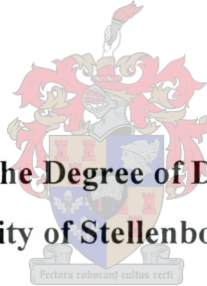


**Physiological plasticity in arthropods from Marion  
Island: indigenous and alien species**

**Sarette Slabber**

**Dissertation presented for the Degree of Doctor of Zoology at the  
University of Stellenbosch**



**Promoter: Professor S.L. Chown**

**December 2005**

## **Declaration**

**I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.**

## Abstract

Climate sets range limits in many taxa, and as climate changes, the ranges that plants and animals occupy are altered. The responses of species to climate change involve either migration or local adaptation. An investigation of the local physiological adaptation shown by indigenous and alien arthropods to temperature acclimation formed the primary focus of this study. Increased environmental temperatures favour the establishment of alien species on Southern Ocean Islands. The first records of *Porcellio scaber* Latreille, 1804 (Isopoda, Porcellionidae) and an *Aphidius* wasp species from Marion Island were documented here. The alien wasp was discovered in 2003 and had a current known distribution along the east coast of Marion Island. Both isopods and wasps were reproducing successfully on Marion Island. The wasp species is an aphid parasitoid and had been found mummifying the alien aphid *Rhopalosiphum padi*. These introductions highlighted the ongoing conflict between use and conservation on the Southern Ocean Islands.

Despite considerable work on the upper and lower lethal limits of insects, several major taxa have received little attention. Here this issue was addressed and the lower and upper thermal tolerances and cold hardiness strategy of *Antarctopsocus jeanneli* Badonnel (Psocoptera: Elipsocidae) from sub-Antarctic Marion Island was investigated. *A. jeanneli* is freeze intolerant, and more specifically, moderately chill tolerant. Field fresh *A. jeanneli* had a mean supercooling point (SCP) of  $-11.1^{\circ}\text{C}$ , whereas LT50 was  $-7.7^{\circ}\text{C}$ , indicating pre-freeze mortality. *A. jeanneli* responded to acclimation: mean SCP increased from  $-15.8^{\circ}\text{C}$  at a treatment temperature of  $0^{\circ}\text{C}$ , to  $-7.3^{\circ}\text{C}$  at  $15^{\circ}\text{C}$ .

Investigations of the responses to acclimation of upper and lower lethal limits and limits to activity in insects have focussed primarily on *Drosophila*. *Halmaeus atriceps* (Staphylinidae) was examined for thermal tolerance responses to acclimation, and seasonal acclimatization. In summer and winter, lower lethal temperatures of adults and larvae were  $c. -7.6 \pm 0.03$  and  $-11.1 \pm 0.06$   $^{\circ}\text{C}$ , respectively. Supercooling points (SCPs) were more variable, with winter SCPs of  $-5.4 \pm 0.4$   $^{\circ}\text{C}$  in larvae and  $-6.3 \pm 0.8$   $^{\circ}\text{C}$  in

adults. The species appeared to be chill susceptible in summer and moderately freeze tolerant in winter, thus showing seasonal acclimatization. Critical thermal minima varied between  $-3.6 \pm 0.2$  and  $-0.6 \pm 0.2$  °C in larvae, and from  $-4.1 \pm 0.1$  to  $-0.8 \pm 0.2$  °C in adults. These findings were in keeping with the general pattern found in insects, although this species differed in several respects from others found on Marion Island.

In this study the differential responses of indigenous and invasive springtails to temperature were explicitly examined in the context of the beneficial acclimation hypothesis (BAH) and its alternatives. In particular, the thermal acclimation responses of desiccation resistance, supercooling ability, lower and upper thermal limits were compared. Invasive springtails (*Pogonognathellus flavescens*, *Isotomurus palustris* and *Ceratophysella denticulata*) did not display greater phenotypic flexibility than indigenous springtails (*Cryptopygus antarcticus* and *Tullbergia bisetosa*), but did perform better under high temperature conditions. Indigenous species, however, performed better under low temperature conditions. In most cases the BAH was not supported, or could not be distinguished from its alternatives. The prediction that invasive species will outperform indigenous species as climates on Southern Ocean Islands warm was supported.

Because temperature plays such a large role in the distribution, abundance and physiological tolerances of invertebrates, microhabitat temperatures along an altitudinal gradient (0 to 800 m above sea level (asl)) were investigated over a two-year period on Marion Island. Mean microhabitat temperatures were comparable to those from previous studies for Southern Ocean Islands, and declined with increasing altitude. The 800 m asl site had the most severe microclimate (highest absolute maximum, lowest absolute minimum and the highest frequency of freeze-thaw cycles). Year one was substantially colder than year two, indicating that interannual variation in microclimate conditions could be responsible for substantial mortality amongst invertebrate populations. Indeed, indigenous species were best able to tolerate the high incidence of low temperatures at high altitudes, whereas alien species appeared to be confined to lower altitudinal sites on Marion Island, possibly as a consequence of extreme low temperatures at higher altitudes.

## Opsomming

Klimaat is een van die grootste faktore wat die verpreiding en digtheid van dier en plantspesies bepaal. Soos wat klimaatsverandering plaasvind, kan spesies migreer om stresvolle klimaatstoestande te vermy, of hulle kan aanpas by plaaslike omstandighede. Dit is die laasgenoemde opsie wat in hierdie tesis ondersoek was. Die opsporing van *Porcellio scaber* Latreille, 1804 (Isopoda Porcellionidae) en 'n *Aphidius* wesp van Marion Eiland word hier aangeteken. Die huidige verspreiding van houtluise in die omgewing van die Navorsingstasie dui daarop dat hulle die eiland waarskynlik vanaf Kaapstad in bou-materiaal bereik het. Die wesp is gedurende 2003 ontdek en is tans versprei langs die oostelike kuslyn van Marion Eiland. Beide hierdie spesies plant suksesvol voort op Marion Eiland. Die wesp spesie parasiteer die uitheemse plantluise *Rhopalosiphum padi*. Uitheemse muise en die inheemse kleinskedebeke weier tot dusver om *P. scaber* te eet. Die ontdekking van twee nuwe uitheemse spesies dui weereens op die konflik tussen bewaring en benutting van die Suidsee Eilande.

