
Brändle *et al.* (2002a)	Habitat	122	0.52	0.01	Comprehensive	Arthropoda: Butterflies	Y
	Diet	122	0.21	0.01	Comprehensive	Arthropoda: Butterflies	Y
	Tolerance	112	0.30	0.01	Comprehensive	Arthropoda: Butterflies	Y
Brändle *et al.* (2002b)	Diet	139	0.04	0.01	Comprehensive	Chordata: Birds	N
	Habitat	139	0.15	0.01	Comprehensive	Chordata: Birds	N
Briers (2003)	Tolerance	32	0.50	0.04	Comprehensive	Mollusca: Gastropods	N
Calosi *et al.* (2008)	Tolerance	4	1.71	1.00	Comprehensive	Arthropoda: Beetles	Y
Calosi *et al.* (2010)	Tolerance	14	0.93	0.09	Comprehensive	Arthropoda: Beetles	Y
Carrascal *et al.* (2008)	Habitat	48	0.61	0.04	Partial	Chordata: Birds	Y

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Table 2.3. Continued from previous page.

Study	Niche category	*n*	*z*	*v(z)*	Scale	Group	Control for sampling effects
Cowley *et al*. (2001a)	Diet	49	0.06	0.02	Comprehensive	Arthropoda: Butterflies	Y
Cowley *et al*. (2001b)	Habitat	26	0.30	0.04	Partial	Arthropoda: Butterflies	Y
Cruz *et al*. (2005)	Tolerance	34	0.02	0.03	Comprehensive	Chordata: Lizards	Y
Dennis *et al*. (2005)	Diet	60	0.63	0.02	Comprehensive	Arthropoda: Butterflies	N
Eeley and Foley (1999)	Habitat	102	1.26	0.01	Comprehensive	Chordata: Primates	N
	Diet	102	0.76	0.01	Comprehensive	Chordata: Primates	N
Eterovick and Barros (2003)	Habitat	12	0.27	0.11	Partial	Chordata: Frogs	Y
Fernandez and Vrba (2005)	Habitat	244	0.73	0.00	Comprehensive	Chordata: Mammals	N
Fonseca *et al*. (2010)	Habitat	15	0.07	0.08	Partial	Chordata: Frogs	N
Forister *et al*. (2011)	Diet	50	0.20	0.04	Comprehensive	Arthropoda: Butterflies	N
Frost *et al*. (2004)	Habitat	39	0.12	0.03	Partial	Arthropoda: Amphipods	N
	Habitat	17	0.20	0.07	Partial	Mollusca: Bivalves	N
Garcia-Barros and Benito (2010)	Diet	205	0.73	0.01	Partial	Arthropoda: Butterflies	N
Goulson *et al*. (2008)	Diet	23	0.38	0.21	Partial	Arthropoda: Bees	N
Gregory and Gaston (2000)	Habitat	85	-0.13	0.02	Partial	Chordata: Birds	Y
Harcourt *et al*. (2002)	Diet	46*	0.56	0.02	Comprehensive	Chordata: Primates	Y
	Habitat	46*	0.37	0.02	Comprehensive	Chordata: Primates	Y
Harley *et al*. (2003)	Habitat	460	0.32	0.00	Comprehensive	Mollusca: Gastropods	Y
Hecnar (1999)	Habitat	230	0.45	0.00	Comprehensive	Chordata: Turtles	N

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Table 2.3. Continued from previous page.

Study	**Niche category**	***n***	*z*	***v(z)***	**Scale**	**Group**	**Control for sampling effects**
Heino (2005)	Habitat	40	0.35	0.02	Partial	Arthropoda: Insects	Y
Hughes (2000)	Diet	11	0.94	0.13	Comprehensive	Arthropoda: Butterflies	N
Hurlbert and White (2007)	Habitat	298	0.49	0.00	Partial	Chordata: Birds	Y
Jahner *et al*. (2011)	Diet	70	0.06	0.02	Comprehensive	Arthropoda: Lepidoptera	N
Kolasa *et al*. (1998)	Habitat	40	1.18	0.03	Partial	Animal†: Invertebrates	N
Kotze *et al*. (2003)	Habitat	449	0.49	0.00	Comprehensive	Arthropoda: Beetles	N
Krasnov *et al*. (2005)	Habitat	341	0.50	0.01	Comprehensive	Arthropoda: Fleas	N
Lappalainen and Soininen (2006)	Habitat	70	0.27	0.02	Partial	Chordata: Fishes	Y
Lehman (2004)	Habitat	8	1.62	0.20	Comprehensive	Chordata: Primates	N
	Diet	8	-0.43	0.20	Comprehensive	Chordata: Primates	N
Pyron (1999)	Habitat	57	0.59	0.02	Comprehensive	Chordata: Fishes	N
Reif *et al*. (2006)	Habitat	49	-0.15	0.02	Comprehensive	Chordata: Birds	Y
Rickart *et al*. (2011)	Diet	16	0.65	0.08	Comprehensive	Chordata: Mammals	Y
Siqueira *et al*. (2009)	Habitat	41*	0.69	0.03	Comprehensive	Arthropoda: Flies	Y
Symonds and Johnson (2006)	Diet	120	0.05	0.01	Comprehensive	Chordata: Birds	Y
	Habitat	120	0.76	0.01	Comprehensive	Chordata: Birds	Y
Williams (2005)	Diet	17	-0.12	0.07	Partial	Arthropoda: Bees	Y
Williams *et al*. (2006)	Diet	8	-0.66	0.20	Comprehensive	Chordata: Frogs	Y

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Table 2.3. Continued from previous page.

Study	Niche category	*n*	*z*	*v(z)*	Scale	Group	Control for sampling effects
Plants							
Baltzer *et al*. (2007)	Habitat	503	-0.04	0.00	Comprehensive	Spermatopsida	Y
Boulangeat *et al*. (2012)	Habitat	1216	0.62	0.00	Comprehensive	Spermatopsida	Y
Burgman (1989)	Habitat	406	0.21	0.00	Partial	Spermatopsida	Y
Callaghan and Ashton (2008)	Habitat	318	0.49	0.00	Comprehensive	Bryophyta: Mosses	Y
	Habitat	111	0.51	0.01	Comprehensive	Bryophyta: Liverworts	Y
Essl *et al.* (2009)	Tolerance	103	0.83	0.01	Comprehensive	Spermatopsida	N
	Habitat	103	0.59	0.01	Comprehensive	Spermatopsida	N
Heino and Soininen (2006)	Habitat	107	0.28	0.01	Partial	Chrysophyta	Y
Kessler (2002)	Tolerance	356	0.15	0.00	Comprehensive	Spermatopsida	N
Köckemann *et al.* (2009)	Habitat	25	0.81	0.05	Comprehensive	Spermatopsida	N
Kolb *et al*. (2006)	Habitat	68	0.81	0.02	Comprehensive	Spermatopsida	Y
Luna and Moreno (2010)	Tolerance	53	0.06	0.02	Comprehensive	Spermatopsida	Y
Luna *et al.* (2012)	Tolerance	31	0.32	0.04	Comprehensive	Spermatopsida	Y
Pither (2003)	Tolerance	103	1.58	0.01	Comprehensive	Spermatopsida	N
Spitale (2012)	Habitat	67	0.22	0.02	Partial	Bryophyta	N
Thompson *et al.* (1998)	Habitat	792	0.68	0.00	Partial	Spermatopsida	Y
Tsiftsis *et al.* (2008)	Habitat	55	0.29	0.02	Partial	Spermatopsida	Y
Williams *et al.* (2010)	Tolerance	319	0.02	0.02	Comprehensive	Spermatopsida	N

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Table 2.3. Continued from previous page.

Study	Niche category	n	z	v(z)	Scale	Group	Control for sampling effects
Youssef *et al.* (2011)	Habitat	32	0.71	0.04	Partial	Spermatopsida	N
Other							
Harley *et al.* (2003)	Habitat	1096	0.36	0.00	Comprehensive	Algae	Y
Heim and Peters (2011)	Habitat	17131*	0.23	0.00	Partial	Fossil	N

* Number of genera.

† Organisms are described only as "aquatic invertebrates", so we were unable to define this group at the phylum level. However, since the study measured environmental tolerance and for this category we only examined taxonomic effects at the level of animal vs plant, this did not affect the analysis.

Table 2.4. Summary statistics from random-effects meta-analyses. *n* is the number of studies, Q_T is total heterogeneity and I^2 is the amount of total heterogeneity attributable to differences among studies.

					Heterogeneity	
Niche category	***n* (studies)**	**Mean effect (*z*)**	**95% CI**	***p***	**Q_T (*p*)**	**I^2 (95% CI)**
Habitat	44	0.45	0.35, 0.54	< 0.001	667.68 (<0.001)	95.39% (92.90, 97.56)
Diet	20	0.28	0.11, 0.45	0.001	115.91 (<0.001)	83.48% (68.60, 94.23)
Tolerance	11	0.49	0.17, 0.81	0.003	203.41 (<0.001)	94.31% (87.45, 98.40)

Table 2.5. Results of a mixed-effects meta-analysis examining the influence of taxonomic group on the relationship between niche breadth and range size. Q_M and the associated *p*-value provide a test for the effect of taxonomic group on the mean effect size, while Q_E provides a test of residual heterogeneity, estimated by τ^2. Results are only shown for taxonomic groups examined by more than one study.

Taxonomic group	***n* (studies)**	**Mean effect (*z*)**	**95% CI**	***p***	**Q_M (*p*)**	**Q_E (*p*)**	**τ^2 (95% CI)**
Habitat					1.69 (0.946)	449.13 (<0.001)	0.10 (0.06, 0.19)
Chordata	18	0.42	0.25, 0.59	<0.001			
Spermatopsida	9	0.50	0.28, 0.72	<0.001			
Arthropoda	8	0.48	0.24, 0.72	<0.001			
Bryophyta	3	0.41	0.04, 0.78	0.006			
Mollusca	2	0.28	-0.22, 0.77	0.275			
Diet					1.11 (0.293)	106.24 (<0.001)	0.11 (0.04, 0.32)
Arthropoda	10	0.36	0.13, 0.60	0.002			
Chordata	10	0.18	-0.08, 0.44	0.182			
Tolerance					0.00 (0.981)	201.29 (<0.001)	0.28 (0.11, 1.11)
Plants	6	0.50	0.07, 0.92	0.024			
Animals	5	0.49	-0.05, 1.02	0.073			

Table 2.6. Results of a mixed-effects meta-analysis examining the effect of the spatial extent of measurement (across entire species range – "Comprehensive"; across some of range – "Partial") on the relationship between niche breadth and range size. Q_M and the associated *p*-value provide a test for the effect of spatial scale on the mean effect size, while Q_E provides a test of residual heterogeneity, estimated by τ^2.

Scale	***n* (studies)**	**Mean effect (*z*)**	**95% CI**	***p***	**Q_M (*p*)**	**Q_E (*p*)**	**τ^2 (95% CI)**
Habitat					1.40 (0.236)	542.37 (<0.001)	0.08 (0.051, 0.158)
Comprehensive	24	0.50	0.37, 0.62	<0.001			
Partial	20	0.38	0.24, 0.53	<0.001			
Diet					1.30 (0.255)	80.87 (<0.001)	0.09 (0.039, 0.340)
Comprehensive	16	0.23	0.05, 0.42	0.014			
Partial	4	0.47	0.11, 0.84	0.011			

Table 2.7. Comparison of effect sizes between studies that do and do not provide some control for sampling effects. Q_M and the associated *p*-value provide a test for the effect of sampling control on the mean effect size (i.e. whether effect sizes differ between the two types of study).

	***n* (studies)**	**Mean effect (*z*)**	**95% CI**	***p***	**Q_M (*p*)**
All					0.45 (0.503)
With control	41	0.38	0.25, 0.51	<0.001	
Without control	34	0.50	0.38, 0.63	<0.001	
Habitat					1.09 (0.297)
With control	25	0.41	0.28, 0.53	<0.001	
Without control	19	0.51	0.36, 0.66	<0.001	
Diet					1.08 (0.300)
With control	10	0.19	-0.05, 0.43	0.125	
Without control	10	0.41	0.18, 0.65	<0.001	
Tolerance					0.15 (0.699)
With control	6	0.30	-0.78, 1.37	0.586	
Without control	5	0.62	0.13, 1.10	0.013	

Table 2.8. Tests for publication bias for study-level analysis. Meta-analysis results are often considered robust if fail-safe numbers are greater than 5*n* + 10, where *n* is the original number of studies (Rosenthal 1991).

Niche category	***n* (studies)**	**z_{bias} (*p*)**	**Trim-and-fill**		**Rosenberg's Fail-safe *N* (5*n* + 10)**
			L	**Corrected *z* (95% CI)**	
Habitat	44	0.37 (0.715)	0	0.45 (0.35, 0.54)	19391 (230)
Diet	20	-1.34 (0.182)	0	0.28 (0.11, 0.45)	549 (110)
Tolerance	11	1.05 (0.292)	0	0.49 (0.17, 0.81)	574 (65)

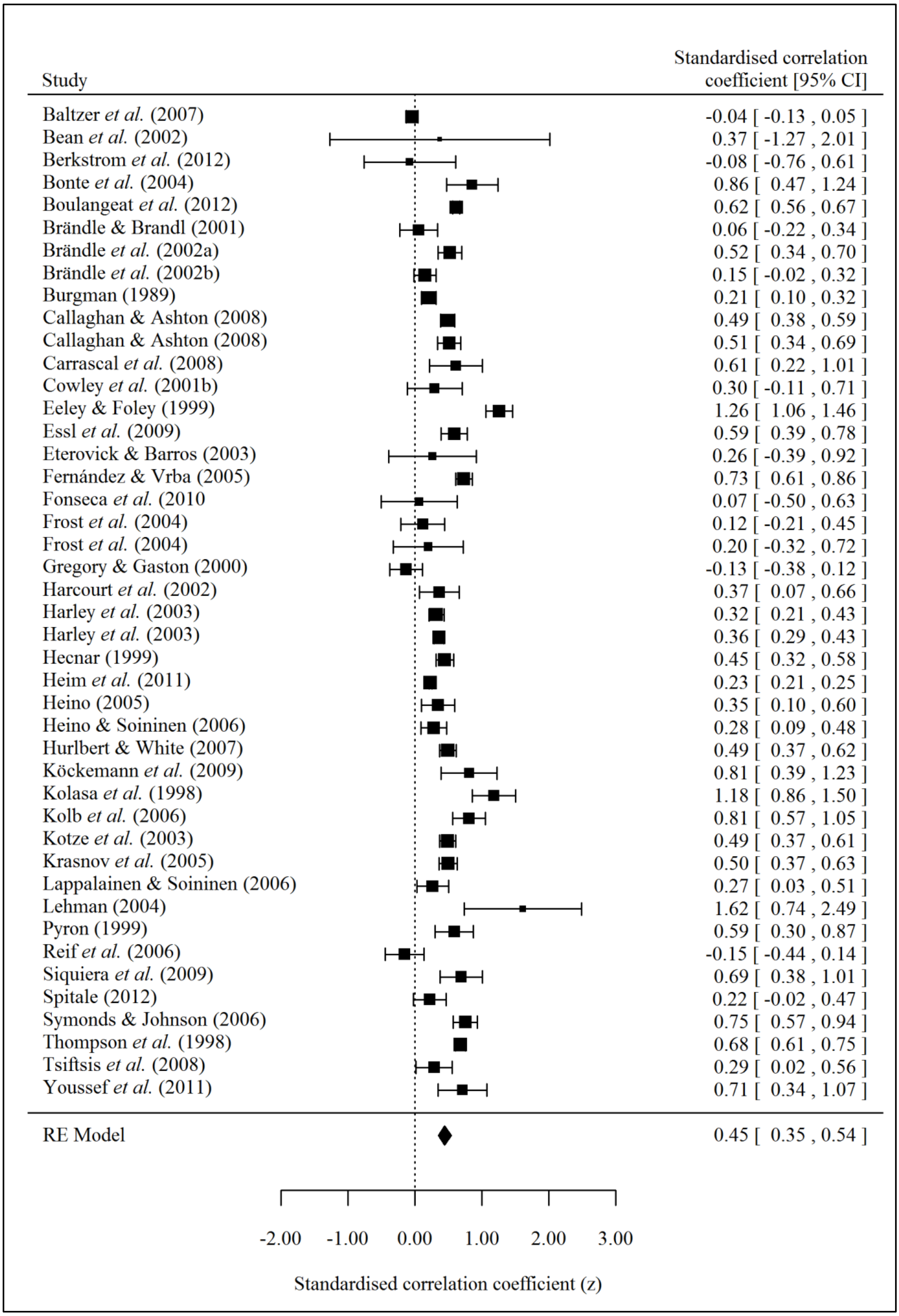

Figure 2.1. Effect sizes and confidence intervals for studies measuring the relationship between habitat breadth and range size, and results of the random-effects meta-analysis (RE model) showing the mean effect size.

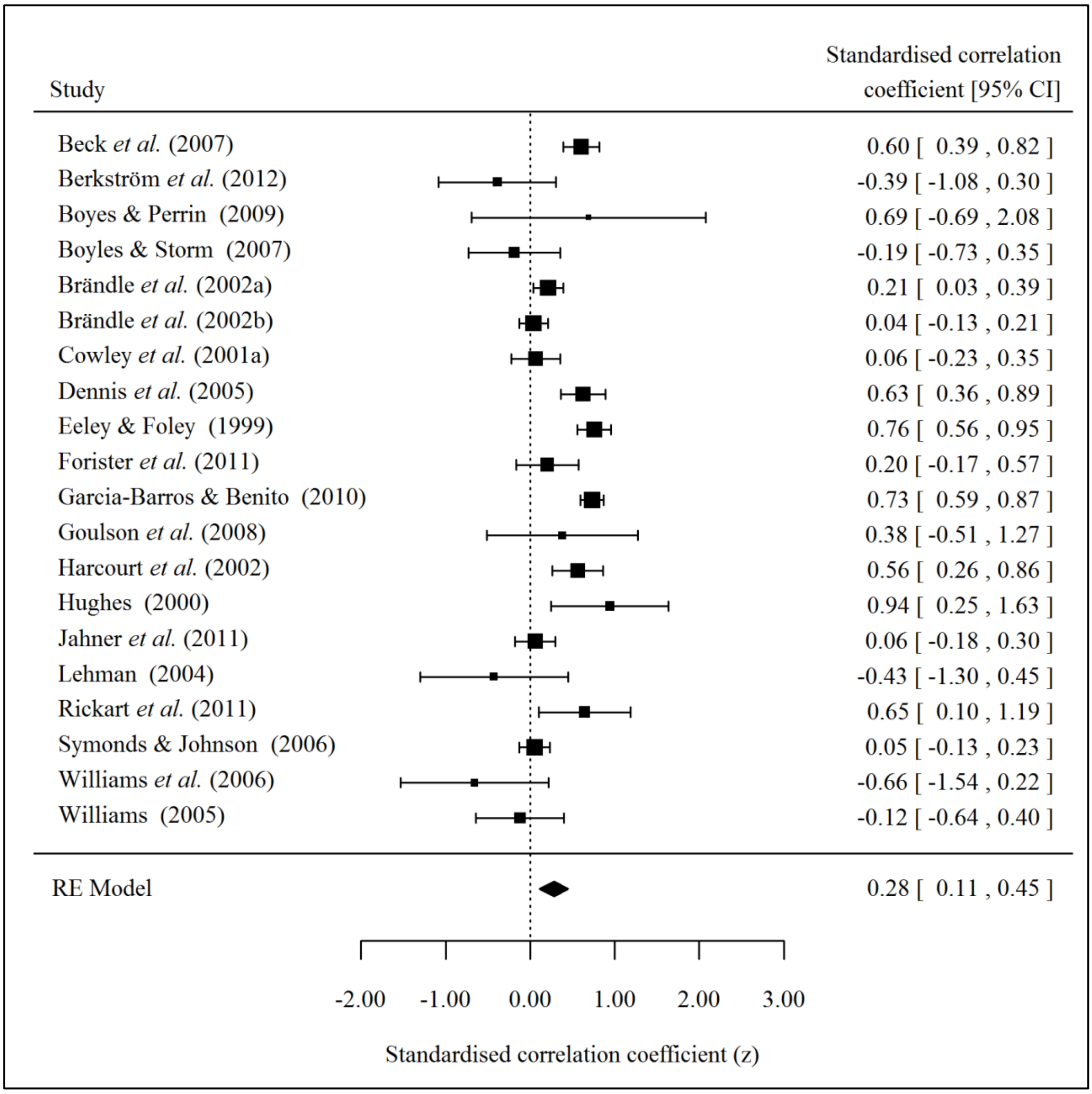

Figure 2.2. Effect sizes and confidence intervals for studies measuring the relationship between diet breadth and range size, and results of the random-effects meta-analysis (RE model) showing the mean effect size.

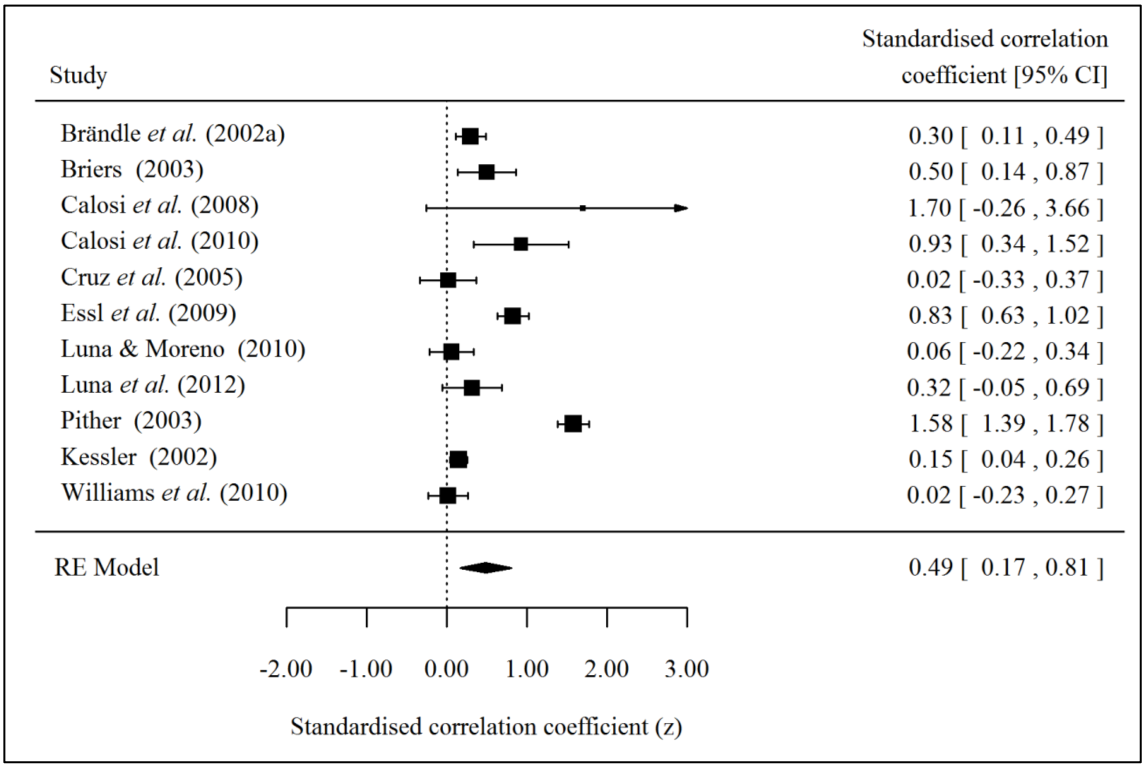

Figure 2.3. Effect sizes and confidence intervals for studies measuring the relationship between environmental tolerance breadth and range size, and results of the random-effects meta-analysis (RE model) showing the mean effect size.

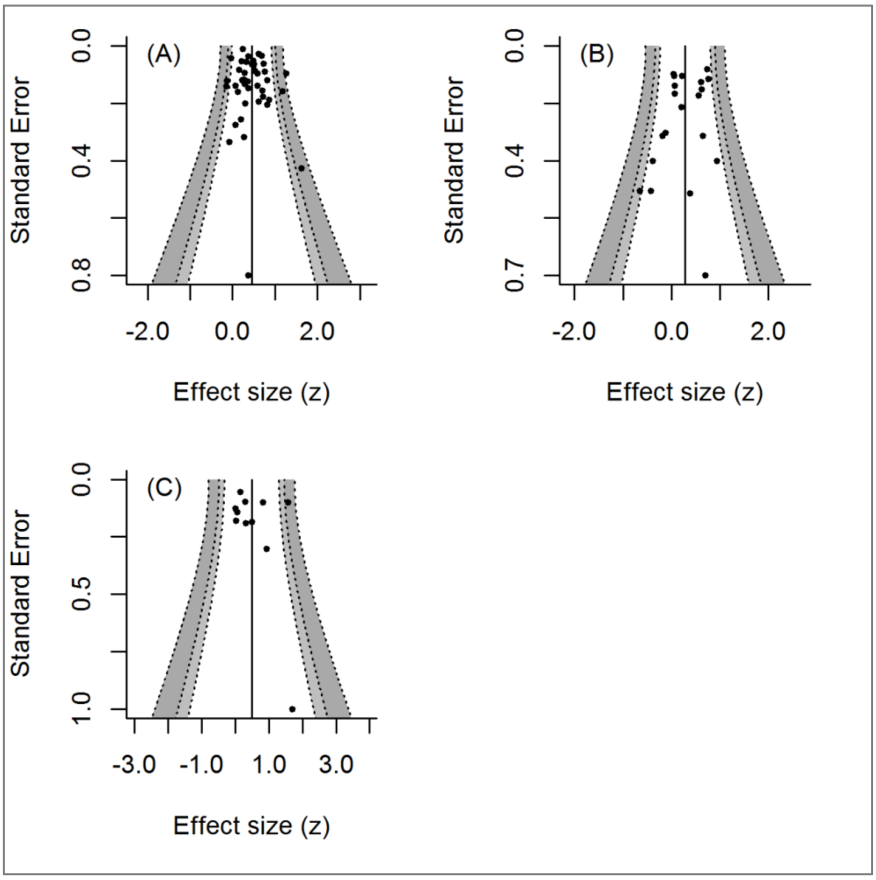

Figure 2.4. Contour-enhanced funnel plots showing the relationship between study effect size and standard error (study precision) for the relationship between range size and (A) habitat breadth, (B) diet breadth and (C) environmental tolerance range. Vertical lines indicate the model estimates (mean effect size). Contour intervals (white: 90%, light grey: 95%, dark grey: 99%) show statistical significance of study effect sizes.

2.4 Discussion

Brown (1984) suggested that species able to utilise a wider range of resources should be able to occupy a larger geographic area, leading to a positive relationship between niche breadth and range size. Yet, due to issues of sampling bias, methods of measuring niche breadth, and spatial scale, it has taken nearly three decades to verify that this pattern exists generally. Here we have shown a consistent relationship between niche breadth and range size, which is independent of broad taxonomic grouping and the scale of range measurement. That we found a highly significant mean effect suggests that the relationship between niche breadth and range size is a general ecological pattern. However, what drives this pattern, and why certain species deviate from it, is in need of further exploration.

The strongest relationship was found when the niche axis examined related to environmental tolerance, suggesting that tolerance of a wide range of abiotic conditions facilitates occupancy of a larger area. Habitat breadth, the most commonly measured metric, was also a good predictor of range size, whereas the weakest relationship (marginally non-significant) was found for diet breadth. The average effect size we estimated for habitat breadth ($z = 0.45$) is remarkably similar to that found in a recent study using path analysis to test drivers of range size variation in passerine birds ($z = 0.43$) (Laube *et al.* 2013). That study also found that diet breadth had a negligible correlation with range size ($z = -0.06$), in broad agreement with our findings here.

In their comprehensive review, Gaston and Blackburn (2000) suggested that niche position is more important than niche breadth in determining range size. Under this hypothesis, widespread species are those that utilise the most common and widespread resources (Hanski 1993). Of the nine studies included in our analysis that reported relationships between niche position and range size, seven found that range size was more strongly related to niche position than niche breadth, with mean effect sizes of $z = 0.54$ (95% CI: 0.33-0.74) and $z = 0.27$ (0.10-0.44), respectively (Appendix A). However, Passy (2012) tested modelled predictions of the relationship between niche breadth and range size using fish and microorganisms and found that adaptation to common environments was insufficient to confer a large geographic range in the absence of a broad niche. There is clearly a need for additional studies that simultaneously compare the strengths of the effects of niche breadth and position on range size and a systematic synthesis of current evidence for the niche position hypothesis.

2.4.1 Sampling and scale effects

A common criticism of the niche breadth-range size hypothesis is that sampling effects might produce a false positive correlation when niche breadth in widespread species is measured from more individuals or more locations (Brown 1984; Hanski 1993; Gaston *et al.* 1997). Burgman (1989) demonstrated the potential strength of this effect by using two measures of niche breadth: one that did not control for sampling effects and one that used statistical methods to negate any potential influence of sampling frequency. While the former gave a significant positive relationship between niche breadth and range size, there was no significant correlation when sampling effects were controlled. Similarly, Kolb *et al.* (2006) found that a positive niche breadth-range size relationship disappeared after controlling for differences in sample size between widespread and restricted species.

Over the last decade, an increasing number of studies have taken steps to explicitly control for sampling effects (e.g. Boulangeat *et al.* 2012; Luna *et al.* 2012). In our analysis, there was no statistical difference in the average effect size for studies that did and did not control for sampling effects, although the latter tended to show a stronger relationship. When examined separately, the niche breadth-range size relationship did, however, lose statistical significance among controlled diet and tolerance studies. For tolerance breadth at least, the effect size remained moderate ($z = 0.3$) and loss of statistical significance might be due to reduced sample size – additional studies are required before we can confidently attribute the initially observed relationship entirely to sampling effects. It does, however, once again highlight the need to control for sampling effects through study design (e.g. Calosi *et al.* 2008) or statistical adjustment (e.g. Burgman 1989). In contrast, when all niche measures were analysed together, or when habitat breadth was considered, the niche breadth-range size relationship remained strongly significant for studies that controlled for sampling effects; habitat breadth had the largest number of studies ($n = 25$) among different niche measures. Thus the observed general niche breadth-range size relationship cannot be explained as a sampling artefact.

The strength of the relationship between niche breadth and range size varied widely among studies. This is not entirely surprising, given the taxonomic diversity (diatoms, algae, plants and animals) included in the dataset, as well as the many different niche breadth indices (Table 2.1) and methods of measuring range size used by the studies. Although we found no statistical differences in the strength of the niche breadth-range size relationship across taxonomic groups, sample sizes for each group were small (only habitat breadth in chordates was measured in more than 10 studies) and we were unable to analyse taxonomic variation below the current taxonomic levels. Intrinsic differences among taxonomic groups might be expected to contribute to variation in the niche breadth-range size pattern if, for example, the relationship is influenced by phylogenetically conserved traits (reviewed in Wiens *et al.* 2010). However, traits such as dispersal ability, which might influence the relationship, can evolve rapidly, even within a species (e.g. Phillips *et al.* 2006), potentially rendering broad taxonomic groupings of little use in explaining among-study heterogeneity. An investigation of the niche breadth-range size hypothesis in a phylogenetic framework, incorporating relevant life-history traits, would shed much-needed light on this issue.

The high diversity in niche breadth and range size measures resulted in substantial variation in both the grain (resolution) and extent (area encompassed) of spatial scale (Wiens 1989) used across studies that might have contributed to the high heterogeneity. For example, studies that measure dietary breadth using coarse categories such as insects and plants (e.g. Brändle *et al.* 2002b) might be less likely to find a positive relationship with range size than one that measures resource use at a finer grain (Shkedy & Safriel 1992).

When considering differences in effect size among different niche axes, grain might also be important: in general, diet is necessarily measured at a finer spatial grain than habitat use. That is, an individual or species can typically access multiple food resources (e.g. different species of plants) within a single habitat type (e.g. forest). Environmental tolerance could be considered a coarser grain, as multiple habitat types (e.g. forest, grassland, heath) are often present within a given climatic zone. Considered in this way, our findings reflect a stronger niche breadth-range size relationship for coarser-grained niche breadth categories. If true, there could be a lower limit of spatial scale at which the relationship between niche breadth and range size no longer holds true.

Several authors (e.g. Cowley *et al.* 2001a; Brändle *et al.* 2002b; Baltzer *et al.* 2007) have suggested that the strength or presence of a relationship between niche breadth and range size will be scale-dependent, with different processes determining geographic ranges as the spatial extent of the investigation changes. In contrast, other studies have found significant correlations between spatial scales (e.g. Briers 2003; Callaghan & Ashton 2008). We did not find any evidence for differences in the niche breadth-range size relationship between studies conducted at a local or regional scale and those that encompassed species' entire geographic ranges, suggesting that niche breadth-range size relationships are not limited to a single spatial scale. The most appropriate scale (in terms of either grain or extent) is not immediately clear. It is therefore important that future studies explicitly define the scale that they are using so that scale-dependency, if present, can be detected in the future.

2.4.2 Evolutionary and historical effects

It is straightforward to understand a link between a species' niche breadth across its entire range and its range size. It would almost be surprising if such a pattern didn't exist. What is less intuitive is whether factors influencing local population dynamics in a single habitat are also relevant for determining global distribution patterns. This is the question being asked when niche breadth is measured from populations or individuals and is at the heart of the niche breadth-range size hypothesis.

Species inhabit environmental (or ecological) space through adaptive differentiation among individuals, phenotypic plasticity within individuals, or both (Ackerly 2003). It is therefore difficult to disentangle whether niche breadth is maintained through an aggregate process of microevolution to multiple environments or broad tolerance (Ackerly 2003). If the former, species having larger niche breadths may be comprised of many locally adapted populations (e.g. Olsson *et al.* 2009), posing a greater risk of local extinction under climate change (Atkins & Travis 2010; Kelly *et al.* 2012). Alternatively, large-niche species may be comprised of individual "generalists", having great environmental tolerance, in which case one would expect lower vulnerability. Of course, a continuum between the above scenarios likely exists, but is one pattern more prevalent than the other? Adaptive differentiation can also evolve quickly and this has been commonly observed during the process of invasion, allowing widespread species to extend their ranges even further (e.g. Phillips *et al.* 2006).

None of the studies we examined sought to disentangle these microevolutionary effects on niche breadth. Although some previous research has explored the underpinnings of niche breadth (e.g. Atkins & Travis 2010; Bolnick *et al.* 2010), further experiments that establish whether niche breadth is more strongly influenced by among-individual variation versus within-individual performance (and equally, among-population versus within-population variation) are sorely needed. Classification of specialists and generalists can depend on whether resource use is measured at the species, population or individual level (Roughgarden 1972). Thus a species might be a local specialist but a regional generalist if it is comprised of locally adapted populations (Hughes 2000). In such a scenario, measuring niche breadth as the average population niche breadth, compared to the species niche breadth, will produce dramatically different results. Similarly, a generalist population can be composed of either specialist or generalist individuals with high among- and within-individual variation respectively (Roughgarden 1972; Bolnick *et al.* 2010). To-date, such research has rarely been applied in the context of large-scale ecological patterns (but see Angert *et al.* 2011b) and consequently, the strength of the influence of adaptive differentiation on these patterns is unclear.

Evolutionary processes as discussed above can obscure the direction of causality in the niche breadth-rage size relationship. The results presented here are unable to address the causal mechanisms driving the observed relationship. Although a broad niche is often assumed to allow expansion into a larger geographic area this direction of causality is not a given. If, for example, species-level niche breadth is maintained through genetic variation in environmental tolerance (i.e. local adaptation), geographic range expansion (accompanied by spatially varying selection) might drive an increase in niche breadth. Similarly, recent work suggests that among butterflies, diet breadth might be influenced by range size, with widely dispersing species encountering a greater range of host plants and thus evolving to utilize a greater variety of species, including exotic species (Jahner *et al.* 2011). Feedback mechanisms might also operate, making causality bi-directional with underlying genetic variation and dispersal capability allowing initial colonisation of new areas, where evolutionary processes promote differentiation and niche expansion. However, until there is research that can determine to what extent the relationship is driven by within versus among population variation, it will be impossible to identify the causal links between local niche breadth and geographic range size. With rapid, modern climate change, whether niche breadth is determined primarily through adaptive differentiation or phenotypic plasticity may produce very different outcomes for species' distributional responses.

Evolutionary effects might also be important if individual-based performance under varying conditions is predictable from close relatives. Phylogenetic conservatism – the tendency for closely related species to have more similar traits (e.g. ecological niche) than distantly related species – is a common observation in ecology (Wiens 2004). When testing ecological patterns, it is therefore important to take relatedness into account to be sure that observed patterns are not merely a phylogenetic artefact (Cooper *et al.* 2010). Although phylogenetic signal in various niche-based traits has been detected (reviewed in Losos 2008), we know of very few studies that have specifically tested for phylogenetic conservatism in niche breadth *per se* (but see Brändle *et al.* 2002b; Emery *et al.* 2012). 13 studies in our analysis used phylogenetically independent contrasts to control for potential effects of phylogenetic relatedness. None found a substantial difference in the magnitude of the niche breadth-range size relationship with and without independent contrasts, and two (Gregory & Gaston 2000; Thompson & Ceriani 2003) found that the strength of the relationship actually increased after controlling for phylogenetic effects. Nevertheless, the above studies are based mainly on descriptive estimates of niche breadth (but see Luna & Moreno 2010; Luna *et al.* 2012). There is a strong need for studies that experimentally compare niche breadth (e.g. environmental tolerance) among close relatives, especially to understand whether rarer relatives actually lack the physiological capacity or genetic variation to tolerate rapidly changing environments. Current evidence suggests that this is the case (e.g. Kellermann *et al.* 2009).

Previous studies have not detected (or only detected weak) phylogenetic signal for range size – sister taxa often have dissimilar range sizes (reviewed in Waldron 2007). Indeed, there are many well-known examples where closely related species vary greatly in their geographic range extent (e.g. the widespread common dandelion (*Taraxacum officinale)* has a congener (*T. pankhurstianum)* restricted to a single Scottish island: Richards & Ferguson-Smyth 2012). A similar lack of phylogenetic signal in niche breadth would suggest that this trait is evolutionary labile. This is supported by recent evidence of rapid shifts in habitat specificity among birds (Barnagaud *et al.* 2011) and niche expansion following invasion in mites (Hill *et al.* 2013).

Invasive species that flourish in new regions are clear evidence that limited dispersal ability can prevent range expansion and filling of the fundamental niche. Similarly, Dullinger *et al.* (2012) suggest that post-glacial migration lags among Austrian alpine flora have caused nearly a third of species to occupy less than half of their predicted suitable range. Historical processes such as these are thus likely to add noise to the observed niche breadth-range size relationship. Dullinger *et al.* (2012) also showed that dispersal capacity greatly increased the amount of range-filling, suggesting that incorporating dispersal ability into niche breadth-range size analyses might provide insights into the underlying variation in this pattern.

2.4.3 Niche breadth, range size and extinction risk

Climate change and habitat loss are key threats to biodiversity worldwide (Schipper *et al.* 2008). Many ecological traits have been shown to correlate with a species' vulnerability to these threats including body size (e.g. Cardillo *et al.* 2005), dispersal ability (e.g. Kotiaho *et al.* 2005) and plant pollination strategy (e.g. Pilgrim *et al.* 2004). Models predict that specialists should decline more quickly than generalists (Thuiller *et al.* 2005), with a greater likelihood of disrupted interspecific interactions (e.g. predator-prey relationships) and loss of required resources. Furthermore, species with broader niches might be able to respond more quickly to climate change (Dullinger *et al.* 2012) and have a faster rate of niche evolution (Lavergne *et al.* 2013). Such rapid niche evolution is associated with lower recent population declines (Lavergne *et al.* 2013).

There is substantial recent evidence, from a variety of taxa, supporting the theory that specialists are declining more quickly, or are more vulnerable to climate change and habitat loss than their broader-niche relatives (e.g. fish: Munday 2004; bats: Boyles & Storm 2007; birds: Seoane & Carrascal 2008; frogs: Botts *et al.* 2013; plants: Ozinga *et al.* 2013; see also Heim & Peters 2011). However, most models predicting vulnerability to climate change assume static niches in both time (i.e. no evolutionary response) and space (i.e. species averages or extrapolation from population traits) (Lavergne *et al.* 2010). Failure to take temporal and spatial variation into account will tend to cause over- and under-estimation of extinction risk respectively (see Harte *et al.* 2004; Atkins & Travis 2010). For example, the widely distributed copepod *Tigriopus californicus* has an upper temperature limit of approximately 38°C, but some populations near the northern (high-latitude) edge of its distribution have upper limits of less than 35°C with limited potential for adaptation to higher temperatures (Kelly *et al.* 2012). This species might therefore be more vulnerable to climatic changes than what would be predicted based on species' averages or overall tolerance breadth.

Geographic range size has similarly been identified as a predictor of species' vulnerability to climate change and disturbance and is perhaps the trait most commonly correlated with extinction risk (see Angert *et al.* 2011a). A recent model predicts a negative relationship between range size and extinction risk, independent of other inter-correlated traits (Birand *et al.* 2012). Large-range species might be less vulnerable to extinction through two non-exclusive mechanisms. First, species with a larger range size might be more resilient to change since they are better able to recolonise areas following localised extinctions (Gaston 2003). Second, large-ranged species might be more resistant to disturbance because they are more likely to occupy "refuge" habitats (Gaston 2003). However, as discussed above, if widely distributed species also show strong local adaptation, range size alone might seriously underestimate extinction risk (Atkins & Travis 2010).

The abundance-occupancy relationship – one of the most general patterns in ecology (reviewed in Gaston *et al.* 2000) – means that species with small range sizes also tend to be locally rare and vice versa. Our results suggest that these same species are most likely to be specialised and these separate effects could disproportionately magnify their extinction risk (Davies *et al.* 2004; Munday 2004; Williams *et al.* 2006; but see Hobbs *et al.* 2010). Consequently, while the presence of a niche breadth-range size relationship provides an initial indicator of potential vulnerability, experiments are urgently needed to understand rarity, especially field experiments under changing conditions where abiotic and biotic factors may interact strongly. Such experiments might also help to explain heterogeneity in the niche breadth-range size relationships, with some species likely to be rare by, for example, low tolerance and others by specialisation (e.g. local mutualisms).

2.4.4 Conclusions

Here we have shown a positive relationship between niche breadth and range size that is maintained across niche breadth measurements, taxonomic groups and spatial scales. This result challenges earlier claims that such a relationship does not exist and is purely a statistical artefact. Instead, we suggest that it is a general ecological pattern and that niche breadth can explain at least some of the variation in geographic range size among taxa. Other species traits, such as niche position, might also be important in determining range size, but systematic review of the current evidence is required. Understanding the underlying causes of this pattern, especially in a phylogenetic context, can tell us about the evolution of niche breadth, the importance of population structure to climate tolerance, and the underpinnings of rarity and commonness in nature. We suggest several directions for future research:

- Additional studies that directly test both the niche position-range size and niche breadth-range size hypotheses are needed to determine the extent to which these patterns are both true and interrelated.
- Experimental comparisons of niche breadth among close relatives will help to determine whether rarity is driven primarily by lack of physiological capacity or genetic variation to tolerate changing conditions. These would ideally be conducted under field conditions where biotic and abiotic factors interact.
- Investigations of the niche breadth-range size relationship in a phylogenetic framework that allows incorporation of life history traits and dispersal ability are needed.
- Tests of niche breadth in narrow and widespread species that incorporate species-, population- and individual-level variation would allow partitioning of overall niche

> breadth between genetic variation and phenotypic plasticity and are crucial to uncovering the causal links between local processes and geographic distribution.

Besides uncovering general, predictive patterns, we should consider how species' distributions could inform us about ecological and evolutionary theory. In this regard, species that are exceptions to the general pattern present opportunities for understanding a wide variety of important effects on distribution.

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Astronomy was a respected science long before ecology, and Copernicus and Galileo never moved a star.

Robert MacArthur

(quoted by J Brown, 1999, The legacy of Robert MacArthur:
from geographical ecology to macroecology)

Chapter 3

Ecological correlates of geographic range size: a case study in North American ground beetles (genus *Nebria*)

Abstract

Geographic range size can differ markedly among closely related species, but an understanding of the underpinnings of this variation remains elusive. Niche breadth and niche position – measures of the range of resources used and the commonness of these resources in the environment, respectively – have both been proposed as general predictors of range size variation. Despite the possibility that both niche traits are important in determining range size, they have rarely been tested simultaneously nor have their interactive effects been considered. I tested the relationship between environmental niche breadth, niche position and range size among 25 ground beetles (genus *Nebria*) from North America in a phylogenetic framework. Niche position was the strongest predictor of range size, with species utilising environments that were common across the study area having larger ranges. In contrast, niche breadth did not show the predicted positive relationship with range size. There was, however, a significant interaction between environmental niche breadth and position: the latter was a strong determinant of range size among species with a narrow niche and its effect became weaker as niche breadth increased. This result suggests that niche position and niche breadth might have synergistic effects on range size variation.

3.1 Introduction

A long-standing challenge in ecology is to understand the processes that structure the geographical distributions of species. As discussed in Chapter 2, range size can differ dramatically among closely related species and several mechanisms have been proposed as drivers of range size variation. These mechanisms include niche characteristics (Brown 1984; Hanski 1993), physical traits (e.g. dispersal ability: Lester *et al.* 2007; Arribas *et al.* 2012), and historical effects (e.g. evolutionary age: Willis 1922; Webb & Gaston 2003). Each of these factors is proposed to control either the size of the potential range (i.e. the geographical area encompassed by the fundamental niche) or the degree to which this potential space is occupied (i.e. range filling) (e.g. Dullinger *et al.* 2004).

Niche characteristics are expected to control range size variation by delimiting the environments in which viable populations of a species can persist (Hutchinson 1957; Brown 1984). In Chapter 2 (Slatyer *et al.* 2013), we found a positive relationship between niche breadth (the number/range of resources used) and range size across a broad spectrum of taxonomic groups, supporting Brown's (1984) hypothesis that species able to utilise a greater array of resources should be able to maintain viable populations within a broader range of conditions and, consequently, become more widespread.

Niche position describes the commonness of the niche within a landscape: species utilising a common resource (low niche position) are expected to occupy a larger geographic area than those using a rare resource (high niche position) (Hanski 1993; Gaston & Blackburn 2000). The predicted negative relationship between niche position and range size has been documented in several taxa, including birds (e.g. Gregory & Gaston 2000; Laube *et al.* 2013), fish (e.g. Tales *et al.* 2004), insects (e.g. Heino 2005; Heino & Grönroos 2014), and plants (Kolb *et al.* 2006). Although the niche breadth and niche position hypotheses are not mutually exclusive, they have rarely been examined in concert. When tested simultaneously, niche position typically emerges as a stronger predictor of range size variation (e.g. Heino & Grönroos 2014; Table A.1), but this is not always the case (e.g. Sheth *et al.* 2014). However, Passy (2012) suggested that a broad niche was a necessary pre-requisite for a positive association between niche position and range size – that is, species with a narrow niche occupy a small geographic range, regardless of niche position. This implies an interaction between niche traits that remains untested.

In contrast to niche characteristics, colonisation ability is expected to control range size primarily through its effects on range filling (e.g. Dullinger *et al.* 2004; Siefert *et al.* 2015). Both species traits (i.e. intrinsic factors governing dispersal ability) and attributes of the landscape (i.e. extrinsic factors governing dispersal opportunity) will determine colonisation ability and, in turn, could influence range size (Lester *et al.* 2007; Gaston 2009). For example, physical barriers (e.g. water bodies, for terrestrial species) might prevent occupation of climatically suitable habitat and disrupt the expected relationship between a species' niche and its geographic range (MacArthur 1972; Baselga *et al.* 2012; Nakazawa 2013). In topographically complex environments, such as mountain regions, dispersal ability might therefore be a more important driver of variation in geographic range size among species than niche characteristics (Siefert *et al.* 2015).

Ground beetles (Coleoptera: Carabidae) in the genus *Nebria* Latreille demonstrate enormous variation in geographic range size. Among 55 North American species, range extent (the distance between the two farthest occurrence points) varies from a mere 10 km to over 22000 km and, for at least half of the sister species pairs, the range size of one species is more than twice as large as the other (Kavanaugh 1979b, 1985). Species in this diverse group primarily occupy cool, moist, temperate environments but occur across a wide range of elevations, with some species restricted to alpine habitats, some found along the coast and at low elevations and others occurring across a broad elevational range (Kavanaugh 1979a). Many of the North American species are brachypterous (have reduced wings), with some degree of hindwing atrophy evolving independently at least 17 times (Kavanaugh 1985). As is common among insects, the incidence of brachyptery in *Nebria* increases with elevation (Kavanaugh 1985; Hodkinson 2005). Kavanaugh (1985) also notes that brachypterous species have significantly smaller geographic ranges than macropterous (fully-winged) forms, suggesting that dispersal ability is an important determinant of realised range size. The variation in range size, elevation and wing structure among North American *Nebria* make them an appropriate group in which to test the factors driving range size variation.

In this study, I examine the relationship between niche traits, dispersal ability and range size, using a macroecological framework to test the relative influence of environmental niche position and breadth in predicting geographic distribution, and explore interaction between these traits.

3.2 Methods

3.2.1 *Occurrence data*

A common tool for measuring niche breadth utilises broad-scale climate data and species distribution information to extract the range of environmental conditions under which a species occurs. This approach relies on occurrence data which is often scarce for insects and other small organisms. I thus used three methods to generate an occurrence database for each species. First, species records were downloaded from the Global Biodiversity Information Facility (GBIF). These records were screened for duplicates and obviously incorrect coordinates (e.g. in the ocean, locality descriptions not matching coordinates). Second, 128 transect surveys were completed over 32 nights, on 13 mountains in northern California, Oregon, and Washington, USA, between May and July, 2014 (Table 3.1; see also Appendix B for a map). Each transect was 100 m long and ran parallel to the slope, and all beetles within 5 metres of the transect line were identified. Transect elevations were between 600 m and 2650 m a.s.l, and were surveyed in the first three hours after last light, when beetles are most active. These mountains cover most of the length of the Cascade Range, as well as mountains in the Coast Range and Olympic Mountains, where *Nebria* are common. Finally, presence data were extracted from the primary literature on each species, where available (Schoville *et al.* 2012). Data from the three methods were combined and sub-sampled to a single record per 1 km^2, yielding a complete dataset of 982 occurrence points from 25 species, with 12 (*N. vandykei*) to 97 (*N.gyllenhalii*) records per species (Table 3.2). It is important to note that several species with very restricted geographic distributions could not be included due to a lack of occurrence data.

The study region was defined by a minimum convex polygon with a 100 km buffer around all the species occurrence points.

3.2.2 *Climate data*

To estimate the climatic niche space occupied by each species within the study region, I obtained 11 variables representing aspects of temperature and precipitation (WorldClim: Hijmans *et al.* 2005), vegetation cover (MODIS44B: DiMiceli *et al.* 2011), and geography (elevation, slope and aspect) (GMTED2010, HYDRO1K: Data available from the U. S. Geological Survey)(see Oke & Thompson 2015 for justification for including geographical variables). From the WorldClim variables, I chose seven representing both temperature and precipitation that were not highly correlated with each other ($r < 0.75$) across the study region: Mean diurnal range (BIO2), isothermality (BIO3), maximum temperature of the warmest month (BIO5), minimum temperature of the coldest month (BIO6), precipitation seasonality (BIO15), precipitation of the warmest quarter (BIO18), and precipitation of the coldest quarter (BIO19). All variables were re-projected into an Albers equal area projection and re-sampled to a 1 km x 1 km grid size. Re-projections and resampling were carried out using ArcMap 10.3.1 (ESRI 2011). For each species, values of environmental variables were extracted for each 1 km x 1 km grid cell for which there was an occurrence point and these data were used to calculate niche breadth and position (see below).

Table 3.1. Locations of transect surveys. Latitudes (ºN) and longitudes (ºE) are the mean values for all the transects in a given mountain/area. Species abbreviations are as follows: *Nebria acuta* (ac), *N. crassicornis* (cr), *N. gebleri* (ge), *N. kincaidi* (ki), *N. mannerheimi* (ma), *N. meanyi* (me), *N. paradisi* (pa), *N. piperi* (pi), *N. sahlbergii* (sa), *N. vandykei* (va).

Mountain/area	Latitude	Longitude	# transects	Elevation span	*Nebria* species detected
Mt Baker	48.72450	-121.83454	7	1350 – 1530	*ac, cr, ge, me*
Cascade Pass	48.46797	-121.06885	7	1050 – 1580	*ac, cr, ge, me, pa, va*
Glacier Peak	48.05070	-121.15871	14	1560 – 1977	*ac, cr, ge, me, pa, va*
Mt Hood	45.37251	-121.65283	14	930 – 2060	*ac, cr, ge, ki, me, pa, sa, va*
Monte Cristo	47.97759	-121.35901	7	1320 – 1430	*ac, me, pa*
Seven Lakes Basin	47.90837	-121.77876	2	1470 – 1550	*ki, va*
Mt Rainier	46.79990	-121.73773	29	960 – 2690	*ac, cr, ge, ki, ma, me, pa, pi, sa, va*
Rainy Lake	48.49937	-120.73801	4	1460 – 1570	*cr, ge, me, va*
Mt Shasta	41.43621	-122.17295	13	2250 – 2930	*me*
Table Mountain	48.85681	-121.69463	5	1240 – 1460	*ac, ge, me, va*
Thompson Peak	41.00714	-123.04818	11	1910 – 2490	*pa, va*
Thornton Lakes	48.68919	-121.33295	1	1415 – 1440	-
White Chuck Pass	48.21440	-121.42803	11	1440 – 1620	*ac, cr, ge, ma, me, pa, pi, va*

3.2.3 Environmental niche breadth, niche position and dispersal ability

The outlying mean index (Doledec *et al.* 2000) is a multivariate method that estimates both niche position and niche breadth by measuring the distance (in multivariate space) between environmental conditions within locations occupied by a species and conditions within a study area. The niche position is represented by the mean, while the variance provides a metric of niche breadth. Species occurring in marginal habitats have a high value for niche position, while those occurring in habitats that are common across the study region have a low niche position value. No assumptions are made about the species response curve, and this method was chosen to provide a simple metric with which to calculate both parameters of interest. The estimation of niche position relies on comparing the niche space occupied by a species to the range of potential environments in the landscape. To characterise the environments within the study region, I generated 10000 random points, each separated by at least 1 km (Hijmans & Elith 2015). Some of these points (n = 612) fell within water (e.g. lakes/rivers) and so data could not be extracted for all environmental layers. After removing these points, background points were combined with the species occurrence points (total n = 10361). Environmental data were extracted for each point and these values were used to construct an estimate of each species' niche breadth and position within the available environmental niche space, based on the first two principal component axes.

A common criticism of studies testing the relationship between niche breadth and range size, particularly methods reliant on occurrence data, is that sampling bias will tend to produce a positive result, irrespective of the underlying relationship (Burgman 1989). In this data set, range size was positively correlated with the number of species occurrence points ($r^2 = 0.33$, $p = 0.002$, $n = 25$), so I used a re-sampling method to test the sensitivity of niche parameters to the number of occurrence points (Siqueira *et al.* 2009). For each species, 12 occurrence points (the minimum number for a species in the dataset) were sampled at random and niche breadth and position calculated from this reduced dataset. This process was repeated 1000 times and the averaged estimates of niche breadth and position were used in subsequent analyses.

Hindwing structure was used as a proxy for dispersal ability, with species designated as either macropterous (fully-winged) or brachypterous (some reduction in hindwing size) (Kavanaugh 1985).

3.2.4 Phylogenetic signal

Traits might not be statistically independent among closely related species due to shared recent evolutionary histories (Felsenstein 1985). I tested the assumption of phylogenetic independence for environmental niche breadth, environmental niche position and range size using three methods: Abouheif's C_{mean} (Abouheif 1999), Pagel's λ (Pagel 1999), and a comparison of Brownian motion (Felsenstein 1985) and 'white noise' (phylogenetic independence) models of trait evolution. These tests were implemented in R 3.2.0 (R Core Team 2014), with the ADEPHYLO 1.16 (Jombart & Dray 2008), PHYTOOLS 0.4.56 (Revell 2012), and GEIGER 2.0.3 (Harmon *et al.* 2008) packages, respectively (Münkemüller *et al.* 2012). There is no molecular phylogeny published for *Nebria*, and I therefore used a morphological phylogeny (Kavanaugh 1978, 1985), with one clade updated based on new molecular analyses (Clarke *et al.* 2001). Branch lengths were generated using the method of Grafen (Grafen 1989) with the APE 3.2 package (Paradis *et al.* 2004) in R.

3.2.5 Statistical analysis

First, to test the effectiveness of the outlying mean index for characterising species' distributions, I tested whether species estimated niche positions were significantly different from the environmental average using a permutation test with 1000 permutations in the ADE4 package in R (Doledec *et al.* 2000; Dray & Dufour 2007).

I used the linear range extent reported by (Kavanaugh 1985) as a measure of geographic range size. This provides an estimate that is independent of the species occurrence points used to generate estimates of niche parameters. I investigated the factors influencing range size variation using a series of regression models including all combinations of environmental niche breadth, niche position and dispersal ability, as well as two-way interactions. Akaike's Information Criteria with correction for small sample size (AICc) was used to select the best-supported models (Symonds & Moussalli 2010) and produce a 95% confidence set of models (cumulative AICc weights = 0.95) (Burnham & Anderson 2002). Model-averaging amongst this set was used to produce parameter estimates. I also calculated predictor weights by summing the AICc weights of all the models with a given predictor (Burnham & Anderson 2002). Model selection and averaging were performed using niche parameters estimated from all occurrence points, and from sub-sampled occurrences. However, as the estimates from all and sub-sampled occurrences were highly correlated (Spearman rank correlation: niche position: ϱ = 1.00; niche breadth: ϱ = 0.99), only the results based on all occurrence points are reported below. Results from sub-sampling are provided in Appendix B. I also repeated the analysis using phylogenetic independent contrasts (Felsenstein 1985), produced with CAPER 0.5.2 (Orme *et al.* 2013) in R. Model selection and model averaging was performed with MUMIN 1.13.4 (BARTON 2015). Range size, niche breadth and niche position data were all log-transformed prior to analysis to improve their fit to a normal distribution (assessed using a Shapiro-Wilks test).

Data are presented as mean ± s.d. unless otherwise stated.

3.3 Results

3.3.1 Predictors of range size variation

The first two principal component axes described 85% of the variation in environmental parameters across the study region. The most important environmental variables contributing to the environmental niche position of species were elevation, slope and winter precipitation, followed by summer precipitation and diurnal temperature range.

All species occupied niche space significantly different from the environmental average (permutation test, all $r < 0.002$) and the two niche characters estimated – niche breadth and niche position – were not correlated (Spearman rank correlation: $\varrho = 0.34$, $p = 0.10$). *Nebria acuta* had the largest environmental niche breadth, followed by *N. meanyi,* while *N. lacustris* and *N. pallipes* had the smallest environmental niche breadths. *Nebria vandykei*, and *N. paradisi,* both alpine-restricted species, had the most marginal environmental niches, while *N. gyllenhali* and *N. hudsonica* occupied the least marginal (i.e. most common) niche space (Table 3.3).

The global model for range size variation, including all predictors, showed a good fit to the data (adjusted $r^2 = 0.77$). The best-supported models for geographic range size all contained both niche position and niche breadth (Table 3.3). As predicted by the niche position-range size hypotheses, niche position was negatively correlated with range size (species occupying more environments that were more common across the landscape were more widespread; Table 3.4, Fig. 3.1). Although niche breadth showed a positive association with range size, this effect was small with a confidence interval overlapping zero (Table 3.4, Fig. 3.1). Macropterous species tended to have larger range sizes (positive effect size) but, as for niche breadth, the model-averaged estimate of the effect size was small (Table 3.4). Two-way interactions between niche position, niche breadth and dispersal ability were included in the 95% confidence model set, but in each case, effect sizes were small with confidence intervals overlapping zero.

Table 3.2. Carabid beetle (genus *Nebria*) species included in the analysis and a summary of key parameters. The number of occurrences is the number of presence points after sub-sampling to a single record per 1 km² for each species.

Species	Occurrences (*n*)	Range size[1] (km)	Niche breadth	Niche position	Fully winged?[2]
N. gyllenhali	97	22550	3.8	2.2	N
N. nivalis	43	17100	1.1	4.3	Y
N. metallica	69	5160	2.1	10.9	Y
N. frigida	17	4870	0.4	3.5	Y
N. obliqua	73	4440	3.4	4	Y
N. sahlbergii	90	4160	4.4	9.2	N
N. acuta	30	3420	8.9	31.2	Y
N. arkansana	37	3390	3.8	13.5	N
N. hudsonica	46	3350	2.2	2.6	N
N. gebleri	61	3100	6.5	13.1	Y
N. meanyi	18	3000	7.8	41.3	Y
N. mannerheimii	46	2870	4.2	15.9	Y
N. crassicornis	74	2830	5.9	13.5	Y
N. pallipes	26	2240	0.4	7.2	Y
N. lacustris	31	2160	0.2	5.3	Y
N. piperi	17	2060	6.7	21.5	Y
N. eschscholtzii	33	1800	1.3	21.7	Y
N. diversa	14	1740	1.8	25.4	N
N. kincaidi	13	1570	7.1	44	N
N. gregaria	20	1540	4	17	N
N. vandykei	12	550	4.4	49.6	N
N. ovipennis	27	480	0.5	32.7	N
N. paradisi	13	320	3.8	58.7	N
N. spatulata	41	280	0.7	36.8	N
N. ingens	25	200	0.8	39.4	N

[1] Maximum linear range extent, data from Kavanaugh (1985).

[2] Proxy for dispersal ability, data from Kavanaugh (1985).

Table 3.3. 95% confidence set of models for range size variation among 25 species of carabid beetle (genus *Nebria*). Models were run with (a) and without (b) phylogenetic independent contrasts (PIC). AICc is the Akaike Information Criteria corrected for small sample size, ΔAICc is the difference in AICc from the model with the lowest AICc and AIC_{weight} is the likelihood of a model being the best model, given the data and the set of candidate models (Burnham & Anderson 2002). Parameters included in the models were environmental niche position (ENP), environmental niche breadth (ENB), and wing structure as a proxy for dispersal ability ("disp").

Analysis/model	AICc	ΔAICc	AIC_{weight}	Cumulative AIC_{weight}	Adj. R^2
(a) *Without PIC*					
ENP + ENB + disp	49.03	0.00	0.36	0.36	0.77
ENP + ENB + disp + ENB:disp	50.74	1.71	0.15	0.51	0.78
ENP + ENB + ENP:ENB	51.41	2.38	0.11	0.62	0.75
ENP + ENB + disp + ENP:ENB	51.46	2.43	0.11	0.72	0.77
ENP + ENB	51.79	2.76	0.09	0.81	0.73
ENP + ENB + disp + ENP:disp	51.97	2.94	0.08	0.89	0.78
ENP + ENB + disp + ENP:disp + ENB:disp	52.96	3.93	0.05	0.94	0.77
(b) *With PIC*					
ENP + ENB + disp + ENP:ENB + ENP:disp	31.58	0	0.60	0.60	0.97
ENP + ENB + disp + ENP:ENB	32.95	1.37	0.30	0.90	0.93
ENP + ENB + ENP*ENB	37.18	5.60	0.04	0.94	0.81
ENP + disp	38.90	7.32	0.02	0.95	0.69

Table 3.4. Parameter estimates for predictors of range size variation in *Nebria*, based on model-averaging over the 95% confidence set of models for (a) all species, and (b) all species with analysis based on phylogenetic independent contrasts (PIC). Parameters are arranged in order of decreasing $\mathrm{AIC}_{\mathrm{weight}}$, which represents the probability that a predictor is a component of the best model and provides an estimate of parameter importance. Parameters included in the models were environmental niche position (ENP), environmental niche breadth (ENB), and wing structure as a proxy for dispersal ability ("disp").