Ten spyte daarvan dat aansienlike aandag gewy is aan die hoë- en lae temperatuur toleransies van insekte, is daar talle taksa wat selde nagevors word. Dié toleransies is dus nagevors vir die boekluis *Antarctopsocus jeanneli* Badonnél (Psocoptera: Elipsocidae) van Marion Eiland. Die spesie is vries vermydend, en meer spesifiek, matig verkoelingsbestand. Veldvars *A. jeanneli* het 'n gemiddelde superverkoelingspunt van  $-11.1$  °C gehad, en letale temperatuur (LT50) van  $-7.7$  °C, wat aandui dat individue vrek voordat hulle vries. Hierdie spesie reageer op akklimasie: gemiddelde superverkoelingspunt het toegeneem van  $-15.8$  °C na akklimasie by  $0$  °C tot  $-7.3$  °C na akklimasie by  $15$  °C.

Die effek van akklimasie op temperatuur toleransie in insekte het dusver meestal op *Drosophila* gefokus. Hier vors ons die effek van akklimasie op die temperatuur toleransie van *Halmaeusia atriceps* (Styphlynidae) na. In die somer en winter was die lae letale temperatuur van kewers en larwes onderskeidelik  $-7.6 \pm 0.03$  en  $-11.1 \pm 0.06$  °C. Superverkoelingspunte (SVP) het meer gevarieer, met SVP van  $-5.4 \pm 0.4$  °C in larwes en

$-6.3 \pm 0.8$  °C in kewers. Die spesie is verkoelingsvatbaar in die somer en matig vriesbestand in die winter, wat dui op akklimatisasie in hierdie spesie. Kritiese termale minima het tussen  $-3.6 \pm 0.2$  en  $-0.6 \pm 0.2$  °C in larwes, en tussen  $-4.1 \pm 0.1$  tot  $-0.8 \pm 0.2$  °C in kewers gewissel. Hierdie spesie blyk dieselfde fisiologiese tendense wat in insekte te vinde is uit te beeld, maar verskil in verskeie opsigte van ander insekte op Marion Eiland.

Hierdie studie het ook die verskillende reaksies van inheemse en uitheemse springsterkte met betrekking tot temperatuur akklimasie bestudeer. Die voordelige-akklimasie hipotese en sy alternatiewe hipoteses is in terme van akklimasiereaksies tot desikkasie weerstand, superverkoeeling en lae- en hoë temperatuur bestandheid in Marion Eiland springsterkte getoets. Die uitheemse springsterkte (*Pogonognathellus flavescens*, *Isotomurus cf palustris* en *Ceratophysella denticulata*) het nie beter fenotipiese plastisiteit getoon as die inheemse spesies (*Cryptopygus antarcticus* en *Tullbergia bisetosa*) nie. Die inheemse spesies het egter beter gevaar onder lae-temperatuur toestande. Die voordelige-akklimasie hipotese ontvang nie veel ondersteuning in die huidige studie nie, maar kon in sommige gevalle nie van die alternatiewe hipoteses onderskei word nie. Klimaatsverandering mag uitheemse spesies bevoordeel.

Die rol wat temperatuur in die verspreiding en fisiologiese toleransies van invertebrate speel word lank reeds waardeur. Daarom vors hierdie studie die mikrohabitat temperature teen 'n gradient tussen seespieël en 800 m bo seespieël (m bs) oor 'n tydperk van twee jaar op Marion Eiland na. Gemiddelde temperature is vergelykbaar met die van vorige studies op Marion Eiland, en neem af soos wat hoogte bo seespieël toeneem. Die 800 m bs studie-gebied het die mees stresvolle mikrohabitat-toestande ondervind (die hoogste absolute maksimum, laagste absolute minimum temperature, en die meeste vries-ontdooi siklusse). Inheemse spesies op Marion Eiland toon hoër toleransies tot lae temperature as uitheemse spesies, laasgenoemde kom slegs voor by laer hoogtes bo seespieël, waar ekstreme temperatuur-toestande dalk minder volop is. Klimaatsverwarming mag egter tot 'n toename in ekstreme weerstoestande lei op Marion Eiland, wat aansienlike mortaliteit in invertebraat populasies kan veroorsaak.

## Acknowledgements

I am grateful to Steven Chown, my mentor, without whom I would not have aimed for the stars. I will always value his opinion and share his love for Marion Island.

Without Erika Nortje, Liezl Wiesner, Wilna Wilkinson and Beneke de Wet, Marion Island would have been a very different place for me. Thank you for contributing to the wonderful memories I have of the 'Southern Jewel'. Erika and Liezl assisted hugely with field and laboratory work, for which I am eternally grateful. J. Deere is thanked for collecting temperature data and collecting cold hardiness data during a short visit to Marion Island in August 2004.

Elrike Marais, Ulrike Irlich and Antoinette Botes read earlier drafts of chapters of this thesis. BJS, Elrike and Willem de Lange, thank you for accommodating me with such incredible hospitality. Jaco Klok and Brent Sinclair assisted in training me in the field of insect physiology. Members of the SPACE Group, Roger Worland, H.P. Leinaas and Pete le Roux are thanked for discussions and assistance.

United States Agency for International Development and the Department of Environmental Affairs and Tourism (USAID are thanked for funding, DEA&T for logistic support on Marion Island).

I am particularly indebted to my parents for the roles they played during my 13-month stay on Marion Island (financial and emotional), as well as during my frequent or extended visits to Stellenbosch or Marion Island for take-over periods.