Analysis/parameter	**Cumulative $\mathrm{AIC}_{\mathrm{weight}}$**	**Estimate**	**95% CI**
(a) All species			
ENP	1	-0.99	-1.31, -0.66
ENB	1	0.39	-0.20, 0.99
Disp	0.80	0.48	-0.49, 1.46
ENP:ENB	0.27	0.17	-0.11, 0.44
ENB:disp	0.23	-0.32	-0.83, 0.20
ENP:disp	0.17	0.26	-0.38, 0.89
(b) PIC			
ENP	1	-3.52	-4.84, -2.21
ENB	0.97	-4.11	-5.68, -2.54
ENP:ENB	0.94	1.44	0.93, 1.95
Disp	0.94	-0.76	-2.82, 1.30
ENP:disp	0.61	0.59	0.26, 0.92

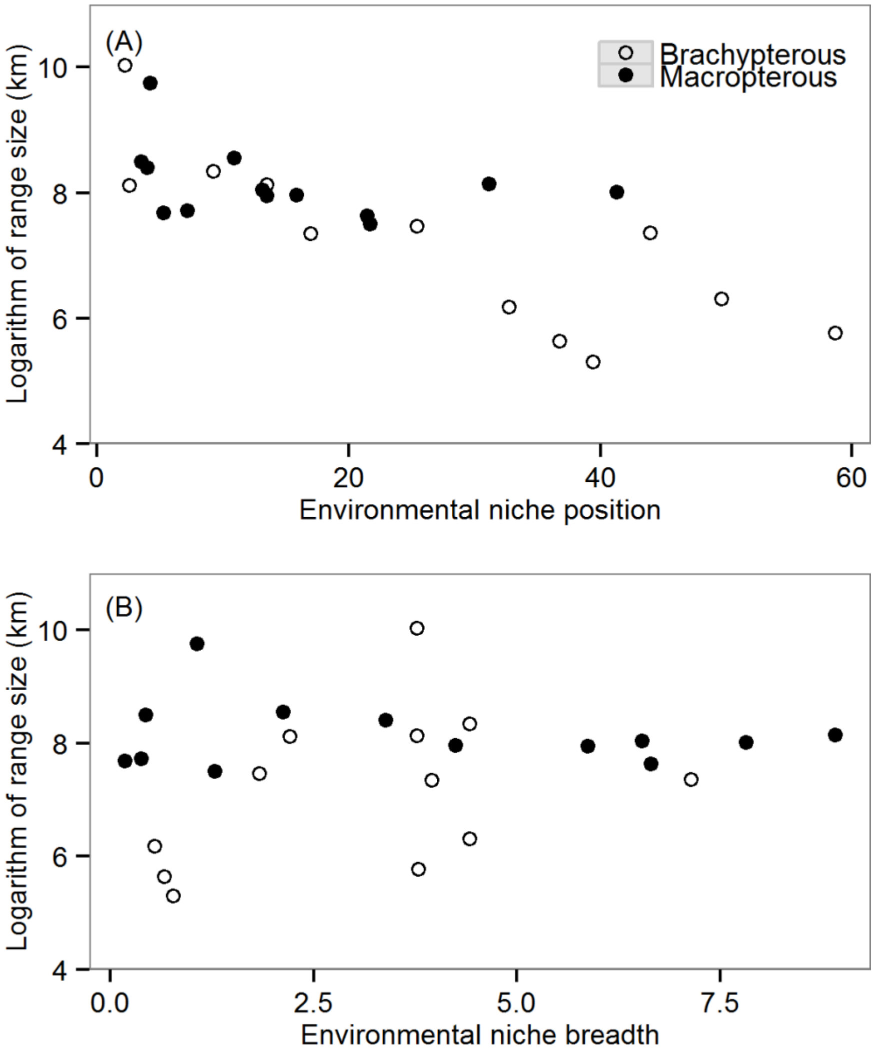

Figure 3.1. The relationship geographic range size (linear range extent; log-transformed) and (A) environmental niche position; (B) environmental niche breadth. In both figures, brachypterous species are represented by unfilled circles and macropterous species by filled circles.

3.3.2 Phylogenetic effects

There was some evidence for phylogenetic signal in both niche position and range size (Table 3.5, Fig. 3.2). After controlling for phylogeny, the four models comprising the 95% confidence set still all contained niche position, and three contained niche breadth (Table 3.3). While niche position was still negatively correlated with range size, the relationship between niche breadth and range size was reversed, suggesting that more widespread species occupy a narrower environmental niche (Table 3.4). Dispersal ability was also included in three of the four best models but, as in the initial analysis, confidence intervals for the effect estimate overlapped zero.

Table 3.5. Tests for phylogenetic signal in niche characteristics and geographic range size among 25 species of carabid beetle (genus *Nebria*) from North America. Phylogenetic non-independence was tested with Abouheif's C_{mean}, Pagel's λ and by comparison of Brownian motion (BM) and "white noise" (phylogenetically independent trait evolution) models of trait evolution. For the former (C_{mean}, λ), significant phylogenetic signal ($p < 0.05$) is highlighted in bold; for model comparisons, the model with the lowest Akaike information criterion (AICc) for each trait is shown in bold.

			Model comparison	
Trait	**Abouheif's C_{mean}**	**Pagel's λ**	**BM**	**White noise**
Environmental niche position	**0.33**	0.37	**213.30**	214.46
Environmental niche breadth	-0.02	$6.90\text{x}10^{-5}$	140.94	**121.96**
Range size	**0.19**	0.17	520.25	**501.19**

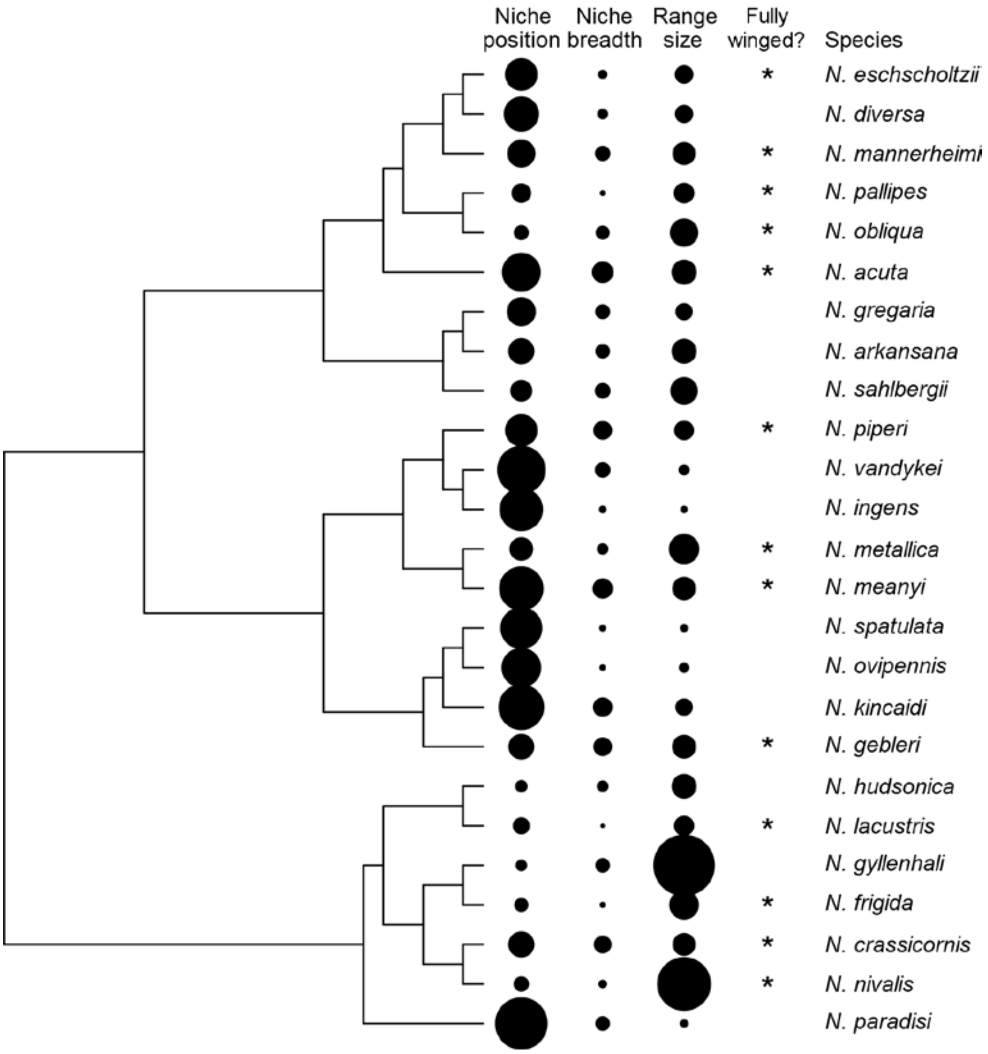

Figure 3.2. Niche characters and range size on a phylogeny of the 25 carabid beetle species (genus *Nebria*) included in this study (Kavanaugh 1985). The size of the circle represents the relative trait value, so that larger circles indicate higher niche position (more marginal niche), greater niche breadth and larger range size. The raw data were square-root-transformed for visualisation. Stars indicate macropterous (fully winged) species.

3.3.3 Trait interactions

The 95% confidence set of models under both a non-phylogenetic and phylogenetically controlled analysis included the interaction between niche position and niche breadth, and model averaging indicated a particularly strong effect of this interaction with PIC (Tables 3.3, 3.4). The relationship between niche position and range size became weaker as niche breadth increased (Fig. 3.2). Conversely, when niche position was low, the relationship between niche breadth and range size was weak; but a strong positive relationship was evident among species with a high niche position (Fig. 3.2).

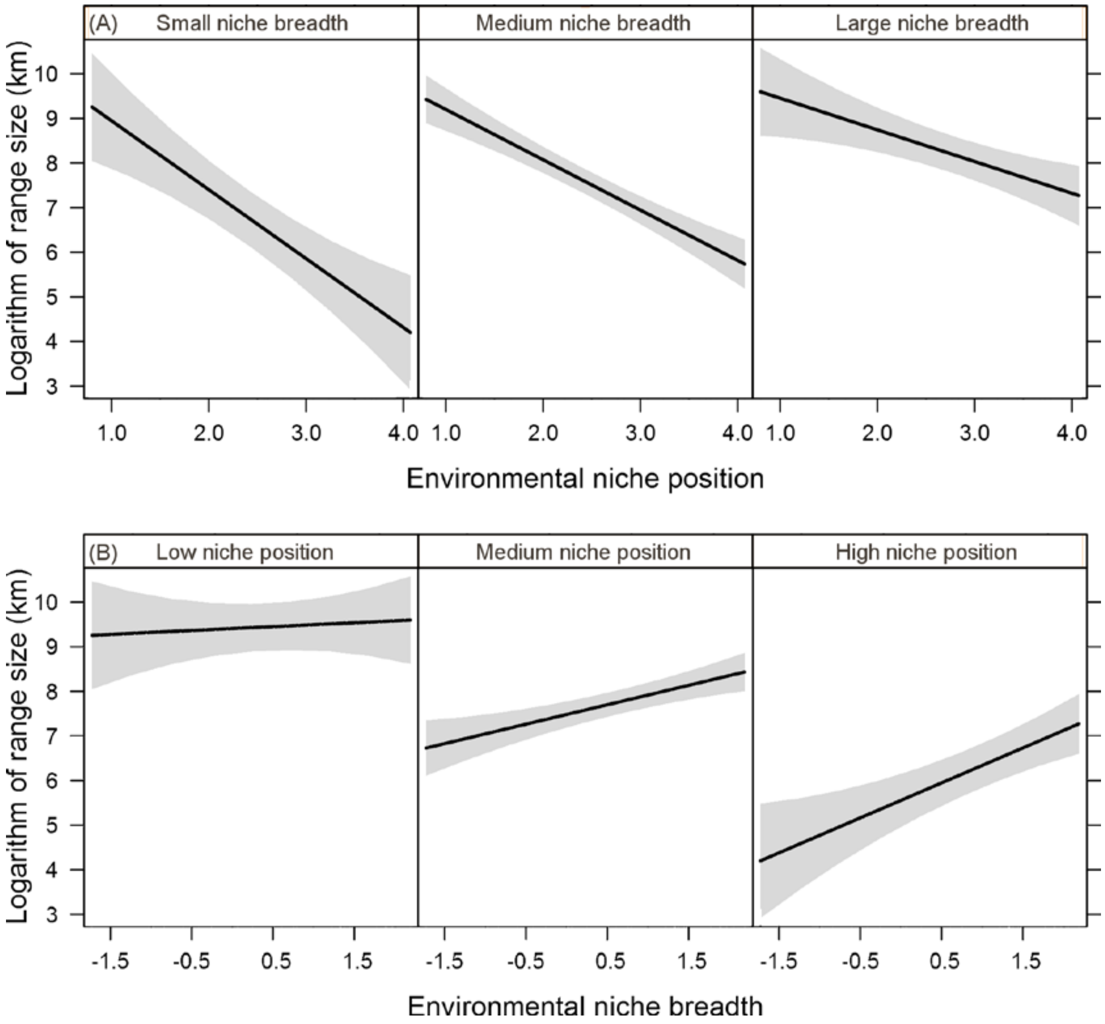

Figure 3.3. Interactions between niche breadth and niche position for 25 species of *Nebria*. (A) shows the relationship between niche position and geographic range size at different levels of niche breadth; (B) shows the alternative view, of the relationship between niche breadth and geographic range size at different levels of niche position. A high niche position indicates occupation of environments that are uncommon across the study region (i.e. high marginality). Range size, niche position and niche breadth are log-transformed.

3.4 Discussion

Why do some species' distributions span whole continents while their close relatives are highly restricted, perhaps occupying just a single mountain, valley or river? This question is central to understanding the speciation process (Grossenbacher *et al.* 2014), global diversity patterns (Rapoport 1982), niche evolution (Jahner *et al.* 2011; Lavergne *et al.* 2013) and extinction risk (Birand *et al.* 2012). Range size can differ by several orders of magnitude among close relatives, yet the underpinnings of this remarkable variation are still poorly understood. Niche breadth and niche position are both expected to show a relationship with geographic range size (Brown 1984; Hanski 1993; Gregory & Gaston 2000). Among North American *Nebria,* it appears that range size is strongly related to niche position, which describes the commonness of suitable environments in the landscape (Gregory & Gaston 2000). However, as originally proposed by Brown (1984), niche breadth, describing the range of environments utilised by a species, also appears to play a role in shaping range size variation in this group.

3.4.1 Predictors of range size variation: one or many?

Niche position and niche breadth need not be related (e.g. Sheth *et al.* 2014) and one or both might influence geographic range size. For example, Passy (2012) suggested that considering interactions between niche position and niche breadth might be necessary for explaining variation in range size; utilisation of common resources may not, on its own, be sufficient to confer a large geographic range in the absence of a broad niche. In this vein, more studies that explore interactions between niche position and niche breadth are needed.

Among *Nebria*, there appears to be an interaction between niche position and niche breadth. For species occupying marginal environments, niche breadth is a strong predictor of range size, but the same is not true for species occupying environments that are common across the study region. The interaction can also be considered from the opposite perspective: for species with a narrow niche, the position of that niche appears to be a more limiting factor for geographic range size. As predicted by the niche position-range size hypothesis, those species that utilise environments that are common across the study region tend to be more widespread, leading to a negative relationship between niche position and range size (Hanski 1993). However, among the *Nebria* species included here, a broad niche is associated with a large geographic range, regardless of niche position. This pattern contrasts with both Passy's (2012) predictions and a recent study of a set of 1200 alpine plants in Europe, where species with a narrow niche breadth almost always had small geographic ranges while those with a broad niche had widely varying range sizes (Boulangeat *et al.* 2012). Although the latter study did not test factors driving range size variation among generalists, all three studies suggest that large-ranged species must have *either* a broad niche *or* utilise non-marginal environments.

When and why different mechanisms might take precedence remains unclear, but could be mediated by additional factors such as habitat use. Many *Nebria* are restricted to montane areas, and their elevational distribution might therefore shape the relative importance of the factors controlling range size. In this study, high-elevation species tended to have higher niche position. Perhaps the geographic range sizes of high-elevation taxa are determined primarily by niche breadth, whereas those of low-elevation species are influenced by niche position. The data are too few to address this hypothesis at present, but interactive effects like this should be considered in further studies if we are to build an understanding of the underpinnings of niche-range size relationships.

Dispersal ability is expected to influence range size through its effects on range filling (e.g. Dullinger *et al.* 2004; Siefert *et al.* 2015). While intuitively appealing, there is inconsistent evidence for a relationship between dispersal ability and range size (Lester *et al.* 2007). Wing structure, as a proxy for dispersal ability, was not a strong predictor of range size among *Nebria*. The dispersal ability hypothesis implicitly assumes a gradient in the isolation of suitable habitat. However, the topography of a region influences the arrangement of environments in geographic space and, in complex terrains (e.g. mountain chains, island archipelagos), this assumption might not be valid (see also Mora *et al.* 2012). When distances between suitable environments are considerably larger than required to provide an effective dispersal barrier even for dispersive forms, dispersal ability is likely to be considerably less important for determining comparative range dynamics (MacArthur & Wilson 1967; Thomas & Harrison 1992; Gaston 2003; Lester *et al.* 2007; or see Mora *et al.* 2012 for the converse case, where distances between suitable habitats are small relative to dispersal distance).

3.4.2 Limitations: measurement of niche characters, phylogeny and scale

This and many previous studies utilise occurrence and spatial environmental data to build environmental models predicting species occurrence or describing a species' environmental niche space (Broennimann *et al.* 2012; Slatyer *et al.* 2013; Guillera-Arroita *et al.* 2015). One limitation of this method is the exclusion of species with few occurrence points; these are often the most geographically-restricted species (Gaston *et al.* 1997), thus truncating the range size distribution. These highly range-restricted species could, however, provide the most valuable insights into the factors limiting range expansion. Of the species included in the current analysis, *N. ingens* had the smallest range extent at 200 km. However, other North American *Nebria* are restricted to just one small mountain range (e.g. *N. praedicta, N. turmaduodecima*) or island (e.g. *N. charlottae*). There was some evidence for phylogenetic conservatism in environmental niche position; it is, perhaps, more than coincidence then, that the closest relatives to *N. praedicta* and *N. turmaduodecima* are, respectively, *N. vandykei* and *N. paradisi*: the two species with the most marginal environmental niches in my analysis. What factors have allowed range expansion? To examine this question in relation to the niche requires good estimates of niche traits as well as a robust phylogeny.

The limiting factor in many comparative analyses is the availability of phylogenetic information (Harvey *et al.* 1995b). Any constructed phylogeny is an estimate of the true evolutionary relationships among taxa, introducing a source of error into comparative analyses (Harvey & Pagel 1991; Felsenstein 2004; Rangel *et al.* 2015). Nevertheless, several theoretical studies have shown that phylogenetic comparative methods are fairly robust to misspecifications of tree branch lengths and local branching patterns (e.g. Díaz-Uriarte & Garland 1996, 1998; Symonds 2002; Stone 2011). In addition, Freckleton (2008) suggests that comparative analysis using even a rough tree should be preferred over a non-phylogenetic model if tests for phylogenetic dependence indicate an improved model fit. Topological errors are likely to lead to conservative results (i.e. type II errors) (Symonds 2002), lending support to the result of a significant relationship between niche traits and range size detected here. Nevertheless, re-analysis with a molecular phylogeny and integrating phylogenetic uncertainty with a set of trees sampled from Bayesian posterior distributions (e.g. Huelsenbeck & Rannala 2003; De Villemereuil *et al.* 2012; Nakagawa & de Villemereuil 2015) would allow a more robust assessment of niche and range size evolution among *Nebria*.

Lastly, I consider the effects of spatial variation. On mountains, environments vary considerably over small spatial scales. This has important implications for estimating niche traits from climate data: 1 km^2 on a mountain is likely to encompass a far greater array of environments than a similar area on flat ground. This is particularly true for small animals (such as beetles) that can utilise microclimatic variation at the scale of metres, or even centimetres (Scherrer & Körner 2010, 2011). In this context, are niche measures based on macroclimatic data relevant for small organisms? Previous research suggests that they can be. For example, two recent studies of monkeyflowers (*Mimulus* spp.) in western North America, one based on correlative macroclimatic niche modelling (Sheth *et al.* 2014) and the other based on experimental measurements of the thermal niche (Sheth & Angert 2014) both revealed a positive relationship between niche breadth and range size. This agreement supports the utility of estimates derived from occurrence and climate data for providing useful descriptions of the environmental niche space occupied. However, an analysis based on microclimate measures (e.g. Heino 2005) would provide a valuable supplement to broad-scale niche estimates.

3.4.3 Conclusions

The results presented here indicate that niche breadth and niche position are not alternative predictors of range size but, rather, might act in synergy. Recognising, and more thoroughly testing interactions between niche traits might help reconcile some of the conflicting reports of the strengths of the niche breadth and niche position hypotheses for range size variation. Integrating phylogeographic information with niche estimates in a macro-ecological framework is a promising approach for examining the underlying drivers of niche evolution and its influence for constraining or, alternatively, facilitating the expansion of species' ranges (Grossenbacher *et al.* 2014; Hidalgo-Galiana *et al.* 2014).

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As we go further away from sea level, the temperature and pressure of the air diminish; at the same time, its dryness and its electrical tension increase: the blue of the sky seems deeper according to one's altitude

-- *Alexander von Humboldt, 1805, Essay on the Geography of Plants*
(trans. S Romanowski, 2009)

Chapter 4

Physiological limits along an elevational gradient in a radiation of montane ground beetles

Abstract
A central challenge in ecology and biogeography is to determine the extent to which physiological traits limit the ranges of species along environmental gradients. This study tests the hypothesis that temperature and desiccation tolerance are associated with the range edges of 12 ground beetle species (genus *Nebria*) occupying different elevational zones on Mt Rainier, Washington, U.S.A. Species from higher elevations did not have greater cold tolerance than lower-elevation species, despite a steep decline in minimum temperature with elevation. Although the lowest-occurring species had elevated heat tolerance, there was not, generally, a decline in heat tolerance with elevation. Thus, temperature clines and acute thermal tolerance do not appear to govern patterns of elevational species turnover in this assemblage. Although atmospheric aridity typically increases with elevation, beetle microhabitats had high relative humidity that varied little with elevation. Desiccation resistance was not associated with a species' elevational distribution and we suggest that a shared behavioural preference among species for humid microhabitats might relax divergent selection pressure on desiccation resistance.

In review as: Slatyer, R. A. & Schoville, S. D., Physiological limits along an elevational gradient in a radiation of montane ground beetles.

4.1 Introduction

Studies of environmental clines are critical to developing a mechanistic understanding of how biotic and abiotic factors regulate species diversity and distributions (Endler, 1977). Some of the steepest environmental clines in nature are found on mountains, where abiotic factors such as temperature, humidity and solar radiation change rapidly with elevation, and these gradients provide a natural laboratory for exploring how populations, species and communities respond to macroclimatic variation (Grinnell 1914; Whittaker 1956; Mani 1968). Across a variety of both plant and animal taxa, traits such as size (reviews: Körner 2003; Chown & Gaston 2010; Shelomi 2012), pigmentation (review: Clusella-Trullas *et al.* 2007), life history (e.g. Morrison & Hero 2003), and physiological capacity (e.g. Slatyer 1978; Gaston & Chown 1999) vary with elevation. Elevational gradients are also associated with considerable changes in community composition (elevational turnover) over small spatial scales (Sundqvist *et al.* 2013). A challenge for ecology and biogeography is identifying which factors underlie species' range limits, and thus species turnover, along such gradients.

In the absence of physical barriers, range limits are expected to reflect the limits of the fundamental niche, describing the resources required for a species to persist in an environment (Hutchinson 1957; Hargreaves *et al.* 2014). Although factors such as dispersal ability and biotic interactions can constrain species to a subset of their potential range (i.e. the realised niche; Hutchinson 1957; MacArthur 1972; Jump *et al.* 2009; Hargreaves *et al.* 2014), physiological limits – as a component of the fundamental niche – are considered to be particularly relevant in mountain environments and are frequently inferred as proximate drivers of elevational range limits (Huntley *et al.* 1989; Normand *et al.* 2009; Cahill *et al.* 2014; Siefert *et al.* 2015). Transplant experiments with plants, for example, show that elevational range limits commonly coincide with niche limits (Hargreaves *et al.* 2014). If physiological constraints determine a species' elevational range, physiological limits should reflect climatic characteristics at the range edge; physiological variation among species occupying different elevational ranges would thus result from clinal variation in environmental conditions (Addo-Bediako *et al.* 2000; Kellermann *et al.* 2012; Hoffmann *et al.* 2013; Siefert *et al.* 2015).

Concurrent clines in temperature and humidity create an increasingly cold and xeric environment towards higher elevations (Mani 1968). These gradients favour lower thermal limits and greater desiccation resistance with increasing elevation (Gaston & Chown 1999; Parkash *et al.* 2008). Insects are likely to be particularly sensitive to such changes in environmental conditions because, due to their small body size and ectothermy, physiological processes are directly impacted by the external environment (Chown & Nicolson 2004; Woods *et al.* 2014). However, mountain environments are characterised by high topographic complexity and, for small organisms, elevational clines in abiotic conditions can be disrupted by microclimatic variation (Scherrer & Körner 2010). For example, Mani (1962) notes that insects occupying snow-covered, high-elevation habitats in the north-west Himalaya are all strongly hygrophilous, restricted to relatively moist microhabitats such as snowfield edges. Factors such as the body size, habitat preference and activity patterns of a species could thus influence both the abiotic conditions experienced as well as how these conditions vary spatially (Buckley *et al.* 2013; Kaspari *et al.* 2014; Schoville *et al.* 2015).

Ground beetles (Coleoptera: Carabidae) are a ubiquitous element of the Nearctic mountain fauna and on Mt Rainier, Washington, USA, 12 species in the genus *Nebria* Latreille (1802) occupy overlapping elevational distributions from sea level to 2750 m (Kavanaugh 1978; Fig. 4.1). The *Nebria* assemblage also occurs on mountains to the north and south of Rainier; species occupy the same relative positions along the elevational gradient, but some species are absent at southern latitudes. This species radiation, in which cryophily is the norm, provides a compelling test of whether physiological tolerance drives species turnover along elevational gradients, and the role that microhabitat selection might play in moderating spatially varying selection. Carabids have been proposed as useful bioindicators for environmental change, with both temperature and humidity being invoked as range-limiting factors for carabids and for *Nebria* specifically (Thiele 1977; Kavanaugh 1979; Koivula 2011).

In this study, we examine whether members of the Mt Rainier *Nebria* assemblage, occupying different positions along a large elevational gradient, vary in their tolerance of temperature and desiccation stress. In particular, we test the hypothesis that environmental tolerance is associated with elevational range limits, incorporating data on microclimate conditions to explore environmental variation at a beetle-relevant scale.

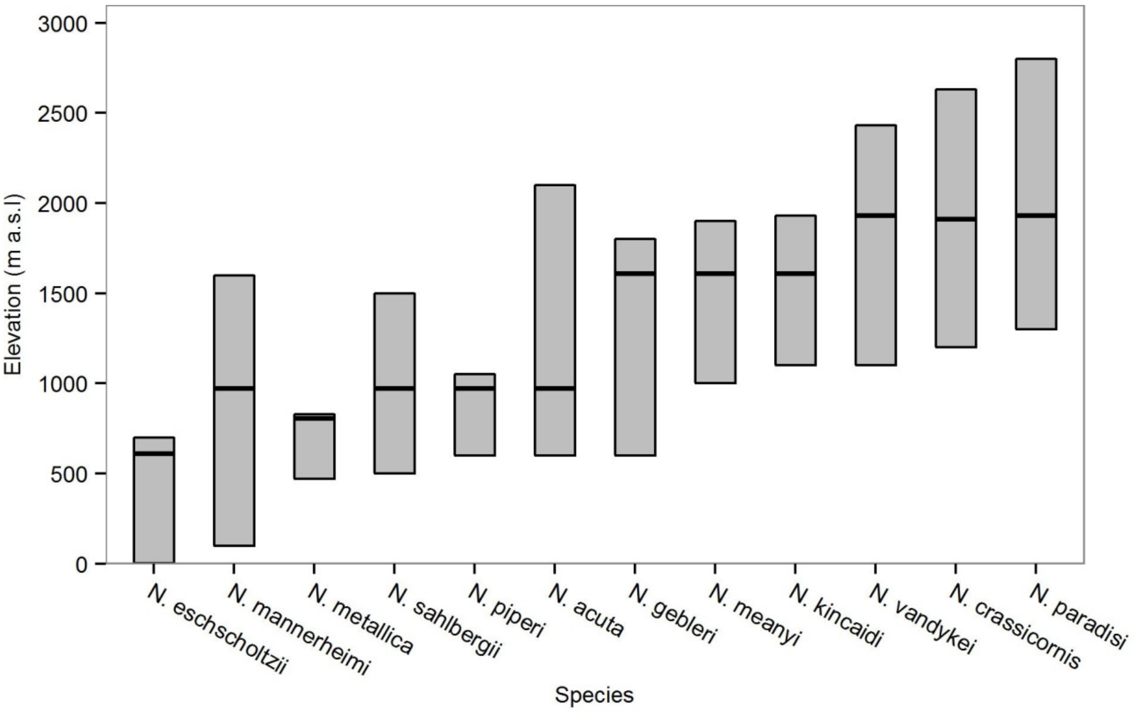

Figure 4.1. Elevational ranges for the 12 *Nebria* (Coleoptera: Carabidae) species on Mt Rainier, Washington. Range edges were derived from Kavanaugh (1979) and our own transect surveys (see Methods). Horizontal lines indicate the elevation of the population used in the study.

4.2 Methods

4.2.1 Study area and species collection

Mt Rainier (4392 m) lies approximately 180 km east of the Pacific Ocean in the northwest USA and supports a succession of biomes from low-elevation deciduous forest, through to glaciers and permanent snow. Mt Rainier's montane environments have been protected as a National Park since 1899, and the environments in which *Nebria* occur are subject to minimal anthropogenic disturbance. The maximum diversity of *Nebria* occurs at 1300 m to 1400 m, where the elevational ranges of nine species overlap (Fig. 4.1); however, only three to five species usually co-occur at any site (Kavanaugh 1978; Table 4.1). High-elevation *Nebria* are associated with both permanent and temporary snowfields, while lower-elevation species are riparian. During the day, adults shelter beneath rocks on the edge of snowfields or streams, where multiple species are often found together. At night, beetles forage on the adjacent snowfields or stream edges; it is during this period that beetles are likely to experience the greatest microclimate variation – both within sites and across elevations. The life-cycles of most species are seasonal, with peak adult activity between May and September (Kavanaugh 1978). The two exceptions are the two lowest-elevation species, *N. mannerheimi* (adults active from March to October) and *N. eschscholtzii* (adults active year-round) (Kavanaugh 1978). Larvae and overwintering adults persist in substrate beneath rocks and snow, in cold (around 0°C: Kamp 1973), but thermally stable and humid microhabitats. As snow cover dampens temperature variation during the late autumn, winter, and spring (Geiger *et al.* 1995), and surface-active adults are exposed to both temperature and desiccation stress, we consider the adult stage the most relevant for testing elevational clines in acute physiological tolerances.

Table 4.1. Summary statistics for night-time temperature and relative humidity at the four primary collecting sites on Mt Rainier from June 20th to August 2nd, 2014 (44 nights). Night was defined, for each 24-hour period, as between the hours of sunset and sunrise. Nightly fluctuations are calculated as the difference between the maximum and minimum temperature on a given night. Species present at each site are listed, and those for which thermal tolerance and desiccation resistance were measured are in bold. *Nebria crassicornis* was collected from 1910 m, *N. metallica* from 805 m (where it co-occurs with *N. piperi*, *N. mannerheimi,* and *N. acuta*), and *N. eschscholtzii* from 610 m (where it co-occurs with *N. mannerheimi*). Data are also shown for Paradise, Mt Rainier (weather station PVC55, Northwest Avalanche Centre), for the same period in 2014 and for a 10-year (2005-2014) period. Data from 2014 are shown as mean ± s.d and 10-year data are shown as mean (range).

	Site 1	**Site 2**	**Site 3**	**Site 4**	**Paradise (2014)**	**Paradise (10-yr)**
Elevation (m a.s.l)	2180	1930	1610	970	1675	
Mean temperature (°C ± s.d)	4.3 ± 3.0	4.7 ± 2.7	5.6 ± 1.9	11.1 ± 2.7	11.4 ± 5.2	9.3 (6.3 – 11.4)
Absolute minimum (°C)	-1.9	-0.9	1.6	3.5	0.6	0.8 (-1.1 – 3.3)
Absolute maximum (°C)	12.3	10.6	13.2	21.7	20.6	19.1 (14.4 – 23.9)
Mean nightly minimum (°C)	3.3 ± 2.7	3.5 ± 2.4	5.6 ± 2.4	10.6 ± 2.9	9.5 ± 4.8	7.8 (4.9 – 9.5)
Mean nightly maximum (°C)	5.4 ± 3.3	6.1 ± 3.0	7.4 ± 2.5	15.0 ± 4.3	12.9 ± 4.8	11.1 (8.2 – 12.9)
Mean nightly fluctuation (°C)	2.1 ± 1	2.6 ± 1.1	1.8 ± 1.0	4.3 ± 1.8	3.5 ± 2.3	3.3 (2.7 – 3.9)
Mean RH (%)	98.2 ± 4.6	-	90.3 ± 9.5	89.0 ± 10.5	70.2 ± 25.2	79.7 (70.2 – 87.6)
Species present	***N. paradisi*** ***N. vandykei*** *N. meanyi*	***N. paradisi*** ***N. vandykei*** *N. kincaidi* *N. acuta*	***N. meanyi*** ***N. kincaidi*** ***N. gebleri*** *N. vandykei* *N. acuta*	***N. sahlbergii*** ***N. acuta*** ***N. mannerheimi*** ***N. piperi***		

The activity of all 12 species in this study is concentrated in the first three hours of darkness (Mann *et al.* 1980). We collected adult beetles at night, by hand, during June and July, 2014. Nine species were collected from one of four sites (Table 4.1). Site 1 (2180 m) and 2 (1930 m) are snowfields with adjacent talus; Site 3 (1610 m) was a snow-covered stream, while Site 4 (970) was a snow-free stream edge. Multiple species were present at all sites, and species were collected at the site where they were most abundant. The remaining three species were not present at any of the primary collection sites and were collected from different snowfield (*N. crassicornis*, 1910 m) or stream-edge sites (*N. metallica*, 805 m; *N. eschscholtzii*, 610 m) (Fig. 4.2).

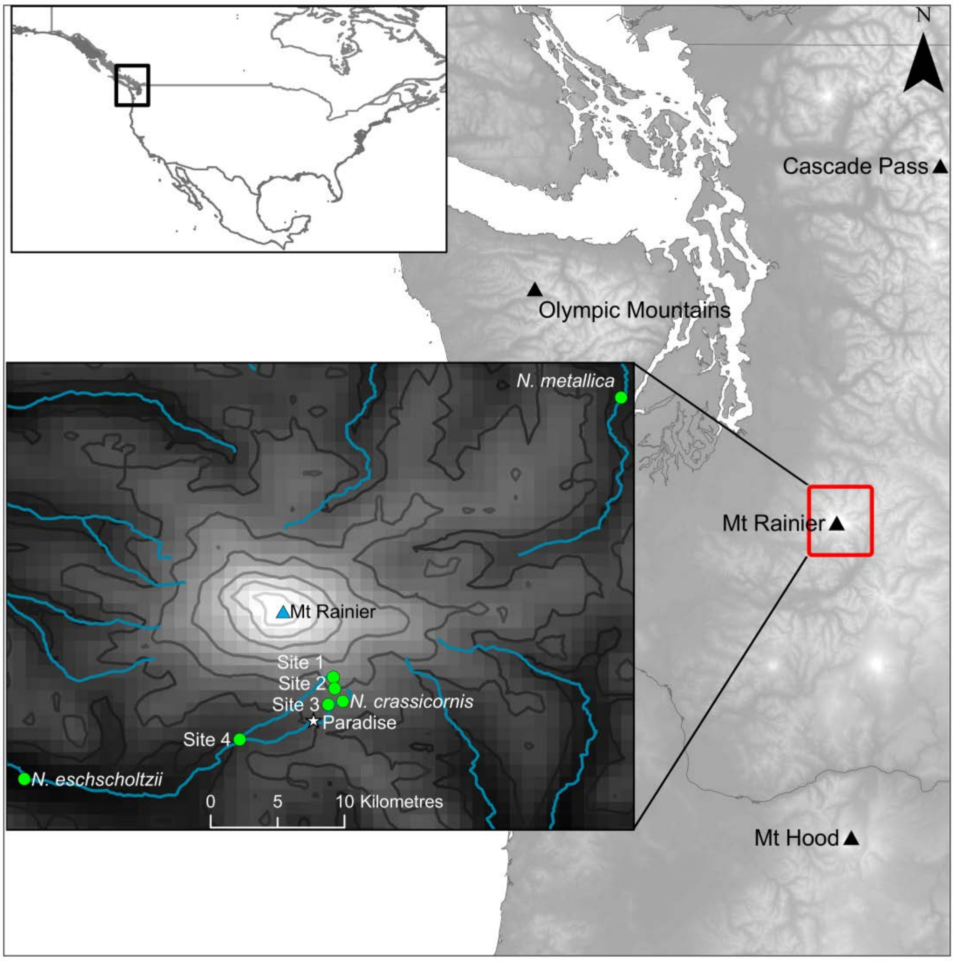

Figure 4.2. Map of the study area and collection sites (inset) at Mt Rainier. Collection sites are indicated by circles; sites labelled with a species name are locations at which only one species was collected; details of the species collected at Sites 1 – 4 are provided in Table 4.1. Sites 1 – 4 had iButtons deployed during the study period. The summit of Mt Rainier and the Paradise weather station are indicated with a triangle and star, respectively, and major rivers are shown with blue lines. Elevation (in metres above sea level) is indicated in grey scale, and with 500 m contour lines starting at 1000 m a.s.l..

Nebria paradisi and *N. vandykei* were abundant at Sites 1 and 2, and were sampled from both locations to assess intraspecific variation (due to plasticity or local adaptation) which might influence conclusions regarding interspecific variation (Schiffer *et al.* 2013). After collection, all beetles were kept in cool conditions (< 10°C) for one to three weeks before being transferred to 4°C for at least five days before experiments began. All the species in our study can be kept at this temperature for long periods of time with low mortality; it was thus deemed a suitable, low-stress temperature. Mortality is noticeably higher at warmer temperatures (e.g. 10°C).

Beetles were kept in single-species groups of three to six individuals (depending on species size) in 60 mL plastic cups with moistened cotton, and were fed weekly with small pieces of mealworm (*Tenebrio molitor*).

4.2.2 Elevational range

Quantification of the elevational ranges of species is a necessary pre-requisite for examining elevational clines. Elevational ranges for *Nebria* on Mt Rainier were described by Kavanaugh (1978). To confirm that these ranges have not shifted substantially (Bässler *et al.* 2013), and that the zonation pattern (i.e. the order of species along the elevational gradient) was the same, in June 2014 we carried out 29 transect surveys (100 m long, 5 m wide) between 960 m and 2650 m a.s.l. Transects ran parallel with the slope and were focused around four primary sites (Fig. 4.2, Table 4.1). Three to five transects were carried out near each of these sites, in addition to 10 transects above 2300 m, one at 2000 m (between sites 1 and 2) and one at 1400 m (between sites 3 and 4). No species were detected outside the ranges described by Kavanaugh (1978), so we used these data for our analysis.

4.2.3 Microclimate

Data loggers (iButtons: DS1922L for temperature and DS1923 for relative humidity; Maxim Integrated Products) were deployed at snowfield or stream edges at the four collection sites, in locations where beetles were commonly found (i.e. 2 loggers per site, see Appendix C). We recorded microclimate temperature (± 0.0625°C) every 30 minutes from June 3rd to September 14th 2014 (104 nights) and relative humidity (± 0.04%) every 30 minutes from June 20th to August 2nd (44 nights). Night-time (interval between sunset and sunrise) temperatures were extracted and used for data analysis as these are the most relevant for *Nebria* activity thresholds. The temperature logger from Site 3 was lost during the sampling period. For purposes of comparison, the data presented correspond to the period in which all loggers were recording (total 44 nights). Summary statistics based on the full data are provided in Appendix C, Table C.2. In addition to these microclimate observations, we obtained hourly temperature and relative humidity data over the study period for the Northwest Avalanche Centre weather station at Paradise (station number: PVC55), which is the closest weather station to collection sites 1 – 3 (MesoWest 2014). We use these data to examine whether microhabitat conditions differ substantially from ambient and whether conditions recorded in 2014 reflect the normal range of temperature and humidity at Mt Rainier.

4.2.4 Thermal tolerance

We used a temperature ramping method to measure both cold and heat tolerance (Terblanche *et al.*, 2011). All experiments were conducted using a temperature-regulated water bath (TX150 R2, Grant Instruments, UK), which circulated 50% propylene glycol around six 50 mL beakers (see Appendix C, Fig. C.1, for a diagram of the experimental apparatus). In each experimental run, one beetle was placed in each beaker. A k-type thermocouple (Jaycar Electronics) was placed in two of the beakers and the beaker temperature was recorded every 10 seconds with a thermocouple data logger (TC-08, Pico Technology, UK).

Cold tolerance was tested with a ramping protocol of 10 minutes at 4°C, followed by cooling at 0.2°C/min. The critical thermal minimum (CT_{min}) was scored as a loss of reactivity to moderate stimulation (tipping the beetle onto its side) ("chill coma"; Mellanby 1939). Beetles were removed from the experiment upon reaching their CT_{min} and placed in individual, 60 mL plastic containers with moistened cotton and a piece of mealworm for food. Beetles were allowed to recover for seven days at 4°C before being tested for heat tolerance.

The heat tolerance protocol started with 10 minutes at 4°C, followed by a fast ramp (0.5°C/min) to 20°C, then a slow ramp (0.2°C/min). Critical thermal maximum (CT_{max}) was scored as a loss of righting ability and onset of twitching (Hazell *et al.*, 2008). Beetles were removed from the experiment upon reaching CT_{max} and returned to their individual container at 4°C. After 24 h, each beetle was weighed and survival (righting and voluntary walking) was scored. Those that did not survive (8 of 302) were deemed to have exceeded their CT_{max} and were excluded from subsequent analyses. For each species, 10 individuals were scored for both cold and heat tolerance.

4.2.5 Desiccation resistance

For insects, water loss rate accounts for most of the variation in desiccation resistance (Chown & Nicolson 2004). After thermal tolerance trials, individuals were allowed to recover for at least one week at 4°C, then randomly assigned to a temperature treatment (5°C or 10°C) for measurements of water loss rates. Five individuals per species were used in each treatment/temperature combination where possible; however sample size limitations meant that not all combinations were tested for each species. Each beetle was weighed to 0.01 mg on an electronic microbalance (Sartorius Research) and transferred to a 12 mL plastic tube. This tube was covered with fine cloth mesh to permit air flow but prevent the beetle escaping. For individuals in the dry treatment (< 5% relative humidity), this tube was then sealed inside a 50 mL tube containing 5 g Indicating Drierite (W.A. Hammond Drierite Co., USA) and placed in an incubator at the assigned temperature. Each beetle was weighed after 12 h and 24 h, and we calculated mass-specific water loss rates ($mgH_2Og^{-1}h^{-1}$) for each period. Proportional water loss was faster in the first 12 h (7.8 ± 3.7% initial mass), compared to the second 12 h (6.9 ± 4.1% 12-h mass) (paired t-test: $t = 9.14$, df = 118, $p < 0.001$), but as the difference was small and similar among species, data were analysed from the whole 24-h period. The ability of the beetle to right itself when placed on its back was recorded after 24 h. Righting ability was scored as "normal" if a beetle immediately righted itself, "slow" if it stayed on its back for between 2 and 5 seconds and "very slow" if righting took more than 5 seconds. However, as only 15 beetles showed a loss of righting ability, these data were not formally analysed. One *N. gebleri* was excluded from the analysis as it died during the experiment and had a water loss rate twice as high as other individuals. All individuals were starved for 24 h prior to the experiment, as faecal water loss can constitute a large and unpredictable component of total water loss in beetles (Ahearn 1970).

4.2.6 Phylogenetic reconstruction

We constructed a molecular phylogeny based on 1488 bp of the cytochrome oxidase subunit I (*CO1*) gene so that trait variation among species could be tested in a phylogenetic framework. The COI locus was amplified following methods in Schoville *et al.* (2012) using two sets of primers,

LCO1490 (5' GGTCAACAAATCATAAAGATATTGG) and HCO2198

(5' TAAACTTCAGGGTGACCAAAAAATCA) (Folmer *et al.* 1994), and Jerry (5' CAACATTTATTTTGATTTTTTGG) and Pat

(5' TCCAATGCACTAATCTGCCATATTA) (Simon *et al.* 1994). PCR products were sequenced with each primer on a 3730 capillary sequencer using BigDye 3.1 chemistry (Applied Biosystems). These data were manually edited and aligned in GENEIOUS v6.1.8 (Biomatters Ltd.), and MRMODELTEST2 v2.3 (Nylander 2008) was used to estimate a substitution model based on the Akaike Information Criterion (Akaike 1973). Model HKY+I+G was selected as the best model and used in separate data partitions representing each codon position. BEAST v3.1.2 (Drummond & Rambaut 2007a) was used to estimate a Bayesian phylogeny of the partitioned dataset based on four independent runs with the following conditions: 100 million steps with genealogies sampled every 10000 steps under a strict molecular clock rate of 1.0. MCMC convergence was assessed using TRACER v1.6 (Drummond & Rambaut 2007b) and effective samples sizes for each parameter were confirmed to have values greater than 200. A 10% burn-in period was selected before calculating the maximum clade credibility tree in TREEANNOTATOR v2.1.2 (Drummond & Rambaut 2007a; Rambaut & Drummond 2009) (Fig. 4.3).

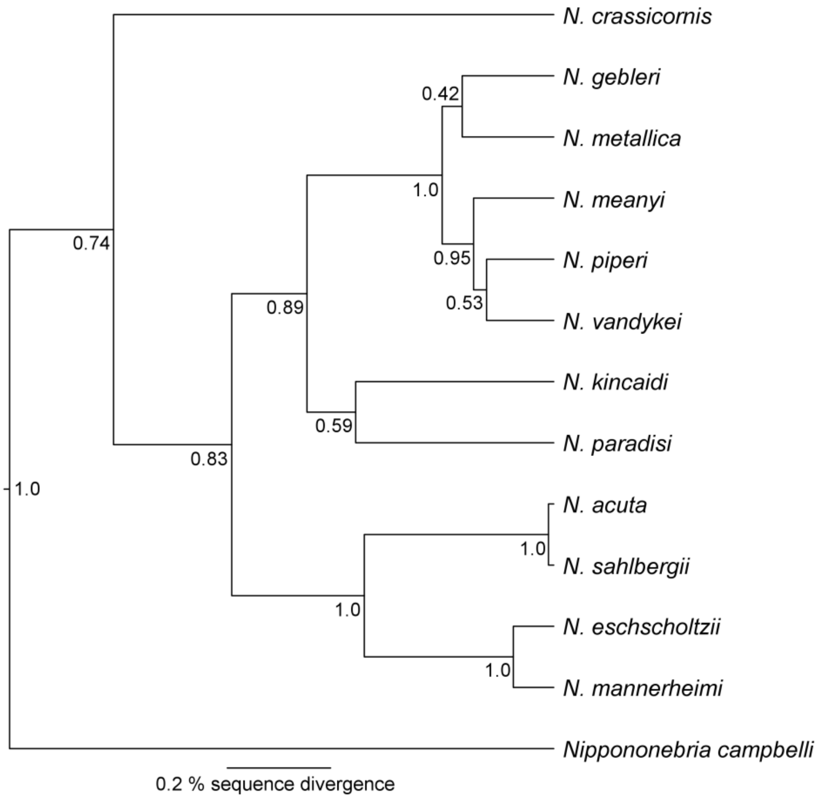

Figure 4.3. Bayesian maximum clade credibility tree for 12 species of carabid beetle (genus *Nebria*) occurring on Mt Rainier, *Nippononebria campbelli* was included as an outgroup. The gene tree is based on 1488 bp of the mitochondrial *CO1* gene and was generated in BEAST v3.1.2 using the HKY + I + G substitution model and a strict molecular clock, with data partitioned by codon position. Nodal support values represent Bayesian posterior probabilities.

4.2.7 Statistical analysis

Using the microclimate data, we calculated mean nightly minimum and maximum temperatures, and nightly fluctuations (the difference between the minimum and maximum temperature on any given night). Ambient temperature lapse rates (°C reduction in temperature for every kilometre increase in elevation) on Mt Rainier have been estimated at 3.5°Ckm^{-1} and 5.1°Ckm^{-1} for minimum and maximum temperatures, respectively (Minder *et al.* 2010). To compare these with our microclimate measures, we calculated minimum and maximum temperature lapse rate using linear regressions of temperature on elevation. We note, however, that these are based on only four data points and should thus be treated cautiously. We used linear regression to test the relationship between hourly microhabitat and weather station temperatures.

CT$_{min}$ and water loss rate data were log-transformed prior to analysis to improve the fit to a normal distribution. If physiological constraints determine range limits in this assemblage, and if elevation is associated with a cline in environmental conditions (in this case, temperature or relative humidity), there should be an association between relative stress tolerance and the relative range edge position (e.g. Calosi *et al.* 2010). We therefore tested for an association between (a) CT$_{min}$ and the high-elevation (cold) range edge, (b) CT$_{max}$ and the low-elevation (warm) range edge and (c) desiccation resistance (water loss rate) and both range edges. Desiccation resistance could be associated with the upper range edge if increasing atmospheric aridity towards higher elevations controls species distributions (Mani 1968); alternatively, as desiccation rates are often very temperature-sensitive, warmer conditions at low elevations might promote greater desiccation resistance among low-elevation species (Addo-Bediako *et al.* 2001). We used linear regressions of trait means on elevational limits for both thermal tolerance measures. For desiccation resistance, we used a general linear model with treatment temperature included as a factor and both range edge limits as predictor variables. Body mass was included as a covariate in all initial analyses but, as it had no significant effect, was not included in the final analyses.

To take into account phylogenetic non-independence, each of the above analyses was repeated using phylogenetic generalized least squares regression (PGLS), implemented in the CAPER 0.5.2 package (Orme *et al.* 2013) in R 3.2.0 (R Core Team 2014). Each regression was fitted using a covariance matrix based on our *COI* phylogeny and using the maximum likelihood method to find the branch length transformation (λ) optimising the fit of the model to the data (Freckleton *et al.* 2002). The value of λ represents the phylogenetic signal in the dataset, with λ = 0 indicating independent trait evolution (no phylogenetic signal) and λ = 1 being consistent with a Brownian motion model of trait evolution (Felsenstein 1985; Pagel 1999). We performed three additional tests for phylogenetic signal (non-independence) in each physiological trait as well as in range edge elevations (Münkemüller *et al.*, 2012). First, we calculated Abouheif's C_{mean} (Abouheif 1999) with 999 permutations in the ADEPHYLO 1.1-6 package (Jombart & Dray 2008) in R. We also calculated Pagel's λ (Pagel 1999) as this metric is robust for small phylogenies (Münkemüller *et al.* 2012). We used likelihood ratios to test the null hypothesis of no phylogenetic signal, with the PHYTOOLS 0.4-56 package (Revell 2012). Lastly, we directly compared the fit of Brownian motion, Ornstein-Uhlenbeck (Butler & King 2004) and "white noise" (phylogenetic independence) models of evolution for each physiological trait and range edge limits using the GEIGER 2.03 package (Harmon *et al.* 2008) in R. For comparison, we also repeated the above tests using a morphological phylogeny constructed by Kavanaugh (1978); these results are presented in Appendix C. We used the sample size-corrected Akaike information criterion (AIC_c) to assess model fit.

Lastly, to directly compare thermal tolerance among species without confounding effects of native site conditions, we tested for interspecific variation within sites at which multiple species were collected (Table 4.1) using analysis of variance in R 3.2.0 (R Core Team 2014).

All data are presented as mean values ± s.d.

4.3 Results

4.3.1 Microclimate

Microclimate temperature data, from the iButtons, covered 44 days of the peak activity season of *Nebria* on Mt Rainier. Across the four sites, temperature decreased approximately linearly with elevation, by 6.4°Ckm^{-1} ($r^2 = 0.96$) and 8.1°Ckm^{-1} ($r^2 = 0.93$) for mean nightly minimum and maximum, respectively (Fig. 4.4). The nocturnal microclimate in snowfield- and stream-edge habitat is very stable, with a maximum nightly temperature fluctuation at a given site of 7.0°C and an average temperature fluctuation of 1.8°C (Site 3) to 4.3°C (Site 4) (Table 4.1). Further, across the whole study period, temperatures at a given site varied by no more than 18.2°C (Table 4.1). Even when recordings collected in September were included, the maximum temperature range was only 22°C (Appendix C). Relative humidity was generally high (mean 92.5% across all sites), and reached a minimum of 57% at Site 4, 61% at Site 2, and 75% at Site 1 (Table 4.1). At these three sites, humidity thus tended to increase with elevation and microhabitats had higher average relative humidity than ambient (weather station) conditions (Table 4.1).

Microhabitat temperatures were moderately to strongly correlated with ambient temperatures measured at the Paradise weather station (adjusted r^2: site 1: 0.84 (n = 608); site 2: 0.83 (n = 608); site 3: 0.82 (n = 290); site 4: 0.63 (n = 610), for Sites 1 – 4, respectively; Appendix C, Fig. C.3). In all cases, the intercept of the regression line was above 0°C and the slope less than 1, indicating reduced temperature variation in *Nebria* microhabitats; in cold conditions, *Nebria* microhabitats tend to be warmer than ambient temperatures at Paradise (1676 m), while in "warm" conditions (e.g. > 5°C for Sites 1 – 3, > 16°C for Site 4) they tend to be cooler. For June-July, 2014 was warmer and drier than 2005 – 2013 (Table 4.1); however, over the whole peak *Nebria* activity period (June – September), conditions in 2014 fell within the 10-year norms (2014 mean: 6.1°C, nightly minimum: 8.1°C, nightly maximum: 11.1°C; 10-year mean: 6.8°C (range: 3.7 – 9.6), nightly minimum: 7.1°C (6.2 – 8.1), nightly maximum: 10.3°C (9.1 – 11.6); Tables 4.1, C.2).

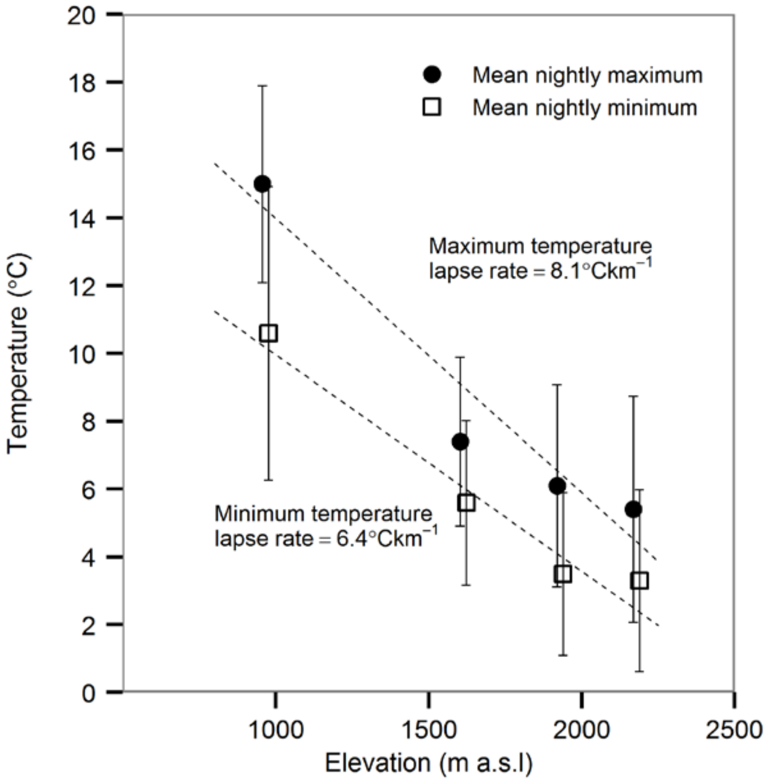

Figure 4.4. Microclimate temperature (± s.d.) variation among four *Nebria* collection sites on Mt Rainier. Temperatures were recorded every 30 minutes for 44 days from June 20th to August 2nd, 2014, with DS1922L iButtons. Mean nightly maximum (filled circles) and minimum (open squares) were calculated for each site using temperatures between the hours of sunset and sunrise.

4.3.2 Elevational clines in thermal tolerance and desiccation resistance

Trait means for all physiological measures are provided in Appendix C. *Nebria* were able to maintain coordinated movements between -3.5 ± 0.8°C and 34.5 ± 1.5°C (n = 120). Cold tolerance varied little among species, ranging from -3.2 (*N. piperi*) to -4.1 (*N. kincaidi*), while heat tolerance ranged between 33.2 (*N. gebleri* and *N. kincaidi*) and 37.0°C (*N. eschscholtzii*) (Fig. 4.4). For all species, these limits exceed both the minimum and maximum microclimate temperatures recorded over the study period (Tables 4.1, C.3).

For *N. vandykei* and *N. paradisi*, there was no significant difference in either cold or heat tolerance among high- and low-elevation populations (CT_{min}: $F_{1,36} = 0.29$, $p = 0.596$; CT_{max}: $F_{1,36} = 1.19$, $p = 0.282$). Among species, there was no association between cold tolerance and the upper elevation limit ($F_{1,10} = 0.31$, $p = 0.589$; Fig. 4.5A), but heat tolerance showed a significant relationship to the lower elevation range edge ($F_{1,10} = 7.90$, $p = 0.018$) (Fig. 4.5B). The latter reflects the greater heat tolerance of *N. eschscholtzii* and *N. mannerheimi,* the two lowest-elevation species. The remaining species had similar upper thermal limits showing no association with the lower range edge ($F_{1,8} = 0.14$, $p = 0.716$). For both measures of thermal tolerance, including genetic relatedness did not improve the model fit. However, we detected weak but significant phylogenetic signal for heat tolerance using Abouheif's C_{mean} and a strong signal of phylogenetic non-independence for the low-elevation range edge (Table 4.2). Under a Brownian motion model of trait evolution, the relationship between heat tolerance and the low-elevation range edge was no longer statistically significant ($F_{1,10} = 3.47$, $p = 0.092$).

Beetles lost 5 – 40% of their body mass over 24 h and began to lose righting ability after losing 20% mass. Four beetles in the 5°C treatment and 11 beetles in the 10°C treatment showed some loss of righting ability after 24 h. These included four *N. gebleri* (21 – 34% mass loss), four *N. paradisi* (22 – 25% mass loss), four *N. acuta* (22 – 34% mass loss), and one each of *N. kincaidi* (21% mass loss), *N. vandykei* (27% mass loss), and *N. mannerheimi* (37% mass loss). Beetles lost water approximately 1.6 times faster at 10°C, compared to 5°C, translating to a mean difference of 0.17 $mgH_2Og^{-1}h^{-1}$ and 7.5% difference in the proportion of body mass lost after 24 h ($F_{1,20} = 32.84$, $p < 0.001$). There was no association between the rate of water loss and either the upper ($F_{1,20} = 3.05$, $p = 0.096$) or lower ($F_{1,20} = 0.15$, $p = 0.708$) range edge (Fig. 4.5C). Including relatedness improved the model fit only when examining the relationship between water loss rate at 5°C and the upper range edge ($\lambda = 0.4$), but this did not change the conclusions of the initial analysis ($F_{1,9} = 1.74$, $p = 0.220$). Further, there was no evidence of phylogenetic signal in this trait (Table 4.2).

4.3.3 Interspecific variation in thermal tolerance at sympatric sites

When we compared species collected within a single site, cold tolerance was highly conserved among species (Site 1: $F_{1,18} = 1.28$, $p = 0.273$; Site 2: $F_{1,18} = 1.23$, $p = 0.282$; Site 3: $F_{2,27} = 1.09$, $p = 0.351$; Site 4: $F_{3,35} = 1.81$, $p = 0.164$; see Appendix C, Table C.4 for pairwise comparisons). There was, however, significant interspecific variation in heat tolerance at the lower-elevation sites: at Site 3 ($F_{2,27} = 10.85$, $p < 0.001$) *N. meanyi* (35.3 ± 0.9°C) had a higher CT_{max} than *N. kincaidi* (33.2 ± 1.0°C; $p = 0.001$) and *N. gebleri* (33.2 ± 1.4°C; $p = 0.001$). At Site 5 ($F_{3,35} = 5.19$, $p = 0.005$), interspecific variation was driven largely by *N. mannerheimi* (35.9 ± 1.1°C), which had a significantly higher CT_{max} than both *N. acuta* (33.7 ± 1.7°C ; $p = 0.003$) and *N. sahlbergii* (34.3 ± 1.3°C; $p = 0.034$) in pairwise comparisons.

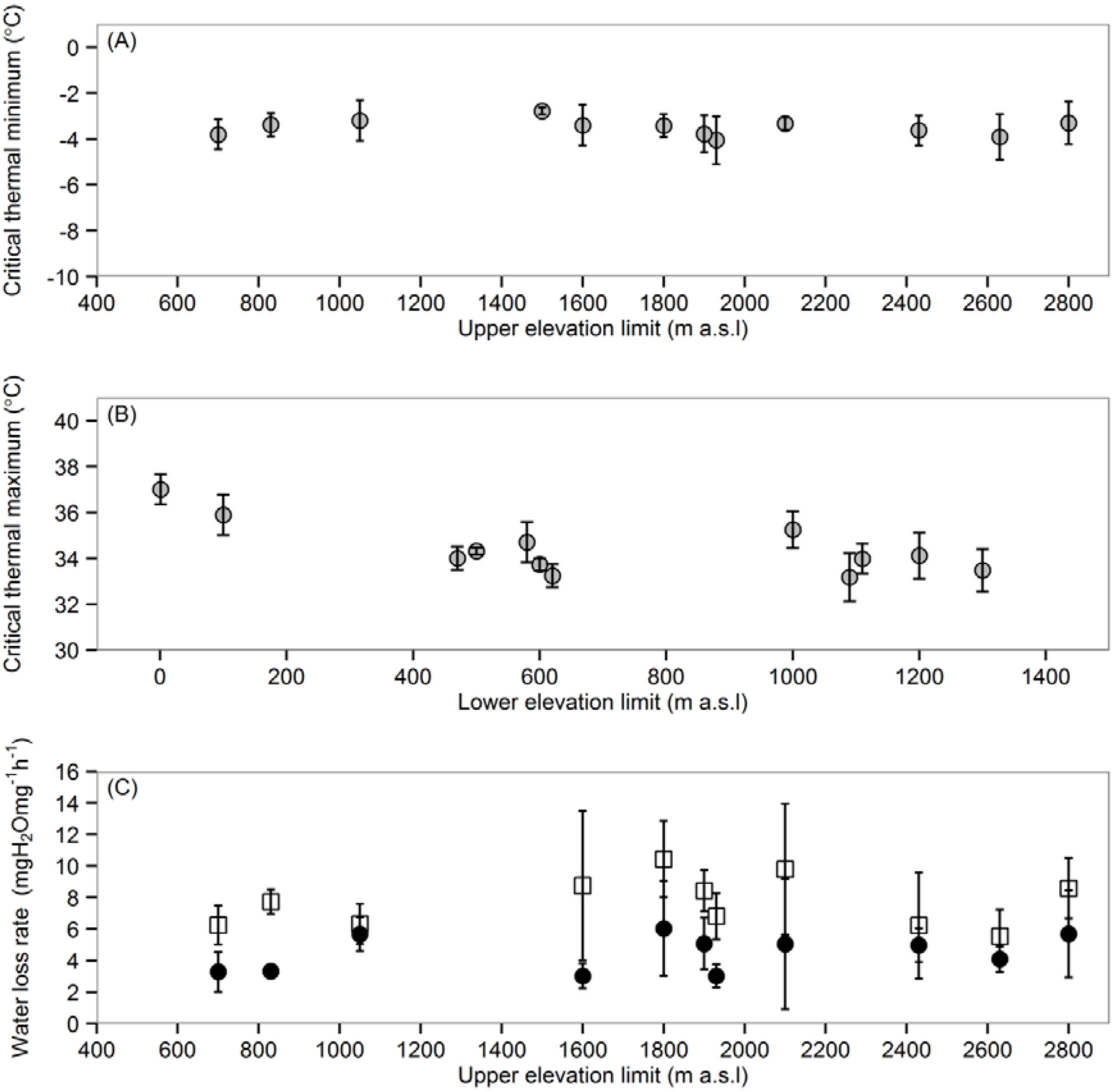

Figure 4.5. Associations between physiological traits and elevational range among 12 species of carabid beetle (genus *Nebria*) from Mt Rainier. (A) cold tolerance and the high-elevation range edge ($F_{1,10}$ = 0.31, *p* = 0.589; all *n* = 10); (B) heat tolerance and the low-elevation range edge ($F_{1,10}$ = 7.90, p = 0.018; all *n* = 10); and (C) desiccation resistance and the high-elevation range edge ($F_{1,20}$ = 3.05, *p* = 0.096; all *n* = 5; filled circles represent water loss rates at 5°C, while open squares are water loss rates at 10°C, measured over 24 h). In (B), three species have their lower elevation limit at 600 m, and two at 1100 m and have been repositioned for graphical purposes only; trait means for each species are also provided in Appendix C, Table C.5. Error bars are ± s.d.

Table 4.2. Tests for phylogenetic non-independence in thermal tolerance, desiccation resistance and range position traits among *Nebria* from Mt Rainier. Non-independence was tested with Abouheif's C_{mean}, Pagel's λ and by comparison of three models of trait evolution: Brownian motion, Ornstein-Uhlenbeck and "white noise" (phylogenetically independent trait evolution). For model comparisons, the model with the lowest Akaike information criterion (AICc) for each trait is shown in bold.

			Model comparison (AICc)		
Trait	**Abouheif's C_{mean}**	**Pagel's λ**	**Brownian motion**	**Ornstein-Uhlenbeck**	**White noise**
Log(CT_{min})	-0.08	6.69×10^{-5}	-16.09	-21.37	**-37.21**
CT_{max}	0.33*	0.89	42.82	42.89	**41.59**
Log(mass-specific water loss rate, 5°C)	0.10	0.28	-12.16	-10.81	**-12.91**
Log(mass-specific water loss rate, 10°C)	0.09	7.28×10^{-5}	-11.59	-11.43	**-17.07**
High-elevation range edge	0.01	6.69×10^{-5}	**178.09**	181.49	183.61
Low-elevation range edge	0.43**	0.99**	205.36	202.58	**194.76**

*$p < 0.05$; **$p < 0.02$

4.4 Discussion

Climate plays a key role in defining the distributions of many species and climatic tolerances are frequently associated with range limits, including the upper and lower elevational bounds of montane species (e.g. Cahill *et al.* 2014; de la Vega *et al.* 2015; Siefert *et al.* 2015). Determining how the environment controls species' range limits and whether this relates directly to measurable physiological traits is a critical challenge for ecologists, with particular importance for predicting the outcomes of ongoing climatic change. We tested the hypothesis that physiological tolerance is associated with elevational range limits in the Mt Rainier *Nebria* assemblage, where component species occupy a 2750 m elevation span. Despite a steep decline in minimum temperatures with elevation, we found no evidence of variation in acute cold tolerance among species. By contrast, in this cryophilic lineage, three species showed elevated heat tolerance and there was some evidence for an association between heat tolerance and the relative elevational distribution of a species.

4.4.1 Does acute thermal tolerance limit elevational range?

Recent syntheses of global variation in thermal tolerance traits highlight close associations between acute cold tolerance and latitude among both vertebrate and invertebrate ectotherms (e.g. Addo-Bediako *et al.* 2000; Sunday *et al.* 2011; Hoffmann *et al.* 2013). Similarly, in a review of transplant experiments across elevational gradients in vascular plants, Hargreaves *et al.* (2014) found that niche limits coincide with range limits in 55% of studies. Among the *Nebria* species tested here, we found remarkably little variation in cold tolerance in both inter- and intra-specific comparisons that aligns with the overwhelming affinity for cold habitats within the *Nebria* radiation (Kavanaugh 1978). For ectotherms, heat tolerance is typically more conserved among lineages (Araújo *et al.* 2013), shows less geographic variation than cold tolerance ("Brett's rule": Brett 1956; Gaston *et al.* 2009), and is less commonly invoked as a primary determinant of range limits (e.g. Machac *et al.* 2011). Nevertheless, temperature (and associated physiological tolerance) has been identified as a dominant factor determining low-elevation range limits in a wide range of taxa (reviewed: Cahill *et al.* 2014; and see Cunningham *et al.* 2015 for an analagous example with latitudinal limits). Among insects, critical thermal limits also coincide with maximum body temperatures predicted by biophysical models (Sunday *et al.* 2014). Heat tolerance variation among *Nebria* is consistent with these results, displaying a negative association with elevation.

Two factors must be considered with respect to the above result. First, species traits are influenced by both the environmental conditions experienced across their range and their evolutionary history, with the latter favouring trait similarity among closely related species (Mayr 1963; Endler 1977; Harvey & Pagel 1991; Wiens & Graham 2005). By incorporating phylogenetic information into comparative analyses, the non-independence of species can be taken into account (Harvey & Pagel 1991). We found some evidence for phylogenetic signal in heat tolerance and a strong signal of phylogenetic non-independence in the low-elevation range edge, suggesting that evolutionary constraints play a role in structuring the Mt Rainier *Nebria* assemblage and shaping the relationship between niche and range limits. Much of the observed variation in heat tolerance was driven by *N. eschscholtzii* and *N. mannerheimi*. These species are close relatives (Kavanaugh 1978; Fig. 4.3) and the association between heat tolerance and elevation was not apparent after accounting for phylogenetic effects. However, the shared sub-montane distribution of these species (Fig. 4.1) makes it difficult to disentangle the effects of common selection regimes from those of a shared evolutionary history with this dataset (Freckleton & Jetz 2009; Kellermann *et al.* 2009).

A second factor to consider is that, among the remaining, strictly montane species, there is no evidence that heat tolerance is associated with range limits, despite significant interspecific variation in this trait in both sympatry and allopatry. This information, coupled with the lack of variation in cold tolerance, strongly suggest that acute thermal limits in the adult life-stage are not responsible for elevational range limits and, as a consequence, elevational species turnover in this system.

A limitation of this study was the absence of measures of intraspecific variation across the elevational range over which each species of *Nebria* occurred. Intraspecific variation represents the outcome of interactions between genetic drift, gene flow and divergent selection (i.e. local adaptation: Lenormand 2002; Kawecki & Ebert 2004) or genotype-by-environment effects (i.e. phenotypic plasticity: Bradshaw 1965; West-Eberhard 2003). Trait variation among species (a product of macro- and microevolutionary processes) is not simply an extension of intraspecific variation (Schiffer *et al.* 2013; Slatyer *et al.* 2015). Among populations on different mountains, and among high- and low-elevation populations of two *Nebria* species on Mt Rainier, intraspecific variation in critical thermal limits appeared to be small, relative to variation among individuals within a population (Appendix C). Nevertheless, an assessment of intraspecific variation along a single elevational gradient, particularly for those species whose range extends below the montane environment, would shed light on the lability (or otherwise) of acute thermal tolerance traits in this genus.

4.4.2 Desiccation resistance and microhabitat selection

All of the *Nebria* species included in our study rapidly desiccated under dry conditions, particularly at higher temperatures. Regulation of water balance is critical for maintaining physiological processes and there is considerable variation in desiccation resistance among insect species and populations (Addo-Bediako *et al.* 2001; Chown *et al.* 2011). Several studies have found elevational variation in desiccation resistance, but while some show increasing desiccation resistance with elevation (e.g. Parkash *et al.*, 2008 for *Drosophila*) – a trend predicted by a general decrease in humidity with elevation (Mani 1968) – there are, equally, several species in which desiccation resistance decreases with elevation (e.g. grasshoppers: Hadley & Massion 1985; *Drosophila*: Sørensen *et al.* 2005). Temperature and moisture have interacting effects on physiology (Addo-Bediako *et al.* 2001). For example, some species show temperature-dependent survival after desiccation stress (e.g. Worland & Block 1986) and temperature-dependent humidity preferences (Hayward *et al.* 2001). Desiccation rate is also strongly temperature-sensitive (e.g. Addo-Bediako *et al.* 2001; this study), complicating the expected relationship between elevation and desiccation resistance.

On Mt Rainier, weather station records reveal a dry high-elevation environment: night-time relative humidity for June and July, 2014, averaged 87% at 595 m a.s.l., 78% at 1680 m and just 41% at 3080 m (MesoWest 2014). These data contrast sharply with the high relative humidity we recorded near the substrate surface at stream and snowfield edges where beetles forage. Many high-elevation insects are closely associated with moist environments (Mani 1968). A shared behavioural preference for humid microhabitats appears to ameliorate the decrease in atmospheric moisture with elevation and could explain the lack of clinal variation in this trait.

4.4.3 Life stage, chronic stress, and competition: missing pieces of the puzzle

With little variation in physiological traits, the question remains: what drives the marked differences in elevational distribution among *Nebria* species on Mt Rainier? While elevational range margins can be a function of acute physiological thresholds (e.g. Lee *et al.* 2009), species turnover might also be driven by variation in other aspects of the physiological niche or biotic interactions which constrain the distribution within the parameters of the physiological limits (MacArthur 1972; Magnuson *et al.* 1979; Chown & Nicolson 2004; MacMillan *et al.* 2014).

In a laboratory study of larval development, Thiele (1969) determined that a forest species, *Nebria brevicollis,* requires cold temperatures (2 – 4 °C) for larval development. How much this requirement varies among *Nebria* species is unknown, but studies in other insects have shown that elevational and latitudinal distributions can be limited by available heat budgets acting on development rates (e.g. Crozier 2004; Bird & Hodkinson 2005). Similarly, chronic exposure to moderately stressful conditions, under which individuals can maintain activity but accumulate sub-lethal injuries, can affect fitness through downstream effects on longevity and reproduction (e.g. Williams *et al.* 2003; Renault 2011). Geographic variation in chronic temperature stress has rarely been examined in the context of species range limits, but offers a clear alternative pathway by which physiology might constrain distributions (Monasterio *et al.* 2011).

For many species, climatic conditions and physiological constraints alone are unable to explain distribution limits, with biotic interactions constraining species to a subset of the environments which they could otherwise occupy (Hutchinson 1957; MacArthur 1972). Spence (1979) found no evidence for direct or indirect adult competition among two partially sympatric *Nebria* (e.g. both species are generalist scavengers, have the same activity patterns, and share diurnal refuges); Mann *et al.* (1980) also found similar activity patterns among *N. vandykei*, *N. paradisi* and *N. crassicornis* on Mt Rainier. However, Spence (1979) suggested that larval competition might be important in structuring *Nebria* communities. When species differ in the conditions under which fitness is maximised, competitive exclusion can drive species turnover along an environmental (e.g. elevational) cline (e.g. MacArthur 1972; Gifford & Kozak 2012). If this is the case, the effect of competitive release should allow a species to expand its environmental (and physical) range when others species are absent (Cadena & Loiselle 2007). On mountains to the south of Mt Rainier, species diversity of *Nebria* declines. It is unclear at present whether range size expands in these less diverse communities. At a broad scale, species persist in the same macroclimatic zone across their geographic range, regardless of the assemblage (Kavanaugh 1978), but more fine-scale study is needed before the effects of competition can be properly assessed.

4.4.4 Conclusions

Physiological traits, in particular thermal tolerance, are frequently inferred as proximal drivers of elevational range edges for insects and other ectotherms. This association has been used to predict and explain recent upslope shifts driven by climate warming and habitat change (La Sorte & Jetz 2010; Larsen 2012; Bässler *et al.* 2013), as well as forming a core component of the age-old question: what factors drive range limits and consequent species turnover along environmental gradients? We found no evidence for clinal variation in either acute cold tolerance or desiccation resistance, nor can heat tolerance variation – whether related to evolutionary constraint or environmental factors – account for elevational turnover among the majority of species. Species turnover in this system is clearly driven by additional, unmeasured factors, which might include physiological constraints such as temperature requirements for development or effects of chronic thermal stress, or biotic interactions.

4.5 Acknowledgements

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Cold is the single most important enemy of life

-- *F. Franks, 1985, Biophysics and Biochemistry and Low Temperature*

Chapter 5

Scale-dependent thermal tolerance variation in Australian mountain grasshoppers

Abstract
Physiological variation among and within species is thought to play a key role in determining distribution patterns across environmental gradients. We tested inter- and intraspecific variation in cold and heat tolerances for three grasshopper species (genus *Kosciuscola*) with overlapping elevation distributions, across their respective ranges in the Australian mountains. Of the three cold tolerance traits measured, the critical thermal minimum was the only trait to vary among species, with greater cold tolerance associated with a distribution extending to a higher elevation. Cold tolerance limits were regularly exceeded in exposed microhabitats, suggesting a role for cold adaptation in structuring species distribution patterns. In contrast to cold tolerance, heat tolerance variation was primarily partitioned within species. For two species, populations from treeless alpine habitat were more heat tolerant than their lower-elevation counterparts, supporting recent models that suggest greater exposure to temperature extremes at higher elevations. These contrasting patterns of physiological variation among and within species emphasise the importance of considering variation within species when attempting to understand how species distributions are affected by thermal extremes.

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5.1 Introduction

Physiological requirements and limits determine the environments in which populations can persist, thus defining the potential geographic distributions of species (Hutchinson 1957; Brown *et al.* 1996). For insects, whose body temperature is intrinsically linked to the thermal environment, large-scale studies have identified strong correlations between thermal tolerance of species and latitude (Addo-Bediako *et al.* 2000; Hoffmann *et al.* 2013). However, many insects have broad geographic distributions, raising the question of whether much of the variation among species from different environments reflects intraspecific variation rather than intrinsic differences among species (Schiffer *et al.* 2013). Few tests of the relationship between inter- and intraspecific variation have so far been undertaken (Chown & Gaston 1999; Chown 2001; Gaston *et al.* 2009). It therefore remains unclear how often apparent species differences reflect plastic or locally adaptive variation among populations, rather than interspecific variation. More generally, can interspecific variation be viewed as an extension of intraspecific variation or separate from it?

Intraspecific variation is a result of interactions between spatially varying selection, gene flow and genetic drift and may be reflected in plastic and/or genetic differentiation, whereas interspecific variation reflects both historical processes (e.g. past environments, evolutionary change) that have driven speciation and population-level effects across the species' range (Mayr 1947; Endler 1977). Thus, while physiological trait variation among populations should reflect environmental conditions within those populations (e.g. Addo-Bediako *et al.* 2000; Hoffmann *et al.* 2013), intrinsic genetic differences among species might not be related to the environment in which a particular population occurs, but rather to the range of conditions experienced across a species' distribution (Sexton *et al.* 2014).

Mountains provide a useful landscape in which to explore inter- and intraspecific variation simultaneously, as populations and species are distributed across comparable spatial scales. Genetic legacies of range shifts in response to past climate cycles (e.g. Knowles 2000; Schoville *et al.* 2012) and recent upslope distribution shifts correlated with warming trends (e.g. Chen *et al.* 2011; Bässler *et al.* 2013) highlight the importance of temperature in determining species' distributions in mountains. Closely related taxa often occupy distinct elevation zones, and thermal limits have repeatedly been proposed as an important factor governing species turnover along elevation clines (Mani 1968; Brown 1996; Cahill *et al.* 2014).

In the Australian alpine region, steep gradients in maximum temperature translate to a nearly 14°C difference in average maximum temperature between the lower edges of montane forests (800 m a.s.l.) and the tops of the highest mountains (~2200 m), while mean and minimum temperatures decrease by roughly 8°C and 3°C, respectively (Slatyer 1978). As such, higher-elevation species are generally expected to have lower thermal limits. Based on the relative lapse rates of maximum and minimum temperature, it is also predicted that populations will vary more in upper than lower thermal limits (which are decoupled in insects), with higher-elevation populations having poorer heat tolerance (Addo-Bediako *et al.* 2000; Hoffmann *et al.* 2013). Recently however, Buckley *et al.* (2013) suggested that, for grasshoppers in the Rocky Mountains of North America, populations occupying high-elevation treeless alpine habitats might actually be exposed to higher thermal extremes than those at lower elevation, leading to the opposite predicted pattern for heat tolerance thresholds.

Grasshoppers are a typically-thermophilic group, but have colonised high-mountain environments worldwide (Alexander 1951). Variation in life-history (e.g. Dearn 1977; Berner *et al.* 2004; Berner & Blanckenhorn 2006), morphology (e.g. Dearn 1978; Roff 1990; Buckley *et al.* 2014), behaviour (e.g. Samietz *et al.* 2005), and physiology (e.g. Buckley *et al.* 2014) along elevation gradients have been documented, but thermal limits have rarely been tested in this context (but see Buckley *et al.* 2014). The flightless *Kosciuscola* grasshoppers (Orthoptera: Acrididae) are endemic to the Australian high country, with three common species occupying overlapping elevation zones between 800 m and the top of Australia's highest mountain, Mt Kosciuszko, at 2228 m (Fig. 5.1).

In this study, we use the *Kosciuscola* as a model system in which to test both inter- and intraspecific variation in thermal tolerance. First, we test whether higher-elevation species have intrinsically lower thermal limits. Second, we test two alternative hypotheses for population variation: (a) higher-elevation populations have lower thermal limits, with greater variation in heat tolerance than cold tolerance, as predicted by relative maximum and minimum temperature gradients, or (b) higher-elevation alpine populations (*K. tristis* and *K. usitatus*) have higher heat tolerance, as predicted by Buckley *et al.*'s (2013) model. Lastly, using microclimate temperature measurements, we assess the role of thermal tolerance limits in structuring the elevation distributions of the three species.

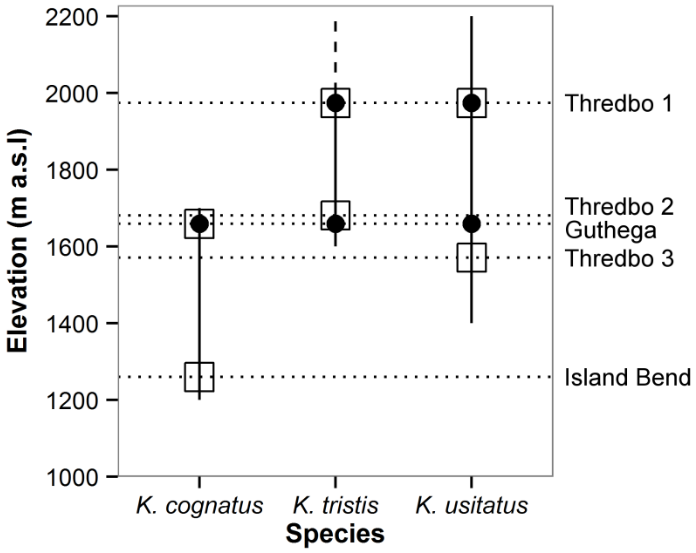

Figure 5.1. Schematic of the distributions of *Kosciuscola* (Orthoptera: Acrididae) in the Mt Kosciuszko region of New South Wales, Australia. Above 2000 m, *Kosciuscola tristis* has a patchy distribution, indicated by the dashed line. Filled circles indicate sites used for interspecific comparisons and open squares indicate sites (populations) for intraspecific comparisons.

5.2 Methods

5.2.1 Study location and species

The study was carried out in the Kosciuszko region of New South Wales – the largest and most continuous area of alpine and sub-alpine habitat on the Australian mainland. Here, temperatures are typically highest in late January and early February. Conditions are highly variable, however, and snow can fall at any time of the year. During February to April, when adult grasshoppers are active, maximum daily ambient temperatures vary between 28°C and -6°C, with a long-term average of 12°C. Minimum temperatures range between 18°C and -11°C, with an average of 3°C (BOM 2014).

Three *Kosciuscola* Sjöst. (Orthoptera: Acrididae) species are common in the Kosciuszko region, and occupy overlapping elevation distributions from the lower edge of montane woodlands to the top of the mountain range (Fig. 5.1). We tested individuals at sites where one, two or three species co-occur (Fig. 5.1). Sympatric sites allow direct comparisons of thermal tolerance among species without confounding effects of native site conditions. For each species, we also tested individuals from one population near the upper edge of their distribution and one population near the lower edge of their distribution (Fig. 5.1). A total of five sites were used in the study, ranging from 1260 m to 1974 m a.s.l (Fig. 5.1). Thredbo 1 is an alpine site (1974 m), above the high-elevation treeline. The vegetation is dominated by *Poa* spp., and both *K. tristis* and *K. usitatus* are abundant here. Thredbo 2, Thredbo 3 and Guthega all have sub-alpine vegetation, dominated by shrubs and snow gum (*Eucalyptus pauciflora niphophila*) woodland. *Kosciuscola usitatus*, *K. tristis* and *K. cognatus* are all common at Guthega, but the latter is not present at either Thredbo site. The lowest site, Island Bend, is tall eucalypt woodland and *K. cognatus* is the only *Kosciuscola* species present. From low to high elevation, body size tends to decrease in females of all species and among males of *K. usitatus* and *K. cognatus,* whereas body size increases with elevation in *K. tristis* (Campbell & Dearn 1980; Appendix D, Fig. D.1). All species are sexually dimorphic in body size, with females approximately three times larger (in live body mass) than males (Appendix D, Fig. D.1).

5.2.2 *Microclimate data*

Temperatures vary markedly over small distances in high-elevation areas (Garrick 2011; Scherrer & Körner 2011). To measure microclimate variation among sites, two iButton data loggers (DS1922L Thermochron®, Maxim Integrated Products) were installed at each collection site. Both were positioned at the soil surface (i.e. with the sensor at the height of a grasshopper): one on bare ground ('exposed') and the other within adjacent vegetation ('sheltered') where grasshoppers were found. The iButtons were positioned so that they did not receive direct sunlight, and were set to record temperature (± 0.0625°C) at 15-minute intervals. These data give an indication of the microclimates available to grasshoppers, rather than operative temperatures, given that grasshoppers will thermoregulate behaviourally and physiologically (Chappell & Whitman 1990).

5.2.3 *Ramping assays for cold and heat tolerance*

All experiments were conducted using a thermoregulator controller (Grant GP200) linked to a water bath (Grant R2) (Grant Instruments, Cambridge, UK), which pumped a 50:50 mix of propylene glycol/water around six 4 cm-diameter beakers (see Appendix C). These were covered to prevent grasshoppers escaping, but a small hole in the lid of each beaker provided fresh air throughout the experiment and prevented the build-up of condensation. Critical thermal limits were measured through ramping assays, which are likely to be more ecologically relevant than static assays (Mitchell & Hoffmann 2010; Overgaard *et al.* 2012). Ramping rates (0.25°C/min and 0.2°C/min for cold and heat tolerance tests, respectively) reflect the upper end of rates of temperature change found at the study sites (e.g. the 98th percentile rate of change was 0.22°C/min over the study period) and take into account that maximum rates of temperature decrease (e.g. mean 0.7°C/min across sites in this study) were higher than maximum rates of increase (e.g. mean 0.5°C/min across sites in this study) in the study area in this and previous years. Grasshopper body temperatures equilibrate rapidly to environmental temperatures (Umbers 2011), and should therefore closely reflect chamber temperatures at the chosen ramping rates. A k-type thermocouple (Jaycar Electronics) was placed in three chambers and connected to a Pico TC-08 Thermocouple Data Logger (Pico Technology) which recorded chamber temperatures averaged over 10 s intervals. As time of day can influence thermal tolerance (Sinclair *et al.* 2003; Kelty 2007), all experiments were carried out during natural daylight hours (9:00 to 17:00) when grasshoppers are normally active. The order in which populations/species were tested during a day was randomised, and time of day did not have an observable effect on thermal tolerance (all $r^2 < 0.05$).

Individual grasshoppers were captured by hand and returned to the laboratory within one hour. For interspecific comparisons, all individuals were separated by sex and kept in mesh cages for five days (Rako & Hoffmann 2006; Terblanche *et al.* 2006) at 12°C under a 14:10-hour light:dark regime before testing. Grasshoppers were fed fresh-cut vegetation (primarily native *Poa* and *Carex* from near the collection sites) every one to two days and provided with water on a moistened cotton wool ball. This treatment was designed to minimise the influence of recent environmental conditions on thermal tolerance (Chown *et al.* 2009), as the experiments were carried out over several weeks. For intraspecific comparisons, individuals were randomly assigned to one of two treatments: 12°C acclimation or no acclimation. Non-acclimated individuals were placed immediately into thermal testing chambers (one per chamber), while individuals for the 12°C acclimation treatment were kept at 12°C for five days, as described above. Different individuals were used for cold tolerance, supercooling point, heat tolerance (ramping) and heat tolerance (plunge) experiments (see below). For interspecific comparisons, in each experiment, we tested five adults/sex/species (total 30 individuals at Guthega, 20 individuals at Thredbo, in each experiment; see Fig. 5.1). For intraspecific comparisons, in each experiment we tested five adults/sex/acclimation treatment/population (total 40 individuals per species in each experiment), for each of the three species (Fig. 5.1). Individuals that died during or within 24-hours of experiments were excluded from statistical analysis and final sample sizes are given in Tables 5.1 and 5.2.

5.2.4 *Cold tolerance*

We used three mechanistically independent measures of cold tolerance: chill coma, chill coma recovery and the supercooling point (SCP) (e.g. MacMillan *et al.* 2014). Chill coma is a state of reversible neuromuscular paralysis (Mellanby 1939; "chill coma 3" in Hazell & Bale 2011) , defined as the temperature at which individuals can no longer maintain coordinated movement (Sinclair *et al.* 2006). This trait often varies across environmental gradients (Gaston & Chown 1999; Keller *et al.* 2013) and was here used to define the critical thermal minimum (CT_{min}). Chill coma and recovery from chill coma ($CT_{min\text{-}recovery}$) were assessed using the ramping set-up described above. In each experimental run, the temperature was held at 12°C for 10 min, decreased at 0.5°C/min to 0°C then decreased at 0.25°C/min to -4°C (*K. tristis, K. usitatus*) or -3°C (*K. cognatus*). Minimum temperatures were chosen to be approximately 1°C below the lowest CT_{min} of each species (Sinclair *et al.* 2006), based on pilot testing, and CT_{min} was scored directly. The temperature was held at the minimum temperature for 5 min then increased at 0.25°C/min until all individuals had recovered, and the temperature of recovery ($CT_{min\text{-}recovery}$) was recorded. There was no correlation between CT_{min} and $CT_{min\text{-}recovery}$ (Pearson correlation test: $r^2 = 0.01$), so we considered these traits as independent estimates of cold tolerance in our analysis.

Many insects reach chill coma and begin to accumulate cold-induced injuries at temperatures much higher than their SCP, when ice forms in the body (Sinclair 1999). Prior to the experiment, individuals were starved for 22 – 26 h as food particles acting as nucleation agents can influence the SCP (Salt 1966; Block & Sømme 1982; Salin *et al.* 2000). Individuals were moved to 4°C for 6 h prior to testing. A k-type thermocouple was affixed to each individual, and individuals were then placed into beakers (described above) and cooled at 0.2°C/min from 0°C. The body temperature of each grasshopper was recorded every 2 s and the SCP was determined as the lowest temperature immediately preceding a rapid rise in temperature. This rise in temperature is caused by release of the latent heat of crystallisation and indicating internal ice formation (Sømme 1982).

5.2.5 *Heat tolerance*

Two traits were used to assess heat tolerance. First, we used the heat movement threshold (HMT) (Alford *et al.* 2012), defined as the temperature at which an organism is unable to maintain voluntary coordinated functions (Hazell *et al.* 2008), as our measure of the critical thermal maximum (CT_{max}). HMT was scored as a loss of righting ability. In each experimental run, using the ramping apparatus described above, the temperature was held at 20°C for 10 min then increased at 0.2°C/min until all individuals had reached their HMT. All runs were filmed with an overhead camera (Sony DSC-HX9V), with timing synchronised to thermocouple recordings. HMT was scored from video playbacks.

In the Australian mountains, exposed dark soils can exceed 45°C on sunny days and be over 30°C warmer than nearby soil shaded by thick vegetation (Figs. 5.2 & 5.3). Even mobile organisms such as grasshoppers could be briefly exposed to extreme temperatures while moving throughout the day. We used a 'plunge' protocol (Terblanche *et al.* 2011) to test tolerance to a sudden high temperature exposure by placing grasshoppers into chambers pre-heated to 50°C. We recorded the time taken for an individual to lose the ability to right itself (time to heat movement threshold, HMT_{time}). All runs were filmed with an overhead camera and HMT_{time} was scored from video playbacks.

As body size often correlates with physiological traits (Chown *et al.* 2002), we measured the hind femur length of all individuals at the completion of experiments (see also Appendix D, Fig. D.2). However, as sex explained 90% of the variation in femur length, we chose not to include leg length as an additional covariate in the statistical analyses.

5.2.6 Thermal safety margins

'Thermal safety margins' and 'warming tolerances' are a common tool for assessing the likely resilience of species to rising temperatures (Deutsch *et al.* 2008; Sunday *et al.* 2011; Diamond *et al.* 2012; Huey *et al.* 2012; Kellermann *et al.* 2012). We used this approach to estimate both warming and cooling tolerances for each species. First, we constructed a microclimate model for each site. We used linear regression to determine the relationship between site microclimate temperature (measured here) and weather station measurements of temperature and solar radiation (Australian Bureau of Meteorology, weather stations 071041, 071032, 071075, and 071003; Appendix D, Table D.1). We used forward and backwards stepwise selection, implemented in the MASS package in R 3.1.2 (R Core Team 2014) to choose the best model based on AIC scores (see Appendix D, Table D.2 for final models). For each site, we fitted separate models for mean daily maximum and minimum, starting with a model that included daily temperature and solar radiation values from each of the four weather stations and first-order interactions. We then used the regression model and 5 years (2010 – 2014) of daily February-April weather station data (BOM 2014) to predict corresponding microclimate temperatures for each site. We extracted the 95th (T_{max}) and 5th (T_{min}) percentile values from daily maximum and minimum estimates respectively, and calculated upper and lower thermal safety margins (TSMs) for each species as CT_{max} - T_{max} (upper TSM) and T_{min} - CT_{min} (lower TSM). Weather station data had poor predictive power for sheltered microhabitats, as has been observed in other systems (e.g. Andrew et al. 2013), so these analyses only considered exposed microhabitats.

5.2.7 Statistical analysis

We compared how cold and heat tolerances varied between species, and between populations (high elevation vs low elevation) within species, using analyses of variance (ANOVA). For the interspecific comparisons, we included species and sex as factors in the model and each site (Thredbo 1, Guthega) was tested separately. For intraspecific comparisons, elevation (high/low), acclimation treatment (yes/no) and sex were included as factors. An elevation*acclimation interaction term was included in these models, to determine whether population differences in thermal tolerance (if any) could be ascribed to short-term plasticity.

Prior to analysis, all data were checked for normality and homogeneity of variance using Shapiro-Wilks tests, q-q plots and Levene's tests. Data for CT_{max} and HMT_{time} were skewed, and hence were log-transformed prior to analysis to attain normality. In the heat tolerance tests, 13 individuals (out of 141) had not reached HMT by the time chamber temperatures had reached 50°C. These individuals (seven *K. cognatus*, three *K. tristis*, three *K. usitatus*) were assigned a CT_{max} of 50°C. CT_{min} and SCP data were normally distributed, thus not transformed for analysis. Generalized linear models with a Gaussian error function and log link were also used to analyse the CT_{max} and HMT_{time} data, however, as the conclusions drawn from both analyses were the same, results are only reported for the ANOVAs.

Unless stated otherwise, all statistical analyses were performed in R 3.1.2 (R Core Team 2014) and data are presented as mean ± 1 SD.

Table 5.1. Cold tolerance trait means, standard deviation (SD), standard error (SE) and sample size (*n*) for each species at each study site.

Trait/Species	Site	Mean	SD	SE	*n*
CT$_{min}$ (°C)					
K. usitatus	Thredbo 1	-3.0	0.6	0.1	20
	Thredbo 3	-2.7	0.7	0.1	20
	Guthega	-3.1	0.4	0.1	10
K. tristis	Thredbo 1	-2.5	0.7	0.2	18
	Thredbo 2	-2.3	0.7	0.2	20
	Guthega	-2.3	0.4	0.1	8
K. cognatus	Guthega	-1.8	0.6	0.1	20
	Island Bend	-2.0	0.9	0.2	20
CT$_{min\text{-}recovery}$ (°C)					
K. usitatus	Thredbo 1	10.7	4.1	0.9	20
	Thredbo 3	11.0	4.4	1.0	20
	Guthega	14.1	3.2	1.0	10
K. tristis	Thredbo 1	14.4	4.1	1.0	18
	Thredbo 2	11.9	4.2	0.9	20
	Guthega	13.4	4.7	1.7	8
K. cognatus	Guthega	10.8	3.5	0.8	20
	Island Bend	11.1	5.0	1.1	20
SCP (°C)					
K. usitatus	Thredbo 1	-3.3	1.1	0.2	22
	Thredbo 3	-3.7	0.9	0.3	7
K. tristis	Thredbo 1	-3.8	1.2	0.3	18
	Thredbo 2	-4.1	1.1	0.4	10
K. cognatus	Guthega	-3.6	1.1	0.2	19
	Island Bend	-3.0	0.7	0.2	10

Table 5.2. Heat tolerance trait means, standard deviation (SD), standard error (SE) and sample size (*n*) for each species at each study site.

Trait/Species	**Site**	**Mean**	**SD**	**SE**	***n***
CT_{max} (°C)					
K. usitatus	Thredbo 1	49.2	0.7	0.2	20
	Thredbo 3	48.6	1.0	0.2	20
	Guthega	49.5	0.5	0.2	11
K. tristis	Thredbo 1	49.1	0.6	0.1	20
	Thredbo 2	48.5	1.2	0.3	20
	Guthega	49.4	0.5	0.1	10
K. cognatus	Guthega	49.0	1.3	0.3	20
	Island Bend	49.1	0.8	0.2	20
HMT_{time} (min)					
K. usitatus	Thredbo 1	16.1	6.1	1.4	20
	Thredbo 3	14.6	4.6	1.0	20
K. tristis	Thredbo 1	18.6	7.0	1.6	20
	Thredbo 2	14.9	5.4	1.2	20
K. cognatus	Guthega	17.1	7.6	1.7	20
	Island Bend	15.1	7.4	1.7	20

5.3 Results

5.3.1 Microclimate variation among sites

Field temperatures (from iButtons) recorded between February to April ranged from -4.7°C to 44.4°C (mean 13.4 ± 4.0°C) (Fig. 5.2). In paired site comparisons, Thredbo 1 (high elevation for *K. usitatus* and *K. tristis*) had a lower minimum temperature than Thredbo 2 and 3 (low elevation) on 79% and 68% of days, respectively, in exposed microhabitats, and on 56% and 75% of days in sheltered microhabitats. Guthega (high elevation for *K. cognatus*) always had a lower daily minimum temperature than Island Bend (low elevation for this species). The upper sites of each species were thus mostly colder than the lower sites, though the absolute difference in temperature was small for comparisons at Thredbo (Fig. 5.2; Table 5.3).

Within sites, exposed habitats had much greater temperature variation (Fig. 5.3), and at any given time they were 6.9°C colder to 27.5°C warmer than adjacent sheltered sites (Table 5.3). Exposed habitats were typically warmer during the day (6 am to 6 pm) (1.3°C to 4.3°C mean temperature difference), while sheltered habitats were warmer at night (7 pm to 5 am) (0.5°C to 1.3°C mean temperature difference). Vegetation thus provides a refuge from high temperature extremes during the day and from cold temperature extremes at night.

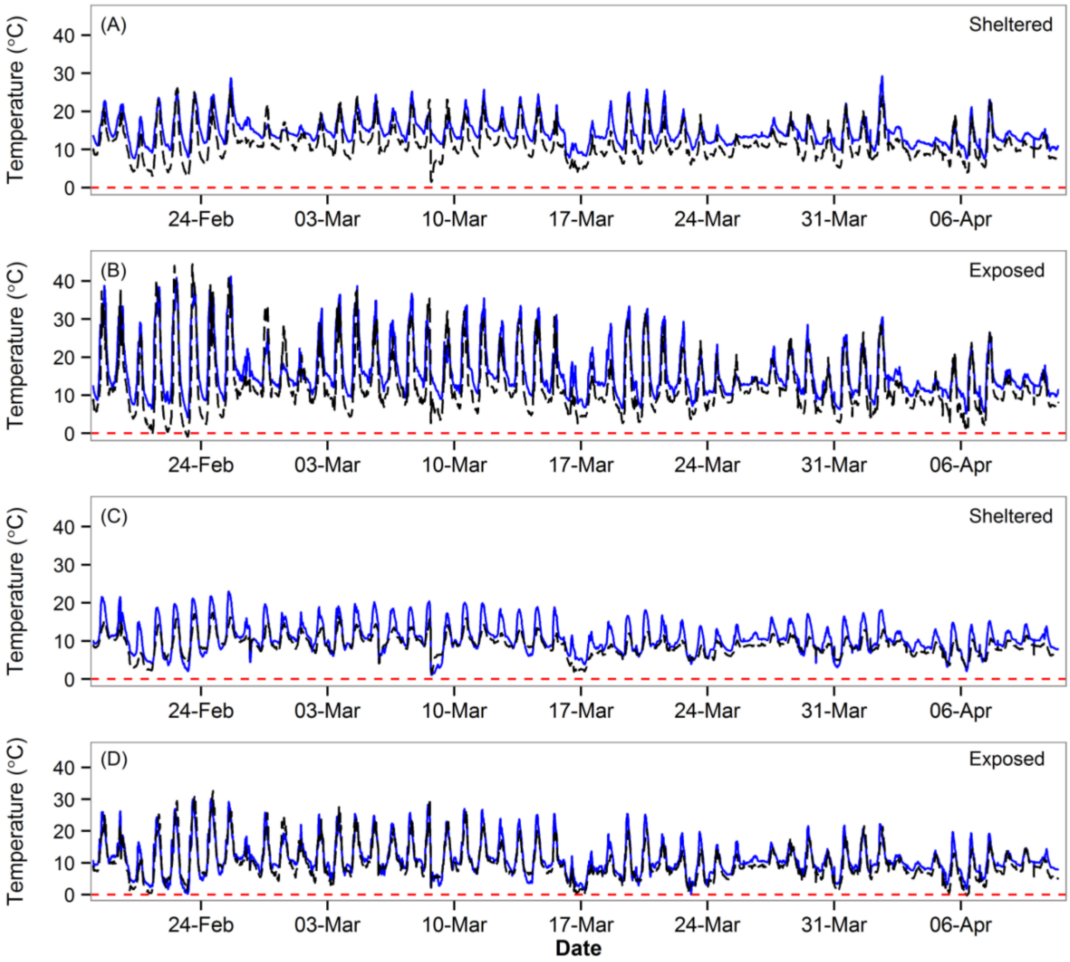

Figure 5.2. Microclimate variation among paired sites between February 18th and March 18th, 2014, with temperatures recorded every 15 min. Temperatures were recorded in two habitat types: sheltered (A, C) and exposed (B, D). *Kosciuscola cognatus* from Island Bend (low elevation; solid line) and Guthega (high elevation; dotted line) (A, B) and *K. tristis* populations from Thredbo 2 (low elevation; solid line) and Thredbo 1 (high elevation; dotted line) (C, D) were tested for thermal tolerance. *Kosciuscola usitatus* were tested from Thredbo 1 and Thredbo 3 (not shown). The horizontal dashed line represents 0°C.

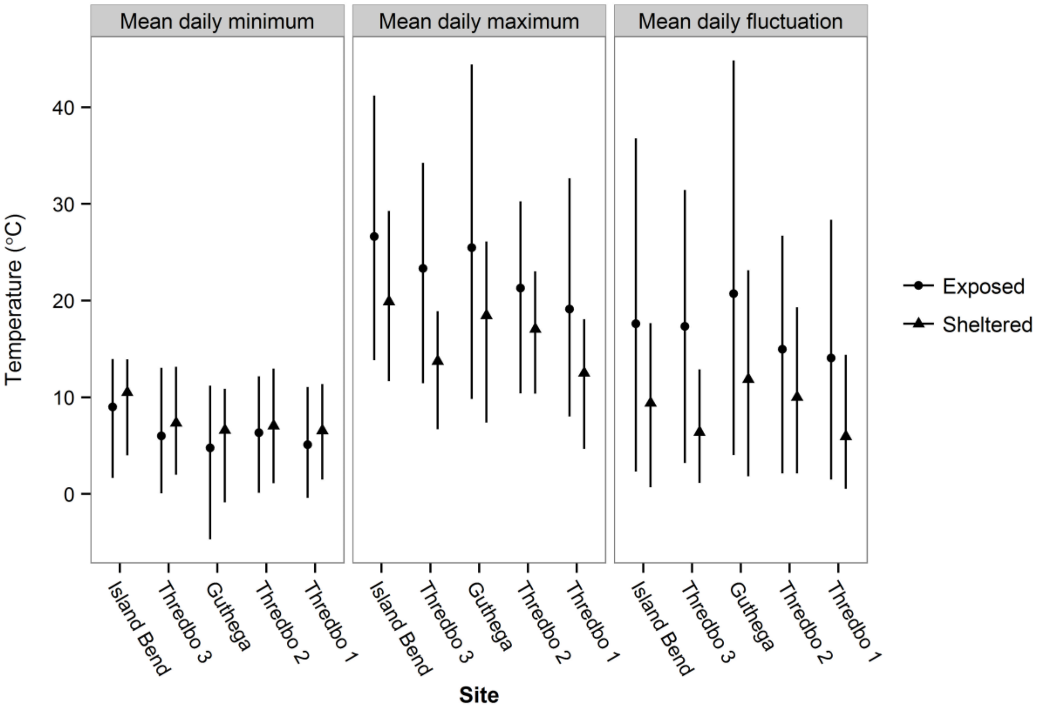

Figure 5.3. Characteristics of the thermal regimes at the five population sites, measured over the study period from February 18th to April 10th, 2014. Points show the mean daily (24-hour) minimum, maximum and fluctuation, and lines show the respective temperature ranges. Within each site, temperatures were recorded in two habitat patches: sheltered underneath vegetation (triangles) or exposed ground (circles). Sites are ordered from lowest (Island Bend) to highest (Thredbo 1) elevation.

5.3.2 *Cold tolerance*

Trait means for all thermal tolerance measures and ANOVA tables are provided in Tables 5.1 - 5.2 and 5.4 – 5.6, respectively. We found intrinsic differences among species in CT_{min} when we compared individuals from the two sympatric sites (Guthega: $F_{2,24} = 21.92$, $p < 0.001$; Thredbo: $F_{1,35} = 6.50$, $p = 0.015$; Fig. 5.4A), with *K. cognatus* – the lowest-elevation species – having the highest CT_{min} (-1.6 ± 0.6°C, $n = 10$), followed by *K. tristis* (Guthega: $CT_{min} = -2.3 \pm 0.4$°C, $n = 8$; Thredbo: -2.5 ± 0.7, $n = 19$) and *K. usitatus* – the highest-elevation species (Guthega: $CT_{min} = -3.1 \pm 0.4$°C, $n = 10$; Thredbo: -3.0 ± 0.6°C, $n = 20$; all pairwise comparisons, $p < 0.05$, see Appendix D, Table D.3 for standardised effect sizes). Recovery from chill coma was less variable among species than entry into it (Fig. 5.4B) and we found significant interspecific variation only at Thredbo ($F_{1,35} = 7.68$, $p = 0.009$; Guthega: $F_{2,24} = 0.24$, $p = 0.792$), where *K. usitatus* recovered at a lower temperature (average 3.7°C) than *K. tristis*. The supercooling point, our third measure of cold tolerance, was the least variable cold tolerance trait (Fig. 5.4A), showing no significant species effects ($F_{1,37}$ = 1.55, p = 0.220). As a result, the SCP was much closer to CT$_{\mathrm{min}}$ for *K. usitatus* than for the other two species (Fig. 5.4A).

Between the high- and low-elevation populations of each species, there was no significant difference in cold tolerance measured as either CT$_{\mathrm{min}}$ (Fig. 5.5A), CT$_{\mathrm{min\text{-}recovery}}$, or SCP (Table 5.5). However, for both *K. usitatus* and *K. tristis* there was a significant independent effect of a 12°C acclimation treatment (*K. usitatus:* $F_{1,35}$ = 8.84, p = 0.005; *K. tristis*: $F_{1,33}$ = 12.41, p = 0.001; *K. cognatus:* $F_{1,35}$ = 0.97, p = 0.335) – acclimated individuals had lower CT$_{\mathrm{min}}$ values by an average 0.5°C and 0.7°C for *K. usitatus* and *K. tristis*, respectively, indicating some plasticity in this trait. Conversely, acclimation increased CT$_{\mathrm{min\text{-}recovery}}$ of *K. tristis* and *K. cognatus* from chill coma by 2.9°C and 5.3°C, respectively (*K. usitatus:* $F_{1,35}$ = 0.58, p = 0.450; *K. tristis*: $F_{1,33}$ = 5.30, p = 0.028; *K. cognatus:* $F_{1,35}$ = 24.77, p < 0.001). In all comparisons, males and females showed similar trait values, with one exception: female *K. tristis* had significantly lower CT$_{\mathrm{min}}$ than males (females: -2.6 ± 0.6°C; males: -2.2 ± 0.7°C; Table 5.5).

5.3.3 *Heat tolerance*

Among species, there was no significant variation in heat tolerance measured via either ramping (Guthega: $F_{2,27}$ = 0.15, p = 0.858; Thredbo: $F_{1,37}$ = 1.43, p = 0.240; Fig. 5.4C) or heat shock ($F_{1,37}$ = 0.73, p = 0.399; Fig. 5.4D) protocols and no influence of sex on either trait. However, for both *K. usitatus* and *K. tristis*, the high-elevation population had higher CT$_{\mathrm{max}}$ than the low-elevation population (*K. usitatus*: $F_{1,35}$ = 5.42, p = 0.026; *K. tristis*: $F_{1,35}$ = 5.83, p = 0.021; Fig. 5.5B). Furthermore, for *K. tristis*, this difference was particularly strong after acclimation (with acclimation, difference = 0.9°C; without acclimation, difference = 0.3°C: $F_{1,35}$ = 5.42, p = 0.026). Neither heat tolerance measure differed between the two *K. cognatus* populations (CT$_{\mathrm{max}}$: $F_{1,35}$ = 0.00, p = 0.949; HMT$_{\mathrm{time}}$: $F_{1,35}$ = 1.18, p = 0.285), though females had higher CT$_{\mathrm{max}}$ than males (females: 49.5 ± 0.5°C; males: 48.6 ± 1.3°C; $F_{1,35}$ = 10.68, p = 0.002) and acclimation increased HMT$_{\mathrm{time}}$ (with: 21.0 ± 7.1 min; without: 11.2 ± 3.6 min; $F_{1,35}$ = 21.76, p < 0.001).

5.3.4 *Warming and cooling tolerances*

For all species, predicted temperatures in their high-elevation habitat were expected to exceed or be within 0.5°C of their critical thermal minimum on least 5% of days during the months when adults are active (see Table 5.7) – species appear to live on the threshold of their capacity to tolerate cold temperatures. In contrast, warming tolerances were all greater than 7°C (Table 5.7).

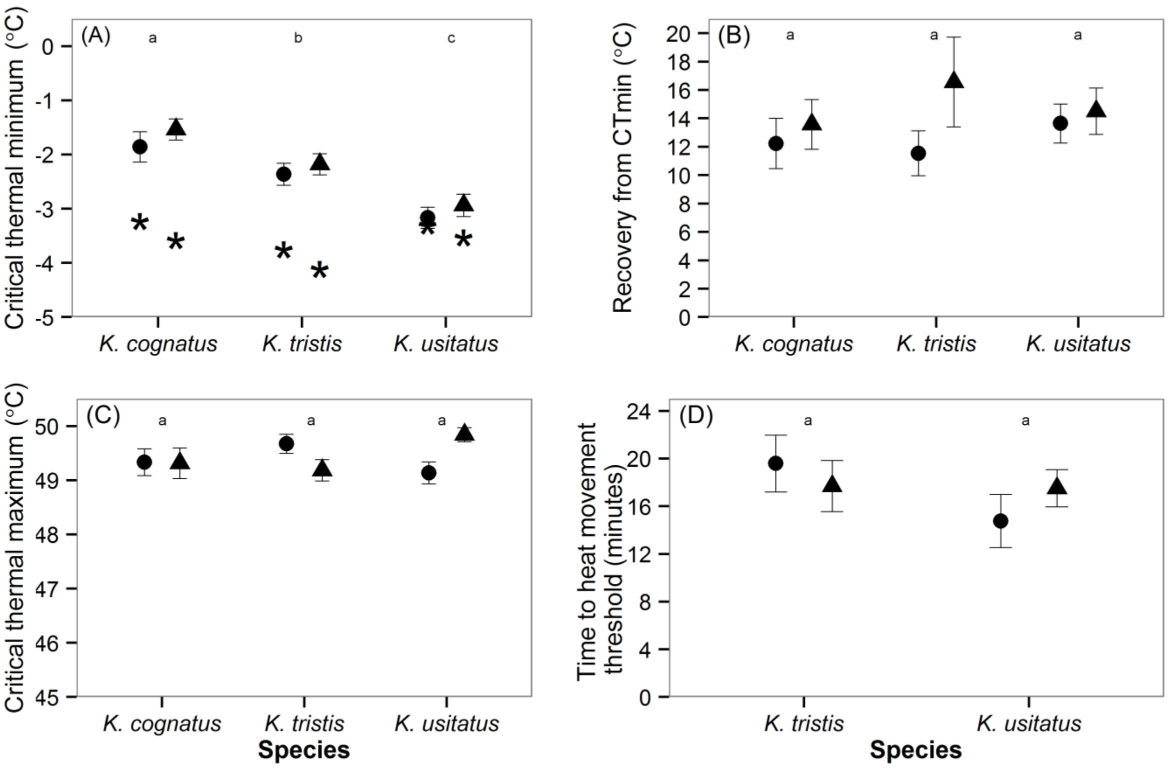

Figure 5.4. Trait means (± standard error) for cold tolerance (A, B) and heat tolerance (C, D) for *Kosciuscola cognatus, K. tristis* and *K. usitatus* individuals from the sympatric sites at Guthega (A – C) and Thredbo 1 (D). Results for females (circles) and males (triangles) are shown separately. Stars in (A) represent the mean supercooling points for each species, averaged across all sites. Different letters indicate a significant ($p < 0.05$) difference among species in pairwise comparisons.

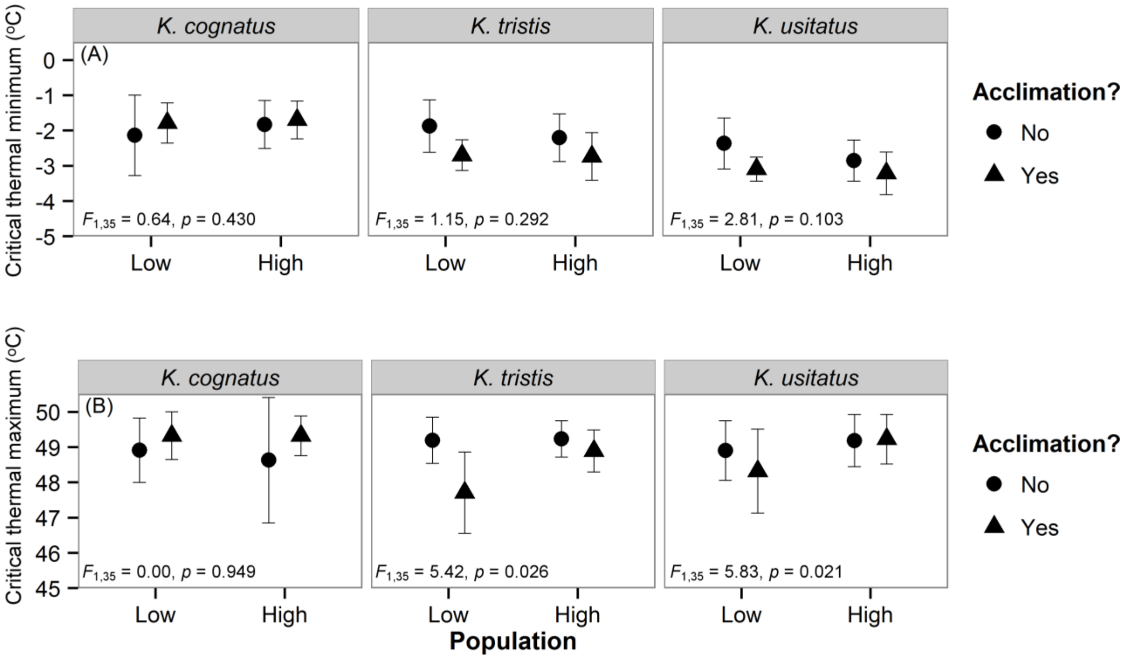

Figure 5.5. Trait means (± standard error) for critical thermal minima (A) and maxima (B) for low- and high-elevation populations of each species. Results with (triangle) and without (circle) acclimation are shown separately. ANOVA results for the main effect of elevation are given for each species and trait.

Table 5.3. Characteristics of the study sites, including temperature conditions measured over the study period. Daily mean, maximum and minimum temperatures, as well as daily temperature ranges (in brackets), were averaged over 52 days of recordings from February 18th to April 10th, 2014 at two microhabitat patches (sheltered within vegetation, or exposed ground) within each site, for a total of 106 daily records per site. Microclimate variation was estimated by calculating the difference in temperature between sheltered and exposed microhabitat patches within each site, for each temperature recording (at 15-minute intervals). For this measure, means are for the absolute difference, and the range is given with positive values indicating that the exposed microhabitat is warmer than the sheltered microhabitat, and negative values indicating that it is colder. Data are given as mean ± SD (minimum, maximum).

Table on next page.

Table 5.3. See caption on previous page.

	Thredbo 1	Thredbo 2	Thredbo 3	Guthega	Island Bend
Elevation (m a.s.l)	1974 m	1681 m	1571 m	1659 m	1260 m
Floristic zone[1]	Alpine	Sub-alpine	Sub-alpine	Sub-alpine	Montane
Overall mean temperature (exposed)	10.17 ± 5.58	11.53 ± 5.37	12.38 ± 6.51	11.66 ± 7.92	15.35 ± 6.94
Overall mean temperature (sheltered)	9.18 ± 2.86	11.07 ± 3.99	10.33 ± 2.75	10.77 ± 4.52	14.09 ± 3.82
Daily minimum (exposed)	5.08 ± 2.95 (-0.42, 11.07)	6.33 ± 2.99 (0.10, 12.17)	6.00 ± 3.17 (0.06, 13.05)	4.76 ± 3.84 (-4.71, 11.20)	9.01 ± 3.05 (1.65, 13.96)
Daily minimum (sheltered)	6.55 ± 2.28 (1.50, 11.37)	7.05 ± 2.60 (1.10, 12.95)	7.34 ± 2.39 (1.97, 13.14)	6.60 ± 3.09 (-0.89, 10.88)	10.50 ± 2.60 (3.98, 13.93)
Daily maximum (exposed)	19.13 ± 6.26 (8.00, 32.65)	21.30 ± 5.13 (10.39, 30.26)	23.32 ± 5.54 (11.44, 34.25)	25.47 ± .842 (9.82, 44.43)	26.63 ± 7.68 (13.83, 41.20)
Daily maximum (sheltered)	12.50 ± 2.92 (4.64, 18.08)	17.06 ± 2.96 (10.36, 23.03)	13.73 ± 2.30 (6.68, 18.91)	18.46 ± 4.44 (7.37, 26.10)	19.89 ± 4.29 (11.67, 29.28)
Daily range (exposed)	14.05 ± 5.97 (1.50, 28.37)	14.97 ± 5.87 (2.13, 26.72)	17.33 ± 6.49 (3.20, 31.45)	20.68 ± 9.04 (4.01, 44.85)	17.62 ± 7.61 (2.32, 36.77)
Daily range (sheltered)	5.94 ± 2.67 (0.53, 14.39)	10.01 ± 3.51 (2.13, 19.30)	6.39 ± 2.54 (1.13, 12.87)	11.78 ± 5.00 (1.82, 23.13)	9.39 ± 4.53 (0.69, 17.66)
Microclimate variation	2.20 ± 2.50 (-6.95, 15.39)	1.28 ± 1.55 (-4.54, 11.75)	3.08 ± 3.69 (-3.80, 17.66)	2.60 ± 3.19 (-4.74, 27.54)	2.53 ± 3.22 (-4.23, 17.39)
Kosciuscola species present	*K. usitatus* *K. tristis*	*K. usitatus* *K. tristis*	*K. usitatus*	*K. usitatus* *K. tristis* *K. cognatus*	*K. cognatus*

[1] Floristic zones are described by Costin (1954).

Table 5.4. ANOVA results for interspecific comparisons of cold and heat tolerance. Six traits were measured: critical thermal minimum (CT_{min}), chill coma recovery ($CT_{min\text{-}recovery}$), supercooling point (SCP), critical thermal maximum (CT_{max}) and the time to heat movement threshold at 50°C (HMT_{time}). Statistically significant effects ($p < 0.05$) are in bold.

	Guthega			Thredbo		
Trait	*df*	*F*	*p*	*df*	*F*	*p*
CT_{min}						
Species	2, 24	**21.92**	**<0.001**	1, 35	**6.50**	**0.015**
Sex	1, 24	2.05	0.165	1, 35	1.72	0.199
$CT_{min\text{-}recovery}$						
Species	2, 24	0.24	0.792	1, 35	**7.68**	**0.009**
Sex	1, 24	2.41	0.147	1, 35	0.29	0.593
SCP						
Species		-		1, 37	1.55	0.220
Sex		-		1, 37	0.08	0.786
CT_{max}						
Species	2, 27	0.15	0.858	1, 37	1.43	0.240
Sex	1, 27	0.10	0.760	1, 37	0.73	0.399
HMT_{time}						
Species		-		1, 37	1.08	0.306
Sex		-		1, 37	0.16	0.688

Table 5.5. ANOVA results for intraspecific comparisons of cold tolerance. Three traits were measured: critical thermal minimum (CT_{min}), chill coma recovery ($CT_{min\text{-}recovery}$), and supercooling point (SCP). Statistically significant effects ($p < 0.05$) are in bold.

	K. usitatus			***K. tristis***			***K. cognatus***		
Trait	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
CT_{min}									
Elevation (high/low)	1, 35	2.81	0.103	1, 33	1.15	0.292	1, 35	0.64	0.430
Acclimation (y/n)	1, 35	**8.84**	**0.005**	1, 33	**12.41**	**0.001**	1, 35	0.968	0.335
Sex	1, 35	1.38	0.248	1, 33	**5.45**	**0.026**	1, 35	0.91	0.347
Elevation*acclimation	1, 35	1.04	0.315	1, 33	0.56	0.460	1, 35	0.20	0.655
$CT_{min\text{-}recovery}$									
Elevation (high/low)	1, 35	0.05	0.828	1, 33	3.76	0.061	1, 35	0.08	0.777
Acclimation (y/n)	1, 35	0.58	0.450	1, 33	**5.30**	**0.028**	1, 35	**24.77**	**<0.001**
Sex	1, 35	0.66	0.421	1, 33	0.05	0.833	1, 35	3.25	0.080
Elevation*acclimation	1, 35	0.63	0.432	1, 33	1.81	0.188	1, 35	0.80	0.378
SCP									
Elevation (high/low)	1, 26	0.63	0.434	1, 25	0.60	0.445	1, 26	2.42	0.132
Sex	1, 26	0.40	0.533	1, 25	0.61	0.441	1, 26	0.91	0.350

Table 5.6. ANOVA results for intraspecific comparisons of heat tolerance. Two traits were measured: critical thermal maximum (CT_{max}) and the time to heat movement threshold at 50°C (HMT_{time}). Statistically significant effects ($p < 0.05$) are in bold.

	K. usitatus			*K. tristis*			*K. cognatus*		
Trait	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
CT_{max}									
Elevation (high/low)	1, 35	**5.83**	**0.021**	1, 35	**5.42**	**0.026**	1, 35	0.00	0.949
Acclimation (y/n)	1, 35	1.93	0.173	1, 35	**16.60**	**<0.001**	1, 35	2.17	0.149
Sex	1, 35	1.55	0.222	1, 35	1.36	0.252	1, 35	**10.68**	**0.002**
Elevation*acclimation	1, 35	0.43	0.515	1, 35	**5.42**	**0.026**	1, 35	0.00	0.949
HMT_{time}									
Elevation (high/low)	1, 35	0.56	0.458	1, 35	2.85	0.101	1, 35	1.18	0.285
Acclimation (y/n)	1, 35	0.37	0.546	1, 35	0.06	0.802	1, 35	**21.76**	**<0.001**
Sex	1, 35	0.10	0.759	1, 35	0.39	0.536	1, 35	0.24	0.628
Elevation*acclimation	1, 35	0.03	0.875	1, 35	2.90	0.098	1, 35	0.41	0.529

Table 5.7. Predicted temperature extremes (T_{min} and T_{max} defined as the 5th and 95th percentiles, respectively, for 5-year predicted microclimate temperatures) in exposed microhabitats at sites where species were found, and safety margins for species at those sites. Cooling or warming tolerances were calculated as the difference between T_{min} or T_{max} and the mean critical thermal limits (CT_{min} or CT_{max}, respectively) for each population. Negative values (in bold) indicate that daily minimum or maximum microclimate conditions are expected to exceed the thermal tolerance limits of the population during at least 5% of the season when adults are active.

			Cooling tolerance (°C)			**Warming tolerance (°C)**		
Site	T_{min}	T_{max}	*K. usitatus*	*K. tristis*	*K. cognatus*	*K. usitatus*	*K. tristis*	*K. cognatus*
Thredbo 1 (1974 m)	-2.67	32.28	0.4	**-0.2**		16.9	16.8	
Thredbo 2 (1681 m)	-0.52	29.18		1.8			19.3	
Guthega (1659 m)	-3.08	41.06	0.0	**-0.8**	**-0.4**	8.4	8.4	7.9
Thredbo 3 (1571 m)	-2.59	32.86	0.1			15.8		
Island Bend (1260 m)	2.34	40.23			5.0			8.9

5.4 Discussion

Patterns of variation in physiological traits within and between species are not necessarily related, with different processes driving trait divergence at these two levels (Chown & Gaston 1999). While variation among populations should reflect spatially varying selection (e.g. along an environmental gradient) tempered by patterns of gene flow among populations (Sexton *et al.* 2014), interspecific variation – when measured in a common environment – will also reflect intrinsic genetic differences resulting from historical processes and selection pressures across the species' range (Mayr 1963; Endler 1977; Peterson *et al.* 2013). Here we examined both scales of variation, and discuss them below with respect to cold and heat tolerance.

5.4.1 *Cold tolerance: variation among species but not among populations*

There are many ways to measure and define a species' cold tolerance, and there is growing evidence that traits such as entry into and recovery from chill coma are mechanistically independent (e.g. Bale 2002; Findsen *et al.* 2014; MacMillan *et al.* 2014). Thus, it is perhaps unsurprising that we found interspecific variation in just one of the three traits measured. Indeed, variance in the supercooling point across insect taxa is almost entirely partitioned above the species level, indicating that this trait is generally conserved at the level of this study; in contrast, the lower lethal temperature – closely related to the critical thermal minimum – is generally variable among species (Addo-Bediako *et al.* 2000).

Experimental studies and broad-scale syntheses suggest that the critical thermal minimum is more plastic (Chown 2001; Chown & Nicolson 2004; Overgaard *et al.* 2011; Alford *et al.* 2012), less evolutionarily conserved (Araújo *et al.* 2013) and varies more across environmental gradients than does the critical thermal maximum (Addo-Bediako *et al.* 2000; Araújo *et al.* 2013). When considered at the interspecific level, our findings support these patterns, with significant variation in critical thermal minima, but not thermal maxima, reflecting the elevation ranges of the three species.

Because we considered populations where one, two or three species occurred, it is possible that trait variation is influenced by competition and subsequent thermal niche partitioning (Magnuson *et al.* 1979; Tracy & Christian 1986). Under this scenario, trait differences among populations of species from locations where the species are sympatric should be reduced in locations where the species are no longer sympatric. However, although intrinsic differences clearly exist among species for cold tolerance, this trait was fixed across populations regardless of species composition, suggesting that thermal niche partitioning did not contribute to interspecific trait variation.

In the study area, maximum temperature – measured at both microclimate and ambient scales – decreases more rapidly and more consistently with elevation than minimum temperatures (Slatyer 1978; this study). A shallow minimum temperature gradient might explain the lack of variation in CT_{min} among populations of *K. tristis* and *K. usitatus,* where higher sites were only 1°C colder, on average, than lower sites. Further, these populations are separated by less than 2 km, and gene flow among populations might overwhelm weak selection for local adaptation (Lenormand 2002; Slatyer *et al.* 2014). The same explanation is, however, unlikely to apply to *K. cognatus*, for which the upper site was, on average 4°C, colder than the lower site and separated by roughly 10 km.

Among insects, documented critical thermal minima vary from well below 0°C in an Antarctic weevil (Klok & Chown 2003) to nearly 20°C in some tropical species (Terblanche *et al.* 2007). Repeated colonisation of cool, mountain environments – even by typically-thermophilic groups such as grasshoppers – suggests that cold tolerance is a readily evolvable trait (Sinclair 1999; Morgan-Richards & Gibbs 2001). For *Kosciuscola*, lower thermal limits to activity were within 4°C of the lowest temperatures in sheltered habitats, while temperatures in exposed habitat patches frequently exceeded cold tolerance limits: each species appears to persist in a climate at the edge of its cold tolerance capacity, and is most likely reliant on vegetation cover to escape cold temperatures. While *K. usitatus* and *K. tristis* are found to the tops of Australia's highest mountains, our results suggest that cold tolerance is likely to have some role in defining the upper range edge of *K. cognatus.* These results also hint at the intriguing possibility of treeless cold air drainages ('frost hollows'), which are common in Australia's alpine region and experience much lower minimum temperatures (Jennings 1979; Davidson & Reid 1985; Costin 1989), influencing patterns of population connectivity for all three species.