I am eternally grateful to David Arnold for his patience, love, and support, for 'allowing' me to put everything we have on hold and enabling me to pursue my dream on Marion Island. I dedicate this thesis to him.

## Table of Contents

<b>Declaration</b>	<b>ii</b>
<b>Abstract</b>	<b>iii</b>
<b>Opsomming</b>	<b>v</b>
<b>Acknowledgements</b>	<b>vii</b>
<b>Table of Contents</b>	<b>viii</b>
<b>Chapter 1. <u>General Introduction</u></b>	<b>1</b>
<b>Chapter 2. <u>Marion Island: the alien species dilemma</u></b>	<b>17</b>
Introduction	18
Materials and Methods	34
Results	36
Discussion	36
References	46
<b>Chapter 3. <u>Thermal tolerance and cold hardiness strategy of the</u></b>	
<b><u>sub-Antarctic psocid <i>Antarctopsocus jeanneli</i> Badonnel</u></b>	<b>52</b>
Introduction	53
Materials and Methods	55
Results and Discussion	58
References	67



<b>Chapter 4.</b>	<b><u>Differential responses of thermal tolerance to acclimation</u></b>	
	<b><u>in the sub-Antarctic rove beetle <i>Halmaeus atriceps</i></u></b>	<b>71</b>
	Introduction	72
	Materials and Methods	75
	Results	80
	Discussion	90
	References	95
<b>Chapter 5.</b>	<b><u>Testing the beneficial acclimation hypothesis:</u></b>	
	<b><u>Indigenous and alien species</u></b>	<b>99</b>
	Introduction	100
	Materials and Methods	103
	Results	112
	Discussion	140
	References	150
<b>Chapter 6.</b>	<b><u>Microhabitat temperature variation across an altitudinal</u></b>	
	<b><u>gradient at sub-Antarctic Marion Island: consequences</u></b>	
	<b><u>for arthropod populations</u></b>	<b>157</b>
	Introduction	158
	Materials and Methods	161
	Results	164
	Discussion	191
	References	200
<b>Chapter 7.</b>	<b><u>Conclusions</u></b>	<b>207</b>
	References	213

# Chapter 1

## General Introduction

*This world, after all our science and sciences, is still a miracle; wonderful, inscrutable, magical and more, to whosoever will think of it.*

- Thomas Carlyle -



Indigenous invertebrates from Marion Island: Top left (clockwise): *Myro paucispinosa* (spider), *Pringleophaga marioni* (Marion flightless moths), *Bothrometopus parvulus* (weevil), and *Notodiscus hookeri* (indigenous snail)

### *Environmental change*

Humans have changed the face of the earth through direct domination of ecosystems (Vitousek *et al.* 1997a; Parmesan *et al.* 2000), in addition to indirect impacts. These impacts have been multiple, and have led to substantial increases in atmospheric CO<sub>2</sub> concentrations (31% - IPCC 2001), alterations of biogeochemical cycles, the production and release of persistent organic compounds into the environment, changes in land use, the extinction of species through direct persecution, and the introduction of invasive species (Vitousek *et al.* 1997b; IPCC 2001). These threats play a pivotal role in determining the effects of global climate change (Parmesan *et al.* 2000; Walther *et al.* 2002) and combined, comprise the most pressing threats to modern biotas (Owens & Bennett 2000).

Habitat transformation is a major force leading to the loss of global biodiversity (Vitousek *et al.* 1997a). Direct human impacts on earth's ecosystems are inevitable; population growth necessitates the development of more extensive agriculture, fishery and forestry activities, and urbanisation is set to increase. The direct influences of humans on landscapes are exacerbated by interactions with other components of global change (Vitousek *et al.* 1997a), such as the warming climate. The world is currently heating up faster than at any time in the past 10 000 years (Edwards 1997; IPCC 2001) with a mean global temperature increase of 0.6 °C during the last decade alone (IPCC 2001). There has also been an increase in minimum global temperatures, leading to fewer cold days. Snow cover and ice extent have decreased and there has been a reduction in the frequency of low temperatures

but an increase in the frequency of high temperatures (IPCC 2001). Changes in the variability in climate have been recorded, in addition to changes in precipitation patterns.

Biota have responded to these changes, and have shown poleward and altitudinal shifts in their distributions (Beaumont & Hughes 2002; Kritikos *et al.* 2003; Karban & Strauss 2004), declines in population density, and phenological alterations (IPCC 2001). Spring and autumnal phenological events have seen temporal shifts, there have been changes in the migration patterns in butterflies, the timing of spawning in amphibians, and earlier breeding of birds (Walther *et al.* 2002). These changes have repercussions; for example, earlier leaf unfolding leads to extended growing seasons, but late frost may cause damage to plant tissues (Walther *et al.* 2002). Global climate change has also led to changes in soil moisture regimes (IPCC 2001), which have had ecological consequences (Walther *et al.* 2002).

### *Alien species*

Global climate change is also exacerbating the effect of alien species on landscapes (see Richardson *et al.* 2000 for a definition of alien species). Humans have been responsible for large-scale changes in the distribution of species that are considered non-native to almost all reaches of the planet. Due to agriculture, tourism, and international commerce, the movement of alien species across biogeographical barriers has become a common occurrence, which has led to a rapid globalisation in biota (Davis 2003). This is also true for islands in general, where the ratio of alien to indigenous species has reached

problematic proportions (Mooney & Cleland 2001). New Zealand, for example, has as many established alien as indigenous plant species (Mooney & Cleland 2001).