5.4.2 Heat tolerance: uncovering variation within species

Although heat tolerance is generally considered to be less variable than cold tolerance (reviewed: Addo-Bediako *et al.* 2000; Hoffmann *et al.* 2013), we found significant variation in heat tolerance traits associated with either elevation (CT_{max}) or acclimation (HMT_{time}) in two of the three species, suggesting locally adaptive and/or plastic intraspecific variation in these traits. High-elevation climates present risks of both cold and heat stress for small ectotherms, as a result of large daily temperature fluctuations and high solar radiation (Mani 1968; Sømme 1989; Buckley *et al.* 2013). Recent models suggest that these thermal challenges may be particularly acute in treeless alpine habitats where a lack of vegetation cover and high radiative heat gain drive high body temperatures in model simulations (Buckley *et al.* 2013). It is therefore notable that the two species to show among-population variation in heat tolerance – *K. tristis* and *K. usitatus* – are those for which the upper population was in alpine habitat.

Dense shrubs are less common at higher elevations (Costin 1954) and the extent to which individuals can thermoregulate by movement between sheltered and exposed habitats might therefore be reduced. Further, although average ambient temperatures decrease with elevation (Slatyer 1978), the incidence of temperature extremes was similar, or even higher at the upper sites (Table 5.7). The combined effects of high-temperature events and increased exposure to these conditions, due to lower vegetation cover (Buckley *et al.* 2013), could explain the variation in heat tolerance among *K. tristis* and *K. usitatus* populations. In comparable studies in the Rocky Mountains, for example, high-elevation populations of three grasshopper species preferred warmer temperatures or were more inclined to actively increase their body temperature above ambient than their lower-elevation counterparts (Samietz *et al.* 2005; Buckley *et al.* 2014).

5.4.3 Temperature extremes and adaptive variation

Growing evidence suggests that maximum and minimum temperatures are more closely associated with species distributions than temperature averages (e.g. Zimmerman *et al.* 2009; Clusella-Trullas *et al.* 2011; Kellermann *et al.* 2012), and that these thermal extremes pose the most significant threat to species under climate change (Bonan 2002; Clusella-Trullas *et al.* 2011; Hoffmann *et al.* 2013). The *Kosciuscola* appear to be well-placed, physiologically, to thrive in a warmer climate: estimates of warming tolerances suggest that high temperatures should not preclude individuals from utilising exposed habitats at any site. This is particularly so when considering trends for increasing shrub cover in current alpine habitat (Myers-Smith *et al.* 2011), which could moderate the effects of thermal (particularly cold) extremes. Fine-scale microclimate measurements coupled with biophysical models would provide a useful assessment of exposure to potentially damaging temperatures and the capacity for behavioural thermoregulation to buffer temperature extremes in the exposed alpine environment (Kearney *et al.* 2009).

5.4.4 Conclusions

Thermal limits are considered to play an important role in structuring the geographic distributions of species and their warm- and cold-edge range limits. The generally low cold tolerance of the *Kosciuscola* puts all species close to their lower thermal safety margin under current climate conditions. Further, this suggests that physiological limits constrain the upper range edge of the montane and sub-alpine species *K. cognatus*. Easing of this constraint due to continued warming is likely to allow upward shifts in the distribution of this species. Our results emphasise that interspecific variation can show different patterns to intraspecific variation, and that when species distributions are linked to variation in physiological traits both population and species levels should be considered. Variation at the intraspecific level provides information on the impact of plasticity and genetic changes on trait variation which can then be compared to the magnitude of any physiological differences among species thought to constrain species distributions.

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And down by Kosciusko, where the pine-clad ridges raise
Their torn and rugged battlements on high,
Where the air is clear as crystal, and the white stars fairly blaze
At midnight in the cold and frosty sky.

-- *Banjo Patterson, 1890, The Man from Snowy River*

Chapter 6

Strong genetic structure corresponds to small-scale geographic breaks in the Australian alpine grasshopper *Kosciuscola tristis*

Abstract

Background

Mountain landscapes are topographically complex, creating discontinuous 'islands' of alpine and sub-alpine habitat with a dynamic history. Changing climatic conditions drive their expansion and contraction, leaving signatures on the genetic structure of their flora and fauna. Australia's high country covers a small, highly fragmented area. Although the area is thought to have experienced periods of relative continuity during Pleistocene glacial periods, small-scale studies suggest deep lineage divergence across low-elevation gaps. Using both DNA sequence data and microsatellite markers, we tested the hypothesis that genetic partitioning reflects observable geographic structuring across Australia's mainland high country, in the widespread alpine grasshopper *Kosciuscola tristis* (Sjösted).

Results

We found broadly congruent patterns of regional structure between the DNA sequence and microsatellite datasets, corresponding to strong divergence among isolated mountain regions. Small and isolated mountains in the south of the range were particularly distinct, with well-supported divergence corresponding to climate cycles during the late Pliocene and Pleistocene. We found mixed support, however, for divergence among other mountain regions. Interestingly, within areas of largely contiguous alpine and subalpine habitat around Mt Kosciuszko, microsatellite data suggested significant population structure, accompanied by a strong signature of isolation-by-distance.

Conclusions

Consistent patterns of strong lineage divergence among different molecular datasets indicate genetic breaks between populations inhabiting geographically distinct mountain regions. Three primary phylogeographic groups were evident in the highly fragmented Victorian high country, while within-region structure detected with microsatellites may reflect more recent population isolation. Despite the small area of Australia's alpine and sub-alpine habitats, their low topographic relief and their lack of extensive glaciation, divergence among populations was on the same scale as that detected in much more extensive Northern hemisphere mountain systems. The processes driving divergence in the Australian mountains might therefore differ from their Northern hemisphere counterparts.

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6.1 Introduction

Mountain landscapes form a matrix of mountain-top 'sky islands', high-elevation ridges and intervening low-elevation habitat. This creates a discontinuous and fragmented landscape with features such as valleys and river drainages forming potential barriers to gene flow for species restricted to high elevations (Magri *et al.* 2006; Schoville *et al.* 2012). The scope of these barriers has changed over time. For example, recent warming and consequent upslope distribution shifts have driven fragmentation of previously continuous alpine populations (Rubidge *et al.* 2012). In contrast, widespread cooling during the Pleistocene glacial periods (0.7 – 0.01 Ma) is thought to have created corridors of suitable habitat between currently isolated mountain ranges (DeChaine & Martin 2004; Galbreath *et al.* 2009). Such large-scale climatic fluctuations have left signatures in the genetic structure of high-elevation taxa worldwide (Knowles 2000; DeChaine & Martin 2004; DeChaine & Martin 2005; Schönswetter *et al.* 2005; Schoville *et al.* 2012).

During Pleistocene climate cycles, the creation of habitat corridors and tracking of cooler climates to low elevations would have reduced the effective distance between populations and enabled migration of alpine species between previously-isolated mountains (Knowles 2001; VanDyke *et al.* 2004). Alternatively, glaciation might have driven population contraction to lowland refugia, driving deep lineage divergence across different mountain ranges (e.g. Schmitt 2007; Schoville & Roderick 2009; Schoville & Roderick 2010; Schoville *et al.* 2012). Different patterns are evident across mountain systems that vary in their continuity, extent and ecological characteristics of (e.g. dispersal ability) of the focal species. These patterns have most frequently been explored in mountain systems of North America and Europe, which extend across hundreds of kilometres. In Australia, high-elevation areas are highly fragmented but distances between distinct mountain regions are much smaller – usually less than 50 km (Fig. 6.1). Alpine and sub-alpine habitats are confined to a relatively small (5200 km^2) area of southeastern Australia, with most of this area within the States of Victoria and New South Wales ('Kosciuszko region') (Fig. 6.1A) (Costin 1989). During the Pleistocene, alpine/sub-alpine conditions would have extended to lower elevations, connecting some, if not all, of these regions (Frakes *et al.* 1987; Mark *et al.* 2005). At the same time, lack of large-scale glacial activity may have allowed persistence of populations in high-elevation areas (Frakes *et al.* 1987). In Victoria (see Fig. 6.1), high endemism and strong divergence among arthropod populations across mountain summits points to a stable system in which populations have persisted on mountain summits through past

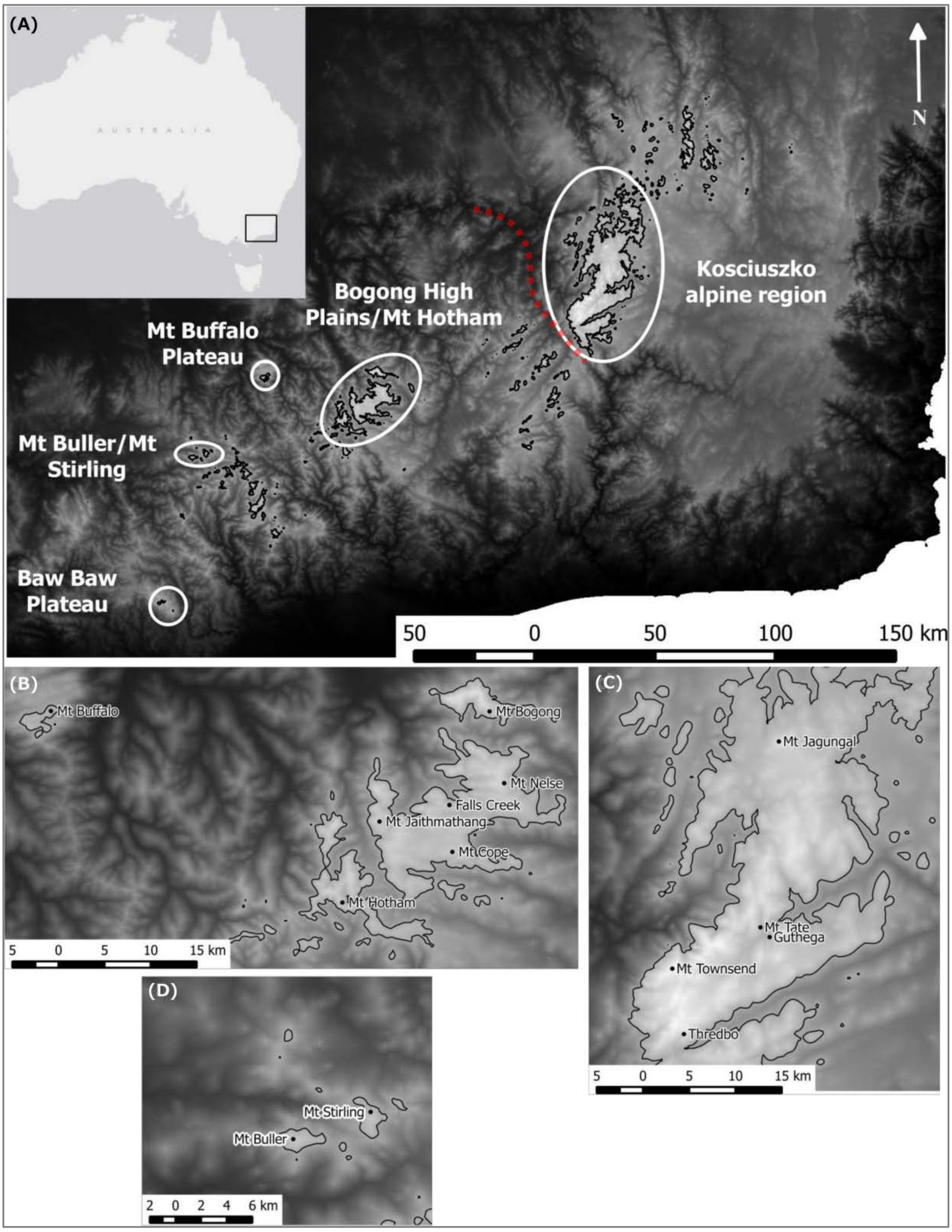

Figure 6.1. The Australian alpine region, showing major mountain areas (A) and sampling locations in the Bogong High Plains, Dargo High Plains and Mt Buffalo Plateau (B), the Kosciuszko region (C), and the Mt Buller/Mt Stirling region (D) (see Table 6.1 for coordinates). Samples were also collected from the Baw Baw Plateau (A). Black lines indicate a 1500 m contour, the approximate low-elevation distribution limit of *Kosciuscola tristis* through most of its range. Shading represents elevation from 0 m a.s.l (black) to 2250 m (white). The red dotted line in (A) indicates the approximate position of the Murray River, where it divides the Kosciuszko region in New South Wales from the Bogong High Plains area in Victoria. The river also marks the State border, between New South Wales (to the north-east) and Victoria (to the south-west).

climate cycles (Endo *et al.* 2014). Nevertheless, the limited geographic scope of genetic studies in the Australian alps area to-date (see Endo *et al.* 2014; Griffin & Hoffmann 2014 for recent exceptions) – a consequence of the highly restricted distributions of most Australian alpine taxa – means that patterns of genetic structure across the remainder of the high country remain poorly understood.

Several features of the Victorian and Kosciuszko mountain regions suggest that patterns of historic and contemporary population structure are likely to differ between them. First, while alpine and subalpine habitat in Victoria is highly fragmented, currently separated into four main fragments by low-elevation agricultural land, the Kosciuszko region comprises largely contiguous alpine/sub-alpine habitat contained within reserved land. Although the Kosciuszko region has few isolated peaks, Mt Jagungal (Fig. 6.1D), which marks the northernmost point of this region, is separated from the main range by approximately 25 km of sub-alpine plains (~ 1500 m elevation). Second, the highest areas of the Kosciuszko region experienced some glaciation during Pleistocene climate cycles – the only area of the Australian mainland to do so (Barrows *et al.* 2001, 2002). A recent study of wind-dispersing *Poa* grasses found no genetic structure within the Kosciuszko region, with significant divergence only apparent among isolated mountains in southern Victoria (Griffin & Hoffmann 2014). However, species with low dispersal ability are likely to show greater structure (e.g. Schoville & Roderick 2009; Schoville *et al.* 2012).

The most extensive areas of alpine and sub-alpine habitat in Australia, lying within the Kosciuszko region in New South Wales and the Bogong High Plains in Victoria, are separated by approximately 100 km of lowland across the Murray River valley, which also marks the State border (Fig. 6.1A). This geographic discontinuity represents a significant divergence point for several alpine reptile and mammalian species (Chapple *et al.* 2005; Mitrovski *et al.* 2007; Koumoundouros *et al.* 2009). For example, the skink *Egernia guthega*, an alpine endemic, shows an average 2.2% mitochondrial (*ND4)* sequence divergence between a population from Kosciuszko and three populations from the Bogong High Plains (Chapple *et al.* 2005). However, no genetic structure across this valley was detected among alpine *Poa* (Griffin & Hoffmann 2014). Thus, with the exception of *Poa*, the restricted distributions of species studied to-date provide no means to assess the context of the Kosciuszko-Bogong High Plains break within the broader alps area.

In this study, we examine the importance of broad-scale geographic discontinuities in shaping patterns of genetic structure in the Australian high country. In particular, we explore the following questions: (1) at the relatively small spatial scale of Australia's high country, what is the extent of lineage divergence among and within regions and does the timing of divergence relate to Pleistocene glacial cycles? (2) Are differences in geographic structure and historic climate between the Victorian and Kosciuszko mountains regions reflected in lower historic and contemporary genetic structure in the latter? (3) To what extent does the gap between Kosciuszko and the Bogong High Plains represent a primary divergence point? We investigate these questions using phylogenetic and population genetic frameworks, with markers of different temporal resolutions to capture signatures of both current range fragmentation and potential distribution shifts in the past.

The grasshopper *Kosciuscola tristis* (Sjösted) was identified as a suitable species in which to explore these genetic patterns. Although restricted to Australia's alpine and sub-alpine habitat, primarily above 1500 m, it is abundant and its distribution stretches from Mt Baw Baw in the south to Mt Jagungal in the north (~ 300 km). This gives it one of the largest ranges of any mountain-endemic animal in Australia, and makes it an ideal model for testing broad-scale phylogeographic patterns in this system. Like many alpine insects, *K. tristis* is flightless and, consequently, is likely to have limited dispersal abilities over the distances separating major mountain regions. Two subspecies are described (Rehn 1957), with *K. tristis tristis* occurring in the Kosciuszko region and *K. tristis restrictus* on the Mt Buffalo plateau (Fig. 6.1). Rehn (1957) also describes Victorian populations from Mt Hotham and the Bogong High Plains as morphologically intermediate between the two proposed subspecific forms. We thus predicted (a) genetic divergence across the Murray River valley, and (b) that *K. tristis* from Mt Buffalo would be genetically distinct from other populations of *K. tristis*. Some study has been made of the genetic structure of this species (Tatarnic *et al.* 2013; Endo *et al.* 2014), providing a basis from which to extend the research to cover a greater geographic extent.

6.2 Methods

6.2.1 Study species and sampling

Kosciuscola tristis Sjösted (1933) (Orthoptera: Acrididae) is a small (15 – 30 mm) grasshopper, endemic to Australia's alpine region (Green 2002). The species has a patchy distribution across small geographic scales, but population density can be very high during the peak adult activity period between February and April. The species is univoltine with discrete generations, all adults dying by late-May and eggs hatching in early summer. Of the four grasshopper species endemic to the sub-alpine and alpine regions of the Australian mainland, *K. tristis* has the narrowest altitudinal range. It is found between 1500 m and 2200 m with a known distribution spanning five distinct mountain areas across two States: New South Wales (Kosciuszko alpine region) and Victoria (Bogong High Plains, Mt Buffalo plateau, Mt Buller/Mt Stirling and the Baw Baw plateau) (Fig. 6.1).

Between January and May of 2012 and 2013, a total of 396 adult *K. tristis* were collected by hand from 15 mountains spanning *K. tristis'* full known geographic distribution (Fig. 6.1, Table 6.1). Some mountains were separated by low-elevation areas, while others represent peaks within continuous alpine/sub-alpine habitat. The geographic distance between sampled locations ranged from 1.7 km to 284 km, but adjacent mountains were always less than 100 km apart. One hind leg was removed from each individual in the field and stored in 100% ethanol until DNA extraction.

6.2.2 Phylogeny reconstruction: DNA extraction, amplification and sequencing

Total genomic DNA was extracted from muscle tissue from the femur. Tissue was placed in a microcentrifuge tube and crushed with a glass bead and mixer mill at 20,000 Hz for 2 min. Samples were centrifuged (30 sec at 13,000 rpm) after which 150 µL of 5 % Chelex-100 resin® (BioRad, Hercules, USA) and 3 µL proteinase K (10mg/mL) (Roche, Basel, Switzerland) were added. Samples were incubated at 56°C for 3 hours, followed by 95°C for 10 min (Walsh *et al.* 1991).

Phylogenetic analyses were performed using DNA sequence data from fragments of the mitochondrial cytochrome oxidase subunit I (*CO1*) gene and the nuclear internal transcribed spacer 1 (*ITS1*) region for five individuals per collection site (total 75 individuals). Polymerase Chain Reactions (PCRs) were used to amplify a 801 base-pair (bp) fragment of the *CO1* gene using primer pairs C1-J-2183 and TL2-N-3014 (Simon *et al.* 1994), and a 573 bp fragment of the *ITS1* gene using primer pairs CAS18sF1 and CAS5p8sB1d (Ji *et al.* 2003). PCR was carried out under the following reaction conditions: *CO1*: 94°C for 4 min, 35 cycles of 94°C for 30 sec, 45°C for 30 sec, 72°C for 30 sec, then 72°C for 5 min; *ITS1*: 94°C for 4 min, 35 cycles of 94°C for 30 sec, 64°C for 40 sec, 72°C for 30 sec, then 72°C for 5 min. PCRs were performed in 25 µL (see Appendix E, Table E.1 for reaction concentrations) and products were sequenced on an ABI 3730 DNA analyser (Macrogen Inc, Korea). *Kosciuscola cognatus,* a congener occupying the sub-alpine zone, was sequenced as an outgroup taxon, using identical PCR conditions.

6.2.3 Phylogeny reconstruction: sequence analysis and divergence time estimation

DNA sequences were aligned using MUSCLE (Edgar 2004) with default settings and refined manually in GENEIOUS 6.1.7 (Biomatters). *CO1* sequences were translated into amino acid sequences and checked for internal stop codons to determine product authenticity and the correct reading frame. Unique haplotypes in each dataset were identified and used for subsequent phylogenetic analyses [Genbank: KJ870103-KJ870137 & KJ870139-KJ870149] (Appendix E, Table E.2). We used two approaches to test phylogeographic structure.

First, we used maximum likelihood (ML) to estimate phylogenetic relationships of *CO1* haplotypes. The data were partitioned by codon position and RAxML 7.4.2 (Stamatakis 2006) was used to estimate a maximum likelihood tree using the GTRGAMMA model and a rapid bootstrapping analysis (Stamatakis 2014) with 1000 iterations. RAxML was implemented in RAXMLGUI 1.3 (Silvestro & Michalak 2012). Two sequences from the congener *K. cognatus* were used to root the tree [Genbank: KJ870138, KM407143].

Second, as there was low variation in the *ITS1* sequences (12 haplotypes), we generated a phylogenetic network using a median-joining approach (Bandelt *et al.* 1999) implemented in NETWORK 4.6.1.1 (Fluxus Engineering), with standard settings. *Kosciuscola cognatus* was again included as an outgroup [Genbank: KJ870150].

We used the mitochondrial *CO1* sequences to estimate divergence time among clades. In particular, we were interested in the timing of divergence among Victorian and Kosciuszko populations. No fossil or geological evidence was available for node calibration. Instead, we estimated divergence times using two divergence rates: 3.5 %/Myr, based on a recent insect molecular clock estimate (Papadopoulou *et al.* 2010) and 2.3 %/Myr, which has been used for grasshoppers in the past (Knowles 2000). Although it is unrealistic to assume a fixed substitution rate across taxa and lineages (Thomas *et al.* 2006), we proceeded with this method to allow comparisons with other studies and to place population divergence in a rough time period. Sequence divergence was measured as uncorrected p-distance, calculated with the APE package (Paradis *et al.* 2004) in R 3.1.0 (R Core Team 2014).

Finally, we calculated average nucleotide diversity (π) for each collection site using DNASP v5.10.1 (Librado & Rozas 2009), and mean p-distance between haplotypes in Victoria and in the Kosciuszko region, to compare levels of diversity among these areas. Diversity statistics were calculated only for the *CO1* data, as *ITS1* sequences showed extremely low diversity.

Table 6.1. Sampling locations and sample sizes for *CO1, ITS1* and microsatellite analyses.

Location	Latitude (ºN)	Longitude (ºE)	Elevation (m a.s.l)	*CO1/ITS1* sample size	Microsatellites sample size
Kosciuszko region					
Mt Jagungal	-36.1481	148.3875	2054	5	31
Mt Tate	-36.3726	148.3652	1671	5	29
Guthega	-36.3843	148.3764	1698	5	30
Mt Townsend	-36.4225	148.2588	2205	5	0
Thredbo 1	-36.5015	148.2728	1901	5	29
Thredbo 2	-36.5093	148.2694	1781	0	30
Thredbo 3	-36.5173	148.2647	1681	0	30
Victoria					
Mt Bogong	-36.7559	147.3266	1791	5	30
Mt Nelse	-36.8434	147.3445	1805	5	0
Falls Creek	-36.8700	147.2777	1744	5	30
Mt Cope	-36.9272	147.2812	1781	5	29
Mt Jaithmathang	-36.8903	147.1923	1844	5	0
Mt Hotham	-36.9890	147.1470	1782	5	0
Mt Buffalo	-36.7553	146.7914	1536	5	29
Mt Buller	-37.1455	146.4308	1774	5	30
Mt Stirling	-37.1216	146.4989	1627	5	30
Mt Baw Baw	-37.8335	146.3081	1476	5	29

6.2.4 Population genetic structure: DNA extraction, amplification and genotyping

Genomic DNA was extracted from femur tissue, which was placed in a 96-well plate with 3 µL proteinase K and 150 µL of 5% Chelex. The plate was then incubated at 56°C for 16 h (Casquet *et al.* 2012). Population genetic analyses were performed using 29 – 31 individuals from 13 collection sites (Table 6.1), three of which were from a single elevation gradient (at elevations of 1901, 1781, and 1681 m a.s.l) near Thredbo, NSW (Thredbo 1, 2, and 3 respectively). Eight microsatellite loci were amplified across two multiplexes, using primers developed by Umbers *et al.* (2012) for *K. tristis* (Multiplex 1: loci *Ktr29*, *Ktr73*, *Ktr76* and *Ktr82*; Multiplex 2: loci *Ktr30*, *Ktr58*, *Ktr60* and *Ktr88*). Microsatellites were designed and tested with *K. tristis* from the Thredbo 2 population and details of the design methodology are given in Umbers *et al.* (2012). PCRs were performed in 11 µL volumes containing 5 µL QIAGEN Multiplex PCR Master Mix (QIAGEN Inc., Valencia, CA, U.S.A), 0.1 µM forward primer, 0.2 µM reverse primer, 0.1 µM fluorescent tags (FAM, VIC, NED, PET), and approximately 7 ng of genomic DNA (Blacket *et al.* 2012). PCR cycling was performed under the following conditions: 95°C for 15 minutes, 40 cycles of 94°C for 30 s, 59°C for 90 s, and 72°C for 60 s; followed by 30 min at 60°C. PCR products were size-separated on an AB3730 DNA analyser, standardised against a GeneScan™ Liz®500 size standard (Life Technologies, CA, USA) at the Australian Genome Research Facility, Melbourne. Fragment sizes were scored manually using GENEIOUS. The loci *Ktr73, Ktr82* and *Ktr60* had high failure rates (> 60 %) for all Victorian populations. Subsequent analyses were therefore run with two datasets (unless indicated otherwise): (1) all populations with the five remaining loci (five-locus dataset), and (2) the six populations from the Kosciuszko region (Kosciuszko dataset).

6.2.5 *Population genetic structure: diversity and differentiation*

Prior to analysis, individual microsatellite loci were checked for linkage disequilibrium, departure from Hardy-Weinberg equilibrium (HWE) and null alleles. The data were initially screened for anomalies using MICROCHECKER (Van Oosterhout *et al.* 2004). GENEPOP 4.2.2 (Rousset 2008) was used to test for linkage disequilibrium (LD) among loci in each population, using default parameters, and to estimate deviations from HWE. Significance values were adjusted for multiple comparisons with False Discovery Rates (Benjamini & Hochberg 1995), implemented in R. The frequency of null alleles at each locus was estimated using FREENA (Chapuis & Estoup 2007) and diversity statistics, including allelic richness and estimates of observed (H_O) and expected (H_E) heterozygosity, were calculated with GENALEX 6.5b3 (Peakall & Smouse 2006, 2012).

We used three methods to examine population structure. First, we used FreeNA to calculate global and pairwise F_{ST} values, correcting for the presence of null alleles with the ENA method (Chapuis & Estoup 2007). We also computed the standardised statistic F'_{ST} in GENODIVE 2.0b23 (Meirmans & Van Tienderen 2004). F_{ST} values are influenced by the amount of genetic variation within populations, with the maximum attainable value of F_{ST} decreasing as heterozygosity increases (Hedrick 2005). Thus, differentiation among populations is often underestimated, particularly for highly variable molecular markers such as microsatellites, and several authors have recommended calculation of standardised statistics, such as F'_{ST}, as a more appropriate measure of genetic differentiation (Jost 2008; Heller & Siegismund 2009; Meirmans & Hedrick 2011). Both statistics were calculated for consistency and comparability with other studies.

Second, we used a discriminant analysis of principal components (DAPC; Jombart *et al.* 2010) to investigate the relationship among geographic regions. This approach does not have underlying assumptions of HWE and was thus appropriate for our dataset (see Results). The method attempts to optimise between-cluster variation whilst minimising variation within clusters, thus producing a clearer distinction of clusters (Jombart *et al.* 2010). As populations were spatially discrete, DAPC was run using *a priori* clusters (Jombart *et al.* 2010): (a) the southern Victorian mountains (Mt Baw Baw, Mt Buller, Mt Stirling), (b) the Bogong High Plains and Mt Buffalo, and (c) Kosciuszko populations. DAPC was run in the ADEGENET 1.4-1 package (Jombart *et al.* 2010) in R, with the first 50 principal components retained (capturing 80% of variation), along with all the discriminant functions. As we were interested in genetic relationships among regions, this analysis was only run using the five-locus dataset.

Third, we examined population structure within the Kosciuszko region and across the study area using a Bayesian clustering approach, implemented in TESS 2.3 (Chen *et al.* 2007; Durand *et al.* 2009). TESS incorporates individual geographic coordinates as *a priori* information and can thus incorporate spatial trends and spatial autocorrelation in estimates of individual ancestry (Durand *et al.* 2009). As TESS only accepts individual coordinates, population coordinates were permuted with a standard deviation of 0.005°. We used the conditional autoregressive (CAR) admixture model, with a burn-in of 50,000 steps followed by 100,000 MCMC steps. The admixture parameter (a) and interaction parameter (ϱ) were initially set to $a = 1$ and $\varrho = 0.6$, then automatically updated. We searched for the optimal number of clusters (K) using 10 runs at $K = 2$ to $K = 10$. The most likely number of clusters was selected from the lowest average Deviance Information Criterion (DIC), averaged over these 10 runs. We then did 100 additional runs at the optimal value of K and the 20 runs with the lowest DIC were averaged using CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) with the 'greedy' algorithm and default settings. The level of admixture in each population was displayed graphically with DISTRUCT 1.1 (Rosenberg 2004).

6.2.6 Spatial patterns of genetic divergence

We examined spatial patterns of divergence using both the *CO1* and microsatellite data. First, we tested for a relationship between genetic distance (p-distances for *CO1* data and F'_{ST} for microsatellites) and log-transformed geographic distance (isolation-by-distance) (Wright 1943). For both mtDNA and microsatellites, we tested for isolation-by-distance (IBD) across all populations, populations from Victorian only, and populations from NSW only. Mantel tests were run using the VEGAN package (Oksanen *et al.* 2013) in R, with 999 permutations to test statistical significance.

6.3 Results

6.3.1 Phylogeographic structure and genetic diversity

In total, 75 individuals were sequenced, representing 35 unique *CO1* haplotypes and 12 unique *ITS1* haplotypes. Of these, 12 *CO1* and 3 *ITS1* haplotypes were represented in the Kosciuszko region, with the remainder from populations in Victoria. Mean *CO1* sequence divergence between haplotypes in Victoria was six times that of the Kosciuszko region (Victoria: mean = 2.4 ± 1.1%; Kosciuszko: 0.4 ± 0.2%), and within-population diversity was also greater in Victoria (Victoria: $\bar{\pi} = 0.006 \pm 0.013$; Kosciuszko: $\bar{\pi} = 0.001 \pm 0.001$).

The maximum likelihood *CO1* phylogeny indicated strong statistical support for a southern Victorian clade incorporating populations from Mt Baw Baw, Mt Buller and Mt Stirling. Within this group, further geographic structure was apparent with good support for the monophyly of haplotypes from Mt Baw Baw and from Mt Buller (Fig. 6.2). We found no statistical support for phylogeographic structure across the remaining mountains, although Mt Buffalo (representing the subspecies *K. tristis restrictus*) did cluster. Mt Hotham showed a particularly interesting pattern, with haplotypes falling out in several places on the phylogeny and two individuals – potentially hybrids – showing strong divergence from the other haplotypes. The *ITS1* data, however, placed Mt Hotham with the southern Victorian mountains (Mts Stirling, Buller and Baw Baw). In contrast to the *CO1* phylogeny, *ITS1* sequences showed divergence between the Kosciuszko region and the Bogong High Plains (separated by the Murray River valley), with populations from each region represented by one primary haplotype (Fig. 6.3).

There was an average 2.9% (range 1.9 – 4.1%) sequence divergence between *CO1* haplotypes from the southern Victorian mountains and those from the Bogong High Plains and Kosciuszko. This corresponds to divergence 0.8 to 1.3 Ma (range 0.5 – 1.8 Ma) using a divergence rate of 2.3% or 3.5% Myr^{-1} respectively. Within these southern mountains, divergence of the Mt Baw Baw clade was estimated at 0.4 to 0.7 Ma (range 0.2 – 0.8 Ma) based on a 1.5% (range 0.8 – 1.9%) mean sequence divergence, and the Mt Buller clade at 0.1 Ma (range 0.1 – 0.2 Ma) with 0.3% (range 0.3 – 0.4%) sequence divergence. We were unable to give an estimated divergence time between populations from the Bogong High Plains and Kosciuszko as there was no phylogenetic split based on *CO1* data.

6.3.2 *Microsatellite data: null alleles and diversity*

Due to poor amplification of some loci in Victoria (see Methods) we split the data into two. The first dataset consisted of all 13 populations sampled (388 individuals) with five microsatellite loci (five-locus dataset) and the second included the six populations from the Kosciuszko region (177 individuals) and eight loci (Kosciuszko dataset).

The two datasets contained 20% and 14% missing data, respectively. After correcting for multiple comparisons, only one pair of loci at one site showed significant linkage disequilibrium. All loci deviated from Hardy-Weinberg Equilibrium (HWE) in at least one of the sampled sites, with locus *Ktr88* showing the highest level of deviation, departing from HWE at all sites. The other loci that deviated from HWE did so in four to seven of the seven Victorian populations and in one to six of the six Kosciuszko populations. MICROCHECKER (Van Oosterhout *et al.* 2004) indicated the presence of null alleles at four loci: *Ktr30, Ktr58, Ktr60* and *Ktr88*. Estimated null allele frequencies for these loci were between 0.12 (*Ktr58*) and 0.3 (*Ktr88*) (Tables E.3, E.4).

The five-locus dataset of 13 populations harboured 136 alleles across the five loci (range 21 to 38 per locus), while 147 alleles were detected across the eight loci in the Kosciuszko dataset (6 – 38 alleles/locus). Although observed heterozygosity was generally low (all $H_O < 0.6$), likely due to the presence of null alleles, genetic diversity measured by expected heterozygosity was high (mean $H_E = 0.82$) and varied little across sites (with the exception of the Baw Baw population, with $H_E = 0.59$) (Table 6.2).

Table 6.2. Genetic variation in markers used for phylogeny reconstruction (*CO1* and *ITS1*) and population genetic analyses (microsatellites). Nucleotide diversity (π) was not calculated for *ITS1* as variation was very low. Observed heterozygosity (H_o), expected heterozygosity (H_E) and mean allelic richness (N_a) were calculated for five microsatellite loci that amplified across all populations, as well as for eight loci typed for the Kosciuszko populations.

	CO1		***ITS1***	**Microsatellites (five shared loci)**			**Microsatellites (all typed loci)**		
Location	**Nucleotide diversity (π)**	**Number of haplotypes**	**Number of haplotypes**	H_O	H_E	N_a	H_O	H_E	N_a
Kosciuszko region									
Mt Jagungal	0.001	2	1	0.61	0.83	11.80	0.56	0.83	11.63
Mt Tate	0.002	4	1	0.51	0.81	12.40	0.51	0.82	11.88
Guthega	0.002	4	3	0.50	0.81	13.60	0.48	0.82	12.00
Mt Townsend	0.001	3	1						
Thredbo 1	0.001	2	1	0.56	0.82	12.40	0.53	0.79	10.25
Thredbo 2				0.51	0.83	13.20	0.48	0.80	11.25
Thredbo 3				0.57	0.81	13.80	0.50	0.77	11.25

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Table 6.2. Continued from previous page.

	CO1		***ITS1***	**Microsatellites (five shared loci)**			**Microsatellites (all typed loci)**		
Location	**Nucleotide diversity (π)**	**Number of haplotypes**	**Number of haplotypes**	H_O	H_E	N_a	H_O	H_E	N_a
Victoria									
Mt Bogong	0.008	2	1	0.46	0.88	13.40			
Mt Nelse	0.001	4	2						
Falls Creek	0.000	1	1	0.51	0.90	14.20			
Mt Cope	0.000	1	1	0.38	0.87	12.20			
Mt Jaithmathang	0.002	2	1						
Mt Hotham	0.041	5	2						
Mt Buffalo	0.002	5	1	0.26	0.80	9.80			
Mt Buller	0.001	2	1	0.49	0.78	8.80			
Mt Stirling	0.000	1	1	0.33	0.69	10.00			
Mt Baw Baw	0.002	3	5	0.27	0.59	7.40			

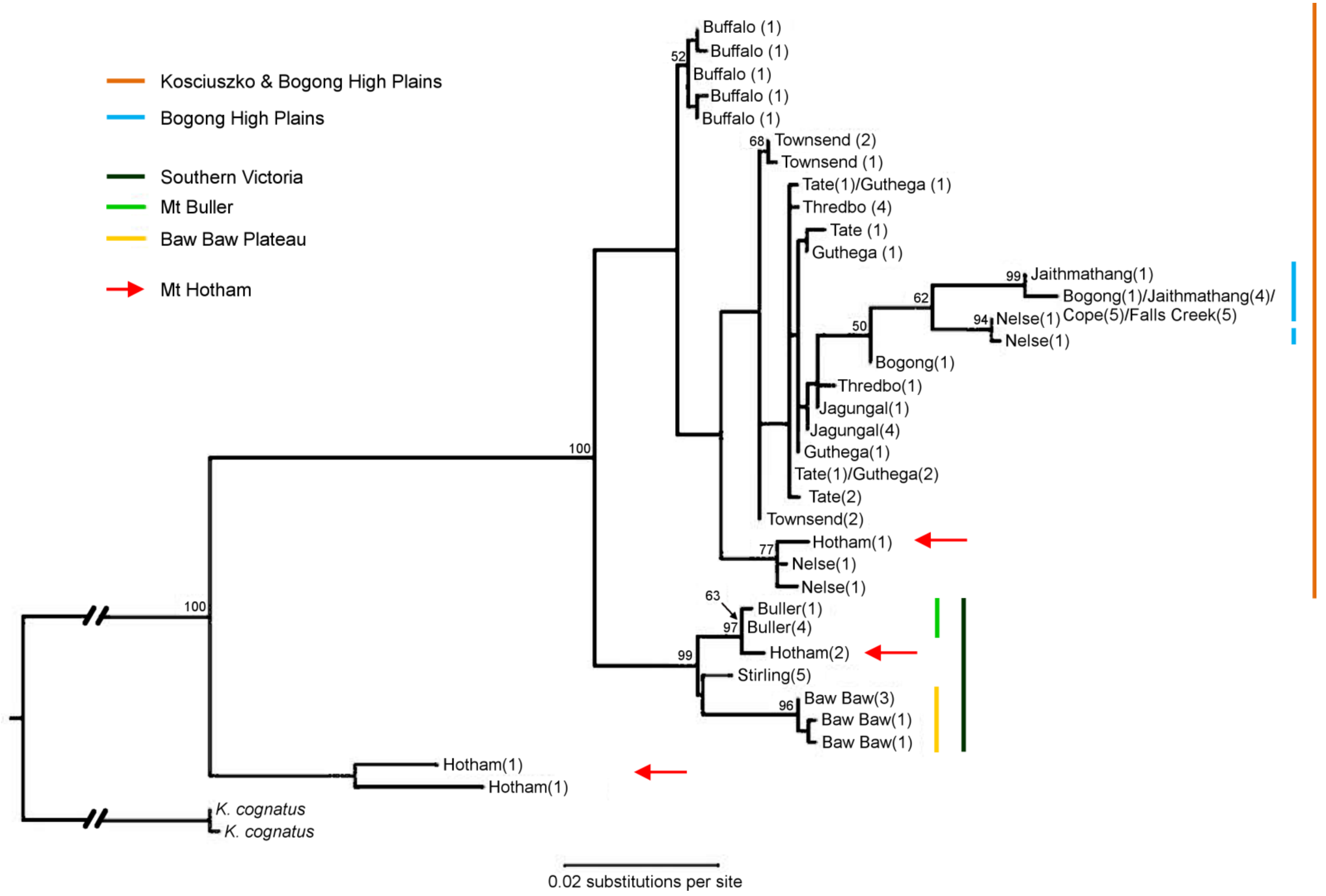

Figure 6.2. Maximum likelihood *CO1* phylogeny for *Kosciuscola tristis*, with the congener *Kosciuscola cognatus* as an outgroup. Populations represented by each haplotype are given in the text next to each tip, with the number of individuals in brackets; coloured bars show phylogeographic clades or (orange bar) geographic affinities, though it should be noted that haplotypes from Mt Hotham (red arrow) occur throughout the phylogeny; bootstrap support values are indicated above the nodes where support was greater than 50%.

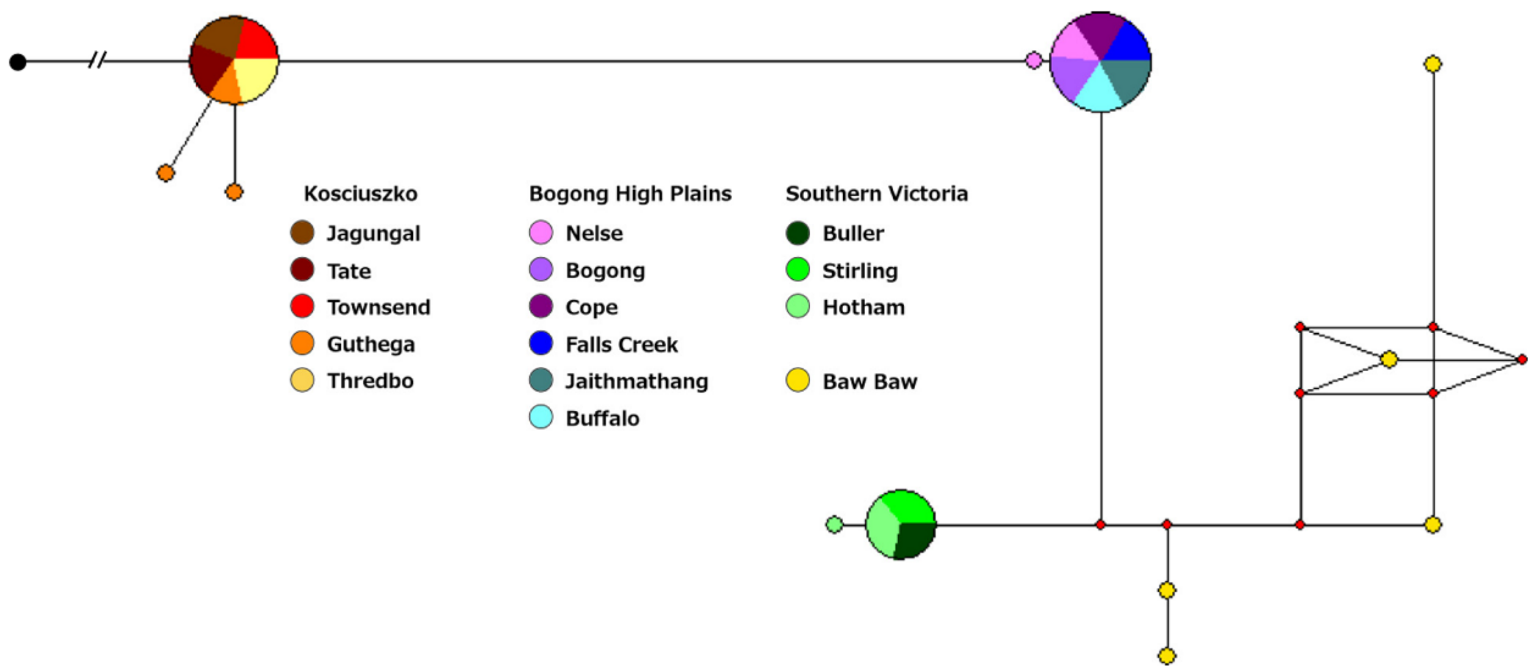

Figure 6.3. Median-joining haplotype network of 12 unique haplotypes from 75 *Kosciuscola tristis ITS1* sequences, rooted with *K. cognatus* (black circle). Circles represent haplotypes, with size proportional to haplotype frequencies; for haplotypes common to multiple populations, slices represent the frequencies of each population; colours indicate the population. Branches are proportional to the number of mutations between them and red circles are median vectors.

6.3.3 Population genetic structure

Deviations from HWE and the presence of null alleles means that estimates of F-statistics are problematic and should be treated cautiously. Nevertheless, we present F_{ST} values, corrected for null alleles, and F'_{ST} values as measures of divergence for comparability. The patterns of structure presented below, detected using F_{ST}, principal components analysis and Bayesian clustering analyses, are concordant.

There was significant pairwise differentiation between 73 of 78 site pairs in the five-locus dataset, and between 13 of 14 pairs in the Kosciuszko dataset, after a correction for null alleles using the ENA method (see Methods for details), although F_{ST} values were generally low (Tables E.5, E.6). The exceptions were Falls Creek – Mt Cope ($F_{ST} = 0.002$, $F'_{ST} = 0$), Mt Buller – Mt Stirling ($F_{ST} = 0.010$, $F'_{ST} = 0.112$), Thredbo 2 – Mt Tate ($F_{ST} = 0.014$, $F'_{ST} = 0.189$), Thredbo 1 – Thredbo 2 ($F_{ST} = 0.002$, $F'_{ST} = 0.017$) and Thredbo 2 – Thredbo 3 ($F_{ST} = 0.013$, $F'_{ST} = 0.057$). The greatest differentiation was observed between populations from the Kosciuszko region and those from Victoria (mean $F_{ST} = 0.100$).

These broad-scale patterns were supported by the discriminant analysis of principal components, which indicated clear differentiation of geographic groups (Fig. 6.4). In particular, the Kosciuszko region represented a robust cluster with 93% of individuals correctly assigned to the region, compared to 83% and 84% for the southern Victorian and Bogong High Plains clusters, respectively.

Population structure was also assessed with the Bayesian assignment method implemented in TESS, which supported five clusters in the five-locus dataset (all populations) and six clusters in the Kosciuszko dataset (Fig. 6.5). Strong regional divergence was again evident: three clusters were almost exclusively assigned to individuals from Victoria (< 4% in the Kosciuszko region), while the remaining clusters were largely exclusive to the Kosciuszko region. The Bogong High Plains region showed equivalent admixture proportions from clusters associated with southern Victoria and the Kosciuszko region (10 – 18% admixture), in addition to a distinct local cluster representing an average 57% of individual admixture proportions. In Victoria, Mt Buffalo and Mt Baw Baw were characterised by very high single-cluster assignment (97% and 89% respectively). Similarly, in the Kosciuszko region, the isolated peak of Mt Jagungal showed high single-cluster assignment (average 93%).

6.3.4 *Spatial patterns of genetic divergence*

We tested for isolation-by-distance (IBD) in both the microsatellite and *CO1* datasets, and examined this pattern in the Victorian and Kosciuszko regions alone, as well as across all populations. There was significant isolation-by-distance in both the microsatellite (all populations (five-locus): $r^2 = 0.640$, p = 0.001; Victoria (five-locus): $r^2 = 0.395$, $p = 0.020$; Kosciuszko: $r^2 = 0.666$, p = 0.001) and *CO1* (all populations: $r^2 = 0.213$, $p = 0.003$; Victoria: $r^2 = 0.304$, $p = 0.005$), datasets. Within the Kosciuszko region, however, *CO1* sequence divergence showed no relationship with geographic distance ($r^2 = 0.009$, $p = 0.367$).

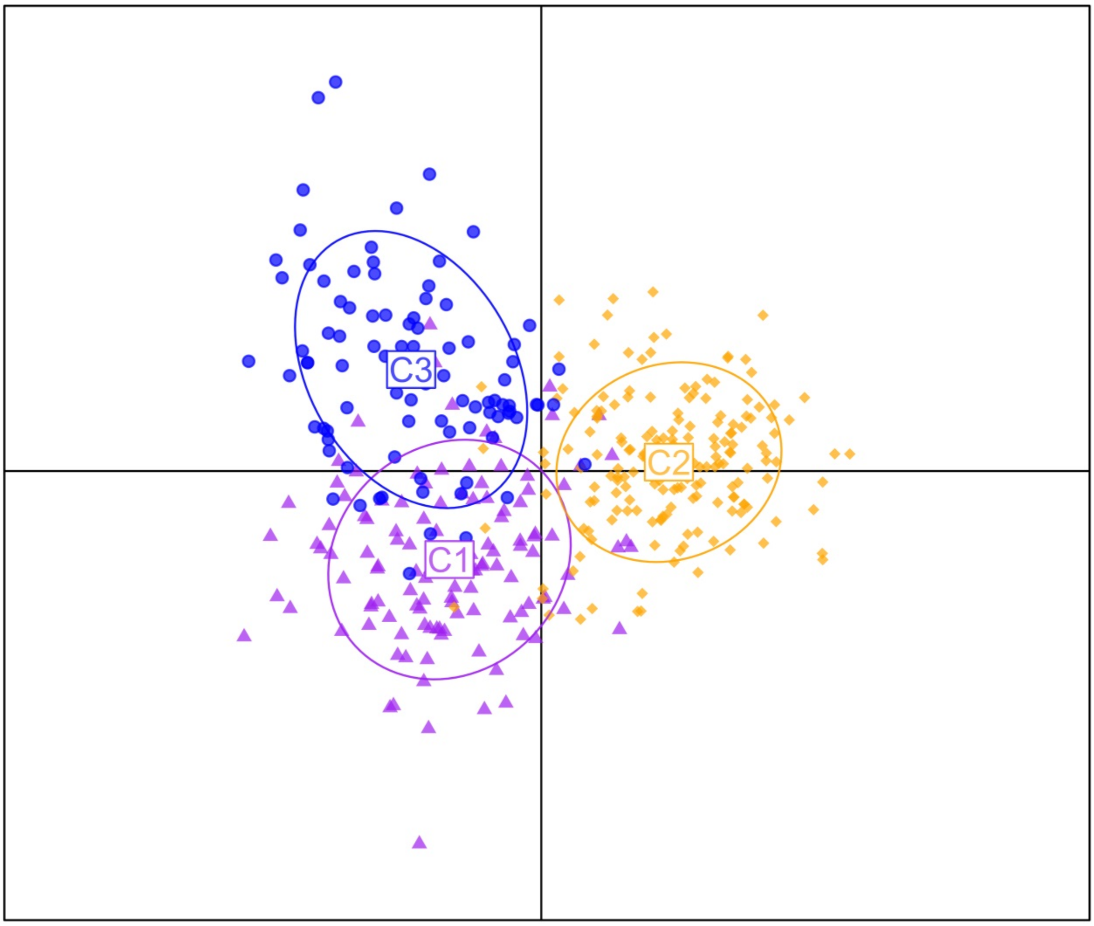

Figure 6.4. Results of a discriminant analysis of principal components based on five microsatellite loci, showing relationships among geographic population clusters. C1 = Bogong High Plains/Mt Buffalo, C2 = Kosciuszko region, C3 = Southern mountains (Mt Baw Baw, Mt Buller, Mt Stirling). Inertia ellipses are a graphical summary of points within each cluster, with the centroid representing the mean coordinates, width equal to the variances and slope equal to the covariance.

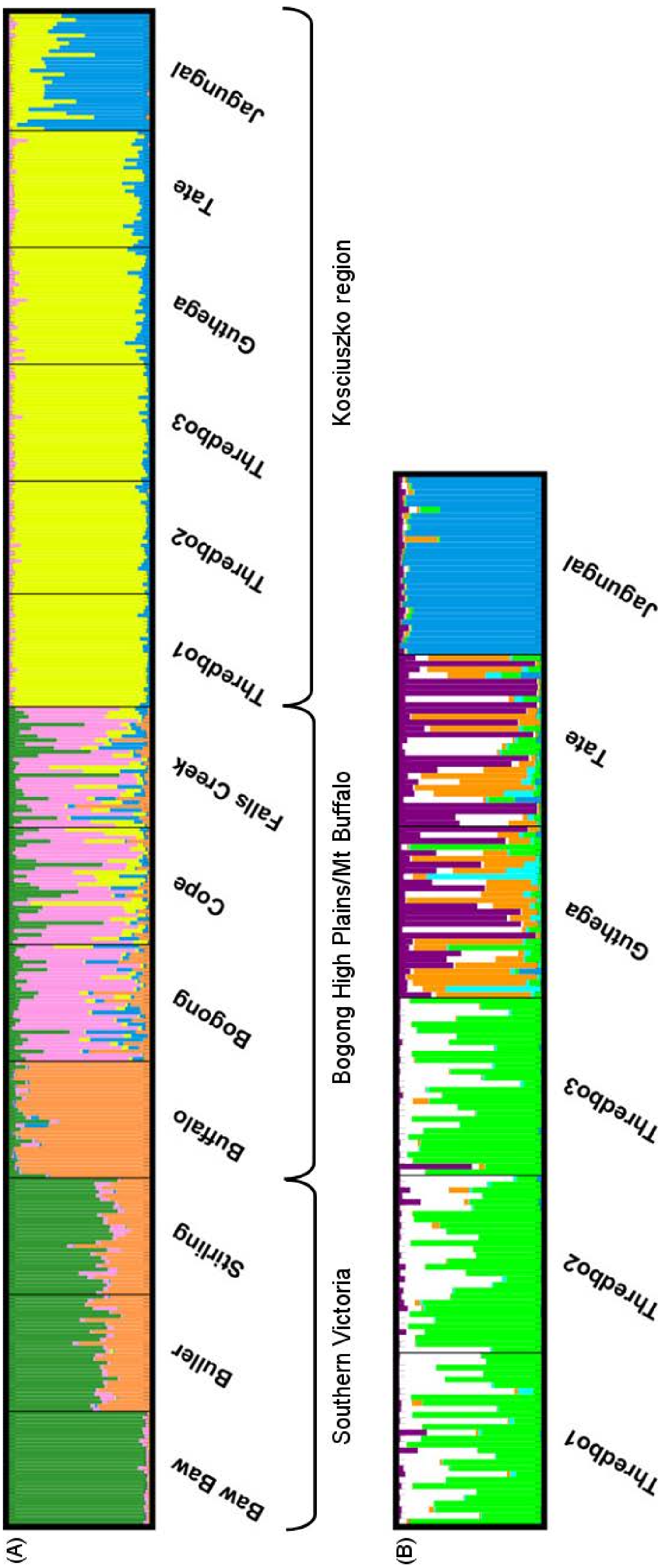

Figure 6.5. Posterior estimates of individual admixture proportions for (A) all populations (five-locus dataset) (K_{max} = 5) and (B) populations from the Kosciuszko region (K_{max} = 6). Admixture proportions represent the estimated proportion of an individual's genome originating in each cluster and different clusters are represented by different colours.

6.4 Discussion

The topographic complexity of mountain landscapes, coupled with large-scale climatic fluctuations, has shaped the evolution and population structure of high-elevation species worldwide (Hewitt 1999; Knowles 2000; Knowles 2001; Schoville & Roderick 2009; Schoville & Roderick 2010; Schoville *et al.* 2012). In the comparatively small (both in area and altitudinal range) and geologically stable Australian high country, we found genetic divergence associated with discontinuities in alpine and subalpine habitats. Four primary genetic groups were identified across the Kosciuszko region of New South Wales and mountain areas of Victoria, with the greatest structure evident in the latter. Here we discuss population structure within Kosciuszko and Victoria, genetic structure across major geographic breaks and the extent to which patterns in Australia parallel (and differ from) those in larger mountain systems.

6.4.1 Within-region structure

The Kosciuszko alpine region contains Australia's highest mountains and the most extensive area of alpine and subalpine habitat. Despite this, previous work in a variety of taxa (e.g. skink: Chapple *et al.* 2005; mammal: Mitrovski *et al.* 2007; insect: Tatarnic *et al.* 2013; grass: Griffin & Hoffmann 2014) has shown low genetic structure within this region. Likewise, we found no phylogeographic structure in *K. tristis*. An absence of isolation-by-distance suggests either very high gene flow over a long period of time or incomplete lineage sorting following relatively recent (re)colonisation (Nielson & Wakeley 2001; Crandall *et al.* 2010). We suggest the latter is more likely. The slopes on and near Mt Kosciuszko are the only areas of mainland Australia where glacial formations during the Pleistocene are known to have occurred (Barrows *et al.* 2001). In addition to concurrent changes in the distribution and structure of vegetation (Hope 1994), *K. tristis* has poor cold tolerance (Chapter 5; Slatyer *et al.* 2015) and may thus have retreated to refugia during glacial periods. Subsequent recolonization during interglacial periods could lead to low diversity and low regional structure – a pattern which is seen among many alpine taxa in the Northern Hemisphere (Taberlet *et al.* 1998; Hewitt 1999; Church *et al.* 2003).

In contrast to the lack of phylogenetic structure, microsatellite data suggested significant population differentiation across small spatial scales (< 20 km) coupled with a strong signature of isolation-by-distance. This could reflect more recent differentiation. In particular, we report a new genetic break, with unambiguous clustering of individuals from

Mt Jagungal, which is separated from Kosciuszko proper by 35 km of subalpine plains at around 1500 m elevation – also the lower-elevation limit of *K. tristis* in the area. Patterns of recent, fine-scale population differentiation (over distances < 50 km) are common across high-elevation taxa – even among species with high dispersal ability (e.g. Britten *et al.* 1995; Keyghobadi *et al.* 1999; Garnier *et al.* 2004; Keyghobadi *et al.* 2005). Habitat features such as forest fragments within high-elevation meadows, topographic relief and water availability have been identified as putative dispersal barriers (Gerber & Templeton 1996; Keyghobadi *et al.* 1999; Henry *et al.* 2012; Castillo *et al.* 2014). Although sub-alpine and alpine habitats are largely contiguous within the Kosciuszko region, as far north as Mt Jagungal, grasshoppers are patchily distributed throughout the landscape (RA Slatyer & KDL Umbers, pers. obs.). This is likely to reflect microhabitat variation which, in turn, could be driving the genetic differentiation across small geographic distances within the Kosciuszko region.

Victoria's high country is highly fragmented, with disjunct mountain regions separated by 50 to 100 km. In line with our predictions that populations from this region would thus show stronger genetic structure than those from Kosciuszko, divergence and diversity were both higher within Victoria. Despite this pattern of generally high regional divergence, however, the Bogong High Plains and Mt Buffalo showed low differentiation. Mt Buffalo contains several endemic flora and fauna species (National Parks Service 1996; Field 2013), showed strong phylogeographic isolation in four other alpine invertebrates (Endo *et al.* 2014) and emerged as one of few distinct genetic clusters in wind-dispersed alpine *Poa* grasses (Griffin & Hoffmann 2014). It was therefore surprising to find no support for a distinct Mt Buffalo clade in our phylogenetic analyses, particularly as this would correspond to the subspecies described from morphological data (Rehn 1957). As for populations within the Kosciuszko region, however, assignment analyses based on microsatellite data suggest that this population is genetically distinct from those of the adjacent Bogong High Plains. Mutations arise rapidly in microsatellites (Whittaker *et al.* 2003), and this differentiation could suggest recent population isolation.

6.4.2 Evolutionary history of the Australian Alps

Rapid cooling and drying in southeastern Australia during the late Miocene to Pliocene (5 – 2.5 Ma) is thought to have promoted the evolution of the cold-adapted sub-alpine and alpine biota, with this species assemblage persisting through subsequent climatic shifts (Galloway & Kemp 1984; Green & Osborne 1994; Gallagher *et al.* 2003). This corresponds to estimated dates of divergence among the four mainland *Kosciuscola* species (Tatarnic *et al.* 2013). Divergence time estimates for the southern Victorian (0.5 – 1.8 Ma) and, subsequently, Mt Baw Baw and Mt Buller clades (0.1 – 0.8 Ma), are consistent with the intense climate cycling of the Pleistocene (Beavis 1959; Barrows *et al.* 2002; Barrows *et al.* 2004). A lack of current gene flow is indicated by an absence of widespread haplotypes and haplotype sharing only between proximate populations (Knowles 2001).

Depression of the snowline during glacial periods is thought to have resulted in continuous alpine/sub-alpine conditions from Mt Jagungal into southern Victoria (Frakes *et al.* 1987; Mark *et al.* 2005). While this depression might have facilitated range expansion and, consequently, low differentiation across the alpine area as a whole (VanDyke *et al.* 2004; Berger *et al.* 2010), previous studies on geographic subsets of Australia's high country have found high endemism within mountain regions, suggesting relatively stable population histories and isolation among mountains (Chapple *et al.* 2005; Koumoundouros *et al.* 2009; Endo *et al.* 2014). In particular, considering both the sequence and microsatellite datasets, we identify four primary genetic groups: two southern Victorian groups comprising Mt Baw Baw and Mt Buller/Mt Stirling; a Bogong High Plains cluster comprising the Bogong High Plains proper and Mt Buffalo; and a Kosciuszko cluster.

One of the most surprising results to emerge in this study was the inconsistent support for the (relatively) well-studied phylogeographic break between the Kosciuszko and Bogong High Plains regions, across the Murray River valley. Approximately 100 km separates alpine/sub-alpine habitats on either side of the valley but two previous studies (Tatarnic *et al.* 2013; Griffin & Hoffmann 2014) have not found significant genetic structure. From both sequences and microsatellite data, we suggest that there is significant divergence between *K. tristis* populations from Mt Kosciuszko and the Bogong High Plains. Perhaps more importantly, however, our data suggest that the Murray River valley (which also forms a State border) may not be the greatest point of divergence among alpine taxa (see also Griffin & Hoffmann 2014).

6.4.3 *The Australian Alps in a global perspective*

For high-elevation taxa in North America and Europe, phylogeographic patterns reflect contrasting population histories of glacial and interglacial expansion and contraction (e.g. Hafner & Sullivan 1995; DeChaine & Martin 2004; Schoville & Roderick 2009; Berger *et al.* 2010; Schoville *et al.* 2012). In many respects, our phylogenetic results mirror those from mountains in the Northern hemisphere – phylogeographic breaks among widespread mountain regions contrast with shallow historical divergence within ranges (Hafner & Sullivan 1995; DeChaine & Martin 2005; Schoville & Roderick 2009). However, mountain regions in Australia are of a different scale to those most often studied – peaks are separated by tens of kilometres, rather than by hundreds of kilometres, and alpine/subalpine habitat is confined to just 500 vertical metres. Further, all current high-mountain regions on mainland Australia are thought to have been connected by alpine/sub-alpine conditions during glacial periods. Given that the processes typically invoked to explain distribution shifts and demographic change – such as ice sheet formation – are not applicable to much of the Australian high country, and that distances among mountain regions are relatively small, it is remarkable that strong lineage divergence (such as for the southern Victorian mountains) is clearly evident. Indeed, sequence divergence was akin to estimates of inter-specific divergence among alpine grasshoppers in the North American Rocky Mountains (Knowles 2000), and across the mountains of Europe (Cooper *et al.* 1995).

6.4.4 Conclusions

Despite the small spatial scale and past intermittent connectivity of Australian alpine and sub-alpine habitats, consistent patterns of divergence among the two molecular datasets indicate differentiation, associated with geographic breaks in alpine/subalpine habitat, that has persisted through glacial cycles and into the present. Populations from peaks within the highly fragmented Victorian high country showed much greater population structure than those from the more continuous Kosciuszko region and structure patterns indicate that the former is comprised of at least three distinct genetic groups. The microsatellite data, although problematic, suggests more fine-scale and possibly more recent structure than either *CO1* or *ITS1,* and genetically distinct populations corresponding to the northern- and southern-most parts of the species' range and to the described sub-species *K. tristis restrictus*. The evolutionary histories of these peaks, particularly the under-studied Mt Jagungal, need to be assessed further. The wide alpine/sub-alpine distribution and ecological characteristics of *K. tristis* make it a good model for exploring associations between genetic markers and adaptive processes across the Australian high country.

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A final word of caution to the prospective alpine ecologist…expect a skeptical response from colleagues when you are encountered leaving for your study site with skis or ice axe in hand.

-- *John S Edwards, 1987, Arthropods of Alpine Aeolian Ecosystems*

Chapter 7

Australia's subnivean invertebrates: characterisation and a test of the impacts of reduced snow cover

Abstract

Snow is one of the most important factors in the ecology of alpine ecosystems. In Australia, both the depth and duration of snow cover have declined significantly in recent decades, and this trend is forecast to continue with progressive global warming. Many small arthropods remain active throughout the winter, within the space beneath the snowpack where the insulating properties of snow create a thermally stable environment. Using field surveys and experimental manipulation of snow depth at two locations in the Australian alpine region, I explored the diversity of winter-active arthropods and their response to reduced snow. Using pitfall traps, individuals from 18 arthropod Orders were detected beneath the snow during winter, with the Collembola, Araneae, Acari and Coleoptera accounting for 85 – 99% of the individuals collected. Removal of the snow layer increased daily temperature fluctuations, increased the number of days below freezing and raised the mean surface temperatures. Community composition was altered by snow removal. This effect was driven by a strong reduction in the abundance of dominant taxa (Collembola: Paronellidae) at Mt Twynam in New South Wales, and by an increase in the numbers of individuals of the dominant taxon (Collembola: Hypogastruridae) at Mt Stirling, Victoria. Subnivean arthropod communities in Australia thus appear sensitive to snow conditions at small spatial scales.

7.1 Introduction

Snow is considered to be one of the most important factors governing the ecology of alpine ecosystems (Billings & Mooney 1968; Mani 1968). It determines the timing and length of the growing season, controls soil nutrient influxes (Woolgrove & Woodin 1996; Körner 2003; Blankinship & Hart 2012) and moderates local and regional hydrology (Vavrus 2007). The snow pack also creates a thermally stable subnivean microclimate, often close to 0°C, in which overwintering flora and fauna are buffered from freezing air temperatures and large daily temperature fluctuations (Körner 2003; Callaghan *et al.* 2011). In this capacity, the snow layer functions as an ecotone between the cold, dry and variable atmospheric conditions and the relatively warm, humid and constant subnivean environment.

Snow, however, has an uncertain future. Since the late 1960s, global snow cover has decreased by 10% (Walther *et al.* 2002). In the last decade, high elevation areas have warmed more rapidly than lower elevation areas and many alpine regions have experienced declines in snow depth and duration (Lemke *et al.* 2007; Stewart 2009). This effect is particularly apparent at middle elevations (1500 – 2000 m a.s.l) and during the spring, when mean air temperatures are close to freezing point and even a small rise in temperature causes a large increase in the proportion of precipitation falling as rain rather than snow (Brown & Mote 2009; Sproles *et al.* 2013; Steger *et al.* 2013). As a consequence, a shorter snow season and earlier snowmelt are expected to be the first and largest effects of climate change across mountain systems (Räisänen 2008; Brown & Mote 2009). Even at high elevations where winter snowfall could increase in the short-term future (due to increased precipitation), spring snow is likely to decline significantly over the next 50 years (Edwards *et al.* 2007; Räisänen 2008; Brown & Mote 2009; Hendrikx *et al.* 2012).

On the Australian mainland, snow seasonally covers an area of roughly 1675 km^2 for two or more months each year (Sanecki *et al.* 2006). The Australian high country has a climate dominated by winter precipitation, with approximately 70% of this precipitation associated with the passage of cold fronts moving eastward, perpendicular to the main axis of the mountain range (Chubb 2010; Dai *et al.* 2014). A shift in the frequency and intensity of cold fronts in recent decades has, consequently, been associated with an overall decline in winter precipitation (Chubb *et al.* 2011; Riseby *et al.* 2013). Several studies have examined recent trends in snow cover and duration across the Australian alpine region. Based on 40 years of snow depth data from the Kosciuszko region in New South Wales (NSW), Nicholls (2005) found a strong decline in spring snow depth (~40%) but with considerable inter-annual variability and no associated decline in maximum snow depth (Fig. 7.1). More recently, Fiddes *et al.* (2015) estimated a decline in snow depth of 15% since 1988 and a reduction in total snow accumulation by 9%. These declines were largely attributable to the loss of small snowfall events (1 – 10 cm), the frequency of which are closely linked to maximum temperatures (Fiddes *et al.* 2015). Since 1989, snowmelt date in the Bogong High Plains, Victoria, has advanced by an average 22 days (Bhend *et al.* 2012). Similarly, snow cover duration in the Kosciuszko region has shortened by an average 18 days since 1954 (Sánchez-Bayo & Green 2013) with snowmelt date advancing by 2 days per decade over this period (Green & Pickering 2009).

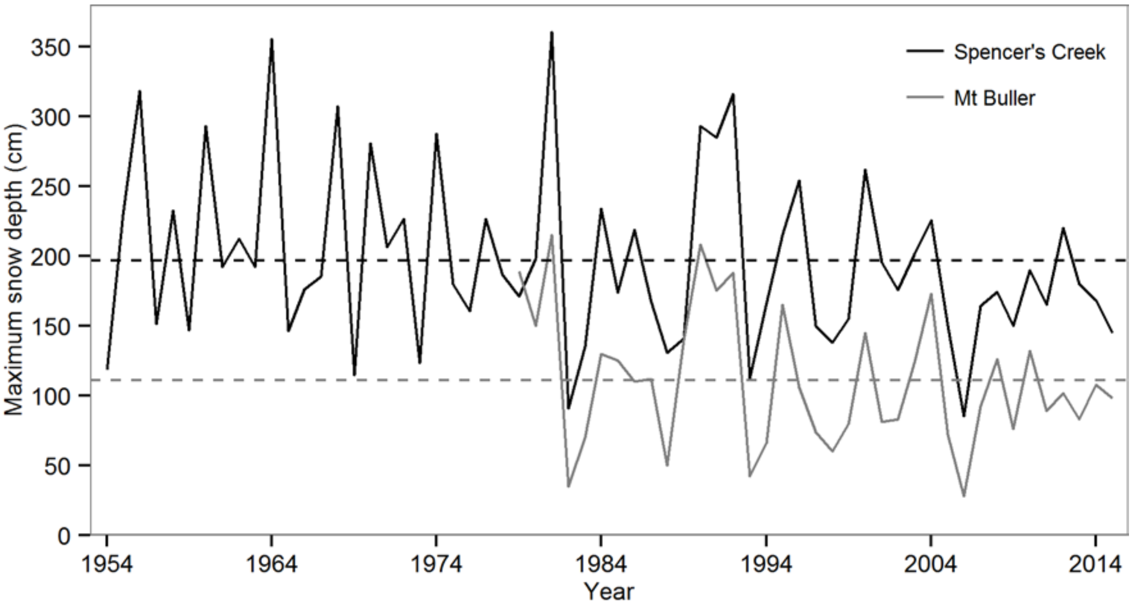

Figure 7.1. Inter-annual variation in maximum snow depth in the Australian alpine region. Snow depths are shown for Spencers Creek (1830 m a.s.l) in the Kosciuszko alpine region (black), and Mt Buller (1700 m) in Victoria (grey). The deepest snow on record was in 1981 (361 cm at Spencer's Creek, 215 cm at Mt Buller) and the shallowest snow year was 2006 (85 cm at Spencer's Creek, 28 cm at Mt Buller). The dashed horizontal lines show the mean maximum snow depth across all years. Data from Snowy Hydro Ltd and Mt Buller Resort.

Approximately 20 cm of snow is required to effectively insulate the subnivean environment from atmospheric temperature fluctuations, with the exact depth required to achieve insulation dependent on snow density (Pruitt 1957, 1970; Marchand 1982; Geiger *et al.* 1995). Simulated long-term (50-year) forecasts for annual mean maximum snow depths at Mt Buller, in Victoria, lie perilously close to this threshold: 10 – 30 cm under a low impact scenario, and 0 – 20 cm under a high impact scenario (Bhend *et al.* 2012). Snow depths at higher elevations are also predicted to decline to between 20 and 105 cm (30 – 87% relative to 1990) by 2059 (Bhend *et al.* 2012). For the Australian alpine region as a whole, climate models predict declines in both snow depth and duration due primarily to an increase in the proportion of precipitation falling as rain rather than snow (CSIRO & BOM 2015). Under a middle-impact scenario, maximum snow depths in the 2040s are expected to be 60% (high-elevation sites, 1830 m) to 80% (low-elevation sites, 1615 m) lower than the current average, corresponding to depths of 1.2 m and 0.8 m, respectively (Hendrikx *et al.* 2013). Similarly, the duration of snow cover is projected to be 9 to 49 days shorter for low- and high-elevation sites, respectively (Hendrikx *et al.* 2013). These estimated changes to the snow regime are accompanied by a predicted rise in the snowline (Table 7.1), representing a loss of 7 – 74% of the elevational range of the sub-alpine/alpine zones (Hennessy *et al.* 2007). In this context, it is critically important to examine how alpine plants and animals – adapted to a snow-mediated winter environment – will respond to changes in the snowpack.

Table 7.1. Predicted changes in snow cover over the Australian alpine region. Numbers are predicted changes in snow conditions for 20-year periods centred on 2020 and 2050, relative to a 20-year period centred on 1990. Projections are for the Australian alpine region as a whole, and for Mt Buller, Victoria, which is adjacent to one of the study sites. The current snowline at Mt Kosciuszko is approximately 1460 m. Data are derived from Hennessy *et al.* (2003), Hennessy *et al.* (2007), and Bhend *et al.* (2012).

	Snow line, Mt Kosciuszko (m)	Area of snow cover (%)		Snow persistence (days)	
		Overall	Mt Buller	Overall	Mt Buller
Low impact (2020)	+ 30	- 10	- 35	- 5	- 20
High impact (2020)	+ 165	- 39	- 35	- 40	- 20
Low impact (2050)	+ 60	- 22	- 63	- 15	- 38
High impact (2050)	+ 570	- 85	- 78	- 100	- 60

7.1.1 *The subnivean space: a winter refuge*

For winter-active arthropods, snow creates three distinct habitats: the snow surface, the snow layer itself, and a space between the soil surface and the base of the snow pack (the "subnivean space") (Aitchison 2001). The subnivean space, the focus of this study, can form in two ways: first, physical objects such as vegetation, stones and branches can hold up the snow. Second, latent heat from the soil can warm the lowest layers of the snow, which either melts (Sanecki *et al.* 2006) or sublimates into water vapour to create a layer of loose snow crystals ("depth hoar") at the base of the snow pack (Marchand 2013). The latter, however, requires a considerable temperature gradient within the snow pack, which does not develop in the shallow and dense snow typical of Australian alpine conditions (Sanecki *et al.* 2006).

Many arthropods remain active beneath the snow during winter. Collembola (springtails), Acari (mites and ticks), Araneae (spiders), Diptera (flies) and Coleoptera (beetles) are particularly common (Aitchison 1978, 1979a, b, c; Green 1997; Aitchison 2001; Vanin & Turchetto 2007). In the sole survey of winter-active invertebrates in the Australian alpine region, Green (1997) found members of 19 arthropod Orders active under the snow, with the dominant groups being Collembola, Diptera, Coleoptera, Araneae, Acari and Diplopoda. The fauna active within the snow layer itself is recruited from the subnivean assemblage and consists primarily of Collembola and Acari, which are small enough to move between snow crystals (Brummer-Korvenkontio & Brummer-Korvenkontio 1980; Leinaas 1981). Reduced snow cover thus has a direct effect of reducing available habitat for these "intranivean" taxa, as well as a suite of potential indirect effects on subnivean taxa (Fig. 7.2).

7.1.2 Ecological effects of reduced snow cover

Beyond the direct effects of physical habitat loss, predictions for the effects of reduced snow on alpine ecosystems fall into two broad categories. On the one hand, higher maximum temperatures and advanced snowmelt could increase productivity in alpine ecosystems (Billings & Bliss 1959; Galen & Stanton 1993). Alternatively, a shallow snow pack or early snowmelt (and consequent loss of the insulating layer) might expose alpine flora and fauna to the damaging effects of frost and cold temperature extremes (Inouye 2008; Schaberg *et al.* 2008; Gerdol *et al.* 2013) (Fig. 7.2).

The duration of the snowpack determines the length of the growing season and snow manipulation experiments from northern hemisphere alpine regions indicate the strongest and most consistent effect of earlier snowmelt is an advance in phenology (reviewed: Wipf & Rixen 2010). The effects of snow duration on community composition are evident from the distinct suite of species that occur in late-lying snow patches, including some species that are restricted to these habitats (Billings & Bliss 1959; Costin *et al.* 2000; Björk & Molau

2007; McDougall & Walsh 2007; Williams *et al.* 2015b). Physiological adaptations to prolonged snow cover are also evident. In the Australian alpine herb *Aciphylla glacialis,* for example, populations from late-lying snow patches have poorer frost resistance than those from areas where snow melts earlier and where they are thus more exposed to spring frost (Briceño *et al.* 2014). Among snow-associated arthropods, adaptations to persistent (but constant) cold are apparent within several taxa including the springtails, spiders, and ice-crawlers (Order Grylloblattodea), with normal activity (locomotion, feeding, development, reproduction) maintained at temperatures near 0°C but, frequently, poor tolerance to extreme cold (Mani 1968; Hågvar 2010; Schoville *et al.* 2015).

A systematic literature survey (see Appendix F for details) identified just 22 studies that have experimentally reduced snow cover (depth and/or duration) in alpine areas, encompassing only 11 unique study sites. These studies showed consistent abiotic effects of reduced snow, including lower average soil temperatures and increased depth of soil freezing (Freppaz *et al.* 2008; Gaul *et al.* 2008; Wipf & Rixen 2010; Gerdol *et al.* 2013). Only two studies (Roy *et al.* 2004; Adler *et al.* 2007), however, measured responses of invertebrates to snow cover decline. Roy *et al.* (2004) found that, in general, earlier snowmelt due to experimental heating in spring was associated with greater summer herbivory/pathogen activity, but effects were idiosyncratic among host plant species. At the same site, long-term spring warming either decreased or had no effect on aphid (Hemiptera: Aphididae) density and population growth (Adler *et al.* 2007). To date and to the best of my knowledge, there are no tests of the effects of a general reduction in snow cover (i.e. without artificial warming) on alpine arthropod communities.

Warming is generally expected to favour ectothermic animals in cold climates by reducing cold stress and accelerating development and reproduction (Crozier 2004; Roy *et al.* 2004; Deutsch *et al.* 2008). Several studies have, accordingly, recorded increases in abundance of alpine invertebrates following experimental warming during the growing season (Liu *et al.* 2011; Nash *et al.* 2013). Physiological theory, supported by empirical studies, suggests that a fluctuating thermal environment should be more energetically costly than a stable one, and that the greatest effects of temperature variation on fitness will be when temperature cycles cross critical physiological thresholds, such as inducing a stress response, freezing or mortality (e.g. Ruel & Ayres 1999; Marshall & Sinclair 2012a; Williams *et al.* 2012; but see Lalouette *et al.* 2011). Under a shallow snowpack (or none at all), temperatures fluctuate more and there are a greater number of freeze-thaw cycles than under a deep snowpack

(Henry 2008). It follows that, when snow is reduced, it is more likely that temperatures will cross critical thresholds, reducing overall fitness (Fig. 7.2).

Laboratory and field experiments from the sub-Arctic, Arctic, and hardwood forests of North America suggest that changes to soil freeze-thaw dynamics can alter the composition of soil arthropod communities (Sulkava & Huhta 2003; Konestabo *et al.* 2007; Bokhorst *et al.* 2012; Templar *et al.* 2012). Increases in the abundance of soil microarthropods following mild freeze-thaw could be due to hatching during benign thaw periods or greater availability of food as the microbial community is activated and then dies (Schimel & Clein 1996; Sulkava & Huhta 2003; Konestabo *et al.* 2007); in contrast, substantial declines in microarthropod abundance associated with more extreme cold are attributable to direct mortality (Sulkava & Huhta 2003; Bokhorst *et al.* 2012).

Springtails appear to be particularly sensitive to small-scale climate manipulations. For example, supplementation of winter ice in the Arctic caused a 50% decline in the abundance of springtails (Coulson *et al.* 2000), while drought experiments (though not specifically in an alpine/winter context) show strong negative effects of dry conditions on springtail biomass (Petersen 2011). Earlier snowmelt reduces soil moisture in the surface layers, and this effect can persist for several months, suggesting a possible indirect effect of reduced snow cover on desiccation-sensitive arthropods (Blankinship *et al.* 2014) (Fig. 7.2). Soil compaction, snow compaction (i.e. through skiing), and artificial snow have also been shown to reduce springtail abundance and diversity (Hopkin 1997). Springtails are common in alpine habitats and many species are known to remain active at temperatures near 0°C (Chapman 1954; Mani 1962; Leinaas 1981). They might therefore serve as sensitive indicators of changes in microhabitat conditions brought about by reduced snow cover (Convey *et al.* 2003; Greenslade 2013).

In addition to a paucity of studies on faunal responses to reduced snowpack, there is a notable geographic bias among snow manipulation studies to date – in my literature survey, all of the studies bar one (Ferrar *et al.* 1988) were from the northern hemisphere (Appendix F, Table F.1). Southern hemisphere alpine climates are typically more moderate (most mountains being close to the ocean and of lower elevation than their northern counterparts) but more variable and unpredictable than those in the northern hemisphere (Bonan 2002). Adaptations to these different climates are expected and observed, including hemispheric differences in cold tolerance strategies among insects (Sinclair & Chown 2005) and plants (Bannister 2007).

Even at the highest elevations, Australia's snowpack is marginal with daytime temperatures often above 0°C (Sanecki *et al.* 2006). With the exception of studies on the overwintering energetics of the iconic mountain pygmy possum (*Burramys parvus*) (Geiser & Broome 1993; Körtner & Geiser 1998) and one study on seedling establishment in the snow gum (*Eucalyptus pauciflora*) (Ferrar *et al.* 1988), the effects of a changing winter snowpack on the Australian alpine biota are restricted to anecdotal reports. For example, Green (1997) ascribed a low number of arthropods in spring of one year to a late snowfall, while the numbers of *Kosciuscola* grasshoppers are apparently reduced following low-snow winters (Green & Osborne 2012). Australia's alpine invertebrate communities are responsive to changes in environmental conditions with, for example, a recent summer warming experiment showing increases in the abundances of several arthropod taxa, including springtails and plant-feeding arthropods (Nash *et al.* 2013). There is, however, little relevant information on either surface-active subnivean taxa or their sensitivity to snow conditions.

7.1.3 Aims

This study focused on taxa that are active in the subnivean space and had two primary goals. The first was to characterise Australia's winter-active subnivean arthropod fauna, adding to the sole published study of this ecosystem to date (Green 1997). The second aim was to test the effects of reduced snow cover on these taxa. With these goals in mind, I explored three key questions: (a) is there a characteristic subnivean arthropod fauna?; (b) does the absence of a snowpack cause a shift in arthropod community composition?; and (c) do the numbers of dominant springtail taxa increase (predicted by greater food availability/higher maximum temperatures) or decrease (reduced habitat availability/colder minimum temperatures; Fig. 7.2) in response to reduced snow cover?

In the work that follows, I will continue the tradition of previous work on snow ecology (Leinaas 1981) by using the term "winter" to refer to the period from the first snowfall to snowmelt and "summer" to refer to the remainder of the year, unless more precise definitions are required.

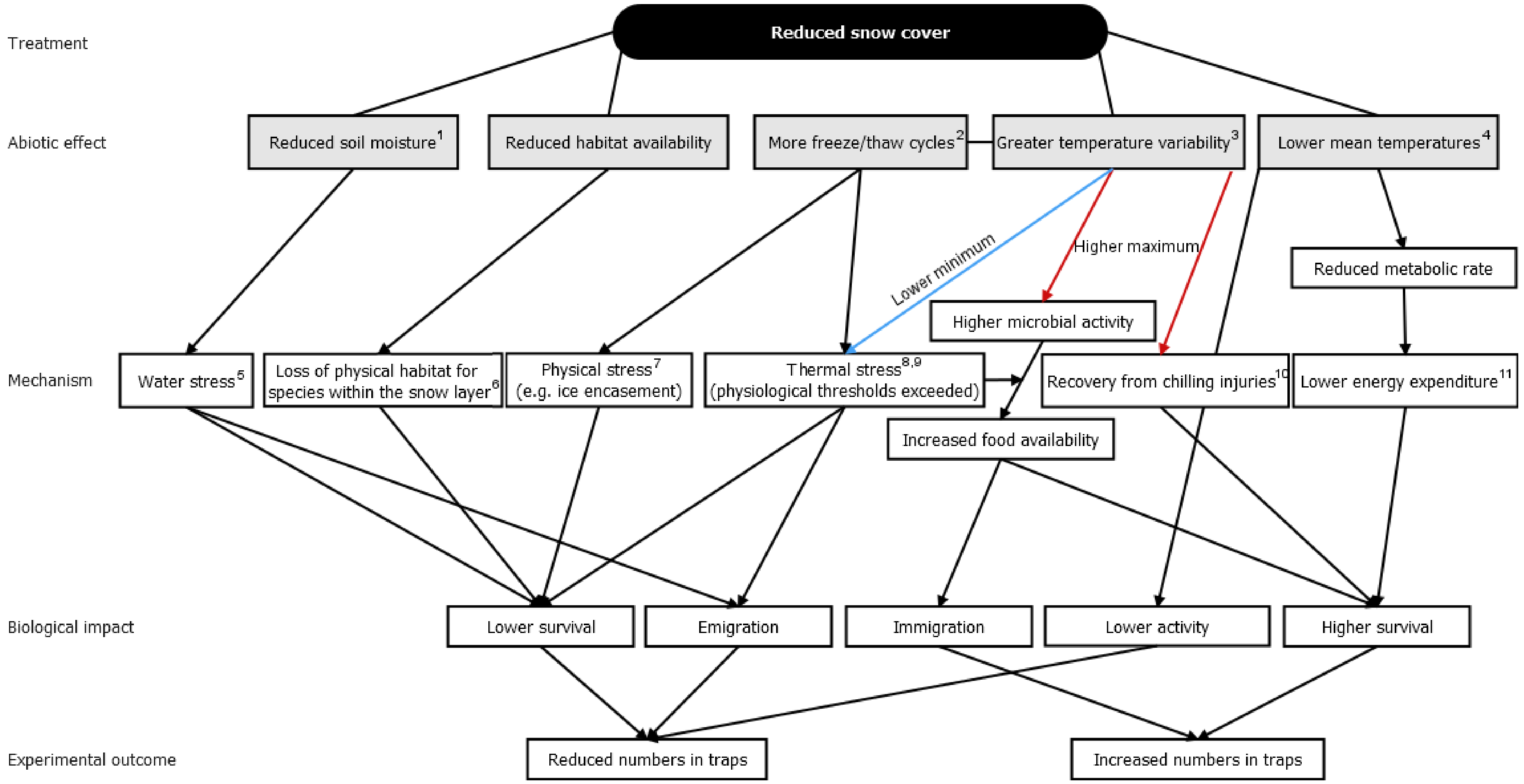

Figure 7.2. Some potential effects of reduced snow cover on alpine arthropods, in relation to experimental snow removal. Example references are referred to with superscript numbers: [1] Adler *et al.* (2007); [2] Gerdol *et al.* (2013); [3] Price and Waser (2000); [4] Freppaz *et al.* (2008); [5] Petersen (2011); [6] Leinaas (1981); [7] Coulson *et al.* (2000); [8] Marshall and Sinclair (2012b); [9] Bokhorst *et al.* (2012); [10] Lalouette *et al.* (2011); [11] Williams *et al.* (2015a).

7.2 Methods

7.2.1 Study area

This study was carried out at two mountains (Fig. 7.3), over three years (2013 – 2015). The first site, used in 2013 and 2015, was at Mt Stirling, Victoria (Fig. 7.4A). The Mt Buller/Mt Stirling region is the southernmost area of true alpine habitat on the mainland. It is geographically isolated from other alpine areas and has a genetically distinct invertebrate fauna (Endo *et al.* 2014; Slatyer *et al.* 2014; Chapter 6). The mountain is part of the Mt Buller/Mt Stirling ski resort and is used for recreational activities (including cross-country skiing and snow-shoeing in winter, and mountain biking, hiking, and horse riding in summer) throughout the year. The study site was on the west/southwest-facing side of the summit, at 1700m. The vegetation community is Snowy Range open heathland dominated by the shrub *Hovea montana* and the tussock grass *Poa fawcettiae* (McDougall & Walsh 2007).

The second site, used in 2014, was at 1700 m on the eastern slopes of Mt Twynam, New South Wales (Fig. 7.4B). This mountain is part of the Kosciuszko Main Range and lies on the western side of the Snowy River, within the Western Fall Wilderness Area of Kosciuszko National Park. The site corresponds to the *Grevillia australis-Nematolepis ovatifolia* open heathland/*Poa fawcettiae-Eurphrasia collina* grassland communities described by McDougall and Walsh (2007). The two sites thus have a similar vegetation structure, with tall herbfield/grassland interspersed with short shrubs.

The study sites at Mt Stirling and Mt Twynam experienced similar mean snow depths over the course of the study, but there was considerable variation in snow depth and duration between years (Appendix F, Fig. F.7). Snow cover persisted at the study plots for an average 64 days at Mt Stirling in 2013, 95 days at Mt Twynam in 2014 and 77 days at Mt Stirling in 2015, with maximum snow depths of 75 cm, 130 cm, and 100 cm, respectively. Once snow began to accumulate, it persisted through to snowmelt in 2014 and 2015. However, the snow pack melted to less than 5 cm twice during the 2013 winter.

Between 1988 and 2013, maximum snow depth in the Australian alpine region averaged 151 cm, with a mean total accumulation of 234 cm (Fiddes *et al.* 2015). Sanecki *et al.* (2006) described snow in Australia (1600 – 1800 m) as “warm-temperate-tropical”. Features of this snow type are a snow-ground interface temperature between 0°C and 1°C, a high density (e.g. mean spring snow density is 0.4 g cm^{-3}), rapid densification over the course of the winter, a weak vertical temperature gradient, and high inter-annual variability (Budin 1985; Sanecki *et al.* 2006; Bormann *et al.* 2013). In addition, at least half of the area experiencing winter snow cover in the Snowy Mountains corresponds to Sturm *et al.* (1995)'s "ephemeral" classification, being less than 50 cm deep and lasting less than 2 months (Sanecki *et al.* 2006). In the absence of a strong vertical temperature gradient, seasonal changes in the structure of the snowpack occur primarily via destructive (equi-temperature) metamorphism, which involves the transformation of snow crystals into larger, more compact snow grains (Sanecki *et al.* 2006; Bormann *et al.* 2013; Marchand 2013).

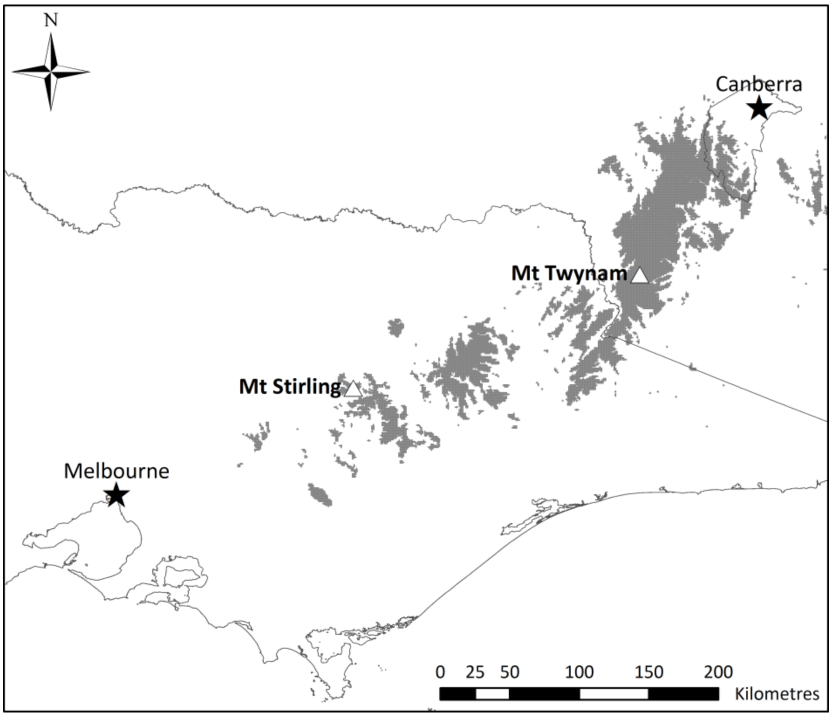

Figure 7.3. Location of the study sites within the Australian alpine region. Grey shading indicates areas above 1500 m and the study locations are indicated with white triangles.

7.2.2 Year 1: 2013, Mt Stirling

Eight 1 m^2 plots were established at Mt Stirling, Victoria to explore seasonal variation in community composition and establish a baseline dataset for winter-active arthropods on this mountain. Three pitfall traps (15 mm diameter glass test tubes) were installed within each plot and were activated at two-monthly intervals for one week (January, March, May, November) or two weeks (July, September). Pitfall traps contained 5 mL of 50:50 propylene glycol:ethanol. Subnivean traps (July, September) were installed and changed by means of an access tube (Fig. 7.5) installed prior to snowfall. In each of four sites, I installed two iButtons (DS1922L Thermochron®, Maxim Integrated Products) – one 2 cm above the soil surface and one 1 cm below the soil surface. These were programmed to record temperature (± 0.0625°C) hourly. Snow depth data were obtained from snow depth gauges at the summit and on the upper trails at Mt Stirling (data provided by Mt Buller/Mt Stirling Resort). Snow depths at the study plots were approximated by an average of the readings from the two gauges, based on personal observations.

Figure 7.4. The study site at (A) Mt Stirling (image taken in autumn) and (B) Mt Twynam (image taken in summer). Study sites are in the foreground of both images.

7.2.3 Year 2: 2014, Mt Twynam

To test the effects of reduced snow cover on subnivean arthropods, in June 2014 I established seven pairs of plots (blocks), each with one treatment and one control plot. Within each block, the two 1 m^2 plots were separated by approximately 2 m. Blocks were arranged 4 – 10 m apart. The experimental period was from the first snowfall (June 19th) until snow had melted from all sites (October 12th). Within this period, treatment plots were kept snow free for two (one block), six (three blocks) or 15 weeks (three blocks). Snow depth varied considerably among blocks, and plots could not be cleared when snow depth was over 1.5 m. Each week, snow was manually cleared from treatment plots, including a 50 cm margin to minimise edge effects (Fig. 7.6). A 5 cm layer of snow was left undisturbed to prevent damage to the underlying vegetation. This snow depth is insufficient to provide thermal buffering effects (Geiger *et al.* 1995). Three pitfall traps were installed within each plot and changed every three weeks over the snow removal period. In each of five blocks, in each plot two iButtons were installed above and below the soil surface as described above. Snow depth was estimated weekly at each snow removal plot, once the depth was below 1.5 m. Based on these estimates, I used snow depth data from the Snowy Hydro Limited gauge at Deep Creek (1620 m a.s.l) to approximate average snow depth at the study site across the whole winter.

7.2.4 Year 3: 2015, Mt Stirling

The 2014 experiment was repeated at Mt Stirling, Victoria in 2015. The experimental design was identical, but with eight blocks and with snow cleared for 11 weeks (the duration of the snow cover) at all treatment plots. Prior to snowfall (May), pitfall traps were activated for 2 weeks at all plots (“autumn”). The snowpack began to develop from July 3rd. All snow removal plots were cleared weekly and traps changed every three weeks from July 19th to September 19th. There were thus three trapping periods during the winter: (1) July 19th – August 8th (“early winter”), (2) August 8th – August 30th (“mid winter”), and (3) August 30th – September 19th (“late winter”). Snow had melted at six of the eight control plots by September 19th and traps were installed for a final two weeks from this date (“spring”). One iButton was installed in the centre of each plot, 1 cm above the soil surface, and recorded temperature hourly from May until the end of the experiment.

7.2.5 Microclimate data analysis

Data from temperature loggers were used to calculate overall mean, mean daily minimum, mean daily maximum, mean daily fluctuation (difference between maximum and minimum), and the number of days where the minimum temperature was below 0°C (a proxy for the number of freeze-thaw cycles) for each year/site and trapping period. Snowmelt date, which marks the end of “winter”, was determined either from direct observation or from the temperature loggers. For the latter, snowmelt was defined as when the temperature first rose above 3°C at midday ± 2 h (Pickering & Green 2009).

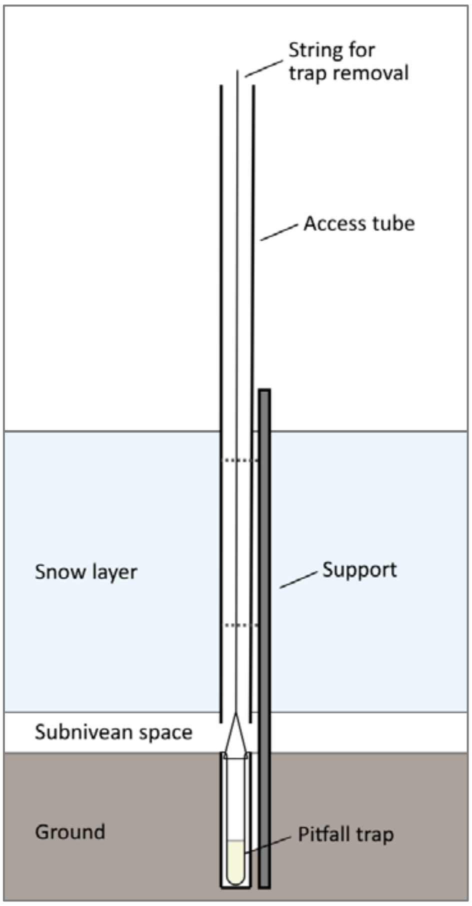

Figure 7.5. Schematic of the subnivean trapping apparatus.

Figure 7.6. Experimental manipulation of snow depth. Experimental snow removal plots (front) were kept clear of snow throughout the winter while control plots (rear) accumulated snow naturally.

7.2.6 Identification and pre-analysis data processing

All individuals captured in pitfall traps were counted and identified to Order level under a stereo microscope (Leica M125) at 8 to 100x magnification. Three dominant winter-active groups (Collembola, Coleoptera, Araneae) were identified to family level or below. The Acari were abundant over the winter, but, as a large proportion of individuals were juveniles and could not be identified to family level, only the Oribatida and Bdellidae were included in subsequent analyses. Identifications were made with the aid of relevant taxonomic keys (Matthews 1980; Goulet & Huber 1993; Raven 2002; CSIRO 2012; Ślipiński & Lawrence 2013), descriptive texts (Brunet 1997; Hangay & Zbrowski 2010; Framenau *et al.* 2014) and expert knowledge (P Greenslade, A Ślipiński, R Leschen, J Reid, M Rix).

For the multivariate analyses of experimental treatments in 2014 and 2015, I focused on taxa that accounted for at least 1% of the total sample. For the 2014 experiment at Mt Twynam, the analysis therefore included: nine springtail taxa (morphospecies) in eight genera (*Paronellides, Pseudoparonella, Drepanura, Lepidophorella, Brachystomella, Triacanthella, Skadisotoma, Katianna*), analysed at the morphospecies level, two mite taxa (Bdellidae, Oribatida), and one spider family (Linyphiidae). At six of the seven blocks, snow had melted prior to, or within one day of, deployment of the final traps in late September. For those blocks, this last trapping period (hereafter referred to as "spring") was analysed separately, with the same taxa as above. For the 2015 experiment, the taxa included (> 1% of the total winter sample) were: five springtail taxa (*Acanthomurus* sp., *Katianna* spp., Hypogastruridae, Brachystomellidae, and one podurid that could not be identified to family level but was clearly distinct), one mite family (Bdellidae), and two spider morphospecies (family Amphinectidae and family Micropholcommatidae, genus *Raveniella*).

Prior to analysis, data were pooled for the three traps within a plot. Traps that failed to catch any arthropods were deemed to be incorrectly installed, and were excluded from analysis (2014: 12/121 traps; 2015: 5/234 traps). Data were then standardised to number per trap-week. In 2014, as numbers during winter were generally very low (with the exception of two common taxa, median number of individuals/trap-week = 3), and the number of sampling periods was uneven between blocks, data were pooled for the whole winter period. In 2015, data were split and analysed in three groups: (1) autumn (May trapping period), (2) winter (three trapping periods between July and September), and (3) spring (one trapping period in September, after most plots were snow free).

7.2.7 Ordination and community dissimilarity

All analyses described below were carried out in R 3.2.2 (R Core Team 2015) with specific packages as detailed below, and plots were constructed with GGPLOT2 1.0.1(Wickham 2009).

I used non-metric multidimensional scaling (NMDS), implemented in VEGAN 2.3-0 (Oksanen *et al.* 2015), to visualise variation in community structure due to experimental effects (Minchin 1987). These analyses were based on the restricted dataset of dominant taxa, described above. NMDS was performed using a Bray-Curtis dissimilarity index (Bray & Curtis 1957), as this index preserves rank order (Faith *et al.* 1987). Transformation (square-root) was limited to groups that were extremely abundant (*Pseudoparonella*, *Paronellides* in 2014) relative to other taxa. I used three dimensions and up to 20 random starts to avoid local optima and find a stable solution with a stress ≤ 0.1 (Clarke 1993). Principal components were rotated so that the variance of points was maximised along the first axis and centred so that the origin was at the average of the axes. Rotation and centering were performed using VEGAN 2.3-0 (Oksanen *et al.* 2015). Axis scores from the final run were used for subsequent exploratory analyses. I tested for an effect of treatment on axis score using (a) paired t-tests (Mt Twynam, and Mt Stirling autumn and spring) or (b) linear mixed models with treatment (two levels) and trapping date (“time”, three levels), and a treatment-time interaction as fixed factors, and block and plot as random factors (Mt Stirling winter). I tested the significance of fixed factors using parametric bootstrap of the likelihood-ratio test statistic, *T*, based on stepwise removal of factors. Bootstrapping was performed with 1000 simulations using PBKRTEST 0.4-2 (Halekoh & Højsgaard 2014), and mixed models were implemented using LME4 1.1-9 (Bates *et al.* 2014).

I used a permutational multivariate analysis of variance (PERMANOVA: Anderson 2001) with a Bray-Curtis distance matrix to test the effects of treatment (2014, autumn and spring 2015) or treatment and trapping date (winter 2015) on community composition. Block was also included in these analyses to allow partitioning of variance due to differences among blocks, and significance was tested with 999 permutations. The 2015 winter data were analysed in a two-step process. First, the treatment and block factors were tested using centroids for each plot (i.e. across the three trapping periods) and significance was tested with 999 permutations. Second, the effects of trapping period and an interaction between treatment and trapping period were tested, with a block-by-treatment factor included to remove between-plot variation from the residual error term. Significance was again tested with 999 permutations. PERMANOVA analyses were carried out using VEGAN 2.3-0 (Oksanen *et al.* 2015).

PERMANOVA is robust to heterogeneity in dispersion for balanced designs (Anderson & Walsh 2013). Nevertheless, I tested for differences in group dispersions using the method of Anderson (2006), implemented in VEGAN 2.3-0 (Oksanen *et al.* 2015). The average dissimilarity of an individual (e.g. plot) to the group (e.g. treatment) centroid can also be used to assess beta diversity (Anderson *et al.* 2006). This estimation method involves three steps. First, a dissimilarity matrix is constructed. For this I used the Bray-Curtis dissimilarity index, which preserves rank order and can be directly related to the PERMANOVA analysis which used the same index. In the second step, the average distance from each plot to the group (representing each trapping period/treatment) centroid is calculated. I used a small-sample bias adjustment which imposes a $\sqrt{(n(n-1))}$ correction (Stier *et al.* 2013) on estimates of dispersion. Third, a permutation test is used to test the null hypothesis of no difference in average dissimilarity among groups. For this step, I used 999 permutations with no restrictions for the 2014 and autumn/spring 2015 analyses. For winter 2015, permutations were permitted among trapping periods within plots (with a restriction that the same permutations were used across plots), and among plots within trapping periods. Permutations were defined using PERMUTE 0.8-4 (Simpson 2015).

The last step in the analysis was to test for an effect of treatment on individual counts of dominant taxa. At both sites, there were two taxa (*Paronellides* and *Pseudoparonella* at Mt Twynam, Brachystomellidae and Hypogastruridae at Mt Stirling) that were markedly more common than others, and these taxa were used for univariate analyses. For Mt Twynam, and for Mt Stirling autumn/spring traps, I tested for an effect of treatment on the number of individuals in traps using paired Wilcoxon tests. For Mt Stirling winter traps, I used a generalised linear mixed model with a negative binomial distribution, implemented with LME4 1.1-9 (Bates *et al.* 2014). As for the mixed models described above, treatment, trapping date, and their interaction were included as fixed factors with block and plot as random factors. The significance of fixed factors was tested using likelihood-ratio tests with stepwise removal of factors. For all tests described above, *p*-values were adjusted for multiple comparisons with a Bonferroni correction where appropriate.

At Mt Stirling, snow removal plots were filled in by fresh snow between one and three days after clearing during the early and mid winter trapping trapping periods (see Results). Only in the late winter (August 30th – September 19th) were snow removal plots snow free for most/all of the trapping period. I therefore ran additional separate ordination, PERMANOVA, dispersion and abundance analyses for this trapping period alone.

Unless otherwise stated, data are presented as mean ± s.d.

7.3 Results

7.3.1 Microclimates

7.3.1.1 Seasonal variation

Seasonal variation in surface and ground temperatures was explored at Mt Stirling in 2013. Surface and ground temperatures, particularly maximum temperatures, varied considerably over the year (Fig. 7.7). The coldest month in terms of overall mean temperature was August, with a mean surface temperature of 0.6 ± 0.7°C and a mean ground temperature of 1.1 ± 0.6°C. However, the coldest temperatures occurred in September (coldest day minimum surface temperature = -5.4°C, averaged across plots) and May (coldest day minimum ground temperature = -0.3°C, averaged across plots). Notably, although surface temperatures passed below 0°C in every month and on 30% of days in total, when averaged across plots the ground temperature was only below 0°C on one of the 333 days of temperature recordings. Summary statistics for each of the trapping periods are provided in Appendix F, Table F.7. Of the six trapping periods, May was the coldest in terms of overall mean (0.5 ± 3.1°C), daily minimum (-2.2 ± 5.9°C), and the number of days below 0°C (seven out of eight days), while July was the coldest in terms of mean daily maximum (2.4 ± 2.5°C). January was the warmest trapping period with respect to overall mean temperature (15.5 ± 10.2°C) and maximum temperature (32.4 ± 7.9°C), but March was the least cold (i.e. higher mean daily minimum: 6.1 ± 2.3°C) and was the only trapping period where surface temperature did not fall below 0°C (Appendix F, Table F.7).

7.3.1.2 Subnivean microclimates

Across sites and years, subnivean surface temperatures varied little (2013: 0.7± 0.6°C, 2014: 0.5 ± 0.3°C, 2015: 0.5 ± 1.4°C; Figs. 7.7, 7.8). Ground temperatures were slightly warmer, but remained similar across sites and years (2013: 1.1 ± 0.5°C; 2014: 0.9 ± 0.4°C; Not measured in 2015). Snow almost completely buffered both air and ground temperature fluctuations (mean daily fluctuation: 0.0 – 0.4°C across years/sites) and the buffering effect induced by the snowpack was clearly apparent: at Mt Twynam, for example, in the two weeks before the snowpack had developed, the mean daily temperature fluctuation was 3°C, compared with 0°C over the snow season (Fig. 7.8). Similarly, mid-winter snowmelt at Mt Stirling in 2013 was associated with higher near-ground and soil temperature fluctuations (Fig. 7.7).

7.3.1.3 Abiotic effects of snow removal

At Mt Twynam (2014), almost a month separated the earliest and latest snowmelt dates of control plots, with the first plot clear of snow by September 10th and the last by October 7th. Snow removal plots were completely snow free an average five days before control plots, with mean melt-out dates of September 18th and September 23rd, respectively. Snow accumulation and melt were more even across plots at Mt Stirling, with only a week's difference in snowmelt among control plots. Snow removal plots were snow free an average four days before control plots, with mean melt-out dates of September 13th and September 17th, respectively.

At Mt Twynam, experimental snow removal plots were warmer, on average, than control plots during the winter (Fig. 7.9; Appendix F, Table F.8). This effect became more pronounced throughout the winter as subnivean temperatures in control plots gradually decreased and maximum temperatures in snow removal plots increased (Figs. 7.8, 7.9; Table F.8). Snow removal plots also experienced considerably more temperature variation with a mean daily fluctuation of 6.0 ± 7.5°C at the soil surface and 1.8 ± 2.0°C in the ground, while temperatures were diurnally constant in control plots (Fig. 7.8). In addition, while control plots never experienced air temperatures below freezing, the minimum temperature at snow removal plots was below 0°C for 39 days over the course of the winter.

At Mt Stirling, average temperatures were similar for control and snow removal plots. Snowfall soon after sites were cleared meant that differences in temperature regimes between snow removal and control plots were small for most of the winter (Figs. 7.8, 7.9). However, in late winter, snow removal plots showed considerably greater temperature fluctuations (5.1 ± 5.1°C compared to 1.1 ± 2.7°C at control plots) and more days with minimum temperatures below 0°C (10 out of 19 days compared to 3 in control plots) (Figs. 7.8, 7.9). In this period, although mean temperatures were similar among snow removal and control plots, maximum temperatures after snow removal (mean daily maximum in late winter: 4.2 ± 4.1°C) were higher than in control plots (1.3 ± 2.1°C) (Fig. 7.8; Appendix F, Table F.9).

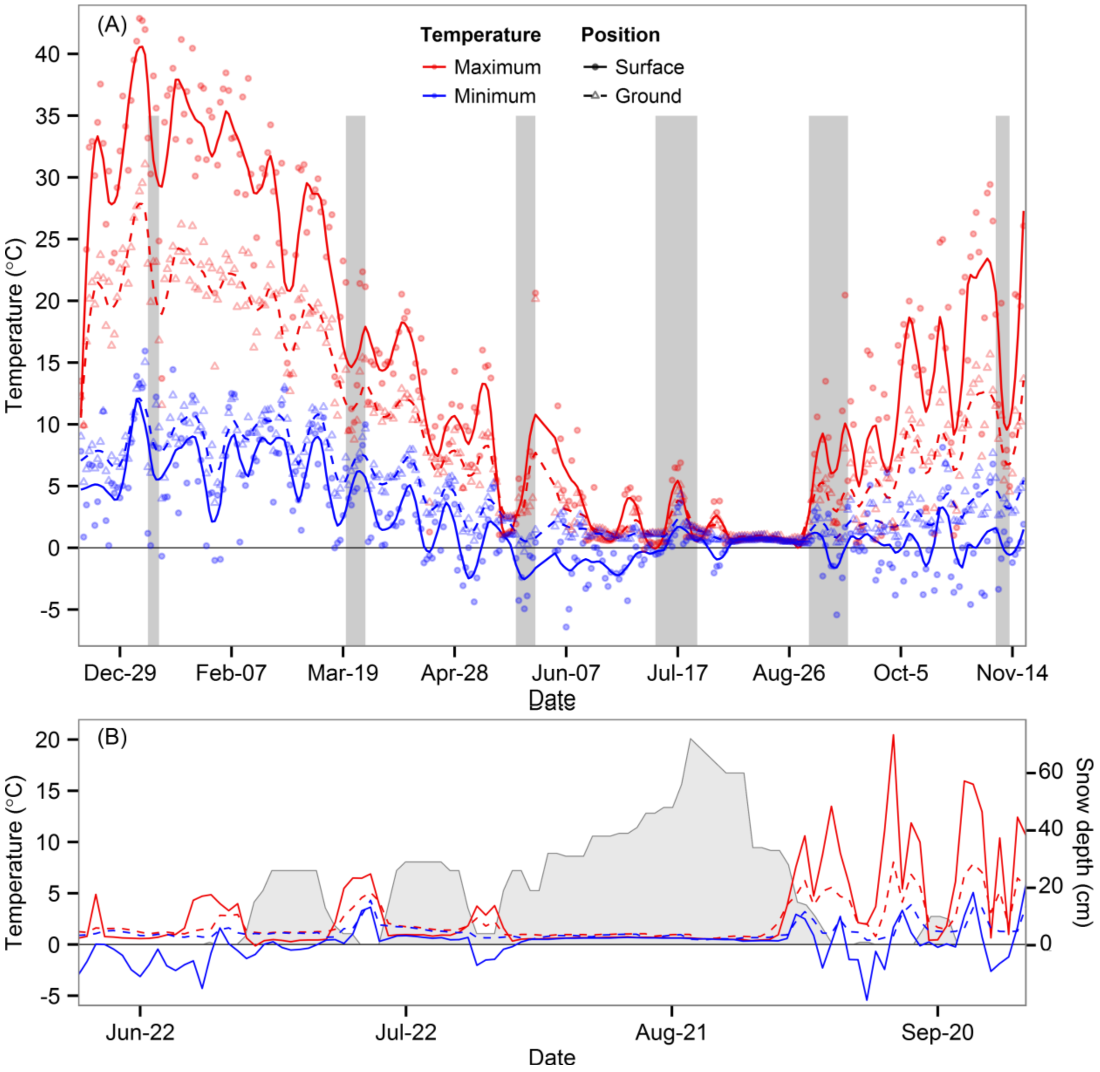

Figure 7.7. Seasonal temperature variation at Mt Stirling in 2013. (A) Daily maximum (red) and minimum (blue) temperatures, averaged across plots, at 1 cm above the soil surface ("surface", solid line, filled circles) and 1 cm below the soil surface ("ground", dashed line, open triangles). A smooth line was fitted to daily data (shown as points) with a locally weighted regression ($\alpha = 0.05$). Grey shading indicates the six trapping periods and the horizontal line is 0°C. (B) Daily maximum (red) and minimum (blue) temperatures over the winter period, and snow depth (grey shading).

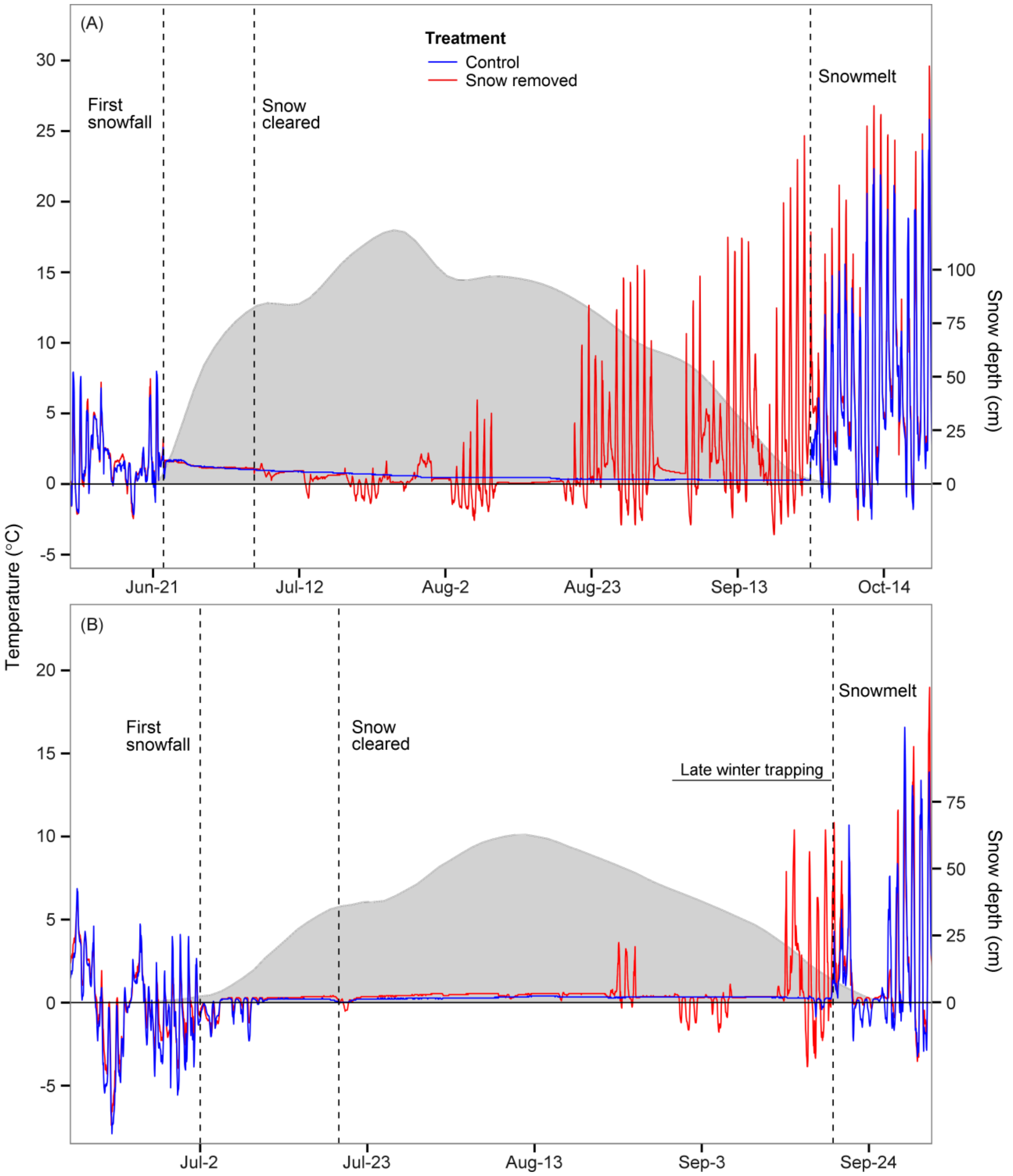

Figure 7.8. The effect of snow removal on surface temperatures at Mt Twynam (A) and Mt Stirling in 2015 (B). Hourly temperatures are shown for a pair of control (blue) and experimental snow removal (red) plots at each site. The horizontal black line is 0°C; vertical dashed lines indicate (from left to right) the date of first snowfall, the date when snow was first cleared, and the date of snowmelt at the control site. Grey shading indicates the snow depth at (A) the Snowy Hydro Deep Creek snow depth gauge, which was close to the average snow depth across plots at the Mt Twynam study site and (B) Mt Stirling upper trail average snow depth. For both, a smooth curve was fitted to weekly (Mt Twynam) or daily (Mt Stirling) snow depth data with a locally weighted regression, α = 0.3). Equivalent figures for the remaining blocks are provided in Appendix F.

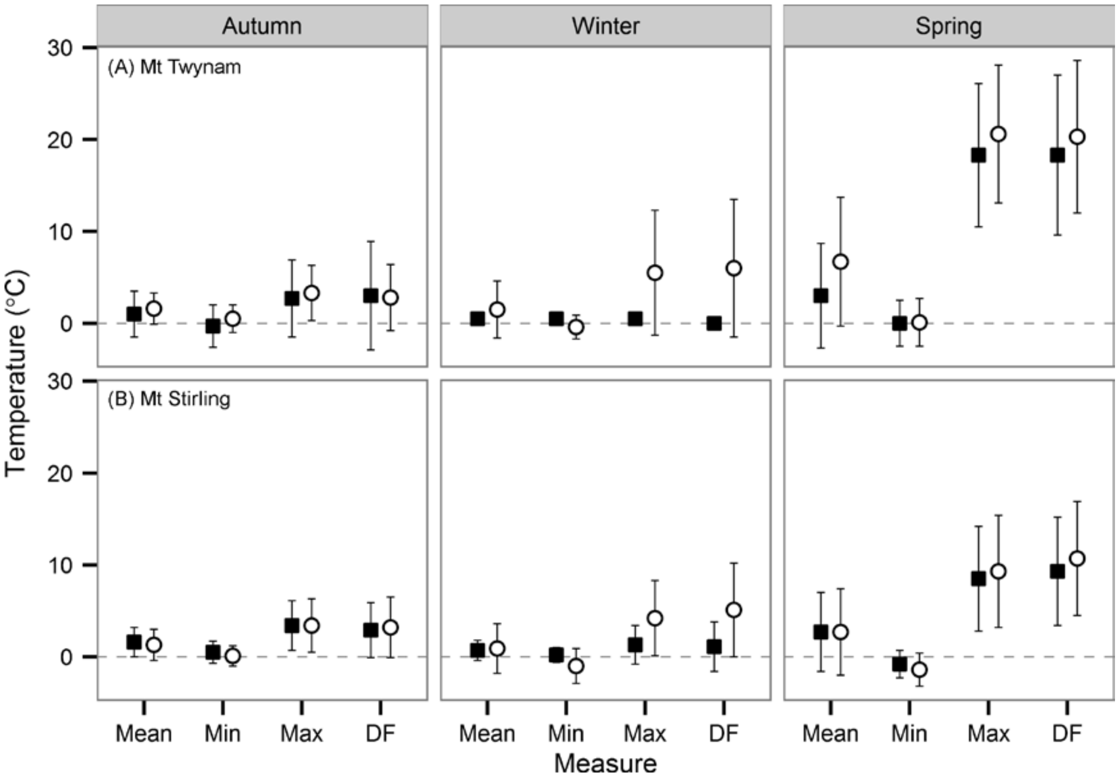

Figure 7.9. Effects of snow removal on overall mean (Mean), mean daily minimum (Min), mean daily maximum (Max), and mean daily temperature fluctuation (DF) of surface temperatures at Mt Twynam (A) and Mt Stirling in 2015 (B). Error bars are ± 1 s.d. Control plots are shown as black squares and experimental snow removal plots are shown as white circles. Winter temperatures in (B) are shown only for late winter (see Appendix F for all summary statistics).

7.3.2 Subnivean communities

Details of the taxa detected in summer and winter at Mt Stirling and Mt Twynam over the three years of the study are provided in Appendix F.

Individuals from 19 major taxa were collected at Mt Stirling over 2013 and 2015. Of these, five groups (Chilopoda, Orthoptera, Thysanoptera, Lepidoptera, Dermaptera) were found only during the summer or when plots were free of snow for at least some of the trapping period. Copepods (Harpacticoidea: Canthocamptidae) were the only group to be found solely during the winter. They were found in a single plot in 2013, where they were highly abundant in both July (*n* = 2848) and September (*n* = 480) but were not detected in any of the other four trapping periods.

For most taxa (and all insects), abundance peaked strongly in either January or November and overall numbers were noticeably reduced during the cooler months, with only 72 individuals (excluding copepods) in all pitfall traps in July in 2013 (Fig 7.10, Appendix F). Notable exceptions were the arachnids (peak abundance in September), Symphypleona (globular springtails; peak abundance in September), and Entomobryomorpha (elongate springtails; peak abundance in March) (Fig. 7.10). One of the strongest seasonal responses was observed in the ants (Hymenoptera: Formicidae, primarily *Iridomyrmex* spp.). Ants were present in high numbers in January (*n* = 969), March (*n* = 278) and November (*n* = 427) and made up 48% and 32% of the pitfall-trapped arthropods in January and March (Fig. 7.11). However, they were almost entirely absent (*n* = 2) between May and September 2013 (Figs. 7.10, 7.11). Ants were similarly scarce in autumn and winter of 2015 with only 24 individuals caught across the 16 plots in May and three individuals detected during winter – all in plots which had been snow free for at least four days (Fig. 7.11). Ants were detected in 15 of 16 plots in the two weeks following snowmelt in 2015.

Individuals from 18 major taxa were collected over the winter and spring at the Mt Twynam study site in 2014 (Appendix F). Of these, only 12 taxa were detected in control plots during the winter and only eight were represented by more than two individuals. The surface activity of ants was sensitive to snow cover: across all fourteen plots, ants were detected in pitfall traps between one and seven days after snowmelt.

Across locations and years, four taxa (springtails, mites, beetles, spiders) accounted for 85 – 99% of the fauna detected in winter pitfall traps (excluding copepods in 2013) (Fig. 7.11).

7.3.2.1 Collembola

A total of 11680 Collembola were caught over the six trapping periods in 2013, and they were the most common group in traps in all months. Individuals from eight families were identified with the highest family diversity (seven families) in January and March (Appendix F, Table F.3). In all months, the fauna was dominated by podurids which accounted for 95% of all of the Collembola caught and 40 – 85% of the overall pitfall-trapped fauna between May and November (Fig. 7.11). In 2015, a similar dominance of podurid forms was apparent: in autumn, individuals of the families Brachystomellidae, Hypogastruridae and Neanuridae made up 91% of the Collembola and over the winter, podurids accounted for 75% – 94% of the Collembola and 62% – 80% of the overall arthropod fauna (Fig. 7.11).

At Mt Twynam in 2014, 12 species from 10 genera and seven families were detected beneath the snow during winter (Appendix F, Table F.3). In contrast to Mt Stirling, the Entomobryomorpha, represented by the families Paronellidae, Isotomidae, Entomobryidae and Tomoceridae, were numerically dominant as well as the most diverse group (seven morphospecies) (Fig. 7.11). In winter, the Collembola fauna was numerically dominated by two morphospecies (genera *Pseudoparonella* & *Paronellides*), which together made up 75% of all Collembola caught. In contrast to most other taxa, which were found in higher numbers (per trap) in spring, *Pseudoparonella* was notably more common in winter (mean 3.1 ± 2.8 individuals/trap-week in control plots in winter, compared to 0.9 ± 1.1 individuals/trap-week in spring).

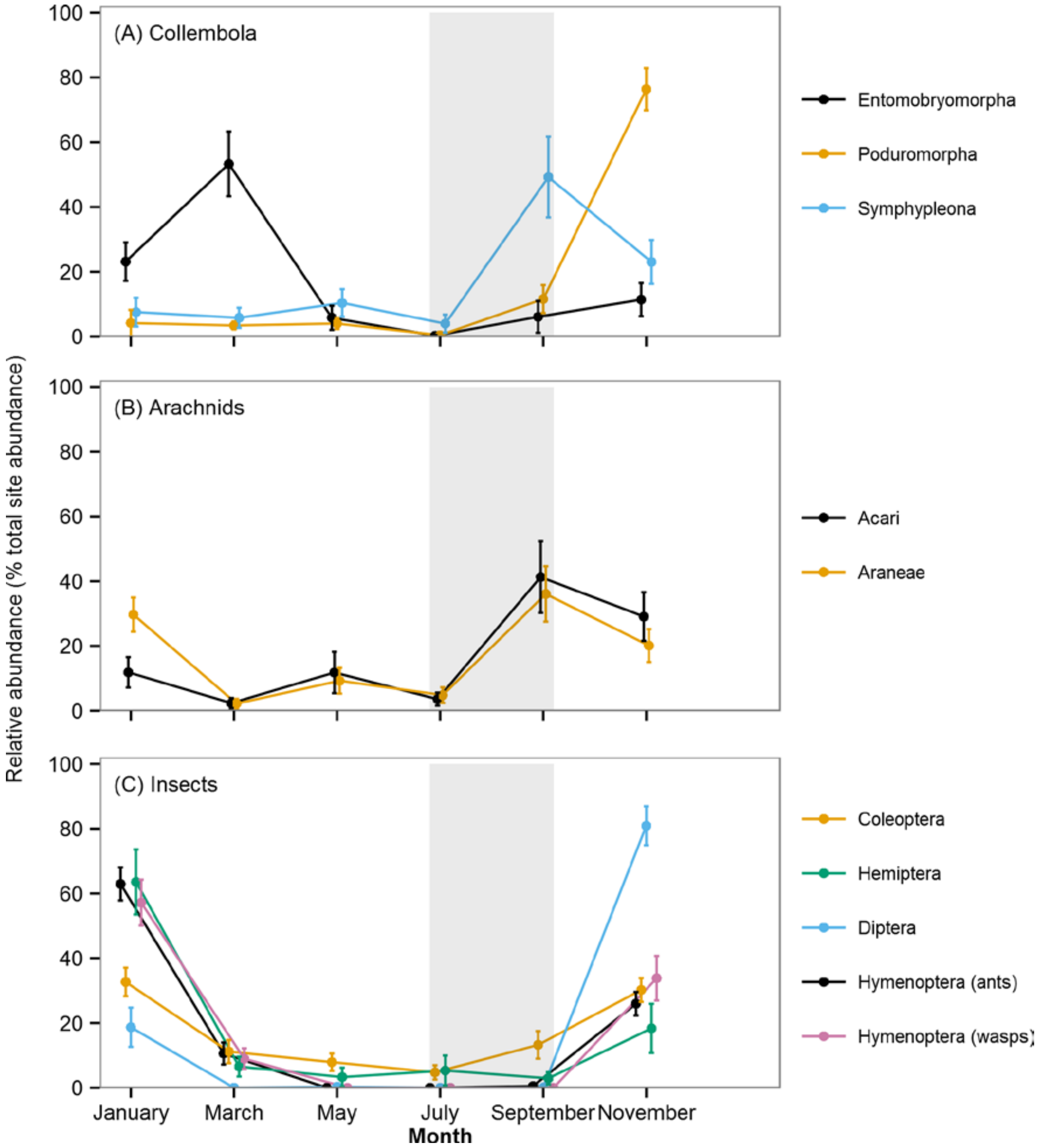

Figure 7.10. Seasonal activity of dominant arthropod taxa at Mt Stirling, 2013. Relative abundance was calculated for each plot as the proportion of individuals in a given taxon that were collected in a given month and thus represents seasonal variation at the plot level. Error bars are ± 1 s.e. Grey shading indicates the period of snow cover. See Appendix F, Fig. F.1 for seasonal activity of taxa not shown here.