Aided by humans, non-indigenous species have colonised natural ecosystems very far removed from their historical ranges, posing serious environmental threats to the sustainable use and integrity of invaded systems (Pimentel 2002; Wiles *et al.* 2003). Alien biotas now comprise a major component of the flora and fauna of both islands and continents (Vitousek *et al.* 1997b; Pimentel 2002). The presence and impacts of invasive species have had dire consequences on the economies of developing countries and have influenced developed countries through their impacts on agriculture, as well as other sectors of the economy (Vitousek *et al.* 1997b). Invasive species are causing profound changes in invaded landscapes (Pimentel *et al.* 2002). A conservative estimate is that more than 120 000 alien species of plants, animals and microbes have become invasive in areas of Brazil, South Africa, India, Australia, the United Kingdom, and the United States. Up to 30 % of these alien species have reached pest status and as such have caused severe environmental damage (Pimentel *et al.* 2002). An estimated 480 000 species have become aliens in ecosystems worldwide, and in South Africa alone more than 8750 plant species have been introduced, 180 of which have invaded ten million ha (Van Wilgen *et al.* 2002). Pimentel *et al.* (2002) estimated the global cost of invasive species to amount to \$1.4 trillion, representing nearly 5 % of the world economy (GNP). In the United States alone, invertebrates cause losses to crops and forestry amounting to \$20 billion annually, whereas annual damages incurred due to the presence and control of cats and rats amount to \$39

billion (Pimentel 2002). In addition to monetary losses, other costs, such as a loss of ecosystem services, are incurred that are more difficult to value (Vitousek *et al.* 1997a).

Global environmental change is causing large scale extinctions, and the rate at which biodiversity is lost at present far exceeds background rates. This is being exacerbated by the introduction of alien and invasive species, and is leading to a homogenization of the global biota (Vitousek *et al.* 1997a). The introduction of alien species often not only influences indigenous species directly, but also impacts on other indigenous species that are associated with those that are directly affected (Pimentel 2002). Taxa from several trophic levels and taxonomic groups have become invasive, and have led to extinctions (Porter & Savignano 1990). Native fauna on oceanic islands are particularly sensitive to alien species, as has recently been shown for avifauna of 220 oceanic islands worldwide, where bird extinctions were strongly correlated with the presence of introduced mammalian predators (Blackburn *et al.* 2004).

#### *The Southern Ocean Islands*

Very few habitats have escaped the effects of these global changes, and the islands of the Southern Ocean (SOIs) are no exception (Croxall 1992; Bergstrom & Chown 1999; Chown *et al.* 2001; Frenot *et al.* 2001; Walther *et al.* 2002; Frenot *et al.* 2005). Islands tend to be more sensitive to the effects of environmental threats due to their isolation, small geographic ranges, and specialized biota (Bergstrom & Chown 1999; Lonsdale 1999; Wiles *et al.* 2003; Blackburn *et al.* 2004). Climate change also is of major concern at high

latitudes (Smith & Steenkamp 1990; IPCC 2001), and changes in temperature and precipitation are marked on the SOIs (Smith & Steenkamp 1990; IPCC 2001; Smith 2002). During the past fifty years alone, mean annual temperature has increased by more than 1.2 °C at Marion Island, and mean annual rainfall has decreased by 500 mm (20 %) (Smith & Steenkamp 1990; Smith 2002). Climatic warming is occurring at an average of 0.025 °C per year on Marion Island, corresponding closely with an increase in sea surface temperature during the same period (Smith & Steenkamp 1990). As a result, the permanent stationary glacier, known as the ice-plateau has decreased substantially in size. As with Marion Island, the Kerguelen Islands are also experiencing ameliorating temperatures (Frenot *et al.* 1997), and Gough Island also has experienced a significant rise in temperature (0.6 °C increase) between 1963 and 2000 (Jones *et al.* 2003).

These islands have also been affected by species introductions. Indeed, only two of the more than 20 Southern Ocean Islands are alien free (McDonald Islands and Pingouin Island), with most being home to at least one alien species, and often to many of which at least a few have become invasive and damaging (Frenot *et al.* 2005). For example, Gough Island has experienced dramatic increases in the numbers of species as a consequence of recent invasions (Gaston *et al.* 2003). At present, of the 99 insect species on the island, 71 are introductions, making the rate of invasion probably three orders of magnitude larger than background colonization levels. At Marion Island, species such as the grass *Agrostis stolonifera* are having a marked impact on indigenous plant diversity in those areas that have been invaded. Moreover, virtually all of the islands have been heavily affected by introduced mammalian carnivores and herbivores (reviewed in Bonner 1984; Frenot *et al.*

2005). Thus, alien species are as much of a problem on these islands as is climate change, and the effects of the two might well be synergistic.

*Climate change, aliens and the Southern Ocean Islands*

Climate sets range limits in many taxa (Chown & Gaston 1999; Sax 2001), and as climate changes, the ranges that plants and animals occupy are altered (Strathdee & Bale 1996; Hodkinson & Bird 1998; Blackburn & Duncan 2001). The responses of species to climate change will involve either migration or local adaptation. Some species are expected to shift their distribution poleward as temperature ameliorates (Pitelka 1997; IPCC 2001; Coulson *et al.* 2002; Walther *et al.* 2002), and indeed many have already done so (Parmesan *et al.* 2000). Those species which are unable to extend their ranges upward in altitude or latitude will be faced with potentially stressful environmental conditions, to which they will have to adapt, or become extinct (Agrawal 2001; Davis & Shaw 2001). Local adaptation, which will characterize some species, will include both genotypic change and phenotypic plasticity (Agrawal 2001). These responses will not only lead to widespread changes in the nature of assemblages of indigenous species, but will also have a major impact on species that are considered alien (Bergstrom & Chown 1999; Stachowicz *et al.* 2002; Kritikos *et al.* 2003). Moreover, it is thought, at least on the SOIs, that alien species are more resilient in their tolerances to high temperatures than indigenous species (Crafford & Chown 1990; Ernsting *et al.* 1995; Klok & Chown 2001). In addition, Barendse & Chown (2001) pointed out that the majority of indigenous species on Marion Island have slow life cycles, often of a year or longer, and seem to be relatively inflexible so far as responses to temperature are



concerned. By contrast the alien species have shorter life cycles, often including several generations per year, and have greater temperature sensitivity. The latter, enhanced phenotypic flexibility in the responses to temperature may well be a consequence of the fact that many alien species are essentially “ruderal, weedy” species (Crafford & Chown 1990; Greve *et al.* 2005). If this is the case, then climate warming and drying at SOIs will have a more pronounced deleterious effect on the indigenous species, than on the aliens. It might also mean that alien species will be capable of faster life cycles and therefore a greater production of offspring. Being at a physiological and life history advantage would mean that the alien species might either simply become more abundant than the indigenous ones, or might displace them. By having different effects on alien and indigenous species, climate change is likely to profoundly alter both the biota and ecosystem functioning of SOIs. These predictions, that alien species will have greater tolerance to high temperature, and possibly greater phenotypic flexibility, than indigenous species, can be explicitly tested, as has already been demonstrated elsewhere (Stachowicz *et al.* 2002; Beckett & Morton 2003).