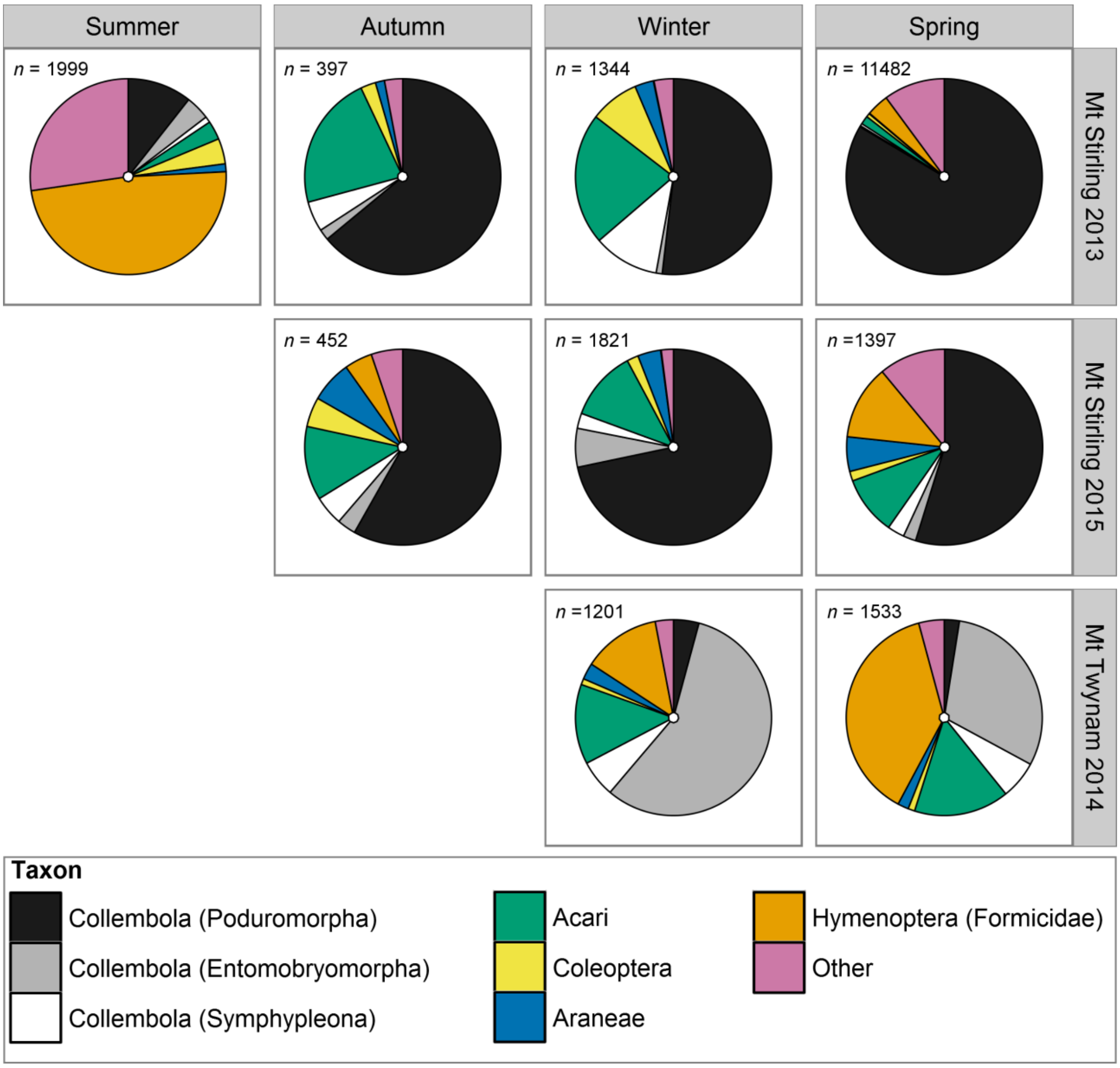

Figure 7.11. The percentage contribution (by numbers) of dominant taxa to the pitfall-trapped arthropod fauna at Mt Stirling and Mt Twynam over the three years of the experiment. Data for Mt Stirling in 2015 and Mt Twynam in 2014 are from control plots only. For Mt Stirling, 2013, summer data are from January (one trapping week), autumn data are from May (two trapping weeks), winter data are pooled for July and September (total six trapping weeks) and spring data are from November (one trapping week). *n* is the total number of individuals collected in each sampling period. Percentage contributions for all taxa are provided in Tables F.4 – F.6. Note that in winter at Mt Twynam in 2014, ants were present in traps only when plots were snow free for some of the trapping period.

7.3.2.2 Coleoptera

Individuals from 19 families were collected at Mt Stirling in 2013 and 2015 (Appendix F, Table F.3). The most common beetles collected in pitfall traps during the snow-free months were from the families Staphylinidae and Curculionidae. The Staphylinidae (three sub-families) were also the most common family represented in subnivean pitfall traps across both years, followed by the Leiodidae (one sub-family) and Carabidae (two sub-families), with a total of nine families detected beneath the snow during at least one trapping period across the two years. While all these families were also found during the snow-free period, the Leiodidae were interesting in that individuals were only present in traps in the colder months (May to September). One new species from an undescribed genus (family Cryptophagidae, tribe Picrotini) was collected in September and November, 2013, and September 2015 (R Leschen, personal communications).

Beetles were uncommon in the Mt Twynam plots, with a total of 41 individuals trapped across both winter and spring periods. 10 families were detected beneath the snow during the winter (Appendix F, Table F.3) but only five (in order of abundance: Curculionidae, Carabidae, Staphylinidae, Latridiidae, Phalacridae) had more than one individual. Over the spring trapping period, only four Families (Carabidae, Staphylinidae, Curculionidae, Melyridae) were observed.

7.3.2.3 Araneae

Spiders from 14 families were detected at Mt Stirling across the two years, but individuals from only six families were found in subnivean traps (Appendix F, Table F.3). Of these, the Amphinectidae (two morphospecies), Micropholcommatidae (one morphospecies, genus *Raveniella*), and Amaurobiidae were the most numerous. In contrast, the Linyphiidae was the most common family in pitfall traps during snow-free periods in both years.

Individuals from seven spider families were found in subnivean pitfall traps during the winter at Mt Twynam (Appendix F, Table F.3). In contrast to Mt Stirling, the Linyphiidae was the most common group in subnivean pitfall traps (18 individuals in winter), followed by the Amaurobiidae (six individuals).

7.3.3 Effects of reduced snow on subnivean invertebrates

7.3.3.1 Mt Twynam

The ordination analysis for winter trapping with three dimensions had a stress value of 0.05, indicating a good representation of the data (Fig. 7.12). Axis scores for individual taxa are shown in Table 7.2. There was a significant effect of treatment on community composition along axis 2 of the NMDS (t = -3.84, p = 0.026 after Bonferroni adjustment for multiple comparisons, Fig. 7.12). Two morphospecies in the genera *Pseudoparonella* and *Paronellides* (Collembola: Paronellidae) accounted for 46% of all the individuals caught, and 70% of the springtails. Both taxa were strongly affected by the snow removal treatment: snow removal plots had 95 ± 4% fewer individuals of *Pseudoparonella* (Wilcoxon test: p = 0.016) and 91% ± 11.7% fewer individuals of *Paronellides* than control plots (p = 0.016) (Fig. 7.13).

Overall, PERMANOVA revealed a significant treatment effect on community composition in winter (Pseudo-F = 3.13, p = 0.002, df = 1, 6; Table 7.3), with treatment explaining 21% of the variation in community composition among plots. Communities from snow removal plots were also more variable (i.e. had higher beta diversity) than those from control plots (Fig. 7.12; $F_{1,12}$ = 7.87, p = 0.019).

In spring, treatments were not separated along any of the NMDS axes (axis 1: t = 0.94, p = 0.392, axis 2: t = -0.99, p = 0.369, axis 3: t = -0.84, p = 0.440). The visual overlap of treatments in the NMDS ordination (Fig. 7.12) was confirmed with PERMANOVA, which showed no difference in community composition among treatments (Pseudo-F = 1.05, p = 0.373, df = 1, 5; Table 7.3). Instead, there was a weak tendency for plots within a pair to share similar community compositions, with block explaining 58% of the variation in communities in spring (Table 7.3). Compared to winter, there was considerably more variation in treatment effects on *Pseudoparonella* and *Paronellides* in spring, and the difference in the number of individuals caught was not statistically significant for either taxon (Fig. 7.13: *Pseudoparonella*: t = 0.91, p = 0.407; *Paronellides*: t = 1.32, p = 0.245). In addition, and in contrast to winter, there was no detectable difference in dispersion among treatment groups ($F_{1,10}$ = 0.25, p = 0.630).

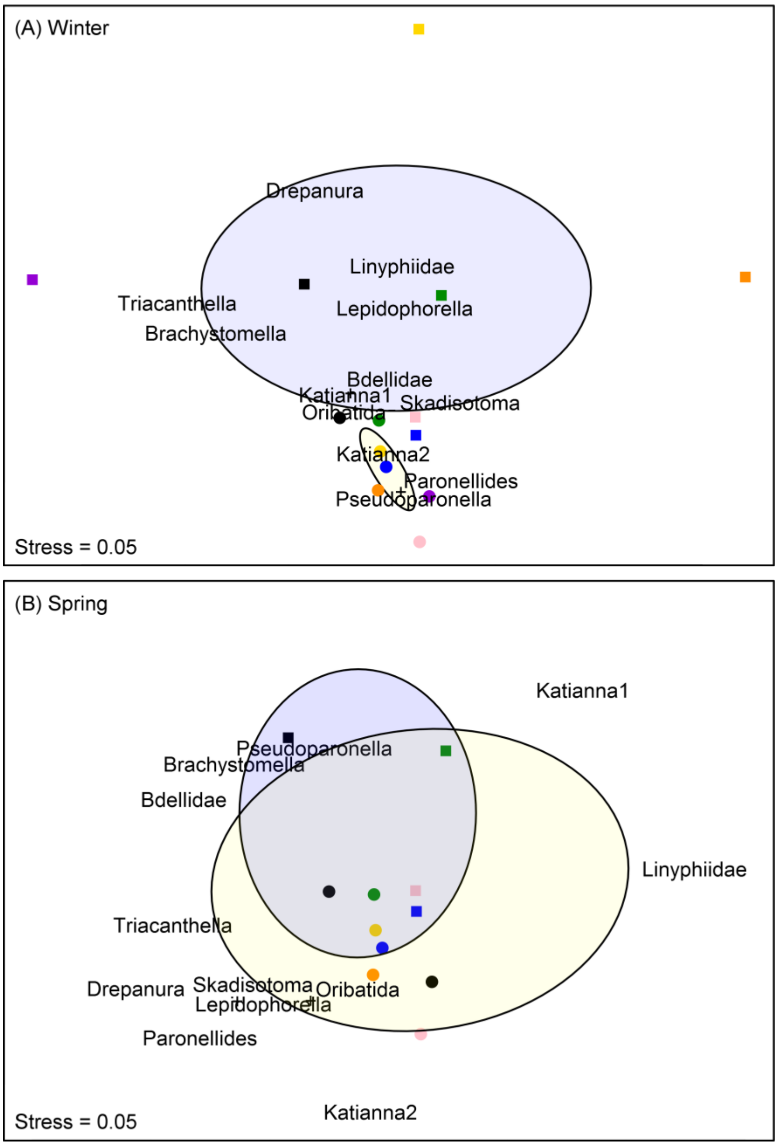

Figure 7.12. Non-metric multidimensional scaling ordination of 12 arthropod taxa sampled in (A) winter, from seven experimental snow removal plots (squares) and seven paired control plots (circles), and (B) spring, from six experimental snow removal plots (squares) and seven paired control plots (circles), at Mt Twynam in 2014. Different colours represent different blocks and ellipses show the 95% confidence limits around the centroids for the two treatments (blue: snow removal, yellow: control). The first two axes (of three) are shown.

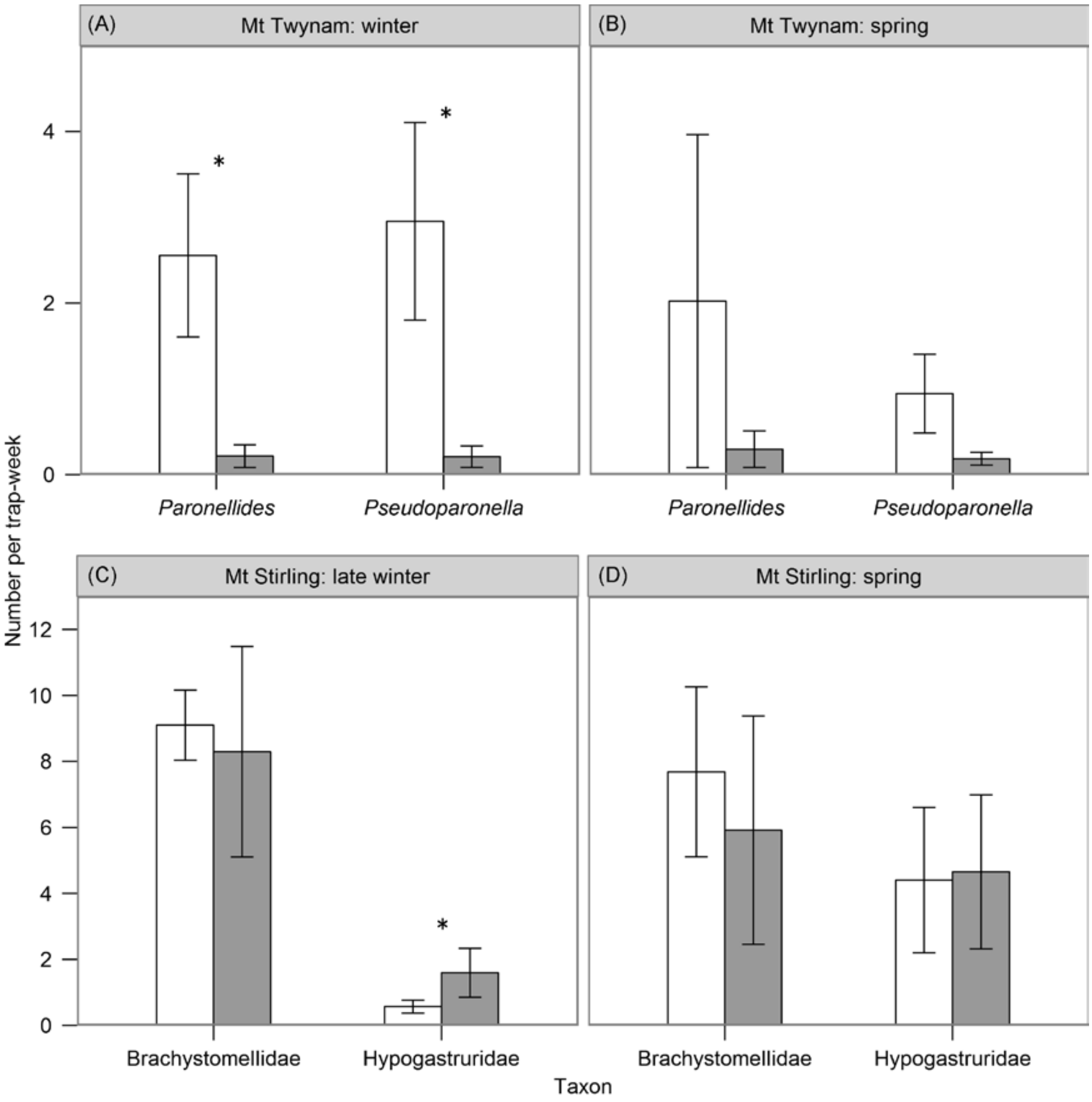

Figure 7.13. Mean (± s.e.) number of individuals per trap-week for the two most common taxa at Mt Twynam (A, B) and Mt Stirling (C, D). Control plots are shown in white and experimental snow removal plots are in grey. Significant differences (Wilcoxon test, $p < 0.05$) among treatments within a site/sampling period are indicated by a star. Results for other taxa are in Appendix F.

Table 7.2. Non-metric multidimensional scaling axis scores for major arthropod taxa sampled from experimental snow removal and control plots in winter (n = 14) and spring (n = 12) at Mt Twynam. Treatment effects on community composition were associated with axis 2 in winter (see Results).

	Winter			**Spring**		
Taxon	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 1**	**Axis 2**	**Axis 3**
Collembola						
Paronellides	0.04	-0.55	-0.12	-0.80	-0.80	-0.27
Pseudoparonella	0.11	-0.59	-0.10	-0.31	0.41	-0.27
Skadisotoma	0.33	-0.11	-0.13	-0.64	-0.65	0.23
Lepidophorella	0.06	0.33	-0.59	0.53	-0.67	0.34
Drepanura	-0.37	0.90	-0.53	-1.06	-0.61	-0.57
Katianna sp. 1	-0.22	-0.08	0.32	0.82	0.66	-0.37
Katianna sp. 2	-0.04	-0.36	-0.88	-0.08	-1.12	1.09
Triacanthella	-1.04	0.36	0.24	-0.91	-0.33	-0.19
Brachystomella	-0.85	0.21	-0.21	-0.65	0.34	0.48
Acarina						
Bdellidae	-0.20	-0.07	0.04	-0.86	0.20	-0.56
Oribatida	-0.20	-0.16	-0.56	-0.33	-0.65	-0.06
Araneae						
Linyphiidae	0.05	0.54	0.98	1.29	-0.10	-0.03

Table 7.3. Results from permutational analysis of variance testing the effects of experimental snow removal (treatment) on arthropod community composition at Mt Twynam in 2014 during the winter and spring (after snowmelt). As plots were arranged in treatment-control pairs, a block factor was also included in the analysis.

Season/effect	df	SS	MSS	Pseudo-*F*	*p*
Winter					
Block	6	1.24	0.21	1.01	0.486
Treatment	1	0.64	0.64	3.13	**0.010**
Residual	6	1.23	0.21		
Total	13	3.12			
Spring					
Block	5	1.85	0.37	1.68	0.087
Treatment	1	0.23	0.23	1.05	0.373
Residual	5	1.10	0.22		
Total	11	3.19			

7.3.3.2 Mt Stirling

In autumn, prior to the experimental treatment, plots assigned to the two treatment groups showed similar community compositions (PERMANOVA: Pseudo-$F = 1.11$, $p = 0.351$; Table 7.4, Fig. 7.14) and beta diversity ($F_{1,14} = 0.69$, $p = 0.418$).

Ordination showed considerable overlap among treatments over the winter period as a whole (Fig. 7.14, and see Table 7.5 for axis scores for individual taxa). This was confirmed by the PERMANOVA, which indicated no significant treatment effect on community composition during the winter (Table 7.4). There was, however, a strong interaction between treatment and trapping period (Table 7.4). When the analysis was restricted to the late winter, in which the treatment effect on abiotic conditions was strongest (Fig. 7.8), there was a significant treatment effect along axis 2 of the NMDS ($t = -4.86$, $p = 0.001$; Fig. 7.14, Table 7.6). Although PERMANOVA suggested that the effects of snow removal on community composition were not statistically significant (Pseudo-$F = 3.36$, $p = 0.053$, df = 1, 15), treatment accounted for 16.9% of variation among sites (Table 7.4). In contrast to Mt Twynam snow removal did not influence beta diversity, when considering either the whole winter ($F_{1,46} = 0.503$, $p = 0.526$) or the late winter only ($F_{1,14} = 3.31$, $p = 0.105$). As at Mt Twynam, treatment effects did not carry over into the spring (Fig. 7.14, Table 7.4).

The number of individuals of Brachystomellidae and Hypogastruridae were similar among treatments when considering the winter as a whole (Table 7.7). However, during the late winter, the Hypogastruridae were significantly more numerous in snow removal plots (14.38 ± 18.8 individuals per plot) than in control plots (5.12 ± 4.97; Wilcoxon paired test: $W = 34.5$, $p = 0.025$) (Fig. 7.13). In spring, there was no difference in the numbers of either taxon among treatment (Brachystomellidae: $W = 25$, $p = 0.383$; Hypogastruridae: $W = 14$, $p = 0.622$).

In autumn and winter, there was a strong tendency for plots within a block to share similar community compositions, with block accounting for most (69% and 63%, respectively) of the variation among plots (Table 7.4).

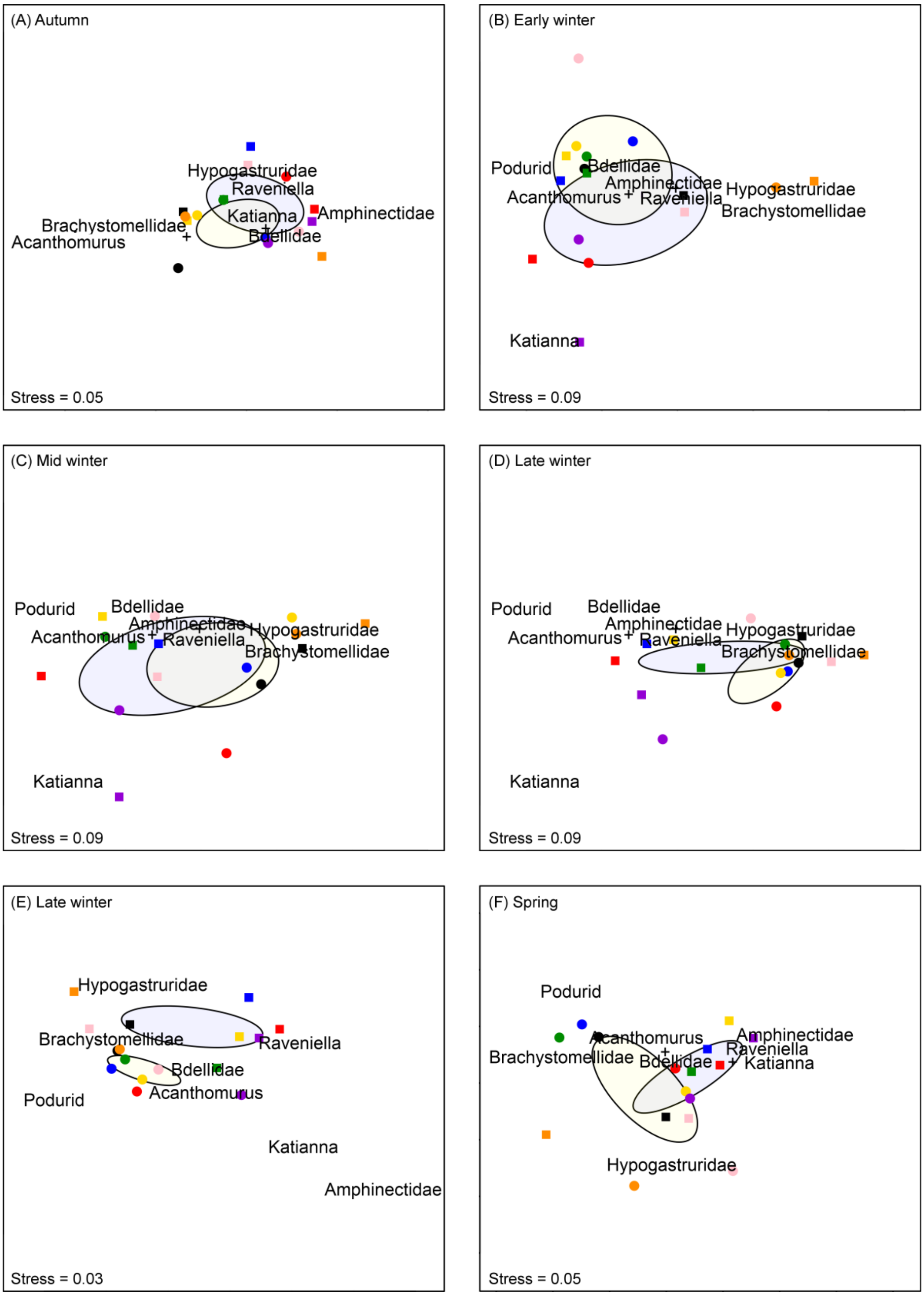

Figure 7.14. Non-metric multidimensional scaling ordination of eight arthropod taxa sampled in (A) autumn, (B – E) winter and (F) spring from eight experimental snow removal plots (squares) and paired control plots (circles) at Mt Stirling in 2015. Different coloured points represent different blocks and ellipses show the 95% confidence limits around the centroids for the two treatments (blue: snow removal, yellow: control). The winter was divided into three trapping periods, each of three weeks and a single ordination was run for the whole winter. In B – D, trapping periods are split for visualisation only. The late winter trapping period was also tested separately (E). The first two axes (of three) are shown.

Table 7.4. Results from permutational analysis of variance testing the effects of experimental snow removal (treatment) on arthropod community composition at Mt Stirling in 2015 during autumn, winter, and spring (after snowmelt). The analysis across the whole winter period (three trapping periods) was carried out in two stages to test (1) the effects of treatment and block and (2) the effects of time and a treatment-time interaction.

Season/effect	df	SS	MSS	Pseudo-*F*	*p*
Autumn					
Block	7	1.43	0.20	2.00	0.054
Treatment	1	0.11	0.11	1.11	0.351
Residual	7	0.72	0.10		
Total	15	2.26			
All winter					
Block	7	1.06	0.15	2.07	**0.018**
Treatment	1	0.10	0.10	1.30	0.255
Residual	7	0.51	0.07		
Total	15	1.67			
Time	2	1.39	0.69	8.86	**0.001**
Treatment:time	2	0.35	0.17	2.23	**0.002**
(Block:treatment)	15				
Residuals	28	2.19	0.08		
Total	47	8.25			
Late winter					
Block	7	0.85	0.12	1.37	0.223
Treatment	1	0.30	0.30	3.36	**0.053**
Residual	7	0.62	0.09		
Total	15	1.78			
Spring					
Block	7	1.41	0.20	1.67	0.098
Treatment	1	0.20	0.20	1.67	1.172
Residual	7	0.84	0.12		
Total	15	2.46			

Table 7.5. Non-metric multidimensional scaling axis scores for major arthropod taxa sampled from experimental snow removal and control plots (*n* = 16) at Mt Stirling in 2015. Results are shown for the analysis with data from all three winter trapping periods and for late winter alone. Treatment effects on community composition were associated with axis 2 in late winter (see Results).

	Whole winter			**Late winter**		
Taxon	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 1**	**Axis 2**	**Axis 3**
Collembola						
Brachystomellidae	0.77	0.01	0.11	-0.46	0.06	-0.11
Hypogastruridae	0.75	0.15	0.02	-0.20	0.50	0.57
Podurid	-1.03	0.30	-1.19	-0.92	-0.42	-0.04
Acanthomurus	-0.32	0.13	0.22	0.31	-0.36	0.11
Katianna	-0.88	-0.84	0.07	1.09	-0.79	-0.43
Acarina						
Bdellidae	-0.35	0.32	-0.13	0.32	-0.17	0.29
Araneae						
Amphinectidae	-0.09	0.19	-0.37	1.74	-1.15	-0.74
Raveniella	-0.01	0.17	-0.54	1.07	0.05	0.70

Table 7.6. Test of the effect of experimental snow removal treatment on winter community composition at Mt Stirling along the three NMDS axes. Models included the fixed effects of treatment (two levels) and time (three levels) and random effects of block and plot. Bootstrapped *p*-values (1000 simulations) were obtained by comparing likelihood ratios from nested reduced models.

Axis/effect	***F***	**df**	***p***
Axis 1			
Treatment	0.72	1, 7	0.399
Time	36.11	2, 30	< **0.001**
Treatment:time	2.37	2, 28	0.116
Axis 2			
Treatment	0.48	1, 7	0.490
Time	8.73	2, 30	**0.003**
Treatment:time	5.12	2, 28	**0.020**
Axis 3			
Treatment	0.57	1, 7	0.427
Time	0.35	2, 30	0.718
Treatment:time	0.69	2, 28	0.518

Table 7.7. Results from generalised linear mixed models testing the effect of experimental snow removal treatment on the number of individuals of Hypogastruridae and Brachystomellidae (Collembola) found in traps during winter. Models included the fixed effects of treatment (two levels) and time (three levels) and random effects of block and plot. Significance was tested using likelihood ratio tests comparing nested reduced models.

Taxon/effect	***F* (full model)**	**df (full model)**	***p***
Hypogastruridae			
Treatment	1.44	1, 7	0.287
Time	3.52	2, 30	0.068
Treatment:time	1.85	2, 28	0.181
Brachystomellidae			
Treatment	0.69	1, 7	0.480
Time	40.04	2, 30	< **0.001**
Treatment:time	3.43	2, 28	**0.044**

7.4 Discussion

Winter snow is a defining feature of alpine ecosystems. The depth and duration of snow cover are, however, declining in many mountain systems with projections for this trend to continue over the coming decades (Räisänen 2008; Brown & Mote 2009). In spite of these predictions, tests of ecological responses to reduced snow cover have rarely included measures of invertebrate responses (but see Roy *et al.* 2004; Adler *et al.* 2007; Leingärtner *et al.* 2014), nor been performed in the southern hemisphere where snow cover is typically less reliable and physiological traits that might underlie species-level responses (e.g. cold/freeze tolerance) could differ (Sinclair & Chown 2005; Bannister 2007; Venn *et al.* 2012). In this study, I found a winter-active subnivean fauna characterised by four arthropod Orders that was consistent across mountains and years. Removal of the snow layer caused significant shifts in community composition, driven by changes in the activity/abundance of three numerically-dominant springtail taxa.

7.4.1 Does Australia have any snow specialists?

The subnivean space is unique for the spatial and temporal homogeneity of abiotic conditions and contrasts sharply with the high variability characteristic of alpine environments during the summer (Geiger *et al.* 1995; Wundram *et al.* 2010; Scherrer & Körner 2011; this study). Springtails (Collembola), mites (Acari), spiders (Araneae) and beetles (Coleoptera) are the most common winter-active arthropods in seasonally snow-covered environments worldwide (Holmquist 1926; Chapman 1954; Aitchison 1978, 1979a, b, c; Green 1997; Vanin & Turchetto 2007; Hågvar & Hågvar 2011) and the results of the current study suggest that Australia is no exception. Although most Orders detected during the summer were also detected in subnivean pitfall traps, the high relative abundance of these four taxa defines a characteristic winter community. Trapping throughout the year at Mt Stirling indicated that this high relative abundance was due primarily to the strong seasonal decline in the numbers of insects.

Following two years of subnivean pitfall trapping at South Ramshead, in New South Wales, Green (1997) noted the absence of winter specialist taxa, with the most common species in summer also being among the most common in winter. Here I found some evidence for three species specialised to the cooler months. One species of springtail (genus *Pseudoparonella*), from Mt Twynam, was found in traps in considerably higher numbers in winter than in spring. This was true even when comparisons were restricted to the three weeks before and after snowmelt, suggesting that this result is unlikely to be simply an artefact of the more limited spring sampling. One leiodid morphospecies (Coleoptera: Leiodidae: Cholevinae) was among the most common beetles at Mt Stirling in both years but was detected only during the cooler months (May – September). Although replication across more years is needed to confirm this result, several species in this sub-family from boreal and temperate regions in the northern hemisphere are cold season specialists, with reproduction and development occurring primarily during autumn and winter and the warmer months spent in dormancy (Topp 1994).

The third species that appeared to be winter-specialised was a copepod (Copepoda: Harpacticoidea: Canthocamptidae), which was highly abundant at a single plot in July and September 2013 but not detected in any other trapping period. This and previous studies have shown that Australian subnivean soil and air temperatures are above freezing for the entire winter, which will cause almost continuous snowmelt from the base of the snowpack (Campbell *et al.* 1986; Sanecki *et al.* 2006). As a consequence, an “aquatic” habitat might exist under the snow in some places which are dry at other times of the year, and which can be utilised by aquatic organisms during the winter (Campbell *et al.* 1986). Indeed, Campbell *et al.* (1986) note that aquatic insects such as mayfly (Ephemeroptera) nymphs have been collected beneath the snow at some distance from standing water bodies. The plot in which copepods were detected was waterlogged for much of the winter; this is likely a result of the aforementioned basal melting of the snowpack as well as the position of the plot in a shallow run-off channel.

In the Australian alpine region, areas of late-lying snow are associated with distinct vegetation communities, even though the overwhelming majority of species also occur outside snowpatches (McDougall & Walsh 2007; Williams *et al.* 2015b). I suggest that there is a similarly distinct subnivean arthropod community, characterised by the disproportionate dominance of four taxa and the presence of species that appear, at this stage, to be cold season specialists.

7.4.2 Ecological effects of reduced snow

Alpine biota are uniquely adapted to winters beneath the snowpack and, among plants, experimental manipulations of snow regimes can have rapid and pronounced effects on community structure (e.g. Galen & Stanton 1995; Gerdol *et al.* 2013; Mark *et al.* 2015). Changes in ground cover that affect microclimate strongly influence both individual taxa and invertebrate communities in many environments (e.g. Molenda *et al.* 2012; Norton *et al.* 2014), but the effects of changing snow cover on alpine arthropod communities have not, to the best of my knowledge, been tested previously. Over the two years of snow manipulation, I found significant short-term effects of snow removal on arthropod communities at a small spatial scale. When considering taxa in isolation, there was a strong negative effect of snow removal on two taxa (morphospecies, Collembola: Paronellidae) at Mt Twynam, but a positive effect on one taxon (Collembola: Hypogastruridae) at Mt Stirling.

In cold climates, increasing mean temperatures should intuitively favour ectotherms by reducing cold stress (e.g. Crozier 2004; Deutsch *et al.* 2008). However, higher rates of energy consumption and thermal fluctuations can also entail considerable fitness cost (e.g. Irwin & Lee 2003; Vasseur *et al.* 2014). Removal of the insulating snow layer increases temperature variability and, in the Australian mountains, also increases mean temperature; the expected direction of effects is thus unclear. With the experimental design used here, I was not able to identify the mechanisms underlying community shifts and taxon-specific responses to snow removal. These mechanisms could include movement of individuals in to/out of the study plots if snow-free microclimates are more/less favourable (Hågvar 1995; Hayward *et al.* 2001; Hugo *et al.* 2004), or changes in activity related to short-term temperature fluctuations and/or food availability (Huey & Stevenson 1979; Hugo *et al.* 2004; Konestabo *et al.* 2007; Hågvar & Klanderud 2009; Tan *et al.* 2014; Vasseur *et al.* 2014). Alternatively, the observed effects could be due to local mortality (from either single extreme events or accumulated sub-lethal injury) or recruitment (Ruel & Ayres 1999; Coulson *et al.* 2000; Sulkava & Huhta 2003; Marshall & Sinclair 2012b; Williams *et al.* 2012a; Colinet *et al.* 2015). Regardless of the mechanism, however, snow removal appears to create a less suitable environment for some taxa (i.e. *Pseudoparonella* & *Paronellides* at Mt Twynam) and a more suitable environment for others (i.e. Hypogastruridae at Mt Stirling). Determining the functional roles of these taxa in the ecosystem and the consistency in their responses across years is therefore an important area for future research.

7.4.2.1 Phenology as an underlying mechanism

Among snow manipulation experiments from the northern hemisphere, a shift in phenology is the strongest and most consistent response of plants to snow removal and earlier snowmelt (Wipf & Rixen 2010). Snowmelt defines the beginning of the alpine growing season (Körner 2003). In this environment, where the period available for growth is limited, the phenology of many taxa – both plants and animals – are linked with snowmelt timing or the accumulation of heat units (“degree days”) (Inouye 2008; e.g.

Forrest & Thomson 2011; Cornelius *et al.* 2013; Iler *et al.* 2013; Leingärtner *et al.* 2014) (Høye & Forchhammer 2008; but see Legault & Weis 2013).

At Mt Twynam, *Pseudoparonella* were three times more common winter than in spring in control plots. By contrast, at Mt Stirling, the Hypogastruridae were six times more common in spring than winter in control plots. If these patterns are indicative of seasonal changes in abundance/activity, then the earlier arrival of "spring" conditions in snow removal plots provides a plausible explanation for the responses to snow removal observed for both taxa: mirroring the winter-spring transition, numbers of *Pseudoparonella* were significantly reduced in winter snow removal plots compared to controls, while the Hypogastruridae were detected in higher numbers in snow removal plots.

7.4.2.2 Springtails as sensitive indicators

Springtails are abundant in alpine habitats worldwide (Mani 1968) and play an important functional role in both above- and below-ground processes including plant litter decomposition, formation of the soil microstructure, and as prey for larger arthropods such as spiders, ants, and many beetles (Hopkin 1997; Rusek 1998). That they were also the most strongly affected by snow manipulations could be due to at least three possible (and not mutually exclusive) reasons. First, springtails are typically sensitive to temperature and desiccation stress (e.g. Mani 1962; Worland & Block 1986; Harrisson *et al.* 1991; Hodkinson *et al.* 1996; Hertzberg & Leinaas 1998; Hayward *et al.* 2001) and the stronger effects of snow removal on these taxa might therefore indicate a greater susceptibility to changing microclimates (Convey *et al.* 2003; Greenslade 2013). Second, the experimental plots were small. Although local site effects can have a strong influence on community composition (e.g. Coulson *et al.* 2003; Hugo *et al.* 2004; Nash *et al.* 2013), larger taxa such as spiders and beetles might be less influenced by conditions at this scale. Finally, the numbers of most taxa were very low during the winter and a lack of observed treatment effects might simply be a statistical artefact. To disentangle these possibilities, greater sampling and experimental tests of the mechanistic basis behind responses to snowpack changes (e.g. measures of physiological tolerances) are needed.

7.4.3 Abiotic effects of reduced snow in an Australian context

Mean global temperatures have increased by 0.7°C over the last 50 years (Stocker *et al.* 2013). Whereas most terrestrial ecosystems experience the effects of climate change directly, in alpine environments, changes in winter precipitation and temperature regimes are mediated by the snowpack (Geiger *et al.* 1995). Subnivean temperatures at Mt Stirling and Mt Twynam were just above 0°C, matching the results of Sanecki *et al.* (2006) who recorded subnivean surface temperatures of 0.4°C to 0.8°C at locations between 1500 m and 1800 m near Mt Koscisuzko, NSW.

Removal of the insulating snow layer completely alters the thermal regime of the ground surface and top layers of the soil (e.g. Groffman *et al.* 2011; this study). In most areas that receive winter snow, including arctic, sub-arctic, boreal and alpine environments, a reduction in snow cover below the hiemal threshold leads to increases in soil freezing and, on average, colder soil and surface temperatures (e.g. Groffman *et al.* 2001; Appendix F; Decker *et al.* 2003; Austnes *et al.* 2008; Cleavitt *et al.* 2008; Pauli *et al.* 2013; Vankoughnett & Henry 2013; Tan *et al.* 2014; Petty *et al.* 2015). Logically, this situation should always occur where mean winter temperatures are below 0°C. Except at the highest elevations in the Australia alpine region, winter temperatures are frequently at or above this threshold (Sanecki *et al.* 2006), especially late in the season (BOM 2015). As a consequence, and in contrast to comparable studies from the northern hemisphere (Appendix F, Table F.2), the effect of snow removal at both Mt Twynam and (to a lesser extent) Mt Stirling was to increase mean temperature. This effect was most pronounced late in the winter, which is notable because it is in this part of the season that declines in the snowpack due to climate change are likely to be strongest and evident earliest (Edwards *et al.* 2007; Räisänen 2008; Brown & Mote 2009; Hendrikx *et al.* 2012).

If the physical effects of reduced snow cover vary among systems, as suggested by the above results, then inferences regarding the ecological effects will necessarily be region-specific. Generalisation of results among mountain systems, or between mountain and other snow-mediated environments (e.g. polar regions) should therefore be done with care and with reference to the snowpack and underlying temperature regimes. In this regard, it is important to consider the ecological or biological response being measured. Those that are mediated primarily by temperature extremes (e.g. frost damage: Wipf *et al.* 2009; Gerdol *et al.* 2013) or snowmelt timing (e.g. phenology of early-developing species: Steltzer *et al.* 2009; Wipf 2010; Cornelius *et al.* 2013) might be more comparable across geographic regions than those influenced by temperature averages or accumulated degree days (e.g. individual/population growth, phenology of late-developing species: Bale *et al.* 2002; Adler *et al.* 2007; Wipf 2010). These considerations are particularly important in light of the geographic bias in snow manipulation studies to-date (Appendix F, Table F.1).

7.4.4 Detecting and predicting responses to reduced snow

Several experimental methods can be used to reduce snow cover, including external heating (e.g. Adler *et al.* 2007), soil heating (e.g. Bokhorst *et al.* 2012), the addition of material that increases albedo and facilitates snowmelt (e.g. Steltzer *et al.* 2009), and physical covering to prevent snow accumulation (e.g. Drescher & Thomas 2013), as well as manual snow removal as used here (see also, e.g. Bombonato & Gerdol 2012). Each of these methods has limitations. I chose to use manual snow removal as it was less intrusive than construction of a covering apparatus or heating mechanism and also avoided the confounding effects that artificial heating produces. However, this method suffers from confounding the effects of reduced snow cover on abiotic conditions with disturbance. This is a particular issue for small arthropods that might inhabit the snow layer itself (Leinaas 1981). The results from Mt Stirling in 2015 in early/mid winter suggest that the disturbance alone is, however, unable to account for community shifts: when snow was cleared (i.e. inducing a disturbance) but re-accumulated rapidly such that microclimates were similar between treatments, there was no effect of treatment on community composition. It was only when snow removal induced differences in microclimate that effects became apparent.

Natural snowmelt gradients offer a complementary field-based approach to studying the effects of snow depth and duration on alpine ecosystems. In contrast to experimental snow removal which is logistically limited in scale, these studies allow assessments of large-scale and long-term effects of growing season duration and winter thermal regimes on community composition and functional traits (e.g. Galen & Stanton 1995; Dunne *et al.* 2003; Shimono & Kudo 2003; Dollery *et al.* 2006; Hülber *et al.* 2006; Baptist & Choler 2008; Hülber *et al.* 2011; Vrba 2012; Briceño *et al.* 2014). At the other end of the spectrum, laboratory tests of physiological tolerances and thresholds (e.g. for activity, mortality) provide a means of testing the mechanisms behind species-specific responses to snow manipulation (e.g. Marshall & Sinclair 2015). A combination of these three methods is needed if we are to understand and predict the effects of landscape-scale variation in snow conditions on individual species and on alpine communities as a whole.

7.4.5 Final notes on Australia's subnivean invertebrates

Despite a similarity in order-level community composition among the study sites, the arthropod communities at Mt Stirling and Mt Twynam differed considerably at lower taxonomic levels. These differences were most apparent within the springtails: podurid forms (primarily families Brachystomellidae and Hypogastruridae) were by far the most numerous in traps at Mt Stirling whereas elongate forms (primarily Paronellidae) were numerically dominant at Mt Twynam. It is unclear how much of this observed variation is due to geographic (local/regional) or temporal effects. At South Ramshead, roughly 15 km from the Mt Twynam study site, Green (1988) found a dominance of globular springtails in one year and podurids in the family Neanuridae the following year; year-to-year variation can clearly be substantial. Similarly, in the Bogong High Plains, Victoria, summer springtail assemblages vary considerably among years, but also across small (< 10 km) spatial scales.

In addition to the springtails, clear differences in the most common spider families were apparent among sites. The Linyphiidae was the most common family in traps at Mt Twynam, and in summer at Mt Stirling, and was the second most common family across the whole year at South Ramshead in the study by Green (1997). This family is common both in the subnivean space and on the snow surface in Finland (Huhta & Viramo 1979), Canada (Aitchison 1984), Norway (Hågvar & Hågvar 2011), and in the mountains of Italy (Vanin & Turchetto 2007). Linyphiids were, however, uncommon over the winter at Mt Stirling, where the spider fauna was dominated by amphinectids (two morphospecies) and one micropholcommatid (genus *Raveniella*). The Micropholcommatidae is a family of minute leaf litter- and moss-dwelling spiders, which has its greatest diversity in south-eastern Australia and New Zealand and is notable because it contains a putative snowpatch specialist (genus *Micropholcomma*) (Rix & Harvey 2010; Williams *et al.* 2015b).

Although the fourth most common taxon in the subnivean space, the absolute number of beetles found in pitfall traps was low. Nevertheless, a high family diversity was observed. Prior to this study, beetles from 13 families were known to remain active beneath the snowpack during winter in alpine and/or boreal environments (Aitchison 1979b; Merriam *et al.* 1983; Green 1997; Aitchison 2001 and references therein). An additional three families, all detected at Mt Twynam, can now be added to this list: Phalacridae, Corylophidae, and Byrrhidae.

Australia's alpine arthropod fauna is unusual among alpine ecosystems in the high abundance of ants (Green & Osborne 2012; Nash *et al.* 2013). Despite their conspicuous presence in traps in summer, this group did not appear to be active in the subnivean space. Marked declines in winter abundance are a common characteristic of ant communities in strongly seasonal environments in Australia (e.g. Briese & Macauley 1980; Andersen 1983, 1986). In some species, this seasonal surface activity is related to temperature thresholds (e.g. Norment & Green 2004). Given that species in the genus *Iridomyrmex* (which accounted for most the ants collected) are typically thermophilic (Andersen 1997), a similar threshold response could account for their plot-specific presence in spring which appeared linked to the timing of snowmelt (see also Appendix F, Fig. F.2).

7.4.6 Conclusions

This study was a first step in assessing the ecological response of alpine taxa in the Australian mountains to changes in snow cover and characterising the diversity of subnivean invertebrates in Australia. I found a characteristic subnivean fauna with strong effects of changing snow conditions on some taxa. However, even within each study site I detected only a subset of the subnivean faunal diversity (e.g. those taxa small enough to be caught in the pitfall traps) and the subnivean is only one aspect of the snow-associated habitat. Assessments of taxa utilising the snow surface and/or the snow layer itself, as well as winter-active soil-dwelling taxa, are therefore desperately needed in Australia. Variation in community composition among locations/years was associated with opposite responses of dominant taxa to snow removal. Hence, although Australia's subnivean arthropod communities are sensitive to changes in snow cover, generalising these results across the mountain region and to larger spatial scales is not yet possible.

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Chapter 8

Summary and future directions

8.1 Background

The interaction between individuals and their environment ultimately determines where populations are able to persist and, as a consequence, the geographic ranges of species (MacArthur 1972). Geographic range size varies by more than 12 orders of magnitude among organisms as a whole (Brown *et al.* 1996) and, globally, species occupy an exceptional array of environments: from deep sea hydrothermal vents to the high Himalaya and from the tropics to the poles. Geographic ranges are also dynamic, shaped internally and externally by interactions between limiting environmental conditions and population dynamics (reviewed: Brown *et al.* 1996). Understanding the factors controlling where a species occurs is thus a key challenge from both an ecological and evolutionary standpoint. At the same time, the particular distribution of a species determines the environmental conditions – and hence the selective pressures – to which it is subject, and the variation in these conditions in space and time.

In the preceding chapters, I first considered ecological factors controlling the size (Chapters 2 and 3) and position (Chapters 4 and 5) of a species' geographic range. Second, I explored how mountain taxa have responded or might respond to past (Chapter 6), contemporary (Chapter 5) and future (Chapter 7) environmental variation. The following sections summarise the major results of the research and discuss future research directions.

8.2 Ecological correlates of range size variation

In Chapters 2 and 3, I examined the relationship between niche characteristics and geographic range size, first using a meta-analytic approach and then by focusing on a single taxon. Meta-analysis revealed a positive relationship between niche breadth and geographic range size, which was particularly consistent when the niche was defined according to habitat use: among closely related species, those with a narrow niche occupy a smaller geographic area than those with a broad niche. Despite having been previously dismissed as a general pattern (Gaston & Blackburn 2000), the results presented here provide support for a general niche breadth-range size relationship, as proposed by Brown (1984). Niche breadth does not, however, explain all of the variation in geographic range size among species, and other ecological traits – such as niche position – might be equally important.

The niche position-range size hypothesis predicts that a species whose niche encompasses resources that are common across a landscape will occupy a larger geographic area than one whose resources are rare (Hanski 1993; Gaston & Blackburn 2000). One of the knowledge gaps identified in Chapter 2 was a paucity of studies that simultaneously test the niche breadth and niche position hypotheses. This issue was addressed in Chapter 3. Among *Nebria* beetles from North America, geographic range size was associated with both niche position and niche breadth, with the former appearing as a stronger effect. The same result was evident among studies included in the meta-analysis that measured both effects: the two niche traits were both associated with range size, but with a somewhat larger effect size for the niche position-range size relationship (Appendix A, Fig. A.1).

With over 60 studies measuring the relationship between niche breadth and range size, the estimated effect size for this association has stabilised and additional studies are unlikely to change the evidence for the existence or strength of the observed effect (Mullen *et al.* 2001; Appendix A, Fig. A.2). Future research can therefore move beyond testing for the existence of a pattern and should instead seek to investigate the underpinnings of the relationship between niche breadth and geographic range size. Whereas estimates of habitat and diet breadth are most often based on observations, environmental tolerance is typically measured experimentally (Chapter 2). Such experimental studies provide an excellent opportunity to test, for example, whether specialisation (i.e. a narrow niche) is driven by a lack of genetic variation (Kellermann *et al.* 2009), the extent to which niche traits are phylogenetically constrained (Emery *et al.* 2012; Kellermann *et al.* 2012a; Kellermann *et al.* 2012b; Hoffmann *et al.* 2013), and the degree to which niche breadth can be scaled up or down the biological hierarchy, from individuals through to species (Roughgarden 1972; Ackerly 2003; Atkins & Travis 2010; Bolnick *et al.* 2011).

A second area that deserves further attention, highlighted in Chapter 3 and supported by earlier studies (Boulangeat *et al.* 2012; Passy 2012), is the interaction between niche breadth and niche position. If either a broad or a common niche is required to allow geographic range expansion or, alternatively, if either a narrow or marginal niche can constrain a species' distribution, a combination of these traits might go some way towards explaining the "exceptions to the rule". This question is particularly interesting in relation to mountain taxa as, by virtue of the decreasing land area towards higher elevations, alpine habitats (and the species they harbour) will always tend to be marginal on a landscape scale.

Finally, meta-analysis has, to-date, been used infrequently to examine macroecological patterns (but see Stein *et al.* 2014). The meta-analysis approach treats the literature as a population of studies, each with one or more effect size (Jennions *et al.* 2013a). These effect sizes are each estimates of the "true" effect, in much the same way that individual replicates provide estimates of the true mean for a statistical population (Jennions *et al.* 2013a). This premise makes meta-analysis a promising approach for statistically testing ecological and evolutionary "rules" (Jennions *et al.* 2013b). The definition of what constitutes a "rule" is the subject of debate (Mayr 1956; Gaston *et al.* 2008) but, regardless of the definition, the assessment of pattern generality is typically approached by (formal or informal) vote-counting: a tally of studies/species providing support for or against a hypothesis. A vote-counting approach to defining support (or lack thereof) is, however, problematic (Koricheva & Gurevitch 2013). Of particular importance for ecology and evolution, where sample sizes tend to be small and statistical power is consequently low (Møller & Jennions 2002), vote-counting relies on statistical significance (usually based on *p*-values). In addition, this approach weights all studies equally, regardless of sample size, and provides no information on the magnitude of the effect (Koricheva & Gurevitch 2013). Meta-analysis provides a means to test rules without relying on vote-counting or subjective assessments and, when competing hypotheses are tested in similar ways (the niche breadth- and niche position-range size hypotheses being a perfect example), the technique can also be employed to directly compare the strengths of the effects.

8.3 Thermal tolerance variation among and within species

Physiological requirements and limits determine a species' fundamental niche and, thus, its potential geographic distribution (Hutchinson 1957; MacArthur 1972). In Chapters 4 and 5, I examined physiological variation associated with differences in elevational distribution among two genera in two very different environments.

The *Nebria* are nocturnal and cryophilic, restricted to cool habitats and with many species that forage on the snow surface at temperatures close to 0°C (Mann *et al.* 1980). Despite a steep decline in microclimate temperatures which paralleled atmospheric temperature lapse rates, I found no consistent clinal variation in thermal tolerance among 12 *Nebria* species occupying different elevational distributions. The only evidence for thermal adaptation came from the two lowest-elevation species, which had the greatest heat tolerance, but it was not possible to separate phylogenetic and environmental effects. The increase in atmospheric aridity towards higher elevations was not apparent for *Nebria* microhabitats where, if anything, the opposite gradient was observed. This is one possible explanation for the lack of elevational variation in desiccation resistance among species.

In contrast to the *Nebria*, the *Kosciuscola* grasshoppers are thermophilic and diurnally active. Among three species, the highest-occurring was the most cold-tolerant and the lowest-occurring the least cold-tolerant – a result predicted based on macroclimatic temperature declines with elevation. With cold tolerance limits regularly exceeded in exposed microhabitats, cold adaptation could play a role in structuring the distributions of these species. Heat tolerance varied within species highlighting, again, the small spatial scales over which adaptation can occur in mountain environments (Byars *et al.* 2007; Briceño *et al.* 2014; Nicotra *et al.* 2015). In support of recent modelled predictions (Buckley *et al.* 2013), but counter to expectations from macroclimatic trends, populations from the treeless alpine zone were more heat tolerant than their lower-elevation counterparts.

Although conducted in different systems, both the *Nebria* and *Kosciuscola* studies highlight the importance of considering climate at the appropriate spatial scale (Storlie *et al.* 2014; Sunday *et al.* 2014). Interactions between abiotic variables and physical characteristics of a given habitat can drive high microclimatic heterogeneity over small spatial scales and large differences between micro- and macroclimates (Gates 1980; Geiger *et al.* 1995; Scherrer & Körner 2010). With the development of technologies and techniques for measuring microclimates and assimilating macroclimate conditions to predict individual body temperatures (e.g. Kearney & Porter 2009; Kearney *et al.* 2014), there has been increasing recognition of the importance of interpreting physiological variation (particularly of insects) with respect to environments actually experienced by the organism in question (Buckley *et al.* 2013; Sunday *et al.* 2014; Buckley *et al.* 2015; Kaspari *et al.* 2015; Pincebourde & Casas 2015). Kaspari *et al.* (2015), for example, found large (3.5 – 5°C) differences in heat tolerance among species of ants occupying different microhabitats within a tropical rainforest. This variation in heat tolerance corresponded to expectations based on microhabitat temperatures, demonstrating that, even within one "habitat", thermal conditions and thermal adaptation can vary considerably (Kaspari *et al.* 2015).

Mountain environments are characterised by strong microclimatic variation (Geiger *et al.* 1995; Scherrer & Körner 2010; Wundram *et al.* 2010). Consider the thermal environment, for example. During the day, high insolation drives large temperature differences between the sun and the shade, while near-ground temperatures can be more than 30°C higher than atmospheric conditions (Geiger *et al.* 1995). The habitat (e.g. sub-alpine or alpine) and activity patterns (e.g. diurnal or nocturnal) will thus determine both (a) exposure to environmental stress, and (b) heterogeneity in environmental conditions; the latter, in turn, defining the potential for behaviour to buffer against abiotic stress (Kearney *et al.* 2009; Sunday *et al.* 2014). Among *Nebria*, for example, choice of humid microhabitats and nocturnal activity might reduce exposure to desiccation stress as well as ameliorating divergent selection for desiccation resistance. Conversely, the occurrence of *Kosciuscola* grasshoppers outside the margins of their cooling tolerance suggests a reliance on behavioural avoidance of cold temperature extremes.

8.4 Genetic structure in the Australian alpine region

In Chapter 6, I examined the phylogeographic and population genetic structure of the alpine endemic grasshopper *Kosciuscola tristis* in the fragmented network of sky islands that form the Australian alpine region. In this region, many taxa have highly restricted distributions, and this has hindered previous assessments of genetic structure across the region as a whole (but see Griffin & Hoffmann 2014). This study identified four primary genetic groups, corresponding to the disjunct Baw Baw, Mt Buller/Stirling and Bogong High Plains regions in Victoria, and the Kosciuszko region in New South Wales. The primary divergence within the Victorian mountains is estimated to have occurred roughly 1 Ma. While molecular clock-based methods of divergence dating are problematic (e.g. Thomas *et al.* 2006), these results point to prolonged genetic isolation and a level of sequence divergence that is on par with inter-specific differentiation in comparable systems (e.g. North American alpine grasshoppers: Knowles 2000).

One geographic area that remains poorly studied is the high country north of Kosciuszko and, in particular, the northernmost point – the Brindabella Range. Chapter 6 included, for the first time, an appraisal of the evolutionary relationship of Mt Jagungal to the main Kosciuszko area. Microsatellites revealed clear, and possibly recent, divergence, which could be a product of distance alone (Wright 1943) or a discontinuous distribution: Mt Jagungal is separated from the main range of the Kosciuszko region by an undulating sub-alpine plateau within which the occurrence of *K. tristis* is undocumented.

Griffin and Hoffmann (2014) found that the Brindabella Range area was one of few distinct genetic clades among alpine *Poa* grasses from across the Australian high country. Chapple *et al.* (2005) also found strong genetic divergence between populations of the montane skink *Egernia montana* from the Brindabella and Kosciuszko areas. To the best of my knowledge, the Brindabella Range has not been included in any other genetic studies of Australian mountain taxa. *Kosciuscola tristis* has not been detected within this area but the three other mainland *Kosciuscola* species are abundant. A comparative study incorporating these species (especially *K. usitatus* which occupies a similar elevational distribution to *K. tristis*) would provide valuable insights into the evolutionary position of populations in the northern mountains and the extent to which the patterns observed in *K. tristis* (e.g. a distinct Mt Jagungal population and relatively weak historic genetic divergence across the Murray River valley) are representative.

The high topographic heterogeneity of mountain landscapes provides an exciting opportunity to examine fine-scale effects of landscape and habitat features on population structure. In North America, these effects have been studied in detail for the endangered alpine butterfly *Parnassius smintheus*, for which patches of forest inhibit dispersal between alpine meadows at both local and regional scales (Keyghobadi *et al.* 1999; Roland *et al.* 2000; Matter *et al.* 2004; Keyghobadi *et al.* 2005; Ross *et al.* 2005). The small cooling tolerance of the *Kosciuscola* (Chapter 5) and their presumably low dispersal ability (being brachypterous) suggests the possibility that large frost hollows, such as the Nungar and Long Plains, might play a role in structuring populations of these species. These sub-alpine grasslands experience regular frosts and temperatures considerably below the critical thermal minima of the *Kosciuscola* (Moore & Williams 1976; Davidson & Reid 1985). Moore and Williams (1976), for example, recorded mid-summer temperatures of -9°C near the Cooleman Plain in New South Wales. Integrating measures of thermal tolerance (ideally across all life stages) with estimates of fine-scale genetic structure would allow this question to be addressed. Using a genomics approach would additionally allow an assessment of adaptive genetic variation and identification of environmental and/or landscape factors responsible for the maintenance of such variation (Joost *et al.* 2007; Manel *et al.* 2012; Manel & Holderegger 2013).

8.5 Snow and the alpine winter

Snow is a critical factor in the ecology of mountain ecosystems and yet there is a marked scarcity of studies on the influence of snow regimes on alpine species and communities. For arthropods, there is an even more striking lack of basic information about the taxa that utilise the snowpack (beneath, within, or on top of it) for winter activity (but see, for example, Aitchison 1978, 1979a, b, c for a thorough appraisal of subnivean winter activity of arthropods on southern Canadian prairies).

In Chapter 6, I studied the subnivean arthropod fauna at two locations in the Australian alpine region. The first goal of the study was to document the subnivean arthropod communities. At both locations, subnivean communities were characterised by the absence of many taxa – most notably ants – but there was also some evidence for a small number of cold season specialists. Experimental reduction of snow depth altered the arthropod community composition at both Mt Twynam and Mt Stirling, though the nature of the effect on dominant taxa was different at the two sites.

Every mountain system is different, but many features of the Australian Alps mark it as particularly unique (Good 1989). These features include an absence of perennial snow and a snowpack structure that aligns poorly with global snow classification systems (Sturm *et al.* 1995; Sanecki *et al.* 2006a). From the snow manipulations in Chapter 6, an additional feature can now be added: by virtue of the relatively warm atmospheric temperatures in the Australian high country, reduced snow cover leads to warmer (or unaltered) average near-surface temperatures, contrasting with the "colder soils in a warmer world" paradigm apparent in other mountain systems (Groffman *et al.* 2001; Edwards *et al.* 2007; Appendix F). Therefore, the impacts of reduced snow cover on Australian alpine ecosystems might well be very different to those from other parts of the world and generalisation of results from other mountain systems (and vice versa) should be done with due caution.

Most snow removal studies from the northern hemisphere have focused on the response of plants and, in particular, phenological responses to earlier snowmelt (Appendix F, Table F.1). Such studies are now needed in Australia, where the most closely related work has shown that frost and extreme cold are detrimental to the establishment of *Eucalyptus* seedlings (Moore & Williams 1976; Davidson & Reid 1985; Ferrar *et al.* 1988), and recent physiological studies suggest fine-scale local adaptation to the length of the snow season in the alpine herb *Aciphylla glacialis* (Briceño *et al.* 2014). Several recent reviews have highlighted the risk of phenological mismatch in interspecific interactions (e.g. plant-pollinator, predator-prey) as a result of climate change and variation in cues for development and/or activity among taxa (Miller-Rushing *et al.* 2010; Thackeray *et al.* 2010; Walther 2010; Forrest 2015; Rafferty *et al.* 2015). In Chapter 7, I found an apparent association between the timing of ant emergence and snowmelt, and it is possible that other taxa (such as springtails) show a similar dependence on the timing of snowmelt for activity. Simultaneous measurements of arthropod and plant responses to reduced snow would provide a powerful test of both phenological drivers and the likelihood of phenological mismatch in this ecosystem. This would complement species-specific physiological measurements (e.g. Briceño *et al.* 2014; Slatyer *et al.* 2015), as well as experimental warming (Jarrad *et al.* 2008; Camac *et al.* 2015) and long-term monitoring programs (Pickering & Green 2009) already established in the region to provide an exciting suite of datasets with which to both detect and predict ecosystem-level responses to climate change.

Finally, while the focus of my study was on winter-active taxa, many species overwinter in inactive life stages and, if we are to make inferences about ecosystem-level responses to shifts in the snow regime, considering these life stages will be crucial. In the North American alpine endemic butterfly *Parnassius smintheus,* for example, survival of overwintering larvae is strongly linked to winter climate; winter conditions are thus the strongest driver of population dynamics in this species (Matter *et al.* 2011; Roland & Matter 2013). Many alpine insects enter diapause over the winter and this life stage is generally robust to environmental stressors (Andrewartha & Birch 1954). The eggs of *K. tristis* and *K. usitatus* overwinter beneath the snow pack in diapause, and Green and Osborne (2012) have suggested that the abundance of the *Kosciuscola* is reduced in years following winters with low snow cover. Although experimental removal of winter snow does not appear to reduce hatching success in *K. tristis* (Appendix G), our knowledge of this aspect of the biology of the *Kosciuscola* remains poor.

With snow cover predicted to decrease substantially in areas where mean winter temperatures are warmer than -10°C (Räisänen 2008), the effects of winter snow on alpine life is a research area that needs urgent attention. In particular, the focus should rest on mid-elevation areas (i.e. 1500 – 2000 m for temperate mountains), where the effects of global warming on the snowpack are likely to be felt first, and felt most (Sproles *et al.* 2013; Steger *et al.* 2013; Vaughan *et al.* 2013).

8.6 Climate change in the Australian alpine region: management and future research

Mountain ecosystems were recently identified as one of 10 biomes in Australia most vulnerable to "tipping points", where moderate changes in the environment cause disproportionately large shifts in ecosystem properties (Laurance *et al.* 2011). This designation was due to their small geographic area, narrow climatic envelope and reliance on seasonal snow, with climatic changes as well as habitat fragmentation identified as major threats (Laurance *et al.* 2011).

Alpine and sub-alpine habitats on mainland Australia are contained almost entirely within a network of seven National Parks and four reserves that span the Australian Capital Territory and the states of Victoria and New South Wales and encompass five separated mountain regions. Within this network, a key question for management of the high country is the extent to which it is considered as one biogeographic entity. Genetic methods are now widely applied to address conservation challenges (reviews: Weeks *et al.* 2011; Aitken & Whitlock 2013; Mijangos *et al.* 2015; Whiteley *et al.* 2015), and one area where they have been put to particular use is in the delineation of distinct evolutionary units (Moritz 2002). Considering sequence and microsatellite data, I detected four discrete genetic clades of *K. tristis,* and a recent study on four other arthropod taxa showed similar patterns of divergence across the Victorian high country (Endo *et al.* 2014). This work suggests that, with respect to conserving evolutionary diversity, each mountain region should be treated as a separate unit for conservation. In particular, deep lineage divergence indicates a long history of population isolation among the *Kosciuscola* from the mountains of southern Victoria, where *K. tristis* is also the only representative of the clade.

Climate change represents an enormous challenge for biodiversity conservation globally (Heller & Zavaleta 2009). At a species level, Williams *et al.* (2008) outlines a framework for assessing vulnerability to climate change that highlights two aspects: exposure and sensitivity. Although high microclimatic heterogeneity might go some way towards ameliorating the exposure of mountain taxa to climate change (Scherrer & Körner 2010), the low topographic relief of the Australian mountains means that the thermal envelope of high alpine climates might disappear altogether by 2085 (Reside *et al.* 2013). As dominant herbivores, grasshoppers play an important functional role in Australia's sub-alpine and alpine ecosystems (Green & Osborne 2012). For the *Kosciuscola*, a high warming tolerance implies a capacity to persist in warmer climates; conversely, limited cold tolerance suggests that warming could, either directly or indirectly (e.g. via shrub encroachment into the alpine zone) facilitate upward range expansions (Myers-Smith *et al.* 2011; Bässler *et al.* 2013; Venn *et al.* 2014). As previously noted however, measures of the sensitivity or robustness of other life stages – particularly the overwintering eggs – to environmental variation are needed.

The strong effects of snow manipulation on arthropod community composition that I found in this study, and the small scales over which they occurred, emphasise the importance of protecting areas which naturally accumulate and retain high levels of snow cover (Williams *et al.* 2015). For this, the effects of locally intensive recreational activity over the winter should also be considered. The Australian alpine region supports 12 ski resorts which receive a high concentration of visitors over the winter months (e.g. ~2500 visitors/day at Mt Buller, Victoria). Skiing, snow grooming and the use of oversnow vehicles (e.g. snowmobiles) compact the snow, reducing the size of the subnivean space as well as increasing the density and thermal conductivity of the snow and thus reducing its insulation capacity (Rixen *et al.* 2004; Rixen *et al.* 2005; Sanecki *et al.* 2006b; Rixen *et al.* 2008). This has significant effects on the subnivean microclimate, akin to removal of the snow layer (Baiderin 1980; Keller *et al.* 2004; Rixen *et al.* 2004; Rixen *et al.* 2008). In Europe and North America, areas groomed for ski runs support a different, and generally less diverse *summer* arthropod community than adjacent habitat (Meyer 1993; Strong *et al.* 2002; Negro *et al.* 2009; Negro *et al.* 2010; Keßler *et al.* 2012; Kašák *et al.* 2013; Negro *et al.* 2013; Rolando *et al.* 2013). The effects of snow compaction and consequent changes in the physical environment on subnivean communities, however, remain untested.

Artificial snow also has a higher density than natural snow (due to different crystal structure) but, in contrast to compacted snow, provides added insulation because of increased snow depth (Jones & Devarennes 1995; Rixen *et al.* 2003; Keller *et al.* 2004; Rixen *et al.* 2005; Meijer zu Schlochtern *et al.* 2014). Artificial snow might therefore provide some degree of short-term and localised buffering against declining snow cover (Keller *et al.* 2004; Rixen *et al.* 2005). In some years, artificial snow doubles the natural snow depth at low-elevation alpine resorts (e.g. Mt Baw Baw: ARCC 2015) but the capacity for snowmaking (Hennessy *et al.* 2008) and its economic and environmental feasibility (Pickering & Buckley 2010) are inversely related to warming trends.

Lastly, in addition to the results presented and discussed above this project detected at least four undescribed species: one beetle (Family Cryptophagidae, tribe Picrotini) (R Leschen, personal communications) and three springtails (Families Entomobryidae and Paronellidae) (P Greenslade, personal communications). I have also provided the first records of the *Kosciuscola* from the Cobberas Range (Victoria) and Mt Jagungal area (New South Wales). For *K. tristis*, this represents an extension of 10 km in linear extent and 350 km^2 in area of its previously documented range. This work highlights that, although Australia has only a small area of alpine habitat, there is still much to learn about the biodiversity it contains.

8.7 Closing remarks

The series of studies presented here approach the challenge of species geographic ranges from several angles. Studies of the geographic range continue to provide insights into the evolutionary process, ecological interactions and, in the context of rapid climate change, extinction risk and the adaptive potential of species and communities. The focus on mountain regions here reflects their utility as a landscape for testing interactions between species and the environment, but also highlights the sensitivity of their component species and communities to environmental change. Overall, this work reveals effects of small-scale environmental and landscape variation on species traits, genetic structure and community composition, which could act to either buffer (e.g. by providing refuge habitat) or exacerbate (e.g. by creating effective habitat fragments) landscape-scale climatic changes. In Australia, and in other mountain areas where snow is currently marginal, alpine winter environments are close to their tipping point and stand to be transformed by snow regime changes induced by global warming. In this context, year-round monitoring and studies of the immediate and long-term consequences of changing snow regimes for alpine flora and fauna are sorely needed.

8.8 References

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APPENDICES

Appendix A

Supporting information for Chapter 2

A.1 The relative strengths of the relationship between range size and niche position/niche breadth

Nine of the studies included in the meta-analysis presented in Chapter 2 measured both niche position and niche breadth. For this subset of studies, I calculated standardised effect sizes (Fisher's z), as described for the main analysis, for both niche breadth and niche position, and ran separate random-effects (RE) meta-analyses for each. The effect sizes and confidence intervals for each study, as well as the mean effect size from the RE model, are shown in Fig. A.1, along with the effect sizes calculated from the analysis with *Nebria* in Chapter 3 (not included in the RE model). Although the effect size for the niche position-range size association is slightly larger, there is considerable overlap in the confidence intervals and both niche position and niche breadth are significantly associated with range size across these studies. In addition, although the confidence interval for the niche breadth-range size relationship estimated in Chapter 3 overlaps zero, the size of the effect is very similar to other studies suggesting that the lack of statistical significance might be a result of small sample size (Jennions *et al.* 2013).

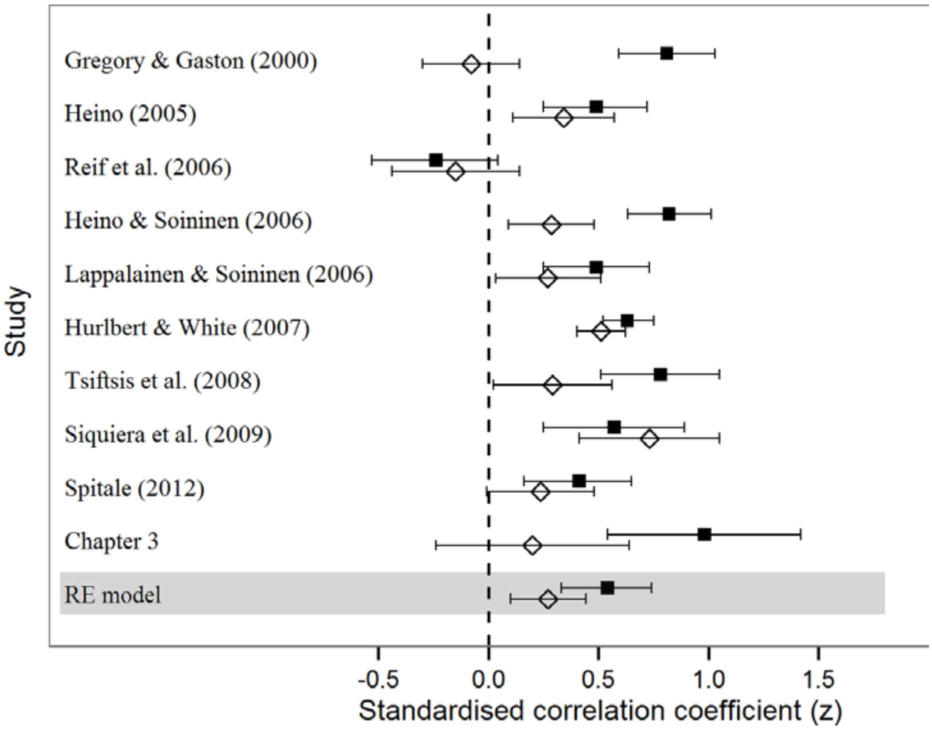

Figure A.1. Effect sizes and 95% confidence intervals for studies measuring the relationship between niche breadth (black squares) or niche position (open diamonds) and range size, and the results of random-effects meta-analyses (RE model) showing the mean effect size across studies. Note that the effect sizes for niche position are shown as the inverse, for comparability.

A.2 Cumulative meta-analysis

Cumulative meta-analysis, introduced by Lau *et al.* (1992), is a method, for examining temporal trends in the data included in a meta-analysis. In this method, studies are ordered chronologically and added to the analysis stepwise. At each step, a mean effect size and 95% confidence interval are re-calculated. Stabilisation of the effect sizes suggests that additional studies are unlikely to change the evidence for the strength/existence of the observed effect (Mullen *et al.* 2001). Using the METAFOR 1.9-7 package in R 3.2.2 (Viechtbauer 2010; R Core Team 2015), I ran a cumulative meta-analysis for the studies measuring the relationship between niche breadth and geographic range size, to establish the stability of the effect size (Fig. A.2). For both habitat breadth and diet breadth, there is clear stabilisation of the mean effect. This threshold does not yet appear to be reached for tolerance breadth, indicating that more studies here could offer additional insights into the generality and strength of the association.

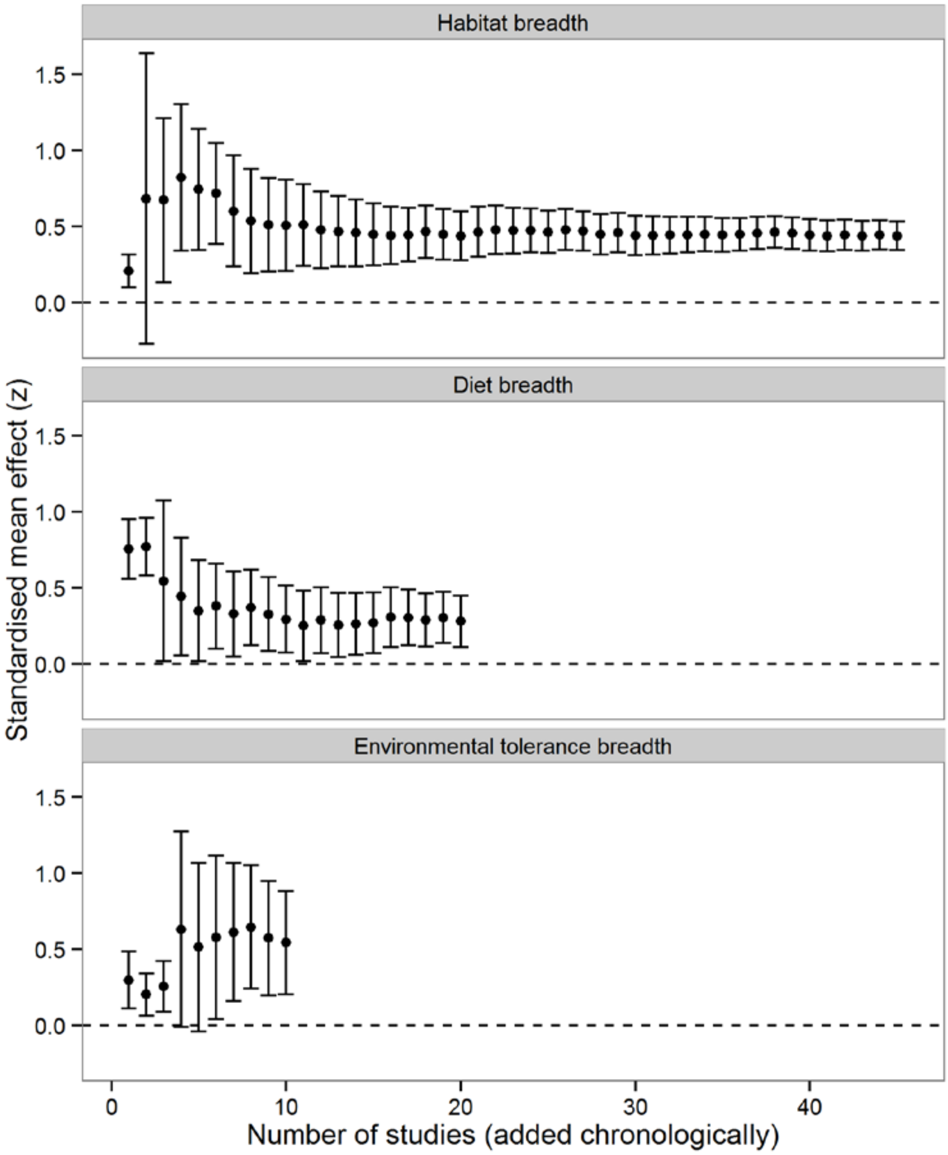

Figure A.2. Cumulative meta-analysis of the relationship between niche breadth and geographic range size, for the three different niche breadth categories used in the meta-analysis. Points show the mean effect (Fisher's *z*) and error bars are 95% confidence intervals.

A.3 References

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Appendix B

Supporting information for Chapter 3

B.1 Model selection based on sub-sampled niche parameter estimates

Table B.1. 95% confidence set of models for range size variation in *Nebria* spp, with niche parameters estimated from sub-sampled occurrence data. AICc is the Akaike Information Criteria corrected for small sample size, ΔAICc is the difference in AICc from the model with the lowest AICc and AICweight is the likelihood of a model being the best model, given the data and the set of candidate models (Burnham & Anderson 2002). Parameters included in the models were environmental niche position (ENP), environmental niche breadth (ENB), and wing structure as a proxy for dispersal ability ("disp"), as well as two-way interactions.

Analysis/model	AICc	ΔAICc	AIC_{weight}	Cumulative AIC_{weight}
ENP + ENB + disp	49.70	0.00	0.36	0.36
ENP + ENB	51.58	1.88	0.14	0.50
ENP + ENB + disp + ENB:disp	51.78	2.07	0.13	0.63
ENP + ENB + ENP:ENB	52.32	2.62	0.10	0.72
ENP + ENB + disp + ENP:ENB	52.46	2.76	0.09	0.81
ENP + ENB + disp + ENP:ENB	52.58	2.87	0.09	0.90
ENP + ENB + disp + ENP:disp + ENB:disp	53.66	3.95	0.05	0.95

Table B.2. Parameter estimates for predictors of range size variation in *Nebria*, based on model-averaging over the 95% confidence set of models, with niche parameters estimated from sub-sampled occurrence data. Parameters are arranged in order of decreasing AICc$_{\text{weight}}$, which represents the probability that a predictor is a component of the best model and provides an estimate of parameter importance. Parameters included in the models were environmental niche position (ENP), environmental niche breadth (ENB), and wing structure as a proxy for dispersal ability ("disp").

Analysis/parameter	**Cumulative AICc$_{\text{weight}}$**	**Estimate**	**95% CI**
ENP	1	-1.05	-1.38, -0.71
ENB	1	0.44	-0.11, 0.98
Disp	0.76	0.40	-0.69, 1.49
ENP:ENB	0.23	0.14	-0.14, 0.43
ENP:disp	0.20	0.30	-0.37, 0.98
ENB:disp	0.17	-0.30	-0.83, 0.23

B.2 References

Burnham, K.P. & Anderson, D.R. (2002). *Model selection and multimodel inference, 2nd edition.* Springer, New York, USA.

Appendix C

Supporting information for Chapter 4

C.1 Experimental set-up for Chapters 4 and 5

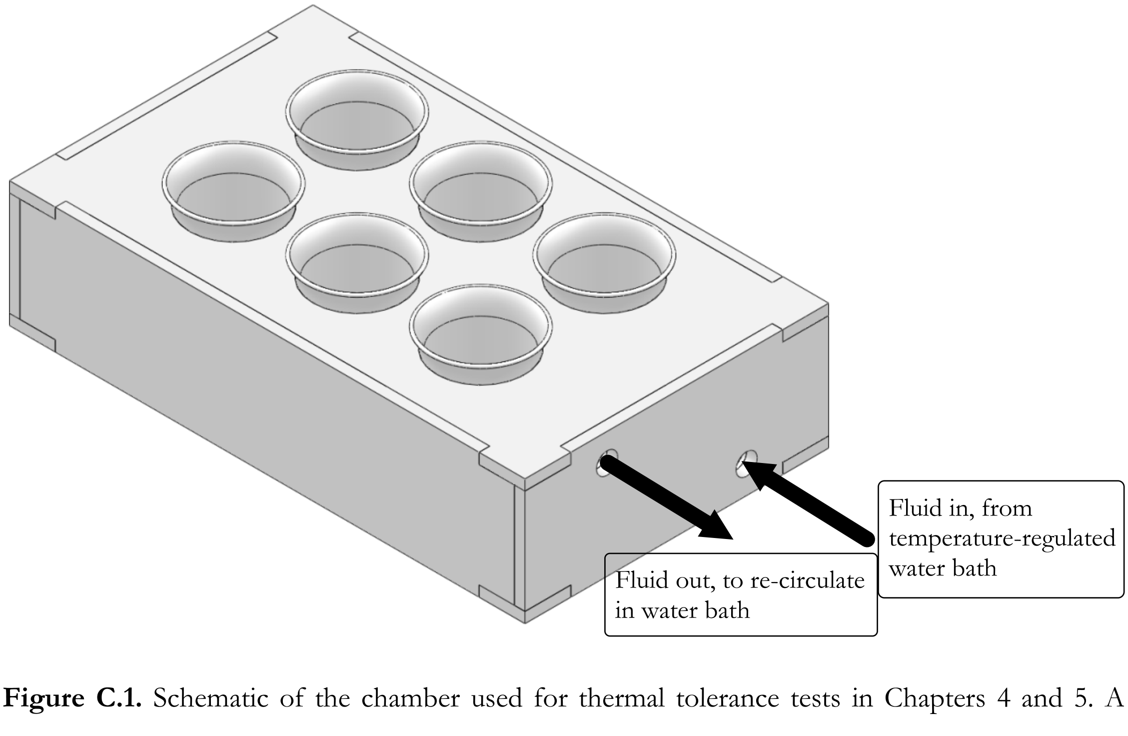

Figure C.1. Schematic of the chamber used for thermal tolerance tests in Chapters 4 and 5. A clear lid with a small hole in the top was placed over each 50 mL beaker, to allow air flow into and out of the chamber but prevent the grasshopper escaping. Design and image by Evan Slatyer.

C.2 Relationships among Mt Rainier *Nebria* species

We constructed a phylogenetic tree for the 12 species of *Nebria* occurring on Mt Rainier, based on the mitochondrial cytochrome oxidase I (*COI*) gene, to examine the influence of shared evolutionary history on the relationships between niche traits and species distributions. A phylogeny is a hypothesis about a clade's evolutionary history, and phylogenetic uncertainty can introduce errors in comparative analyses (Rangel *et al.* 2015). Several nodes in our *COI* phylogeny had low support values (Fig. 4.3), so we also ran our analysis using a phylogeny constructed from morphological data by Kavanaugh (1978) (Fig. C.2). Branch lengths for this tree were generated using the Grafen (1989) method in the APE 3.2 package (Paradis *et al.* 2004) in R 3.2.0 (R Core Team 2014). Most relationships were concordant between the two trees, with the most significant difference being the placement of *N. paradisi* (in a clade with *N. kincaidi* in the *COI* tree and with *N. crassicornis* in the morphological tree). Both tree topologies produced qualitatively similar results, however, with support for phylogenetic signal in the lower range edge (Table C.1).

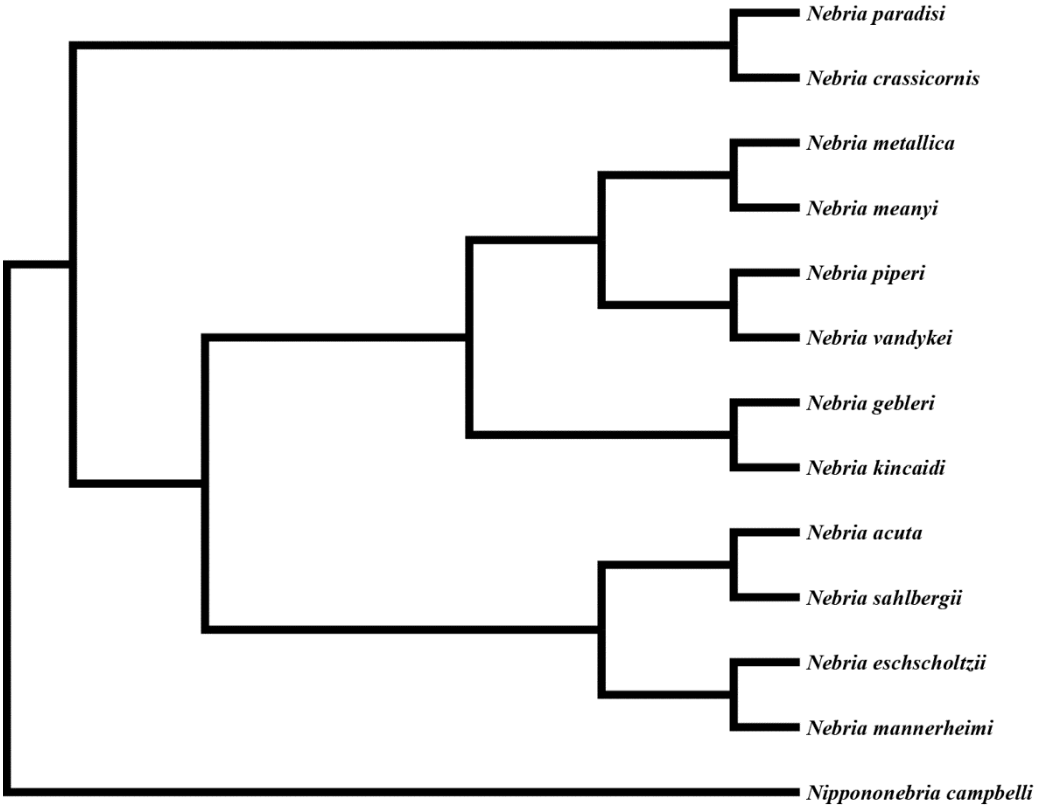

Figure C.2. Phylogeny for the *Nebria* on Mt Rainier, and *Nippononebria campbelli,* using morphological data, as proposed by Kavanaugh (1979).

Table C.1. Tests for phylogenetic signal in physiological traits and range limits among *Nebria* from Mt Rainier, based on both the *COI* and morphological phylogenies. Phylogenetic non-independence was tested with Abouheif's C_{mean}, Pagel's λ and by comparison of three models of trait evolution: Brownian motion, Ornstein-Uhlenbeck and "white noise" (phylogenetically independent trait evolution). For model comparisons, the model with the lowest Akaike information criterion (AICc) for each trait is shown in bold.

Variable	**Abouheif's C_{mean}**	**Pagel's λ**	**Model comparison**		
			Brownian motion	**Ornstein–Uhlenbeck**	**White noise**
Molecular phylogeny					
Log(CT_{min})	-0.08	$6.69\text{x}10^{-5}$	-16.09	-21.37	**-37.21**
CT_{max}	**0.33***	0.89	42.82	42.89	**41.59**
Log(mass-specific water loss rate, 5°C)	0.10	0.28	-12.16	-10.81	**-12.91**
Log(mass-specific water loss rate, 10°C)	0.09	$7.28\text{x}10^{-5}$	-11.59	-11.43	**-17.07**
High-elevation range edge	0.01	$6.69\text{x}10^{-5}$	**178.09**	181.49	183.61
Low-elevation range edge	**0.43****	**0.99****	205.36	202.58	**194.76**
Morphological phylogeny					
Log(CT_{min})	-0.08	$7.27\text{x}10^{-5}$	-26.04	-28.75	**-37.21**
CT_{max}	0.33	0.91	**40.95**	41.99	41.59
Log(mass-specific water loss rate, 5°C)	0.05	$7.27\text{x}10^{-5}$	-4.15	-6.06	**-12.91**
Log(mass-specific water loss rate, 10°C)	-0.06	$7.27\text{x}10^{-5}$	-7.06	-9.12	**-17.07**
High-elevation range edge	0.16	0.24	**183.22**	184.28	183.61
Low-elevation range edge	**0.41****	0.69	201.58	200.15	**194.76**

*$p < 0.05$; **$p < 0.02$

C.3 Microclimate data summaries and pairwise comparisons

Table C.2. Summary statistics for night-time temperature and relative humidity during June – September at Paradise (1675 m), Mt Rainier (weather station PVC55, Northwest Avalanche Centre). Data shown are for 2014, and for a 10-year period (2005 – 2014), based on night-time observations of temperature and relative humidity. 2014 data are shown as mean ± sd and 10-year data are shown as mean (range).

	2014	10-year
Mean temperature (°C)	6.1 ± 6.5	6.8 (3.7 – 9.6)
Absolute minimum (°C)	-8.3	-5.3 (-10.0 – 0.0)
Absolute maximum (°C)	21.7	21.1 (17.8 – 23.9)
Mean nightly minimum (°C)	8.1 ± 4.6	7.1 (6.2 – 8.1)
Mean nightly maximum (°C)	11.1 ± 4.9	10.3 (9.1 – 11.6)
Mean nightly fluctuation (°C)	3.1 ± 2.1	3.3 (2.6 – 3.9)
Mean RH (%)	80.5 ± 22.9	80.9 (75.4 – 86.1)

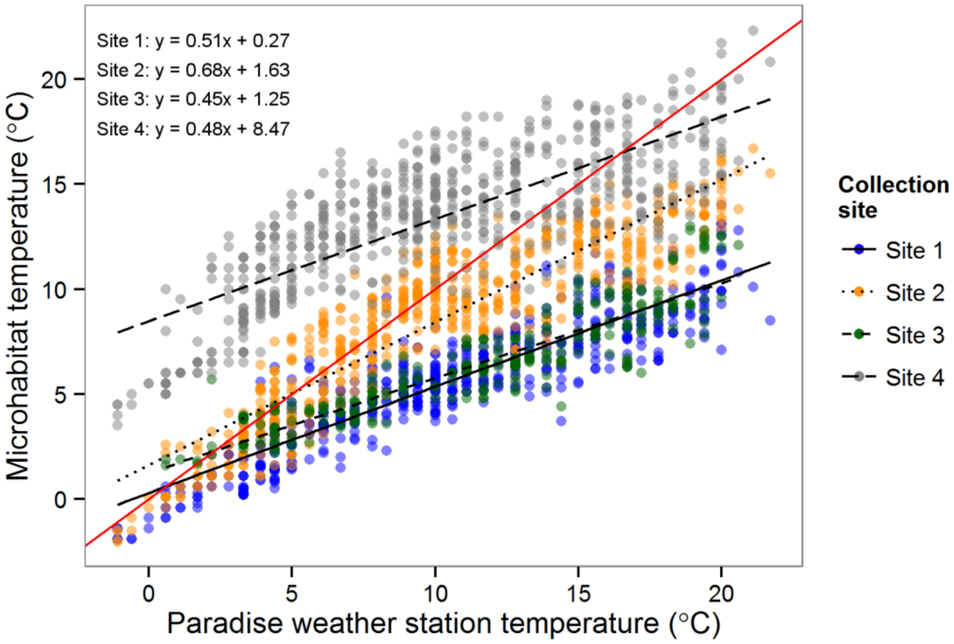

Figure C.3. Correlations between weather station data at Paradise (1675 m), Mt Rainier (weather station PVC55, Northwest Avalanche Centre), and *Nebria* collection sites, as well as the intercept and slope of the regression line, fitted by linear regression. The red line shows y = x.

Table C.3. Summary statistics for night-time temperature and relative humidity at the four primary collecting sites on Mt Rainier during June – September 2014. Logger A at each site was the primary data logger (iButton DS1922L), placed in a microhabitat location where beetles are commonly found. Logger B was a secondary logger, placed approximately 1 m away from Logger A, in an exposed microhabitat. Logger C at Sites 1 and 4 was an additional thermometer/hygrometer (iButton DS1923) placed in a similar microhabitat to Logger A. Species present at each site are listed, and those for which thermal tolerance and desiccation resistance were measured are in bold. *Nebria crassicornis* was collected from 1911 m, N. *metallica* from 804 m (where it co-occurs with N. *piperi*, N. *mannerheimi,* and N. *acuta*), and N. *eschscholtzii* from 610 m (co-occurring with N. *mannerheimi*).

	Site 1			**Site 2**		**Site 3**		**Site 4**		
Elevation (m a.s.l)	2179 m			1929 m		1613 m		967 m		
Logger	**A**	**B**	**C**	**A**	**B**	**A**	**B**	**A**	**B**	**C**
Mean temperature (°C ± s.d)	5.3 ± 2.9	7.6 ± 4.2	3.4 ± 1.6	6.5 ± 2.8	8.5 ± 3.8	6.5 ± 2.5	7.0 ± 3.5	13.6 ± 3.4	9.6 ± 2.7	12.0 ± 3.3
Absolute minimum (°C)	-1.9	-0.9	-0.6	-0.9	-2.0	1.6	1.0	3.5	4.1	1.1
Absolute maximum (°C)	14.0	18.2	8.0	14.5	17.9	13.2	13.6	25.1	16.7	22.2
Mean nightly minimum (°C ± s.d)	4.0 ± 2.5	6.1 ± 3.9	1.9 ± 1.0	5.1 ± 2.5	7.2 ± 3.7	5.5 ± 2.5	5.5 ± 2.4	11.4 ± 2.6	7.9 ± 1.9	9.7 ± 2.2
Mean nightly maximum (°C ± s.d)	6.7 ± 3.2	9.6 ± 4.6	5.1 ± 2.0	8.1 ± 3.3	10.5 ± 3.8	7.4 ± 2.5	8.9 ± 3.4	16.4 ± 4.0	11.8 ± 3.5	15.1 ± 4.1
Mean nightly fluctuation (°C ± s.d)	2.7 ± 1.2	3.5 ± 1.8	3.2 ± 1.5	3.1 ± 1.3	3.3 ± 2.4	1.9 ± 1	3.5 ± 1.2	4.9 ± 2.1	3.9 ± 2.0	5.4 ± 3.1
Number of nights recorded	103	103	32	104	104	44	17	104	18	44
Mean RH (% ± s.d)	98.2 ± 4.6			-		90.3 ± 9.5		89.0 ± 10.6		
Species present	***N. paradisi*** ***N. vandykei*** *N. meanyi*			***N. paradisi*** ***N. vandykei***	*N. kincaidi* *N. acuta*	***N. gebleri*** ***N. meanyi*** ***N. kincaidi***	*N. vandykei* *N. acuta*	***N. sahlbergii*** ***N. acuta***	***N. piperi*** ***N. mannerheimi***	

Table C.4. Pairwise comparisons of thermal tolerance limits at sites 3 and 5. Mean differences (absolute values) among species for cold tolerance (log-transformed) are shown below the line, and for heat tolerance above the line. Significant differences (*p* < 0.05), after adjusting for multiple comparisons, are in bold.

Site 3

CT_{max} / CT_{min}	*N. kincaidi*	*N. meanyi*	*N. gebleri*
N. kincaidi		**2.08**	0.07
N. meanyi	0.03		**2.01**
N. gebleri	0.06	0.04	

Site 5

CT_{max} / CT_{min}	*N. mannerheimi*	*N. acuta*	*N. piperi*	*N. sahlbergi*
N. mannerheimi		**2.16**	1.19	**1.69**
N. acuta	0.00		0.97	0.47
N. piperi	0.03	0.03		0.50
N. sahlbergii	0.08	0.08	0.05	

Table C.5. Trait means (± s.d.) for thermal tolerance and desiccation resistance for each *Nebria* species included in the study; "Elevation" is the elevation of the population for which traits were measured. Mass-specific water loss rates ($mgH_2Og^{-1}h^{-1}$) and total desiccation (% initial mass, in brackets) at 5°C and 10°C at < 5% relative humidity, calculated after 24 hours. All $n = 10$ for CT_{min} and CT_{max}, and $n = 5$ for all water loss data.

				Water loss rates [$mgH_2Og^{-1}h^{-1}$ (% initial mass loss)]	
Species	**Elevation (m a.s.l)**	**CT_{min} (°C)**	**CT_{max} (°C)**	**5°C**	**10°C**
N. eschscholtzii	610	-3.8 ± 0.7	37.0 ± 1.2	3.3 ± 1.3 (7.9 ± 3.0)	6.2 ± 1.2 (14.0 ± 3.0)
N. metallica	805	-3.4 ± 0.5	34.0 ± 0.8	3.3 ± 0.4 (8.0 ± 1.0)	7.7 ± 0.8 (18.6 ± 1.9)
N. piperi	970	-3.2 ± 0.9	34.7 ± 0.8	5.7 ± 1.1 (13.6 ± 2.6)	6.3 ± 1.3 (15.2 ± 3.1)
N. mannerheimi	970	-3.4 ± 0.9	35.9 ± 1.1	3.0 ± 0.8 (7.3 ± 1.9)	8.8 ± 4.7 (21.0 ± 11.4)
N. sahlbergii	970	-2.8 ± 0.1	34.3 ± 1.3	5.7 ± 2.7 (13.8 ± 6.6)	
N. acuta	970	-3.3 ± 0.3	33.7 ± 1.7	5.1 ± 4.1 (12.1 ± 9.9)	9.8 ± 4.2 (23.5 ± 10.0)
N. gebleri	1610	-3.4 ± 0.5	33.2 ± 1.4	6.0 ± 3.0 (14.5 ± 7.3)	10.4 ± 2.4 (25.0 ± 5.8)
N. meanyi	1610	-3.8 ± 0.8	35.3 ± 0.9	5.1 ± 1.6 (12.2 ± 3.9)	8.4 ± 1.3 (20.2 ± 3.1)
N. kincaidi	1610	-4.1 ± 1.0	33.2 ± 1.0	3.0 ± 0.7 (7.2 ± 1.8)	6.8 ± 1.5 (16.4 ± 3.5)
N. crassicornis	1910	-3.9 ± 1.0	34.1 ± 1.3	4.1 ± 0.8 (9.8 ± 1.9)	5.5 ± 1.7 (13.3 ± 4.1)
N. vandykei	1930	-3.6 ± 0.7	34.0 ± 1.9	5.0 ± 1.1 (11.9 ± 2.6)	6.2 ± 3.4 (14.9 ± 8.1)
	2180	-3.8 ± 0.8	34.5 ± 0.8		
N. paradisi	1930	-3.3 ± 0.9	33.5 ± 1.5	5.7 ± 2.8 (13.7 ± 6.7)	8.6 ± 1.9 (20.6 ± 4.6)
	2180	-3.4 ± 0.9	34.4 ± 1.1		

C.4 Rapid cold hardening in *Nebria*

Short-term plasticity in thermal tolerance can considerably widen the thermal niche (e.g. Terblanche *et al.* 2005; Chidawanyika & Terblanche 2011). Rapid cold hardening is common in insects, where a short pre-exposure to cold significantly increases cold tolerance (Lee *et al.* 1987). I tested the extent to which rapid cold hardening might increase thermal niche breadth estimates and whether it varied among species, using seven *Nebria* species from Mt Rainier. The experimental set-up is described in the main text (Chapter 4). Individuals were placed in separate beakers and cooled at 0.2°C/minute to -2.5°C. After five minutes, the temperature was increased (0.2°C/minute) to 4°C before CT_{min} was tested using the protocol described in Chapter 4. One individual (*N. gebleri*) was unable to walk normally after the initial cold treatment and was therefore excluded from analysis. Survival was checked after 24 h and all beetles survived the experiment. All species showed rapid cold hardening ($F_{1,124} = 11.78$, $p = 0.001$). However, this effect was small, improving cold tolerance by an average 0.6°C (Table C.6). The effect of cold pre-exposure was also similar among species (species*treatment: $F_{6,125} = 1.27$, $p = 0.272$), indicating that estimates of relative thermal niche breadth are unlikely to be biased by differential plasticity, at least with respect to cold tolerance.

Table C.6. Estimates of cold tolerance (mean ± s.d.) and cold hardening for seven *Nebria* species from Mt Rainier. All $n = 10$ (except $n = 9$ for *N. gebleri* with cold pre-exposure; see methods above).

	CT_{min} (°C)		
Species	**Without cold pre-exposure**	**With cold pre-exposure**	**Difference**
N. acuta	-3.4 ± 0.3	-4.0 ± 1.3	0.6
N. crassicornis	-3.9 ± 1.0	-4.3 ± 1.1	0.4
N. gebleri	-3.4 ± 0.5	-4.3 ± 0.7	0.9
N. kincaidi	-4.1 ± 1.0	-3.6 ± 0.7	-0.4
N. metallica	-3.4 ± 0.5	-4.2 ± 1.3	0.8
N. paradisi	-3.4 ± 0.9	-4.2 ± 0.4	0.8
N. piperi	-3.2 ± 0.9	-3.6 ± 0.8	0.4

C.5 Intraspecific variation in thermal tolerance in *Nebria*

To examine the extent to which a single population's thermal limits are representative for the species, I measured thermal limits for five *Nebria* species (*N. paradisi*, *N. vandykei*, *N. gebleri, N. kincaidi* and *N. meanyi*) from three to four mountains (Fig. C.4). 10 individuals of each species were tested from each mountain, with the exception of *N. gebleri* and *N. kincaidi* from Mt Hood (5 and 7 individuals, respectively). Lower thermal limits had a weak negative skew, so the absolute values were log-transformed. Upper thermal limits had a strong negative skew, so were transformed with the logarithm of $((K+1)-x)$, where *K* was the maximum value recorded (Tabachnick & Fidell 2007). To test variation in thermal limits among populations and species, I used a linear mixed model with population (mountain) nested within species and body mass as a covariate, implemented using LME4 1.1-7 (Bates *et al.* 2014) in R (R Core Team 2014). The amount of variance explained by species and population (mountain) was calculated as the Intraclass Correlation Coefficient 1 in the MULTILEVEL 2.5 package in R (Bliese 2013). Significance of individual terms was tested using a likelihood-ratio test with sequentially reduced models.

When thermal tolerance was measured in multiple populations of a species, the species-level thermal tolerance range (CT_{max} - CT_{min}) was between 37.9°C (*N. kincaidi*) and 39.4°C (*N. meanyi*). This was only marginally greater than the thermal tolerance range estimated from single populations, which was between 36.7°C (*N. gebleri* from Mt Rainier) and 39.3°C (*N. meanyi* from Mt Hood) (Fig. C.5). Most of the variation in thermal limits was partitioned at the individual level (cold tolerance: 85.3%; heat tolerance: 58.1%). Although population-level effects accounted for a considerable proportion (37.4%) of variation in heat tolerance (compared to only 4.3% for cold tolerance), the actual size of this effect was small and not statistically significant (likelihood-ratio test: $\chi^2 = 1.93$, $p = 0.165$).

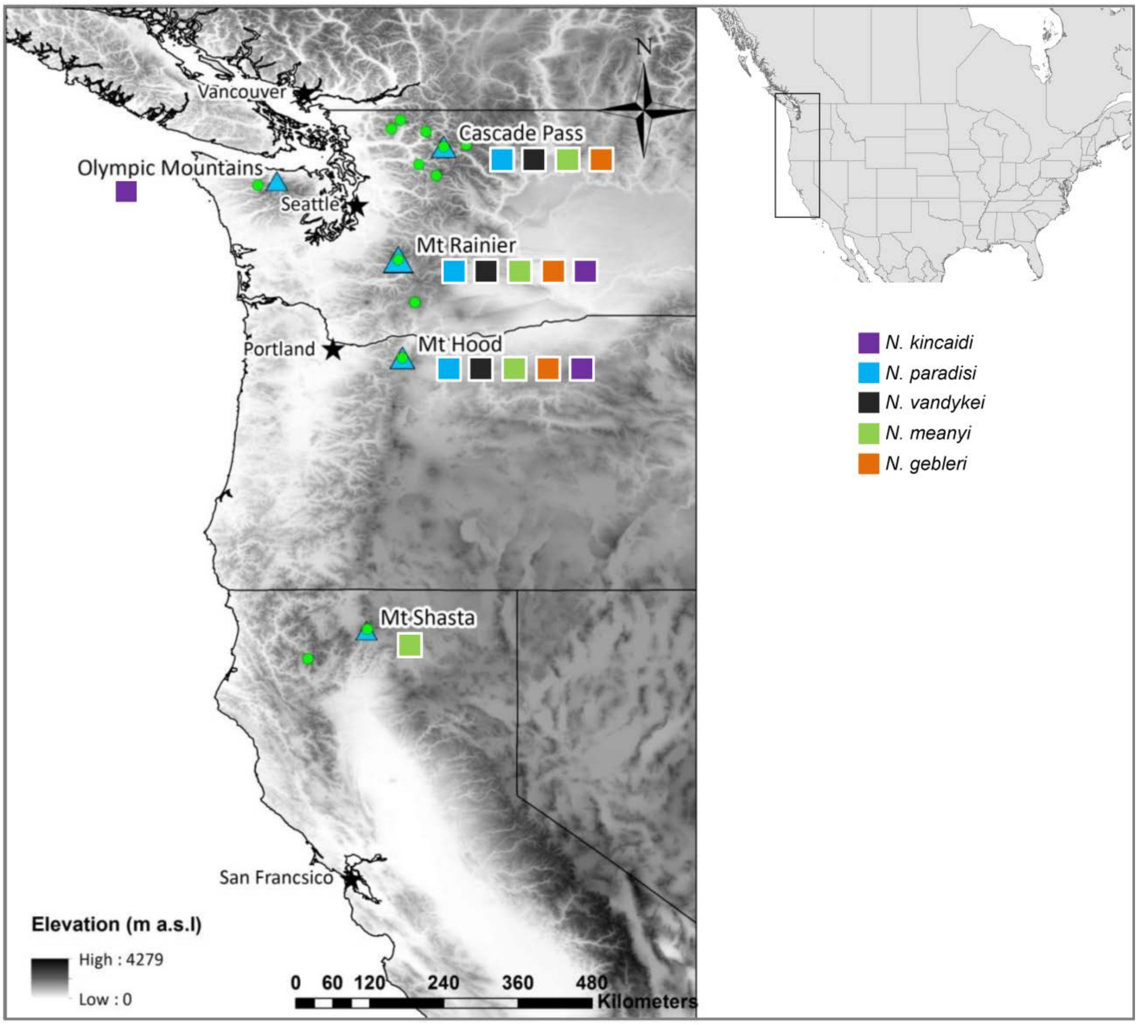

Figure C.4. Map of the Northwest USA, showing locations of transects sampled for Chapter 3 (green circles) and mountains from which beetles were sampled for thermal tolerance measurements (blue triangles). Coloured squares indicate the species for which thermal tolerance was measured at each mountain.

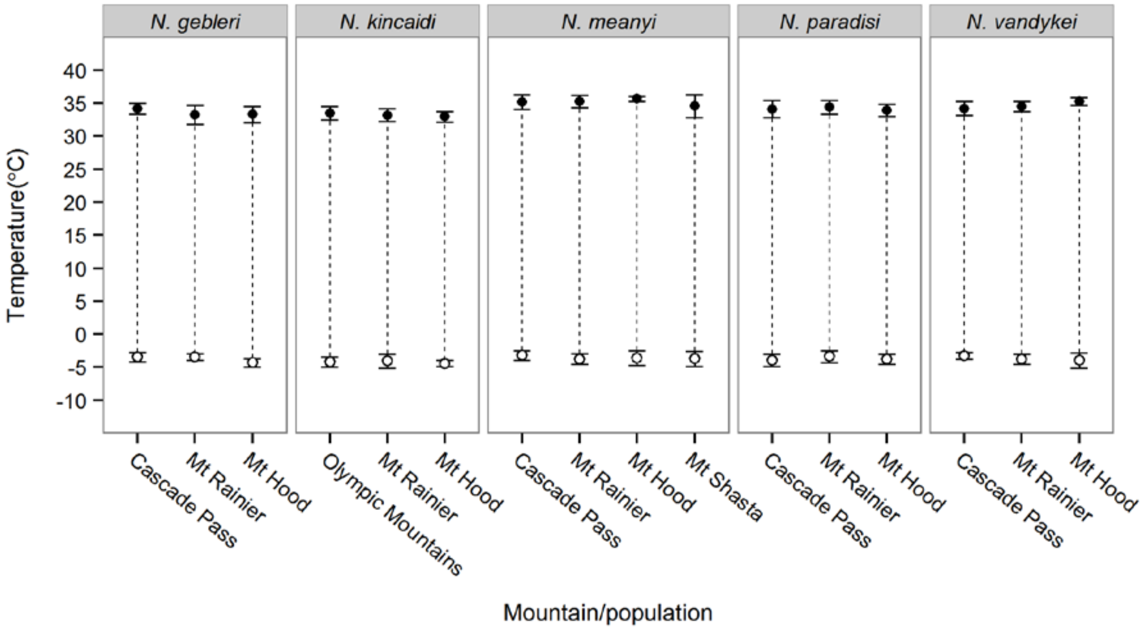

Figure C.5. Among-population variation in thermal tolerance for five *Nebria* species. Filled circles represent the upper thermal limits (± s.d.), unfilled circles show the lower thermal limits (± s.d.). The difference between these limits (dashed line) is the thermal tolerance range. Mountains are ordered from northernmost (left) to southernmost (right) (see Fig. C.4).

C.6 References

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Appendix D

Supporting information for Chapter 5

D.1 Size variation in the *Kosciuscola*

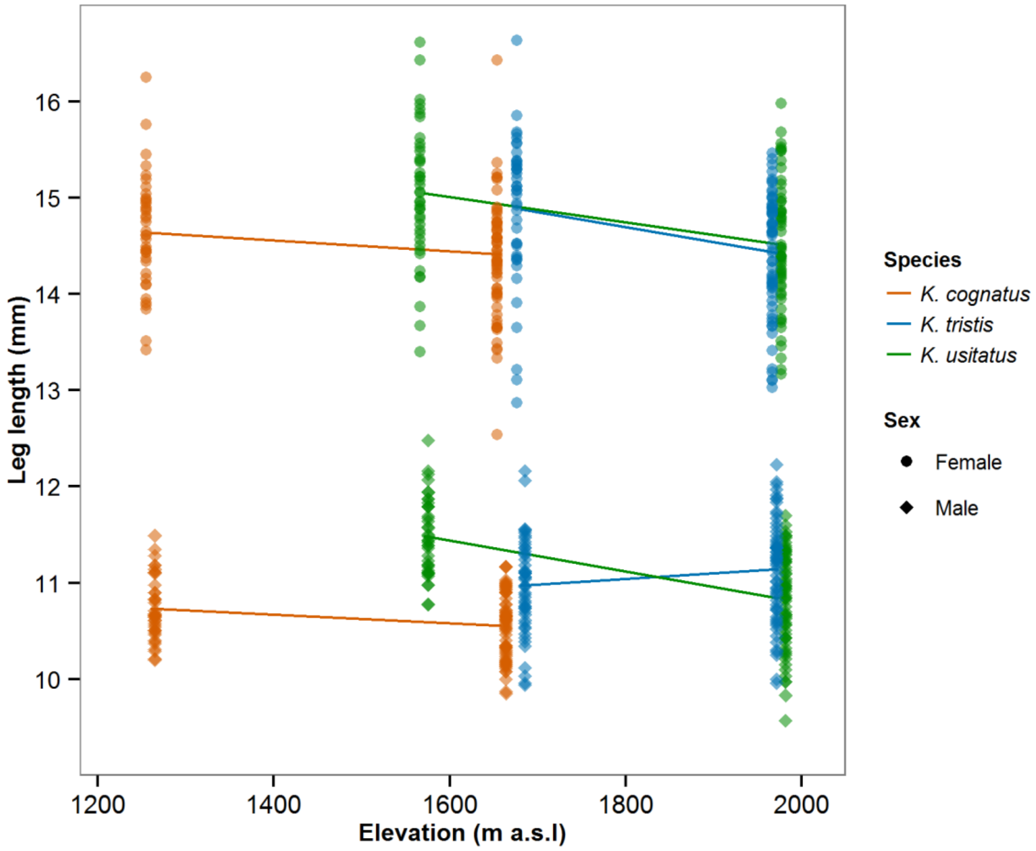

Figure D.1. Leg lengths (mm) for three species of *Kosciuscola* grasshoppers from the low- and high-elevation populations used for thermal tolerance measurements in Chapter 5. High-elevation *K. tristis* and *K. usitatus* were collected from the same location, and are offset in the figure for visualisation only.

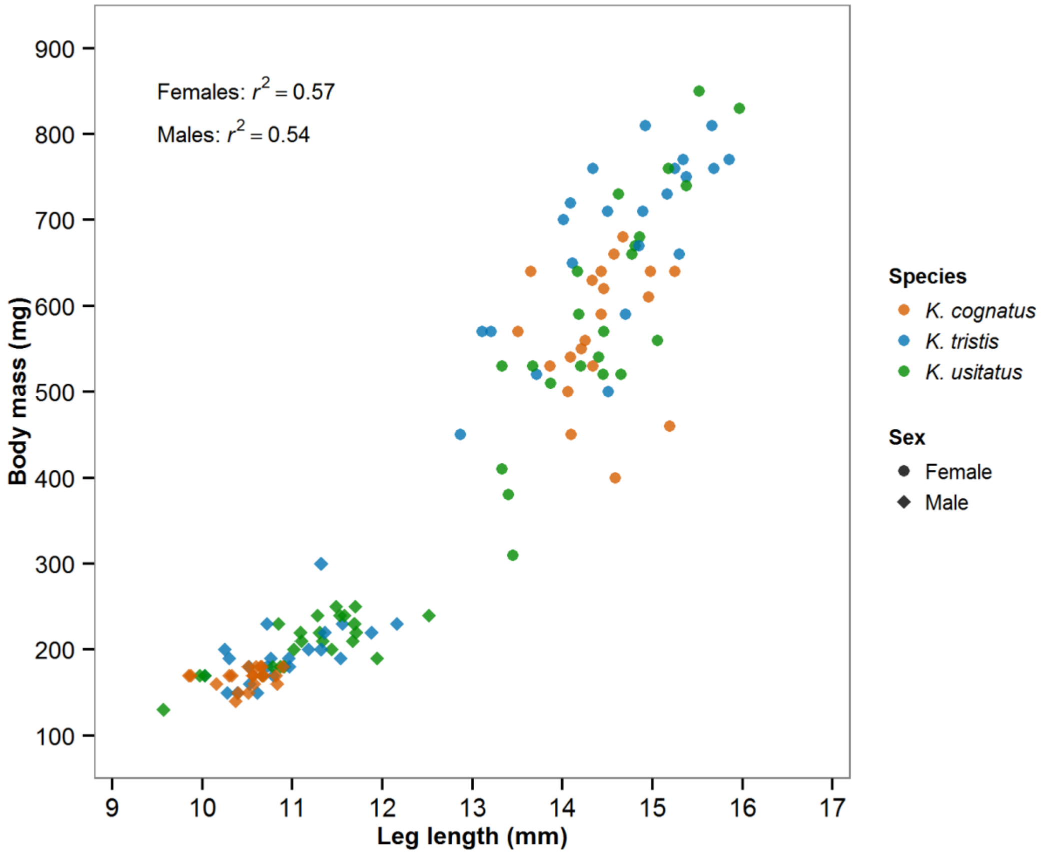

Figure D.2. The correlation (Pearson's r^2) between leg length and body mass among three species of *Kosciuscola*. Leg length was used as a proxy for overall body size for analyses in Chapter 5.All data were collected in April, 2013.

D.2 Weather station information and microclimate models

Table D.1. Australian Bureau of Meteorology weather stations in the Kosciuszko region used for microclimate predictions.

Station name (number)	Weather observations (abbreviation)
Thredbo Village (071041)	Maximum (TV_{max}), minimum (TV_{min})
Thredbo Top Station (071032)	Maximum (TT_{max}), minimum (TT_{min}), solar radiation (TT_{solar})
Perisher Valley (071075)	Solar radiation (PV_{solar})
Charlotte Pass (071003)	Solar radiation (CP_{solar})

Table D.2. Final models for microclimate predictions at each of the five study sites, identified by the lowest AIC values following stepwise model selection. Daily maximum and minimum values were modelled separately.

Site	Temperature	Model
Thredbo 1	Maximum	$TT_{max} + TT_{solar} + TT_{max}*TT_{solar}$
	Minimum	$TV_{min} + TT_{solar} + TT_{min} + CP_{solar}$
Thredbo 2	Maximum	$TV_{max} + TT_{solar}$
	Minimum	$TT_{min} + PV_{solar} + TV_{min} + TT_{min}*TV_{min}$
Thredbo 3	Maximum	$TT_{max} + TV_{max} + TT_{solar} + PV_{solar}$
	Minimum	$TV_{min} + TT_{solar} + PV_{solar} + CP_{solar} + TV_{min}*TT_{solar} + TV_{min}*CP_{solar}$
Guthega	Maximum	$TT_{max} + TT_{solar} + PV_{solar} + CP_{solar}$
	Minimum	$TV_{min} + PV_{solar} + CP_{solar}$
Island Bend	Maximum	$TT_{max} + TV_{max} + PV_{solar}$
	Minimum	$TV_{min} + TT_{solar}$

D.3 Standardised effect sizes and pairwise comparisons

Table D.3. Standardised mean effect sizes (Hedges' *g* with small-sample correction). Interspecific comparisons are shown below (Guthega) and above (Thredbo 1) the diagonal, and intra-specific comparisons (for the main effect of elevation) are shown on the diagonal. Values in bold are statistically significant after correction for multiple comparisons using false discovery rates.

CT$_{\text{min}}$	*K. usitatus*	*K. tristis*	*K. cognatus*
K. usitatus	0.5	**0.8**	
K. tristis	**2.0**	0.3	
K. cognatus	**3.1**	**1.3**	0.3
CT$_{\text{min-recovery}}$			
K. usitatus	0.1		
K. tristis	0.2	0.6	
K. cognatus	0.3	0.1	0.1
SCP			
K. usitatus	0.4	0.9	
K. tristis		0.3	
K. cognatus			0.6
CT$_{\text{max}}$			
K. usitatus	**0.7**	0.2	
K. tristis	0.1	**0.7**	
K. cognatus	0.2	0.2	0.1
HMT$_{\text{time}}$			
K. usitatus	0.3	0.4	
K. tristis		0.6	
K. cognatus			0.3

D.4 Thermal tolerance of *Kosciuscola cuneatus*

In Chapter 5, I measured cold and heat tolerances for three of the four mainland *Kosciuscola* species. The fourth species, *K. cuneatus*, is uncommon in the Kosciuszko region where the study was completed. For this species, I measured critical thermal limits and recovery from chill coma using individuals from Mt Gingera, Australian Capital Territory (ACT) (35.57593°S, 148.77913°E) at elevations of 1600 m a.s.l (21 individuals) and 1855 m (7 individuals). Using the methods described in Chapter 5, I tested cold tolerance (CT_{min} and $CT_{min\text{-}recovery}$) for five females and four males and heat tolerance (CT_{max}) for seven females and six males. All individuals were acclimated for one week at 12°C prior to the experiment (see Chapter 5 for details). Grasshoppers were collected with permission from ACT Territory and Municipal Services. As shown in Chapter 5, there can be significant variation among populations in thermal limits. The results are provided here (Table D.4) as a first estimate of the thermal limits of this species, but are not directly comparable to measurements from the other three species, which were made from populations in the Kosciuszko region.

Table D.4. Summary of cold tolerance (CT_{min}, $CT_{min\text{-}recovery}$) and heat tolerance for *Kosciuscola cuneatus* from Mt Gingera, ACT.

Measure	Mean	s.d.	*n*
CT_{min}	-2.5	0.4	9
$CT_{min\text{-}recovery}$	10.7	1.5	9
CT_{max}	49.5	0.7	12

Appendix E

Supporting information for Chapter 6

Table E.1. Details of reagent concentrations for polymerase chain reactions to amplify *CO1* and *ITS1* sequences.

Reagent	PCR concentration	Volume (µl) in 25 µL reaction
CO1		
BSA	0.5 µg/µL	1.25
MgCl2	0.75 mM	0.75
10x Thermo pro buffer	1 x	2.5
dNTPs	200 µM	5
Forward primer	0.016 µM	1
Reverse primer	0.016 µM	1
NEB taq	1.25 u	0.25
DNA	~0.7 ng/µL	5
ITS1		
BSA	0.5 µg/µL	1.25
MgCl2	0.75 mM	0.75
10x Thermo pro buffer	1 x	2.5
dNTPs	200 µM	5
Forward primer	0.016 µM	1
Reverse primer	0.016 µM	1
NEB taq	1.25 u	0.25
DNA	~0.7 ng/µL	5

Table E.2. Genbank accession numbers for *Kosciuscola tristis* sequences used to construct the *CO1* and *ITS1* phylogenies. The collection sites represented by each haplotype, and number of individuals, are also provided.

Haplotype number	Genbank accession number	Collection site (*N* individuals)
CO1		
1	KJ870103	Mt Bogong (4)
		Mt Jaithmathang (4)
		Mt Cope (5)
		Falls Creek (5)
2	KJ870104	Mt Buller (4)
3	KJ870105	Thredbo (4)
4	KJ870106	Mt Jaithmathang (1)
5	KJ870107	Mt Hotham (2)
6	KJ870108	Mt Buller (1)
7	KJ870109	Mt Stirling (5)
8	KJ870110	Baw Baw plateau (1)
9	KJ870111	Baw Baw plateau (1)
10	KJ870112	Baw Baw plateau (3)
11	KJ870113	Mt Tate (1)
12	KJ870114	Mt Jagungal (4)
13	KJ870115	Mt Tate (1)
		Guthega (2)
14	KJ870116	Thredbo (1)
15	KJ870117	Mt Tate (1)
		Guthega (1)
16	KJ870118	Mt Tate (2)
17	KJ870119	Mt Nelse (1)
18	KJ870120	Mt Nelse (2)
19	KJ870121	Mt Bogong (1)
20	KJ870122	Mt Jagungal (1)
21	KJ870123	Mt Buffalo (1)
22	KJ870124	Mt Buffalo (1)
23	KJ870125	Mt Buffalo (1)
24	KJ870126	Mt Buffalo (1)
25	KJ870127	Mt Buffalo (1)
26	KJ870128	Mt Nelse (1)
27	KJ870129	Mt Nelse (1)
28	KJ870130	Mt Townsend (2)
29	KJ870131	Mt Townsend (2)

Continued on next page.

Table E.2. Continued from previous page.

Haplotype number	Genbank accession number	Collection site (*N* individuals)
COI		
30	KJ870132	Mt Townsend (1)
31	KJ870133	Guthega (1)
32	KJ870134	Guthega (1)
33	KJ870135	Mt Hotham (1)
34	KJ870136	Mt Hotham (1)
39	KJ870137	Mt Hotham (1)
ITS1		
1	KJ870139	Baw Baw plateau (1)
2	KJ870140	Baw Baw plateau (1)
3	KJ870141	Baw Baw plateau (1)
4	KJ870142	Baw Baw plateau (1)
5	KJ870143	Baw Baw plateau (1)
6	KJ870144	Mt Bogong (4)
		Mt Buffalo (5)
		Mt Cope (5)
		Falls Creek (5)
		Mt Jaithmathang (5)
		Mt Nelse (4)
7	KJ870145	Mt Buller (5)
		Mt Hotham (4)
		Mt Stirling (5)
8	KJ870146	Guthega (1)
9	KJ870147	Guthega (4)
		Mt Jagungal (5)
		Mt Tate (5)
		Thredbo (5)
		Mt Townsend (5)
10	KJ870148	Mt Nelse
11	KJ870149	Mt Hotham

Table E.3. Average population-specific null allele frequencies

Population	Null allele frequency	
	mean	S.D.
Five-locus dataset		
Thredbo 1	0.12	0.08
Thredbo 2	0.11	0.10
Thredbo 3	0.09	0.09
Guthega	0.12	0.11
Mt Jagungal	0.10	0.12
Mt Tate	0.12	0.11
Mt Buffalo	0.21	0.09
Mt Bogong	0.20	0.13
Mt Cope	0.20	0.10
Falls Creek	0.19	0.07
Mt Buller	0.19	0.10
Mt Stirling	0.18	0.09
Baw Baw plateau	0.19	0.14
Kosciuszko dataset		
Thredbo 1	0.16	0.07
Thredbo 2	0.20	0.07
Thredbo 3	0.17	0.12
Guthega	0.21	0.12
Mt Tate	0.19	0.12
Mt Jagungal	0.20	0.11

Table E.4. Average locus-specific null allele frequencies.

Locus	Null allele frequency	
	mean	S.D.
Five-locus dataset		
Ktr29	0.09	0.03
Ktr76	0.04	0.05
Ktr73	0.18	0.04
Ktr82	0.18	0.08
Ktr58	0.20	0.08
Ktr88	0.30	0.03
Ktr30	0.25	0.06
Ktr60	0.30	0.05
Kosciuszko dataset		
Ktr29	0.05	0.04
Ktr76	0.12	0.10
Ktr58	0.14	0.08
Ktr88	0.30	0.05
Ktr30	0.17	0.07

Table E.5. Pairwise F_{ST} (below diagonal) and F'_{ST} (above diagonal) matrices for the 5-locus dataset. F_{ST} values were corrected for null alleles with the ENA method (Chapuis & Estoup 2007). Statistical significance was tested for F_{ST} values, using 1000 bootstrap iterations and corrected for multiple comparisons using the False Discovery Rates method. All pairwise differences are statistically significant ($p < 0.05$) except those in bold.

	Buffalo	**Bogong**	**Cope**	**Falls**	**Baw Baw**	**Buller**	**Stirling**	**Guthega**	**Jagungal**	**Tate**	**Thredbo 1**	**Thredbo 2**	**Thredbo 3**
Buffalo		0.535	0.484	0.497	0.408	0.38	0.568	0.456	0.561	0.469	0.524	0.495	0.515
Bogong	0.079		0.172	0.227	0.456	0.432	0.284	0.643	0.623	0.556	0.514	0.485	0.636
Cope	0.093	0.015		0	0.295	0.309	0.355	0.402	0.540	0.344	0.388	0.364	0.433
Falls	0.083	0.013	**0.002**		0.424	0.313	0.327	0.422	0.401	0.384	0.397	0.321	0.434
Baw Baw	0.108	0.050	0.057	0.056		0.207	0.249	0.357	0.485	0.34	0.508	0.419	0.503
Buller	0.070	0.044	0.039	0.035	0.047		0.112	0.571	0.505	0.516	0.585	0.527	0.555
Stirling	0.077	0.036	0.043	0.034	0.055	**0.010**		0.657	0.559	0.573	0.644	0.549	0.672
Guthega	0.156	0.083	0.057	0.057	0.118	0.102	0.109		0.414	0.028	0.112	0.095	0.074
Jagungal	0.148	0.080	0.066	0.056	0.111	0.093	0.096	0.059		0.409	0.463	0.376	0.427
Tate	0.158	0.081	0.056	0.059	0.108	0.099	0.100	0.010	0.064		0.189	0.117	0.15
Thredbo 1	0.160	0.079	0.062	0.060	0.127	0.106	0.110	0.013	0.068	0.027		0.023	0.085
Thredbo 2	0.149	0.067	0.049	0.047	0.105	0.089	0.092	0.015	0.055	**0.014**	0.005		0.022
Thredbo 3	0.172	0.096	0.068	0.070	0.137	0.112	0.121	0.007	0.069	0.021	0.012	0.008	

Table E.6. Pairwise F_{ST} (below diagonal) and F'_{ST} (above diagonal) matrices for the Kosciuszko dataset. F_{ST} values were corrected for null alleles with the ENA method (Chapuis & Estoup 2007). Statistical significance was tested for F_{ST} values, using 1000 bootstrap iterations and corrected for multiple comparisons using the False Discovery Rates method. All pairwise differences are statistically significant ($p < 0.05$) except those in bold.

	Guthega	**Jagungal**	**Tate**	**Thredbo 1**	**Thredbo 2**	**Thredbo 3**
Guthega		0.312	0.048	0.162	0.123	0.144
Jagungal	0.048		0.307	0.326	0.291	0.298
Tate	0.011	0.044		0.166	0.079	0.181
Thredbo 1	0.029	0.065	0.031		0.017	0.106
Thredbo 2	0.023	0.048	0.013	**0.002**		0.057
Thredbo 3	0.023	0.055	0.028	0.018	**0.013**	

Appendix F

Supporting information for Chapter 7

F.1 Systematic literature survey to identify snow manipulation studies

I carried out a systematic search of the literature to identify snow manipulation experiments in alpine or montane environments. I used the Web of Science database and the search terms [winter warming AND snow], [snow manipulation] and [snow AND alpine AND climate change AND (experiment OR reduced snow)] within the life sciences literature to retrieve 451 articles in November 2013. The search was updated in August 2015 with an additional 141 articles. I also identified recent reviews on related topics (Edwards et al., 2007, Groffman et al., 2001, Kreyling, 2010, Wipf, 2009, Zhang, 2005) and retrieved all references citing or cited by these papers, yielding a total of 695 unique references. From this set of articles, I scanned the abstracts to identify potentially relevant papers, then read the methods of those shortlisted to determine which studies had experimentally reduced snow cover. In doing so, I excluded studies that used natural snow depth gradients and laboratory-based freeze-thaw experiments. I found 124 studies that had manipulated snow cover and measured biotic and/or abiotic responses. However, these covered only 60 unique sites and snow cover was experimentally reduced at only 40 of those sites. The 22 alpine studies covered 11 sites. The ecological effects of reduced snow cover measured by these studies are summarised in Table F.1, while effects on the abiotic environment are summarised in Table F.2.