Therefore, the major goal of this study is to test these predictions for the arthropods of Marion Island by examining the physiological limits of indigenous and alien taxa. In other words, the primary aim of the thesis is to investigate the responses of several indigenous and alien arthropods to high and low temperature, their responses to acclimation, and the relationships between upper and lower temperature responses in these species.

This study specifically addresses the following research topics:

- First, the baseline thermal tolerances and desiccation resistance of indigenous and alien species are investigated to provide information on what the physiological tolerances of a variety of species are. These experiments are thus conducted on ‘field fresh’ organisms that have been kept under ambient temperature and photoperiod conditions, and are supplemented by published work on a variety of other species from Marion Island (Klok & Chown 1997; Van der Merwe *et al.* 1997; Klok & Chown 1998; 2001).
- Second, the extent to which phenotypic plasticity is displayed by indigenous and alien species is tested and discussed in terms of physiological tolerances. Here investigations focus on springtails, which have not been well investigated from a physiological perspective. Baseline tolerances of indigenous and alien species are investigated, and then the effects of acclimation on desiccation and thermal tolerance are examined.
- Finally, temperature plays a large role in the physiological tolerances displayed by organisms (Cannon 1987), and the ways in which ectotherms respond to their surrounding thermal environments may vary over space and time (Watson & Hoffmann 1996; Kingsolver & Huey 1998). Thus, the microhabitat temperatures experienced by Marion Island invertebrates are examined along an altitudinal transect over a two-year period. In conjunction with physiological data, knowledge

of spatial and temporal changes in microhabitat temperatures will provide insight into the extent to which physiological tolerances enable survival and therefore influence the abundance and distribution of the species concerned (Chown & Gaston 1999; Sinclair 2001; Chown & Nicolson 2004).

Because there are several major threats facing the Southern Ocean Islands, and particularly the Prince Edward Islands (Chown *et al.* 2002; Greenslade 2002), Chapter 2 of this thesis focuses on these threats, focusing specifically on the indigenous and alien fauna of the Prince Edward Islands. The recent discovery of the first recorded terrestrial crustacean for Marion Island, the alien *Porcellio scaber* (Slabber & Chown 2002) and an unknown wasp species is documented as part of this review. Chapters 3 and 4 provide information on the upper and lower thermal tolerances, and their responses to acclimation, of two insects indigenous to Marion Island. Previous physiological studies on Marion Island invertebrates have mainly focused on macro invertebrates (e.g. Chown *et al.* 1997; Klok & Chown 1997; Van der Merwe *et al.* 1997; Klok & Chown 1998; 2001; Sinclair & Chown 2003), while neglecting smaller species. These chapters are aimed at remedying this situation, and provide information on the thermal tolerance limits and plasticity of two smaller indigenous invertebrates from Marion Island. Chapter 5 focuses on the physiological tolerances (in terms of high and low temperature responses, as well as desiccation resistance responses to acclimation) in two indigenous and three introduced springtail species from Marion Island. Chapter 6 investigates microhabitat temperatures experienced by invertebrate fauna on Marion Island along an altitudinal transect, and physiological tolerances displayed by several species are discussed in this context. Chapter 7 sets out the lessons learned from

these investigations, especially in the context of what the responses to climate change of the indigenous and alien species will be, whether predictions that alien species will benefit at the expense of indigenous ones are supported, and what actions should be taken, from a management perspective, in light of these findings.

## References

- Agrawal A. A.** (2001) Phenotypic plasticity in the interaction and evolution of species. *Science* **294**: 321-326
- Barendse J. & Chown S. L.** (2001) Abundance and seasonality of mid-altitude fellfield arthropods from Marion Island. *Polar Biology* **24**: 73-82
- Beaumont L. J. & Hughes L.** (2002) Potential changes in the distributions of latitudinally restricted Australian butterfly species in response to climate change. *Global Change Biology* **8**: 954-971
- Beckett S. J. & Morton R.** (2003) The mortality of three species of Psocoptera, *Liposcelis bostrychophila* Badonnel, *Liposcelis decolor* Pearman and *Liposcelis paeta* Pearman, at moderately elevated temperatures. *Journal of Stored Product Research* **39**: 103-115
- Bergstrom D. M. & Chown S. L.** (1999) Life at the front: history, ecology and change on southern ocean islands. *Trends in Ecology and Evolution* **14**: 472-477
- Blackburn T. M., Cassey P., Duncan R. P., Evans K. L. & Gaston K. J.** (2004) Avian extinction and mammalian introductions on oceanic islands. *Science* **305**: 1955-1958
- Blackburn T. M. & Duncan R. P.** (2001) Determinants of establishment success in introduced birds. *Nature* **414**: 195-197
- Bonner W. N.** (1984) Introduced mammals. *Antarctic ecology*. Ed. Laws R. M., Academic Press, London. 1: pp 237-278
- Cannon R. J. C.** (1987) Effects of low-temperature acclimation on the survival and cold tolerance of an Antarctic mite. *Journal of Insect Physiology* **33**: 509-521
- Chown S. L. & Gaston K. J.** (1999) Exploring links between physiology and ecology at macro scales: the role of respiratory metabolism in insects. *Biological Reviews* **74**: 87-120
- Chown S. L., McGeoch M. A. & Marshall D. J.** (2002) Diversity and conservation of invertebrates on the sub-Antarctic Prince Edward Islands. *African Entomology* **10**: 67-82
- Chown S. L. & Nicolson S. W.** (2004) *Insect physiological ecology. Mechanisms and patterns*. Oxford University Press, Oxford
- Chown S. L., Rodrigues A. S., Gremmen N. J. M. & Gaston K. J.** (2001) World heritage status and the conservation of southern ocean islands. *Conservation Biology* **15**: 550-557

- Chown S. L., van der Merwe M. & Smith V. R.** (1997) The influence of habitat and altitude on oxygen uptake in sub-Antarctic weevils. *Physiological Zoology* **70**: 116-124
- Coulson S. J., Hodkinson I. D., Webb N. R., Mikkola K., Harrison J. A. & Pedgley D. E.** (2002) Aerial colonization of high Arctic islands by invertebrates: the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae) as a potential indicator species. *Diversity and Distributions* **8**: 327-334
- Crafford J. E. & Chown S. L.** (1990) The introduction and establishment of the diamondback moth (*Plutella xylostella* L., Plutellidae) on Marion Island. *Antarctic ecosystems. Ecological change and conservation*. Ed. K.R. K. & Hempel G. pp 354-358
- Croxall J. P.** (1992) Southern ocean environmental changes: effects on seabird, seal and whale populations. *Philosophical Transactions of the Royal Society of London B* **338**: 319-328
- Davis M. A.** (2003) Biotic globalization: does competition from introduced species threaten biodiversity? *Bioscience* **53**: 481-489
- Davis M. B. & Shaw R. G.** (2001) Range shifts and adaptive responses to quaternary climate change. *Science* **292**: 673-679
- Edwards D.** (1997) Global warming and the political economy of threats. *The Ecologist* **27**: 2-4
- Ernsting G., Block W., MacAlister H. & Todd C.** (1995) The invasion of the carnivorous carabid beetle *Trechisibus antarcticus* on South Georgia (sub-Antarctic) and its effect on the endemic herbivorous beetle *Hydromedion sparsutum*. *Oecologia* **103**: 34-42
- Frenot Y., Chown S. L., Whinam J., Selkirk P. M., Convey P., Skotnicki M. & Bergstrom D. M.** (2005) Biological invasions in the Antarctic: extent, impacts and implications. *Biological Reviews* **80**: 45-72
- Frenot Y., Gloaguen J. C., Massé L. & Lebouvier M.** (2001) Human activities, ecosystem disturbance and plant invasions in subantarctic Crozet, Kerguelen and Amsterdam Islands. *Biological Conservation* **101**: 33-50
- Frenot Y., Gloaguen J.-C. & Trehen P.** (1997) Climate change in Kerguelen Islands and colonization of recently deglaciated areas by *Poa kerguelensis* and *P. annua*. *Antarctic communities: species, structure and survival*. Ed. Battaglia B., Valencia J. & Walton D. W. H., Cambridge University Press, Cambridge. pp 358-366
- Gaston K. J., Jones A. G., Hänel C. & Chown S. L.** (2003) Rates of species introductions to a remote island. *Proceedings of the Royal Society of London B*: 1091-1098

- Greenslade P.** (2002) Assessing the risk of exotic Collembola invading subantarctic islands: prioritising quarantine management. *Pedobiologia* **46**: 338-344
- Greve M., Gremmen N. J. M., Gaston K. J. & Chown S. L.** (2005) Nestedness of Southern Ocean Island biotas: Ecological perspectives on a biogeographic conundrum. *Journal of Biogeography* **32**: 155-168
- Hodkinson I. D. & Bird J.** (1998) Host-specific insect herbivores as sensors of climate change in Arctic and alpine environments. *Arctic and Alpine Research* **30**: 78-83
- IPCC** (2001) *Climate change 2001: synthesis report. A contribution of working groups I, II, and III to the third assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge, United Kingdom and New York
- Jones A. G., Chown S. L., Ryan P. G., Gremmen N. J. M. & Gaston K. J.** (2003) A review of conservation threats on Gough Island: a case study for terrestrial conservation in the Southern Oceans. *Biological Conservation* **113**: 75-87
- Karban R. & Strauss S. Y.** (2004) Physiological tolerance, climate change, and a northward range shift in the spittlebug, *Philaenus spumarius*. *Ecological Entomology* **29**: 251-254
- Kingsolver J. G. & Huey R. B.** (1998) Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *American Zoologist* **38**: 545-560
- Klok C. J. & Chown S. L.** (1997) Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic caterpillar, *Pringleophaga marioni* Viette (Lepidoptera: Tineidae). *Journal of Insect Physiology* **43**: 685-694
- Klok C. J. & Chown S. L.** (1998) Interactions between desiccation resistance, host-plant contact and the thermal biology of a leaf-dwelling sub-Antarctic caterpillar, *Embryonopsis halticella* (Lepidoptera: Yponomeutidae). *Journal of Insect Physiology* **44**: 615-628
- Klok C. J. & Chown S. L.** (2001) Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic kelp fly, *Paractora dreuxi* (Diptera: Helcomyzidae). *Journal of Insect Physiology* **47**: 95-109
- Kritikos D. J., Sutherst R. W., Brown J. R., Adkins S. W. & Maywald G. W.** (2003) Climate change and the potential distribution of an invasive alien plant: *Acacia nilotica* ssp. *indica* in Australia. *Journal of Applied Ecology* **40**: 111-124
- Lonsdale W. M.** (1999) Global patterns of plant invasions and the concept of invasibility. *Ecology* **80**: 1522-1536
- Mooney H. A. & Cleland E.** (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences of the USA* **98**: 5446-5451