Table F.1. Primary results for studies testing ecological effects of reduced snow cover in alpine/sub-alpine environments. All responses are for plants, unless otherwise indicated. Symbols indicate the response variable measured was higher (+), lower (-) or equivalent (=) in the reduced snow treatment, compared to controls. For phenology, symbols indicate earlier (+) or later (-) timing of phenophases.

Study	Location	Method of snow removal	Effect of snow removal	Response measured	Result		
Adler *et al.* (2007)	West Elk Mountains, Rocky Mountains, USA	Heat	↓ duration (spring)	Growth (arthropod)	+	-	=
				Abundance (arthropod)	+	-	=
				Leaf N		-	
				Leaf C			=
				Leaf C:N	+		
			↓ duration (spring)	Abundance (arthropod)			=
				Density (arthropod)	+	-	=
				Plant C:N	+		
				Plant C			=
				Plant size	+		
Baptist *et al.* (2009)	Agnel Pass, France	Unspecified	↓ duration (spring)	Productivity		-	
				Biomass			=
				Leaf area			=
Bombonato and Gerdol (2012)	Trento, Italian Alps, Italy	Shovel	↑ duration (spring)	Plant N			=
				Plant P			=
			↑ duration (autumn)	Plant N			=
				Plant P			=

Study	Location	Method of snow removal	Effect of snow removal	Response measured	Result
		Shovel/cloth	↓ duration (spring)	Plant N	=
				Plant P	=
Cornelius *et al.* (2013)	Berchtesgaden, Bavarian Alps, Germany	Shovel	↓ duration (spring)	Phenology	+
			↑ duration (spring)	Phenology	=
Dunne *et al.* (2003)	West Elk Mountains, Rocky Mountains, USA	Heat	↓ duration (spring)	Phenology	+
		Shovel	↓ duration (spring)	Phenology	+
Ferrar *et al.* (1988)	Snowy Mountains, Australia	Unspecified	↓ depth	Seedling survival	=
Galen and Stanton (1993)	Pennsylvania Mountain, Rocky Mountains, USA	Cloth cover	↑ duration (spring)	Seed maturation interval	-
				% cover of target species	=
				Seed mass	-
		Shovel	↓ duration (spring)	Seed maturation interval	+
				% cover of target species	+
				Seed mass	+
Galen and Stanton (1995)	Pennsylvania Mountain, Rocky Mountains, USA	Cloth cover	↑ duration (spring)	Phenology	-
				Community composition	change
		Shovel	↓ duration (spring)	Phenology	-
				Community composition	change
Galen and Stanton (1999)	Pennsylvania Mountain, Colorado, USA	Cloth cover	↑ duration (spring)	Seedling establishment	=
		Shovel	↓ duration (spring)	Seedling establishment	+
Gaul *et al.* (2008)	Fichtel Mountains, Germany	Shovel	↓ depth	Root biomass	=

Study	Location	Method of snow removal	Effect of snow removal	Response measured	Result
				Root mortality	+
				Root production	+ - =
				Root length	+
				Root longevity	-
Gerdol *et al.* (2013)	Apennines, Italy	Shovel	↓ depth, ↓ duration (spring)	Leaf area	- =
				# leaves	- =
				Shoot length	=
				# flowers	-
				Leaf C	-
				Leaf N	=
			↑ depth, ↑ duration (spring)	Community composition	change
				Leaf area	=
				# leaves	=
				Shoot length	=
				# flowers	- =
				Leaf C	=
				Leaf N	=
Loik *et al.* (2000)	West Elk Mountains, Rocky Mountains, USA	Heat	↓ duration (spring)	CO2 assimilation rate	=
				CO2 uptake	=
				Leaf temperature	=

Study	Location	Method of snow removal	Effect of snow removal	Response measured	Result		
				Leaf water potential			=
				Photosynthesis			=
Petraglia *et al.* (2014)	Gavia Valley, Rhaetian Alps, Italy	Shovel	↓ duration (spring)	Phenology	+		=
				Shoot mortality			=
Price and Waser (2000)	West Elk Mountains, Rocky Mountains, USA	Heat	↓ depth, ↓ duration (spring)	Community composition			=
				Species richness			=
Rixen *et al.* (2012)	Davos, Central Alps, Switzerland	Shovel	↓ depth, ↓ duration (spring)	Age of ramets			=
				Xylem ring width			=
				Shoot length	+		
				Community composition			=
			↑ depth, ↑ duration (spring)	Age of ramets			=
				Xylem ring width	+	-	=
				Shoot length			=
				Community composition			=
Robroek *et al.* (2012)	Vallée de Joux, Jura Mountains, Switzerland	Shovel	↓ depth	Leaf N			=
				Leaf P			=
				Leaf N:P			=
				Root enzyme activity			=
Roy *et al.* (2004)	West Elk Mountains, Rocky Mountains, USA	Heat	↓ duration (spring)	Damage to plants (arthropods)	+	-	=
				Species richness (arthropod)	+	-	=

Study	**Location**	**Method of snow removal**	**Effect of snow removal**	**Response measured**	**Result**
Saavedra *et al.* (2003)	West Elk Mountains, Rocky Mountains, USA	Heat	↓ duration (spring)	# plants	-
				# flowering plants	-
				# flowers per plants	=
				% aborted flower buds	+ =
Saleska et al (2002)	West Elk Mountains, Rocky Mountains, USA	Heat	↓ duration (spring)	Aboveground biomass	=
				Community composition	change
Steltzer *et al.* (2009)	San Juan Mountains, Rocky Mountains, USA	Black shade cloth; dust	↓ duration (spring)	Phenology	+
			↑ duration (spring)	Phenology	=
Wipf *et al.* (2009)	Davos, Central Alps, Switzerland	Shovel	↓ depth	Phenology	+ =
				Annual growth	=
				Root mass	-
				Fecundity	- =
			↓ depth, ↓ duration (spring)	Phenology	+ =
				Annual growth	- =
				Frost damage to roots	+
				Bud mortality	+
				Fecundity	=

Table F.2. Effects of reduced snow cover on abiotic conditions, from studies in alpine/sub-alpine environments.

Study	Location	Method of snow removal	Effect of snow removal	Response measured	Result
Adler *et al.* (2007)	West Elk Mountains, Rocky Mountains, USA	Heat	↓ duration (spring)	Soil moisture	-
				Soil temperature	+
Baptist *et al.* (2009)	Agnel Pass, France	Unspecified	↓ duration (spring)	Soil moisture	=
				Soil temperature	=
				# frosts	+
Blankinship *et al.* (2014)	Sierra Nevada, California, USA	Black sand/white shade cloth	↓ duration (spring)	Soil moisture	-
Cornelius *et al.* (2013)	Berchtesgaden, Germany	Shovel	↓ duration (spring)	Soil moisture	=
Dunne *et al.* (2003)	Rocky Mountains, Colorado, USA	Shovel	↓ duration (spring)	Soil moisture	=
				Soil temperature	+
		Heat	↓ duration (spring)	Soil moisture	-
Dunne *et al.* (2004)	Rocky Mountains, Colorado, USA	Shovel	↓ duration (spring)	Soil moisture	=
		Heat		Soil moisture	-
Freppaz *et al.* (2008)	Vallee d'Aoste, Italy	Shovel	↓ depth, ↓ duration (spring)	Soil temperature	-
Gaul *et al.* (2008)	Fichtelgebirge Mountains, Germany	Shovel	↓ depth	Soil temperature	-
Gerdol *et al.* (2013)	Apennines, Italy	Shovel	↓ duration (spring)	# frosts	+
Petraglia *et al.* (2014)	Gavia Valley, Rhaetian Alps, Italy	Shovel	↓ duration (spring)	# frosts	+
Price and Waser (2000)	Rocky Mountains, Colorado, USA	Heat	↓ depth, ↓ duration (spring)	Soil temperature	+
				Soil temperature fluctuations	+

Study	Location	Method of snow removal	Effect of snow removal	Response measured	Result
				Soil moisture	-
				Air temperature	=
Robroek *et al.* (2012)	Vallée de Joux, Jura Mountains, Switzerland	Shovel	↓ depth	Soil temperature fluctuations	+
				# frosts	=
				Soil moisture	=
Schmitt and Glaser (2011)	Fichtel Mountains, Germany	Shovel	↓ depth	Soil temperature	-
Wipf *et al.* (2009)	Davos, Switzerland	Shovel	↓ depth, ↓ duration (spring)	Soil moisture	=
				Soil temperature	-

F.2 Details of taxa collected in pitfall traps at Mt Stirling and Mt Twynam

Table F.3. Details of the taxa collected during pitfall trapping at Mt Stirling (years 2013 and 2015) and Mt Twynam (year 2014). Shading indicates the presence of a taxon in at least one pitfall trap during a season. At Mt Stirling, "summer" indicates the snow-free period and "winter" is the period under snow.

Phylum/Class	Order/Sub-order	Superfamily/Family/Genus/Species		Mt Stirling (summer)	Mt Stirling (winter)	Mt Twynam (winter)	Mt Twynam (spring)
Annelida							
Oligochaeta						🟩	
Arthropoda							
Chelicerata							
Arachnida	Acari						
	Trombidiformes	Bdellidae		🟩	🟩	🟩	🟩
	Sarcoptiformes						
	Oribatida			🟩	🟩	🟩	🟩
	Araneae						
	Araneomorphae	Amaurobiidae		🟩	🟩	🟩	🟩
		Amphinectidae		🟩	🟩	🟩	🟩
		Micropholcommatidae	*Raveniella*	🟩	🟩	🟩	
			Micropholcomma		🟩		
		Malkaridae	*Perissopmeros*	🟩			
		Linyphiidae		🟩	🟩	🟩	🟩
		Lycosidae		🟩		🟩	🟩
		Ctenidae				🟩	🟩
		Pisauridae			🟩		
		Pararchaeidae	*Flavarchaea*	🟩		🟩	🟩

Phylum/Class	Order/Sub-order	Superfamily/Family/Genus/Species		Mt Stirling (summer)	Mt Stirling (winter)	Mt Twynam (winter)	Mt Twynam (spring)
		Theridiidae		🟩			
		Prodidomidae					🟩
		Gnaphosidae		🟩	🟩		
		Oonopidae			🟩		🟩
		Clubionidae		🟩			
		Salticidae		🟩			
	Mygalomorphae	Idiopodidae		🟩			
	Opiliones					🟩	🟩
	Pseudoscorpiones				🟩	🟩	
Crustacea							
Malacostraca	Amphipoda	Talitridae		🟩	🟩	🟩	🟩
	Isopoda			🟩	🟩	🟩	
Maxillopoda	Copepoda						
	Harpacticoida	Canthocamptidae			🟩		
Myriapoda							
Chilopoda				🟩	🟩	🟩	🟩
Diplopoda					🟩	🟩	🟩
Symphyla	Cephalostigmata	Scutigerelloidea	Scutigerellidae		🟩	🟩	
Hexapoda	Collembola						
	Symphypleona	Katiannidae	*Katianna*	🟩	🟩	🟩	🟩
			Polykatianna	🟩			
			Sminthurinus	🟩	🟩	🟩	🟩

Phylum/Class	Order/Sub-order	Superfamily/Family/Genus/Species			Mt Stirling (summer)	Mt Stirling (winter)	Mt Twynam (winter)	Mt Twynam (spring)
		Bourletiellidae	*Corynephoria*		🟩			
	Entomobryomorpha	Tomoceridae	*Lepidophorella*				🟩	🟩
		Entomobryidae	*Entomobrya*				🟩	🟩
			Entomobrya cf. *marginata*					🟩
			Lepidocyrtoides		🟩	🟩		
			Drepanura		🟩	🟩	🟩	🟩
			Australotomurus		🟩		🟩	🟩
			Setogaster		🟩	🟩		
		Paronellidae	*Paronellides* sp.		🟩			
			Paronellides sp. nov.				🟩	🟩
			Paronellides sp. nr. *tasmaniae*				🟩	🟩
			Paronellides cf. *dandenongensis*		🟩	🟩		
			Pseudoparonella sp.				🟩	🟩
		Isotomidae	*Acanthomurus*		🟩	🟩		
			Skadisotoma cf. *impericulosa*		🟩	🟩	🟩	🟩
	Poduromorpha	Hypogastruridae			🟩	🟩		
			Triacanthella				🟩	🟩
		Neanuridae	*Acanthanura*		🟩	🟩	🟩	
		Brachystomellidae			🟩	🟩		
			Brachystomella sp. nr *solidaria*				🟩	🟩
Insecta	Coleoptera	Caraboidea	Carabidae					
			Broscinae	*Promecoderus masteri*			🟩	🟩
				Promecoderus inornatus			🟩	🟩
				Chylnus montanum	🟩			

Phylum/Class	Order/Sub-order	Superfamily/Family/Genus/Species				Mt Stirling (summer)	Mt Stirling (winter)	Mt Twynam (winter)	Mt Twynam (spring)
				Trechinae		🟩	🟩		
				Moriomorphinae		🟩	🟩		
				Harpalinae		🟩			
		Byrrhoidea	Byrrhidae						
				Syncalyptinae	*Microchaetes*	🟩		🟩	
		Chrysomeloidea	Chrysomellidae						
				Chrysomelinae			🟩		
				Galerucerinae		🟩	🟩		
				Hispinae		🟩			
			Cerambycidae			🟩			
		Cleroidea	Melyridae						
				Malachiinae		🟩		🟩	🟩
		Cucujoidea	Anthicidae			🟩			
			Coccinellidae			🟩	🟩	🟩	
			Corylophidae			🟩			
			Cryptophagidae		Picrotini gen. 1. sp. 1	🟩	🟩		
			Latridiidae			🟩	🟩	🟩	
			Nitidulidae				🟩	🟩	
			Phalacridae			🟩		🟩	
		Curculionoidea	Curculionidae						
				Molytinae		🟩	🟩	🟩	🟩
				Brachycerinae		🟩			
				Dryophthorinae		🟩	🟩		

Phylum/Class	Order/Sub-order	Superfamily/Family/Genus/Species			Mt Stirling (summer)	Mt Stirling (winter)	Mt Twynam (winter)	Mt Twynam (spring)
		Elateroidea	Cantharidae					
			Cauliognathinae	*Cauliognathus* cf. *lugubris*	🟩			
			Elateridae					
			Denticollinae			🟩		
		Scarabaeoidea	Scarabaeidae					
			Melolinthinae		🟩			
		Staphylinoidea	Leiodidae					
			Cholevinae		🟩	🟩		
			Staphylinidae					
			Aleocharinae		🟩	🟩	🟩	🟩
			Scydmaeninae		🟩	🟩		
			Pselaphinae		🟩		🟩	
		Tenebrionoidea	Melandryidae					
			Melandryinae		🟩	🟩		
			Mycetophagidae		🟩	🟩		
	Hymenoptera	Vespoidea	Formicidae	*Iridomyrmex* sp.	🟩	🟩		
		Ceraphronoidea	Ceraphonidae		🟩			🟩
			Megaspilidae		🟩			🟩
			Eulophidae		🟩			🟩
		Ichneumonoidea	Ichneumonidae				🟩	
			Braconidae		🟩	🟩		
		Platygastroidea	Scelionidae		🟩	🟩	🟩	🟩
			Platygastruridae		🟩			
		Proctotrupoidea	Diapriidae		🟩	🟩	🟩	🟩

Phylum/Class	Order/Sub-order	Superfamily/Family/Genus/Species			Mt Stirling (summer)	Mt Stirling (winter)	Mt Twynam (winter)	Mt Twynam (spring)
		Chalcidoidea	Myrmaridae		🟩			
		Cynipoidea	Figitidae		🟩			
	Diptera				🟩	🟩	🟩	🟩
	Lepidoptera				🟩			
	Trichoptera				🟩			
	Thysanoptera				🟩	🟩	🟩	
	Blattodea				🟩	🟩		
	Orthoptera	Grylloidea	Trigonidiidae	*Bobilla victoriae*	🟩			
	Dermaptera				🟩			
	Hemiptera	Aphidoidea	Aphididae		🟩	🟩	🟩	
		Lygaeoidea	Lygaeidae		🟩	🟩		
		Membracoidea	Cicadellidae		🟩	🟩	🟩	
		Coccoidea			🟩	🟩	🟩	
	Neuroptera						🟩	

F.3 Summary data

F.3.1 Percentage contributions of invertebrate taxa to the pitfall-trapped fauna

Table F.4. The percentage contribution by numbers to the pitfall-trapped invertebrate fauna of Mt Stirling, 2013. Calculations were made after excluding copepods, which occurred in high abundance in a single plot (see Chapter 7 for details). The total number of individuals collected in each sampling period is included at the bottom of the table.

Taxon	January	March	May	July	September	November
Collembola (Poduromorpha)	10.6	19.4	64.0	40.3	63.4	83.5
Collembola (Entomobryomorpha)	4.0	36.1	1.8	1.4	0.6	0.2
Collembola (Symphypleona)	1.0	0.8	5.0	15.3	6.4	0.4
Acari	3.1	1.7	22.2	19.4	24.1	1.4
Coleoptera (adults)	4.2	3.5	2.5	13.9	2.4	0.6
Coleoptera (larvae)	1.2	1.8	0.8	1.4	0.2	0.0
Araneae	1.3	0.2	1.5	4.2	2.0	0.1
Hemiptera	10.7	1.6	0.5	4.2	0.5	0.2
Hymenoptera (ants)	48.5	31.5	0.0	0.0	0.2	3.7
Hymenoptera (wasps)	3.0	1.6	0.0	0.0	0.0	0.3
Diptera	8.6	0.0	1.3	0.0	0.2	9.4
Amphipoda	2.8	0.0	0.3	0.0	0.0	0.0
Isopoda	0.2	0.0	0.3	0.0	0.0	0.0
Blattodea	0.2	0.0	0.0	0.0	0.1	0.0
Lepidoptera	0.2	0.2	0.0	0.0	0.1	0.1
Orthoptera	0.0	0.1	0.0	0.0	0.0	0.0
Dermaptera	0.3	0.7	0.0	0.0	0.0	0.0
Diplopoda	0.2	0.8	0.0	0.0	0.0	0.0
Chilopoda	0.3	0.0	0.0	0.0	0.0	0.0
Thysanoptera	0.2	0.0	0.0	0.0	0.0	0.0
Total #	1999	882	397	72	1272	11482

Table F.5. The percentage contribution by numbers to the pitfall-trapped invertebrate fauna of Mt Twynam, 2014, in control (C) and snow removal (E) plots. The total number of individuals collected in each sampling period is included at the bottom of the table.

Taxon	**Winter**		**Spring**	
	C	E	C	E
Collembola (Poduromorpha)	4.2	4.6	2.5	3.8
Collembola (Entomobryomorpha)	57.0	25.0	30.4	20.5
Collembola (Symphypleona)	6.1	4.8	6.3	0.9
Acari	13.2	12.7	15.7	19.4
Coleoptera (adults)	1.0	1.6	1.1	1.1
Coleoptera (larvae)	0.6	1.8	0.5	0.2
Araneae	2.7	5.2	1.8	1.1
Hemiptera	1.0	0.6	0.5	1.8
Hymenoptera (ants)[1]	12.8	40.8	38.0	49.1
Hymenoptera (wasps)	0.4	0.4	0.3	0.7
Diptera	0.2	1.2	2.3	1.1
Amphipoda	0.3	0.0	0.1	0.0
Isopoda	0.2	0.0	0.0	0.0
Pseudoscorpiones	0.1	0.2	0.0	0.0
Symphyla	0.1	0.0	0.0	0.0
Thysanoptera	0.1	0.0	0.0	0.0
Lepidoptera	0.0	0.0	0.1	0.0
Chilopoda	0.0	0.4	0.1	0.2
Opiliones	0.0	0.2	0.1	0.0
Annelida	0.0	0.2	0.0	0.0
Diplopoda	0.0	0.4	0.4	0.2
Neuroptera	0.0	0.0	0.1	0.0
Total #	1201	503	1533	556

[1] Ants were present in traps only when the trapping period was partially snow-free.

Table F.6. The percentage contribution by numbers to the pitfall-trapped invertebrate fauna of Mt Stirling, 2015, in control (C) and snow removal (E) plots. The total number of individuals collected in each sampling period is included at the bottom of the table.

Taxon	**Autumn**		**Winter**		**Spring**	
	C	E	C	E	C	E
Collembola (Poduromorpha)	58.2	52.1	71.7	70.9	54.8	55.9
Collembola (Entomobryomorpha)	3.1	7.8	6.4	7.4	2.1	1.8
Collembola (Symphypleona)	4.9	3.4	2.5	3.2	2.8	1.2
Acari	12.2	9.1	11.6	10.0	9.7	10.9
Coleoptera (adults)	4.9	4.9	1.9	1.5	1.6	1.4
Coleoptera (larvae)	1.1	3.4	0.1	0.2	0.1	0.2
Araneae	6.9	9.6	3.8	4.5	5.7	5.9
Hemiptera	0.9	3.1	0.6	0.5	5.8	3.9
Hymenoptera (ants)[1]	4.6	0.8	0.1	0.0	12.3	9.8
Hymenoptera (wasps)	0.2	0.8	0.2	0.1	0.6	0.7
Diptera	0.0	1.0	0.4	0.9	4.0	7.2
Amphipoda	1.5	0.0	0.1	0.2	0.0	0.1
Isopoda	0.4	1.8	0.1	0.1	0.0	0.1
Blattodea	0.2	1.6	0.1	0.0	0.0	0.3
Lepidoptera	0.2	0.0	0.0	0.0	0.1	0.1
Dermaptera	0.4	0.3	0.1	0.0	0.0	0.1
Trichoptera	0.0	0.0	0.1	0.0	0.1	0.0
Diplopoda	0.2	0.3	0.1	0.0	0.0	0.1
Chilopoda	0.0	0.3	0.0	0.0	0.0	0.0
Pseudoscorpiones	0.0	0.0	0.1	0.0	0.0	0.0
Symphyla	0.0	0.0	0.0	0.1	0.0	0.0
Thysanoptera	0.0	0.0	0.0	0.1	0.0	0.0
Total #	452	386	1821	2127	1397	1375

[1] Ants were present only in traps when the trapping period was partially snow-free.

F.3.2 Seasonal activity of alpine arthropods

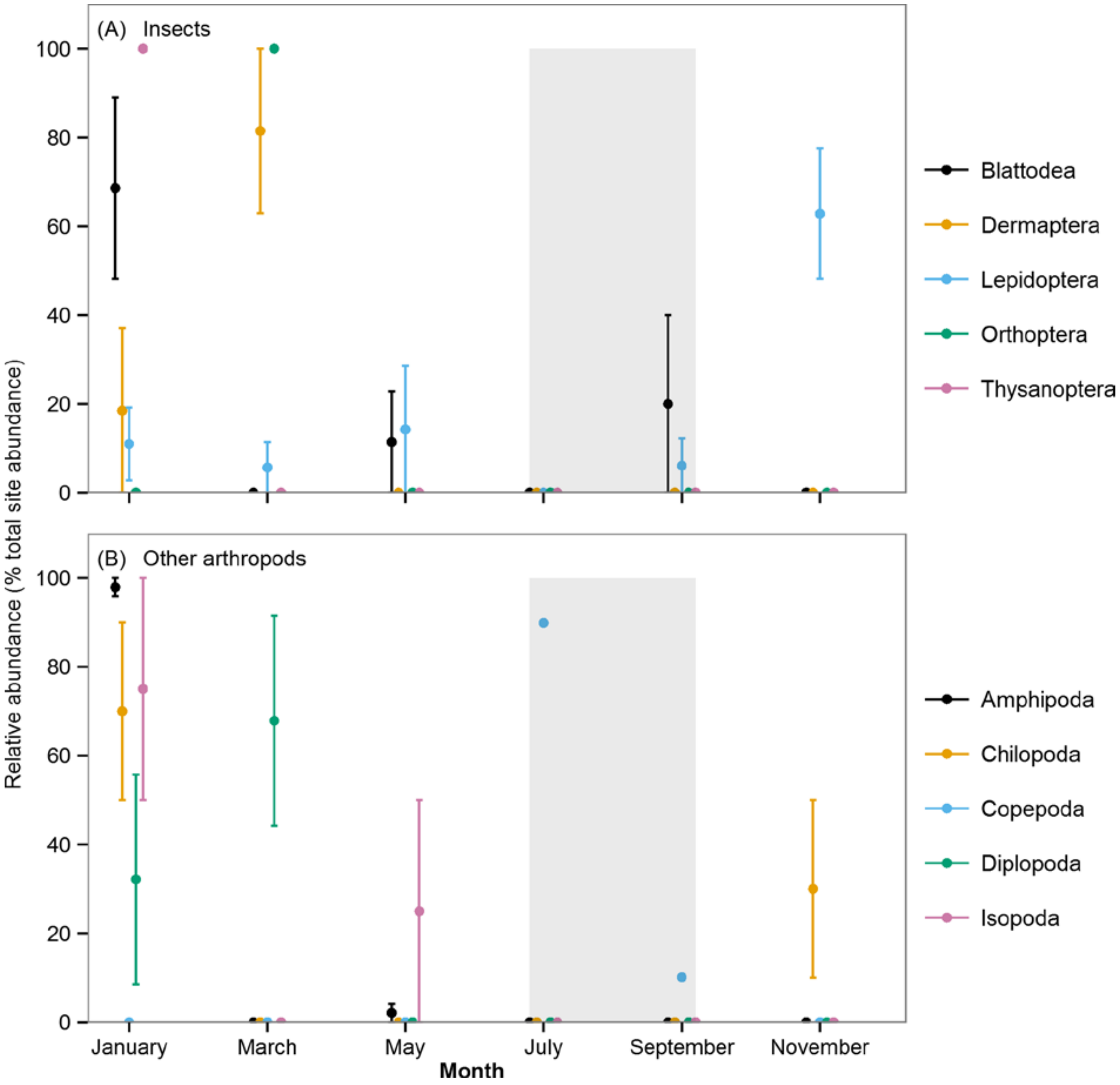

Figure F.1. Seasonal activity of taxa that made a minor contribution (by numbers) to the Mt Stirling arthropod community in 2013. Relative abundance was calculated for each plot as the proportion of individuals in a given taxon that were collected in a given month and thus represents seasonal variation at the plot level. Error bars are ± 1 s.e. Where no error bars are shown, individuals were detected in a single plot. Grey shading indicates the period of snow cover, though plots were snow free for at least one day in every trapping period.

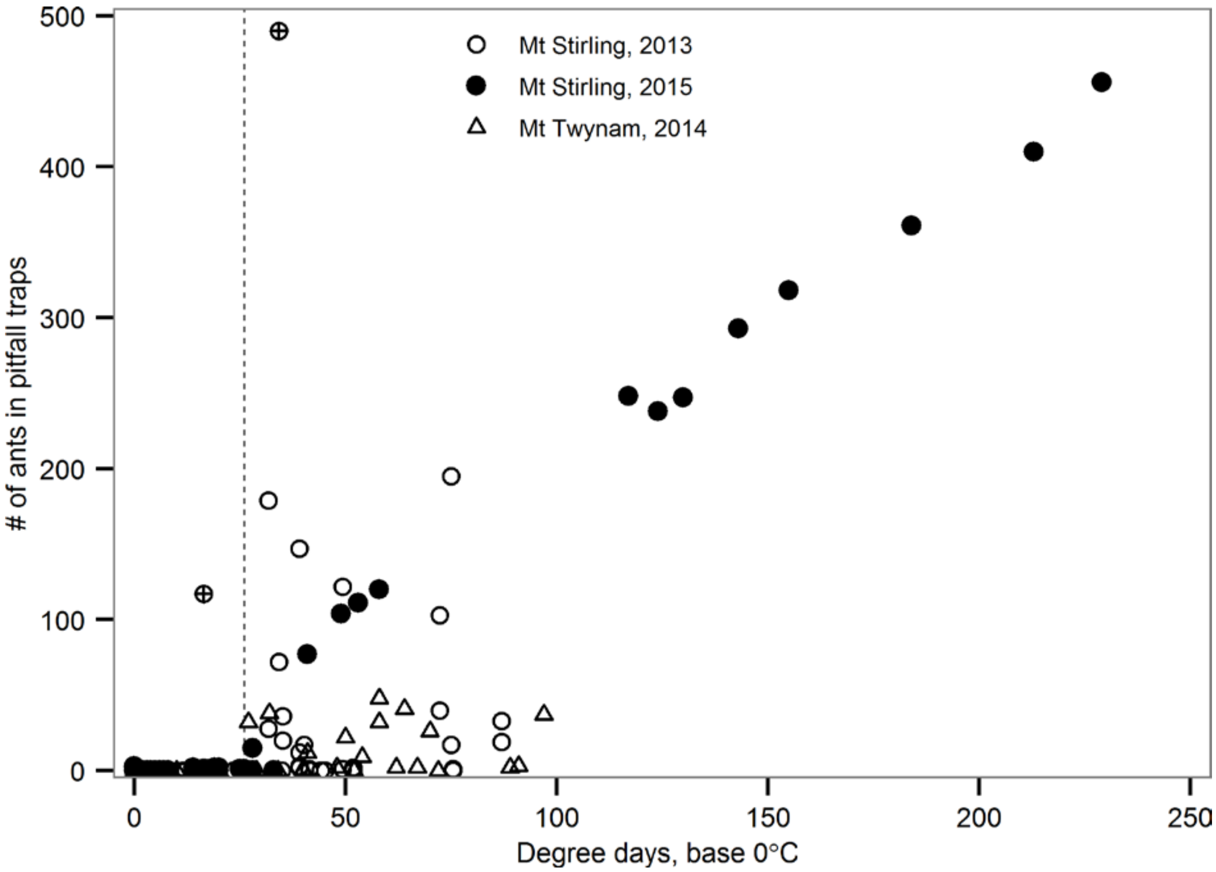

Figure F.2. The relationship between the number of ants caught in pitfall traps and the number of degree days (base 0°C: dd = ∑ ((maximum – minimum)/2) within the trapping period. Data are shown for the three years of study, but emphasis is placed on the results from Mt Stirling, 2015, where temperature was recorded in every plot. For 2013 and 2014, degree day calculations for plots without data loggers were performed using temperatures from the closest plot of the same treatment type. The vertical dashed line is at 26 degree days. Two outliers (from Mt Stirling, 2013) are shown with crossed circles.

F.3.3 Effects of snow removal on the numbers of dominant arthropods in pitfall traps

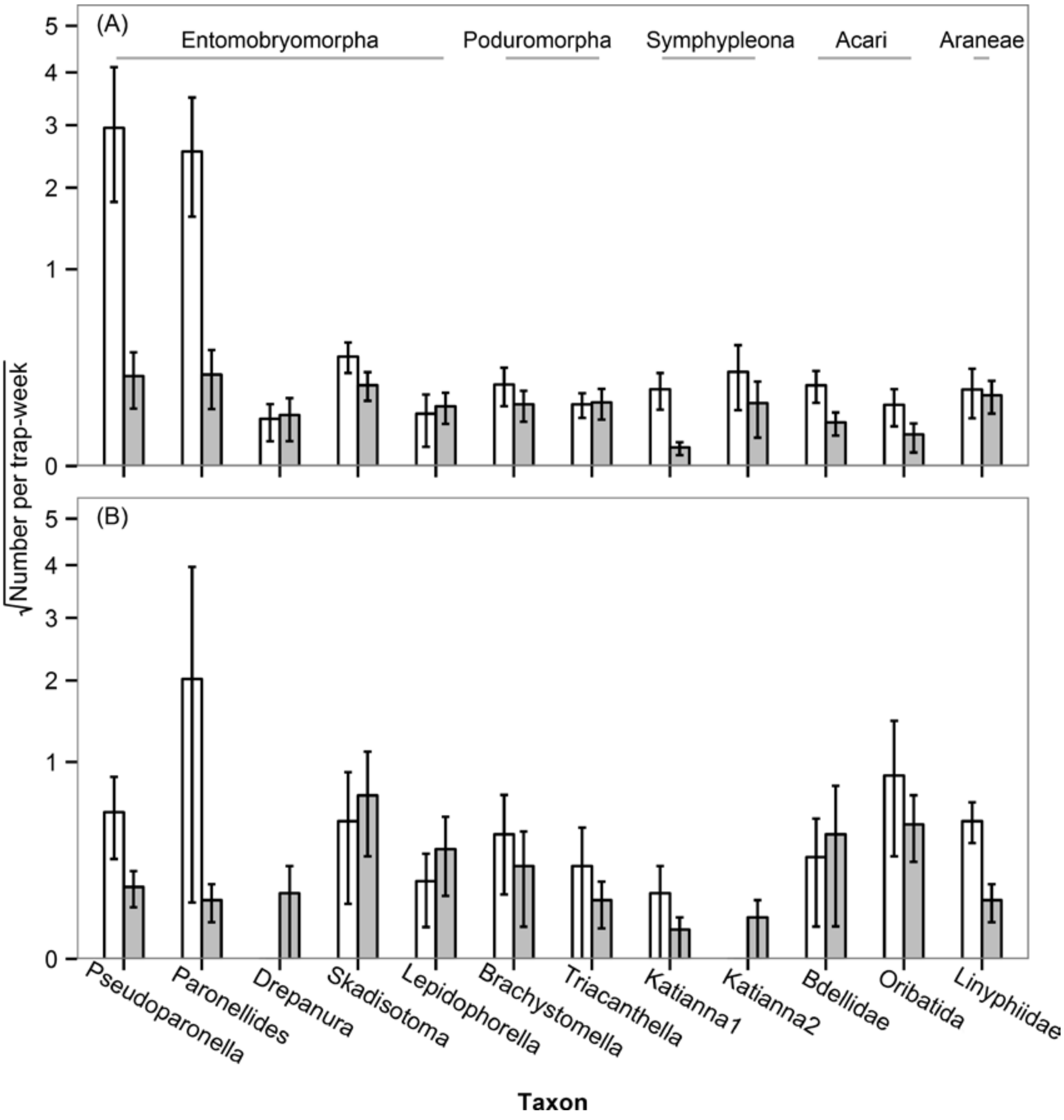

Figure F.3. Mean (± s.e.) number of individuals per trap-week for numerically dominant taxa in (A) winter and (B) spring at Mt Twynam in 2014. For winter, data are from seven experimental snow removal plots (grey bars) and seven paired control plots (white bars). For spring, data are from six experimental snow removal plots and six paired control plots.

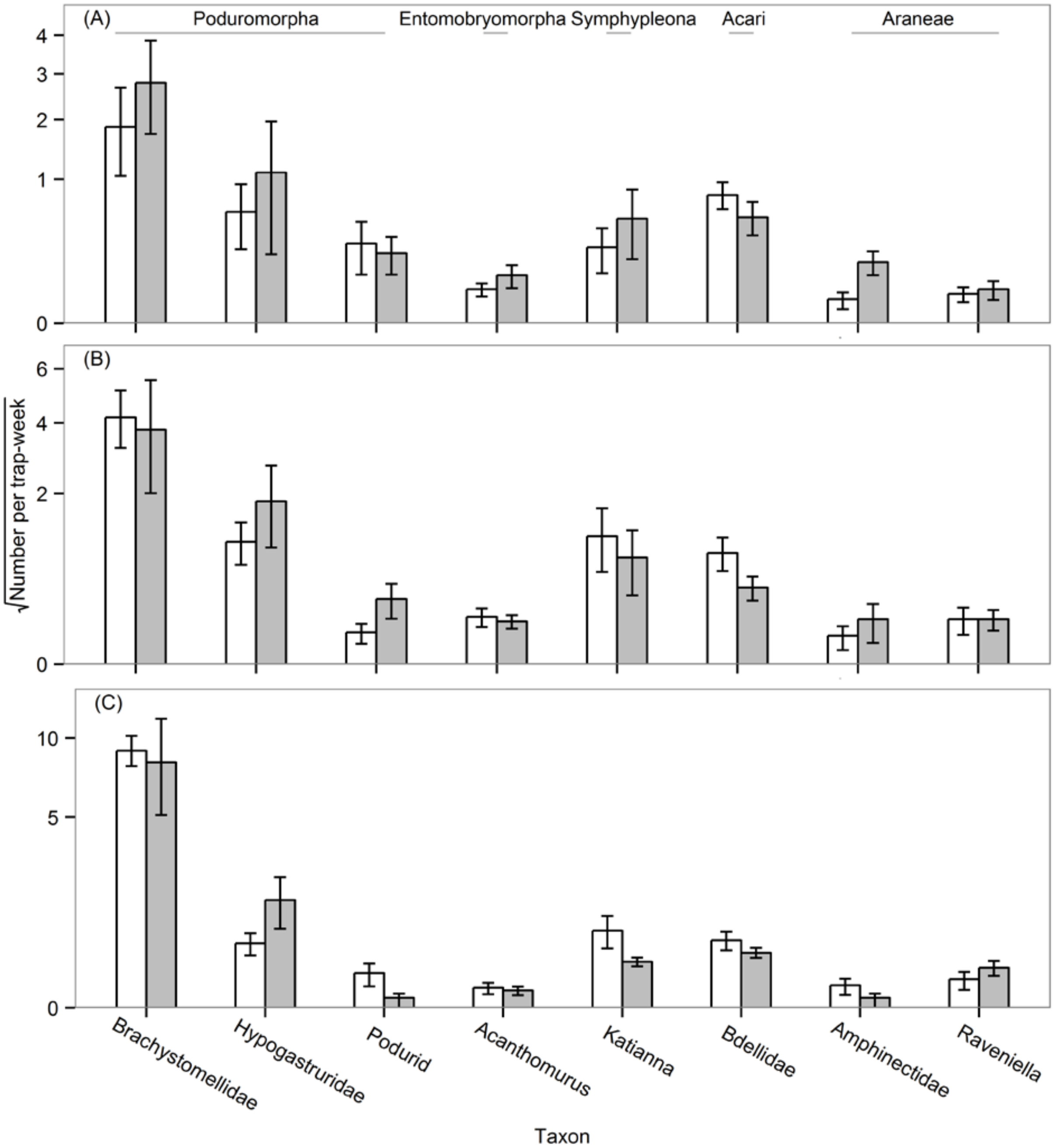

Figure F.4. Mean (± s.e.) number of individuals per trap-week for numerically dominant taxa over the winter at Mt Stirling in 2015. The winter is divided into the three trapping periods: (A) early winter, (B) mid winter, and (C) late winter. Data are from eight experimental snow removal plots (grey bars) and eight control plots (white bars).

F.3.4 Microclimate and snow cover

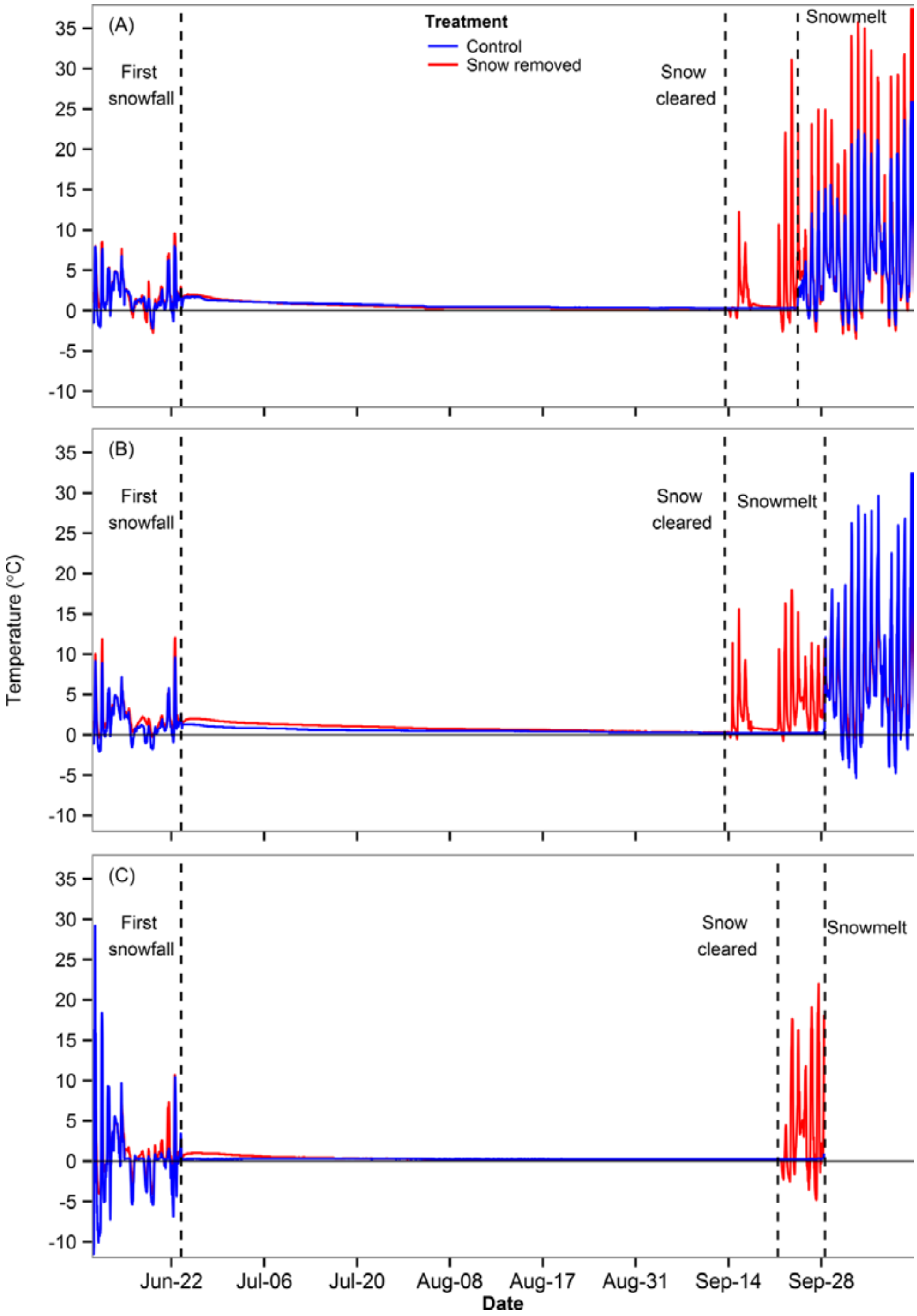

Figure F.5. The effect of snow removal on surface temperatures at Mt Twynam in 2014. Hourly temperatures are shown for three blocks, each with one control (blue) and one experimental snow removal (red) plot. The horizontal black line is 0°C, vertical dashed lines indicate (from left to right) the date of first snowfall, the date when snow was first cleared, and the date of snowmelt at the control site. Note that in (C), temperatures were not recorded after snowmelt.

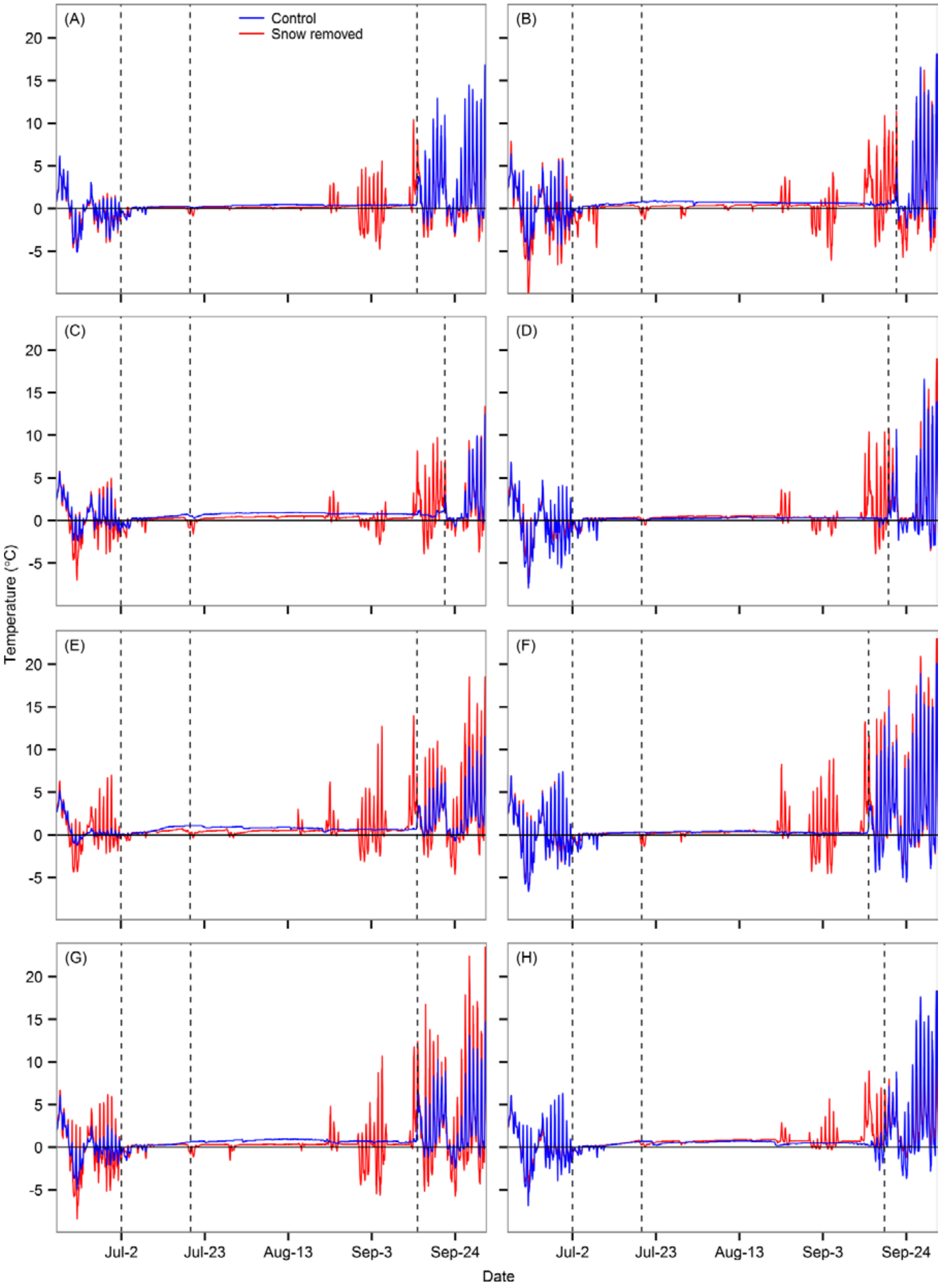

Figure F.6. The effect of snow removal on surface temperatures at Mt Stirling in 2015. Hourly temperatures are shown for all eight blocks, each with one control (blue) and one experimental snow removal (red) plot. The horizontal black line is 0°C, vertical dashed lines indicate (from left to right) the date of first snowfall, the date when snow was first cleared, and the date of snowmelt at the control site.

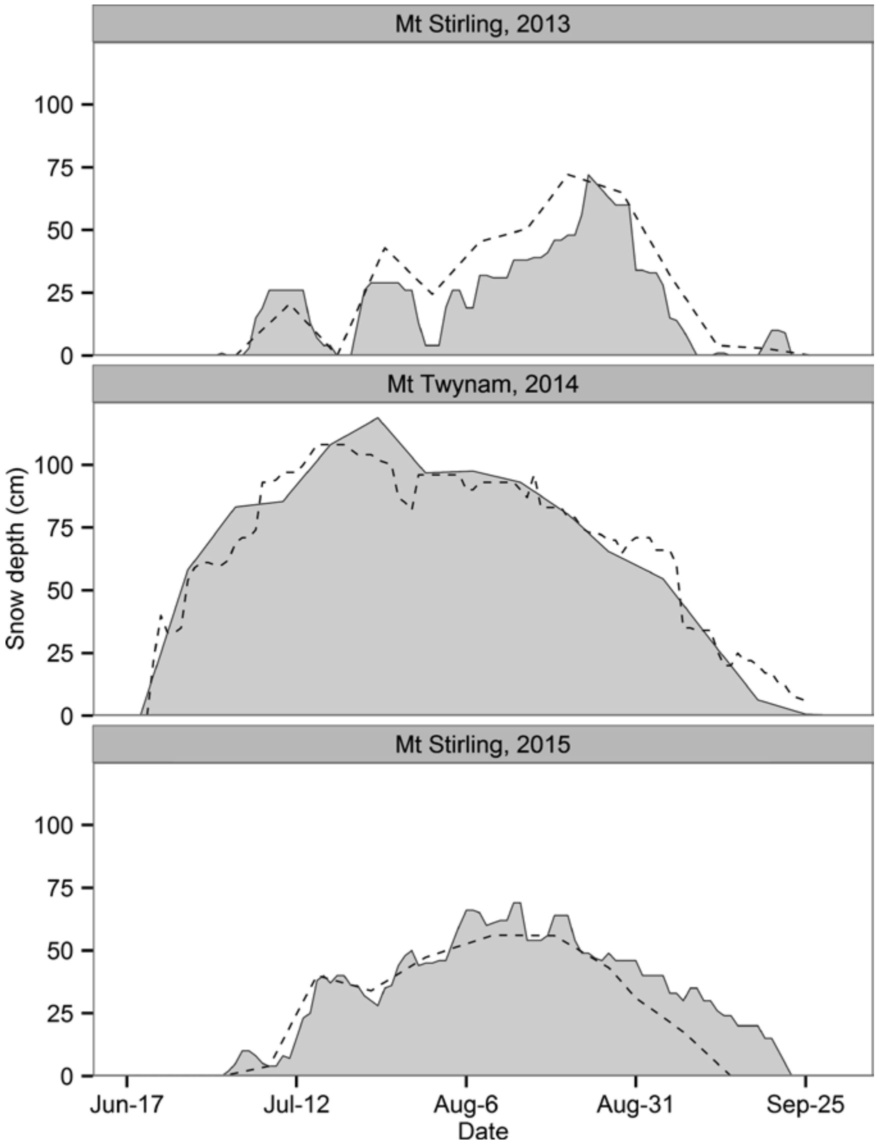

Figure F.7. Snow depths over the three years of the study. Snow depths shown for Mt Stirling are daily recordings of snow depths on the "upper trails" (data from Mt Buller/Mt Stirling resort). Snow depths shown for Mt Twynam are from weekly recordings at the Snowy Hydro Deep Creek depth gauge, which approximates the average snow depth at the Mt Twynam study site. Plot labels indicate the site studied in each year, and snow depth for this site is shaded grey. The dashed line shows the snow depth at the inactive site (i.e. Mt Twynam in 2013, 2015; Mt Stirling in 2014), showing close similarity in snow patterns between locations over the three years of the study.

Table F.7. Summary of surface (1 cm above soil surface) and ground (1 cm below soil surface) temperatures for the six trapping periods at Mt Stirling, 2013. Data are shown as mean ± s.d.

	January	**March**	**May**	**July**	**September**	**November**
Surface						
Overall mean	15.5 ± 10.2	9.7 ± 4.8	0.5 ± 3.1	1.5 ± 1.9	2.7 ± 3.8	4.3 ± 4.8
Mean daily minimum	6.0 ± 5.1	6.1 ± 2.3	-2.2 ± 5.9	0.7 ± 1.2	0.1 ± 2.5	-0.1 ± 1.8
Mean daily maximum	32.4 ± 7.9	16.3 ± 6.3	7.2 ± 6.1	2.4 ± 2.5	7.3 ± 5.7	11.7 ± 6.2
Mean daily fluctuation	26.4 ± 9.6	10.2 ± 5.2	9.4 ± 0.6	1.6 ± 1.9	7.2 ± 5.5	11.8 ± 6.6
# days with mean min < 0	2	0	7	3	6	2
# loggers	5	5	5	3	3	3
Ground						
Overall mean	13.6 ± 5.4	9.4 ± 2.7	1.6 ± 1.7	2.0 ± 1.1	2.7 ± 1.8	5.6 ± 2.2
Mean daily minimum	8.5 ± 2.9	7.3 ± 1.7	0.6 ± 2.0	1.6 ± 0.9	1.5 ± 1.1	3.6 ± 1.1
Mean daily maximum	21.2 ± 5.8	12.2 ± 3.8	5.4 ± 5.8	2.3 ± 1.3	4.1 ± 2.5	7.9 ± 2.8
Mean daily fluctuation	12.7 ± 6.9	4.8 ± 3.2	4.7 ± 6.1	0.6 ± 0.8	2.6 ± 2.1	4.3 ± 2.7
# days with mean min < 0	0	0	0	0	0	0
# loggers	7	7	6	3	3	3
Total # days	5	8	8	16	15	6

Table F.8. Summary of surface (1 cm above soil surface) and ground (1 cm below soil surface) temperatures at Mt Twynam in 2014 for control (C) and snow removal (E) plots. Temperatures "after snowmelt" are from the date of snowmelt at control plots. Data are shown as mean ± s.d.

	Before snowfall		**Under snow**		**After snowmelt**	
	C	**E**	**C**	**E**	**C**	**E**
Surface						
Overall mean	1.0 ± 2.5	1.6 ± 1.7	0.5 ± 0.3	1.5 ± 3.1	3.0 ± 5.7	6.7 ± 7.0
Mean daily minimum	-0.3 ± 2.3	0.5 ± 1.5	0.5 ± 0.3	-0.4 ± 1.3	0.0 ± 2.5	0.1 ± 2.6
Mean daily maximum	2.7 ± 4.2	3.3 ± 3.0	0.5 ± 0.3	5.5 ± 6.8	18.3 ± 7.8	20.6 ± 7.5
Mean daily fluctuation	3.0 ± 5.9	2.8 ± 3.6	0.0 ± 0.0	6.0 ± 7.5	18.3 ± 8.7	20.6 ± 8.3
# days with mean min < 0	13	10	0	39	9	8
# loggers	4	4	7	4	3	4
Ground						
Overall mean	2.4 ± 0.9	2.6 ± 0.9	0.9 ± 0.4	1.6 ± 1.5	3.2 ± 3.4	5.8 ± 2.7
Mean daily minimum	1.7 ± 0.9	2.5 ± 0.9	0.9 ± 0.4	1.5 ± 2.1	3.2 ± 1.7	5.5 ± 3.7
Mean daily maximum	2.2 ± 1.5	2.8 ± 1.2	0.9 ± 0.4	2.4 ± 2.3	9.7 ± 3.9	7.5 ± 3.1
Mean daily fluctuation	0.6 ± 0.8	0.8 ± 0.9	0.1 ± 0.1	1.8 ± 2.5	6.6 ± 3.6	5.5 ± 2.8
# days with mean min < 0	9	6	0	2	4	3
# loggers	3	4	5	4	2	4

Table F.9. Summary of surface (1 cm above soil surface) temperatures at Mt Stirling in 2015 at control (C) and snow removal (E) plots over the five periods of pitfall trapping. Data are shown as mean ± s.d.

	Autumn		**Early winter**		**Mid winter**	
	C	**E**	**C**	**E**	**C**	**E**
Surface						
Overall mean	1.6 ± 1.6	1.3 ± 1.7	0.6 ± 0.3	0.3 ± 0.3	0.6 ± 0.2	0.5 ± 0.6
Mean daily minimum	0.5 ± 1.2	0.1 ± 1.1	0.6 ± 0.3	0.2 ± 0.4	0.6 ± 0.2	0.3 ± 0.3
Mean daily maximum	3.4 ± 2.7	3.4 ± 2.9	0.6 ± 0.3	0.4 ± 0.2	0.6 ± 0.2	0.9 ± 1.2
Mean daily fluctuation	2.9 ± 3.0	3.2 ± 3.3	0.1 ± 0.1	0.2 ± 0.3	0.1 ± 0.1	0.6 ± 1.3
# days with mean min < 0	3	4	0	3	0	2
# days	14		19		21	

	Late winter		**Spring**	
	C	**E**	**C**	**E**
Surface				
Overall mean	0.7 ± 1.1	0.9 ± 2.7	2.7 ± 4.3	2.7 ± 4.7
Mean daily minimum	0.2 ± 0.8	-1.0 ± 1.9	-0.8 ± 1.5	-1.4 ± 1.8
Mean daily maximum	1.3 ± 2.1	4.2 ± 4.1	8.5 ± 5.7	9.3 ± 6.1
Mean daily fluctuation	1.1 ± 2.7	5.1 ± 5.1	9.3 ± 5.9	10.7 ± 6.2
Mean # days with min < 0	3	10	9	9
# days	19		12	

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Appendix G

Effects of reduced snow cover on the hatching success of *Kosciuscola tristis*

G.1 Background

Many insects enter a state of arrested development and metabolic suppression during their life cycle. This state, termed "diapause", occurs in the egg stage of many acridid grasshoppers and usually confers high resistance to environmental extremes (reviewed: Andrewartha & Birch 1954). The *Kosciuscola* overwinter as eggs and are thought to undergo a one-year diapause (Green 1983). As a sub-alpine/alpine-endemic, *K. tristis* eggs overwinter under the snow pack, which provides a thermally buffered microclimate, just above 0°C (Chapter 7). The Australian snowpack is predicted to decrease considerably in the coming decades and this change will expose eggs to more extreme temperatures (see Chapter 7). Shallow snow is associated with high mortality in overwintering larvae of the moth *Mamestra configurata* (Lepidoptera: Noctuidae) (Lamb *et al.* 1985) and eggs of the grasshopper *Camnula pellucida* (Orthoptera: Acrididae) in Canada (Pickford 1966). In addition, anecdotal reports of reduced abundance of adult *Kosciuscola* in summers following winters with shallow snow (Green & Osborne 2012) suggest that this life stage could be sensitive to overwinter conditions. In this study, I tested the effects of reduced snow cover on the hatching success of *Kosciuscola tristis*.

G.2 Methods

Between March 17th and April 13th, 2014, I collected 19 egg pods of *Kosciuscola tristis* from a high sub-alpine (1930 m, *n* = 6) and alpine (1974 m, *n* = 13) site adjacent to the Dead Horse Gap walking trail near Thredbo, New South Wales. This trail is on the south-eastfacing slope of the Ramshead Range. Eggs were collected either (a) by observing a female laying, marking the position and carefully extracting the egg pod the following day (n = 14), or (b) digging in the soil near marked egg pods (n = 5). Egg pods were kept at 4°C in individual 1 mL tubes with a small amount of vermiculite until being transferred to the Mt Twynam experiment site described in Chapter 7. Egg pods were randomly assigned to one of two treatments: (a) natural snow accumulation or (b) snow removal. The plots are the same as those described in Chapter 7. Egg pods (between one and three per plot) were placed in a small (2 cm diameter sphere) wire mesh cage, lined with soil from the collection site to reduce the risk of abrasion to the eggs. These cages were then buried so that the egg pod was positioned approximately 1 cm below the soil surface. As much as possible, egg pods were paired by collection date, collection location, and collection method (Table G.1).

Eggs were removed from all plots on October 5th 2014, and each pod was transferred to a 70 mL plastic container half-filled with lightly moistened vermiculite. Eggs were kept at 4°C until December 21st, then transferred to 700 mL plastic cups (BioPak, Australia) with 4 cm lightly moistened vermiculite on the bottom. Egg pods were covered in a thin layer of vermiculite, and the cups were covered with Parafilm (Bemis, USA) and kept at 19°C under a 12:12 light:dark cycle. Eggs were monitored daily for hatching. As previous authors have suggested that *K. tristis* might have a 2-year diapause (Green 1983), when no eggs had hatched by March 3rd, 2015, I simulated a second winter by transferring the eggs to 4°C and constant dark. The eggs were transferred back to 19°C (12:12 light cycle) on May 7th, at which time they were also transferred to fresh vermiculite. On June 16th, the eggs were transferred back to 4°C for two months, before the final transfer to 19°C on August 16th, 2015. Over this period, two additional egg pods, which were placed in a control plot during the field experiment, were used to intermittently check egg development. This was done by separating single eggs from the pod and removing the chorion under a dissecting microscope (Leica M125). Eggs were monitored weekly until September 13th, 2015, when the first nymphs had emerged. Nymphs were transferred to 3000 mL plastic containers (one container per egg pod) with mesh side walls, a thin layer of sawdust on the bottom and wheat bran for food. I recorded the number of hatched nymphs (alive or dead). Eggs were monitored for a further 5 days, then the eggs in each pod were counted and the number of unhatched eggs recorded. Unhatched eggs were classed as “healthy” if they were turgid and a golden/pale brown, or “dead” if they were shrivelled and/or darkly coloured. I removed the chorion of 10 eggs, chosen at random, to check the developmental stage of unhatched embryos.

Table G.1. Details of egg pods included in the experiment, including the date collected from the field, location of the collection and the method of collection (a: female observed laying, b: digging in soil). Note that for method (b), the laying date of the egg pod is unknown; for method (a), eggs were laid 1 – 2 days before collection.

	Control plot			**Snow removal plot**		
Block	**Date**	**Location**	**Method**	**Date**	**Location**	**Method**
1	Apr-8	Alpine	a	Apr-8	Alpine	a
	Apr-9	Alpine	b	Apr-7	Alpine	b
2	Apr-9	Alpine	a	Apr-9	Alpine	a
3	Apr-9	Alpine	a	Apr-9	Alpine	a
	Mar-17	Sub-alpine	a	Mar-17	Sub-alpine	a
	Apr-13	Sub-alpine	a	Apr-13	Sub-alpine	a
4	Apr-9	Alpine	b	Apr-9	Alpine	b
5	Apr-9	Alpine	a	Apr-9	Alpine	a
6	Apr-13	Sub-alpine	a	Apr-8	Sub-alpine	a
				Apr-9	Alpine	b

G.3 Results and discussion

After removal from the field, eggs spent a total of 203 days at 4°C and 135 days at 19°C before hatching. Embryos were observed in two developmental stages. In June, after 140 days at 4°C and 203 days at 19°C, embryos had developed clear eye spots (Fig. G.1). This is likely to correspond to 40-55% development, and Chapman & Whitham's stages IV-VI (Chapman & Whitham 1968). In September, when the first eggs had hatched, unhatched eggs had reached 90-100% development (Bentley *et al.* 1979) (Fig. G.1). This stage corresponds to Chapman & Whitham's stage VIIIb (Chapman & Whitham 1968). A two-year diapause is common among New Zealand sub-alpine and alpine grasshoppers (Green 1983). Although the current study cannot confidently ascribe a diapause length, the lack of development after a single winter suggests that a two-year diapause is a plausible scenario for *K. tristis*.

Figure G.1. Embryo development. (a) June 8th, (b) September 17th. The chorion has been removed.

Egg pods contained an average of 19 eggs, but this number varied considerably among pods (s.d. = 6, range = 7 - 29, n = 19). This is considerably fewer than the number of eggs estimated for *Kosciuscola usitatus* from similar elevations (1780 m: 25 ± 0.5 eggs per pod, 2140 m: 27 ± 0.4 eggs per pod; both n = 15; Dearn 1977). A total of 44% of eggs had hatched by September 13th and a full range of hatching successes was observed: 100% of eggs hatched in one egg pod and 0% hatched in two egg pods.

There was no difference in hatching success between treatments (control: 40 ± 25 %, n = 9, snow removal: 44 ± 35 %, n = 10; Mann-Whitney U-test: W = 41, p = 0.775), nor was there a difference in egg mortality (control: 20 ± 19 %, snow removal: 30 ± 40 %; W = 49, p = 0.743) (Fig. G.2). From these results, it appears that eggs of *K. tristis* are robust to variable environmental conditions, at least in their first year of diapause. This experiment should be followed-up with tests of the temperature-sensitivity of eggs in autumn (Pickford 1966), and during later developmental stages.

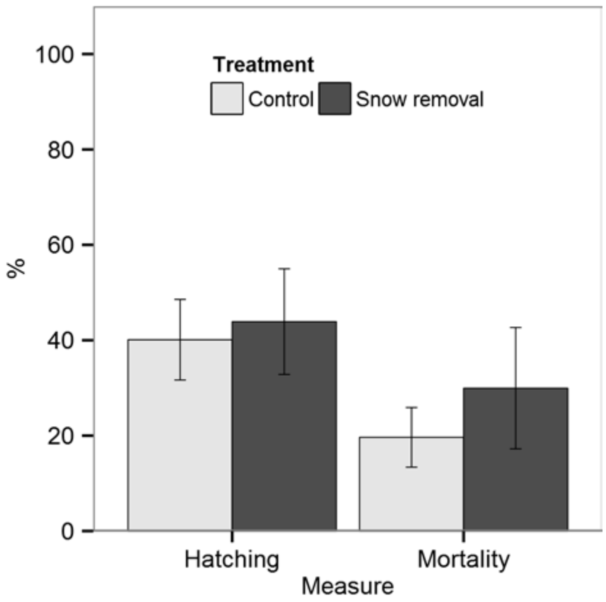

Figure G.2. The effect of snow removal on the hatching success and egg mortality of *Kosciuscola tristis*. Error bars are ± s.e.

G.4 References

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A mountain

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