- Owens I. P. F. & Bennett P. M.** (2000) Ecological basis of extinction risk in birds: Habitat loss versus human persecution and introduced predators. *Proceedings of the National Academy of Sciences of the USA* **97**: 12144-12148
- Parmesan C., Root T. L. & Willig M. R.** (2000) Impacts of extreme weather and climate on terrestrial biota. *Bulletin of the American Meteorological Society* **81**: 443-450
- Pimentel D.** (2002) *Economic and environmental threats of alien plant, animal, and microbe invasions. Biological invasions.* CRC Press LCC, Boca Raton
- Pimentel D., McNair W., Jancecka J., Wightman J., Simmonds C., O'Connell E., Wong E., Russel L., Zern J., Aquino T. & Tsomondo T.** (2002) Economic and environmental threats of alien plant, animal and microbe invasions. *Biological invasions. Economic and environmental costs of alien plant, animal and microbe species.* Ed. Pimentel D., CRC Press LCC, Boca Raton. pp 307-329
- Pitelka L. F.** (1997) Plant migration and climate change. *American Scientist* **85**: 464-473
- Porter S. D. & Savignano D. A.** (1990) Invasion of polygyne ants decimates native ants and disrupts arthropod community. *Ecology* **71**: 2095-2106
- Richardson D. M., Pyšek P., Rejmánek M., Barbour M. G., Panetta F. D. & West C. J.** (2000) Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions* **6**: 93-107
- Sax D. F.** (2001) Latitudinal gradients and geographic ranges of exotic species: implications for biogeography. *Journal of Biogeography* **28**: 139-150
- Sinclair B. J.** (2001) Field ecology of freeze tolerance: interannual variation in cooling rates, freeze-thaw and thermal stress in the microhabitat of the alpine cockroach *Celatoblatta quinquemaculata*. *Oikos* **93**: 286-293
- Sinclair B. J. & Chown S. L.** (2003) Rapid responses to high temperature and desiccation but not to low temperature in the freeze tolerant sub-Antarctic caterpillar *Pringleophaga marioni* (Lepidoptera, Tineidae). *Journal of Insect Physiology* **49**: 45-52
- Slabber S. & Chown S. L.** (2002) The first record of a terrestrial crustacean, *Porcellio scaber* (Isopoda, Porcellionidae), from sub-Antarctic Marion Island. *Polar Biology* **25**: 855-858
- Smith V. R.** (2002) Climate change in the sub-Antarctic: An illustration from Marion Island. *Climatic Change* **52**: 345-357
- Smith V. R. & Steenkamp M.** (1990) Climatic change and its ecological implications at a sub-Antarctic island. *Oecologia* **85**: 14-24



- Stachowicz J. J., Terwin J. R., Whitlatch R. B. & Osman R. W.** (2002) Linking climate change and biological invasions: ocean warming facilitates nonindigenous species invasions. *Proceedings of the National Academy of Sciences of the USA* **99**: 15497-15500
- Strathdee A. T. & Bale J. S.** (1996) Life cycle and morph production in the Arctic aphid *Acyrosiphon brevicorne*. *Polar Biology* **16**: 293-300
- Van der Merwe M., Chown S. L. & Smith V. R.** (1997) Thermal tolerance limits in six weevil species (Coleoptera, Curculionidae) from sub-Antarctic Marion Island. *Polar Biology* **18**: 331-336
- Van Wilgen B. W., Richardson D. M., Le Maitre D. C., Marais C. & Magadlela D.** (2002) The economic consequences of alien plant invasions: examples of impacts and approaches to sustainable management in South Africa. *Biological Invasions. Economic and environmental costs of alien plant, animal and microbe species*. Ed. Pimentel D., CRC Press LCC, Boca Raton. pp 243-265
- Vitousek P. M., D'Antonio C. M., Loope L. L., Rejmánek M. & Westbrooks R.** (1997b) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology* **21**: 1-16
- Vitousek P. M., Mooney H. A., Lubchenco J. & Melillo J. M.** (1997a) Human domination of earth's ecosystems. *Science* **277**: 494-499
- Walther G.-R., Post E., Convey P., Menzel A., Parmesan C., Beebee T. J. C., Fromentin J. M., Hoegh-Guldberg O. & Bairlein F.** (2002) Ecological responses to recent climate change. *Nature* **416**: 389-395
- Watson M. J. O. & Hoffmann A. A.** (1996) Acclimation, cross-generation effects, and the response to selection for increased cold resistance in *Drosophila*. *Evolution* **50**: 1182-1192
- Wiles G. J., Bart J., Beck R. E. & Aguon C. F.** (2003) Impacts of the brown tree snake: patterns of decline and species persistence in Guam's avifauna. *Conservation Biology* **17**: 1350-1360

## Chapter 2

### Marion Island: the alien species dilemma

*Discovery consists of seeing what everybody has seen and thinking what nobody has thought.*

- Albert Szent-Györgyi -



Aliens from Marion Island: Top left (clockwise): *Deroceras panormitanum* (slug: previously *D. caruanae*), *Hypochoeris radicata* (mouse-ear chickweed), *Cerastium fontanum* (cats-ear), and *Mus musculus* (young). A specimen of the recently discovered alien wasp (*Aphidius* sp.) is shown in the centre of the image.

The paucity of land in the Southern Ocean means that the small islands found there represent some of the only terrestrial habitats at these latitudes (Bergstrom & Chown 1999). Due to their characteristically small size, the Southern Ocean Islands (SOIs) are strongly influenced by the ocean, and their climates are typically oceanic (Smith & Steenkamp 1990). Marion Island (46°54'S, 37°45'E) forms part of the Prince Edward Islands Group in the Southern Ocean (Fig. 1), lies about 2000 km south of South Africa, and is thought to be ~275 000 years old (McDougall 1971). Marion Island's climate is characterised by low annual air temperature (5.7 °C), high precipitation (c. 2000 mm per annum), considerable cloud cover, high wind speed, and high relative humidity (year average 83 % RH) (Schulze 1971; Smith 1992a; 2002). There is little diurnal and seasonal variation in temperature, both in terms of air and microhabitat temperature (Chown & Crafford 1992; Smith 1992a; Blake 1996; Smith 2002). Despite the rather stable, oceanic climate, recent climatic changes have been rapid (Smith 2002). This change is thought to constitute a considerable threat to the indigenous biota (Smith 2002; Pakhomov & Chown 2003). However, it is not only a warming climate that is threatening and likely to threaten the indigenous biota of Marion Island. As with other SOIs, the Prince Edward Islands, and particularly Marion Island, have had a reasonable share of human impacts (see Gremmen 1981; Hänel & Chown 1999; Frenot *et al.* 2005). The direct impacts humans have had on these ecosystems (Van Aarde 1980; Crafford & Chown 1990; Ernsting *et al.* 1995; Gremmen & Smith 1999) are perhaps more visible than the indirect ones, such as our contribution to the impacts of global climate change (Vitousek *et al.* 1997; IPCC 2001). Indeed, humans have been responsible for a number of accidental and deliberate introductions of species to the SOIs

(Watkins & Cooper 1986; Ernsting *et al.* 1995; Gremmen 1997; Gremmen & Smith 1999; Frenot *et al.* 2001), as they have been elsewhere (Vitousek *et al.* 1997; Pimentel 2002).

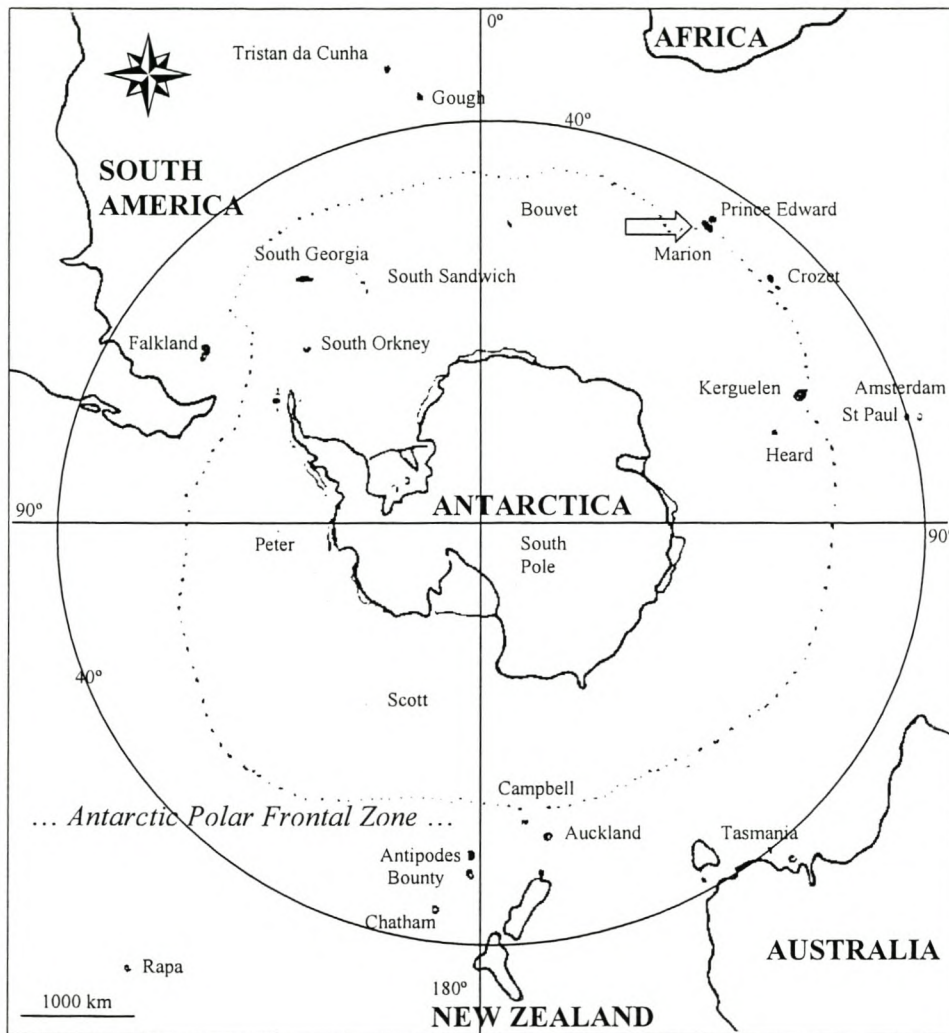


Figure 1. Map of Antarctica and sub-Antarctic Islands, showing the location of the Prince Edward Islands

The geographic location, isolation and small size of Marion Island render its unique biota (Bergstrom & Chown 1999) vulnerable to the direct and indirect effects of invasive species and human impacts (Chown *et al.* 2001). In addition, due to the harsh climate, recent origin, past glaciations and remoteness of Marion Island, its biota is relatively species poor.

### *Vegetation*

Several detailed studies report on the vegetation of the Prince Edward Islands (Smith 1978; Gremmen 1981; Gremmen 1997; Gremmen & Smith 1999), which consists of 40 vascular plant species, of which 18 are introduced (Smith 1978; Hänel & Chown 1999) (Table 1). Of the 18 introduced species, 12 are naturalised, and comprise grasses and flowering plants only. Some introduced species have to date been unable to produce viable seed, but with an ameliorating macroclimate and a subsequent rise in microhabitat temperatures, invaded habitats may expand (see Parmesan *et al.* 1999; Beaumont & Hughes 2002; Kritikos *et al.* 2003; Karban & Strauss 2004 for examples of range expansions in response to climate change), and previously unaffected areas may be invaded (see Frenot *et al.* 1997 for a sub-Antarctic example; and Kritikos *et al.* 2003 for a continental example). Other invasive plant species, especially some species belonging to the Poaceae, have caused rapid changes in the landscapes they have invaded. Of all alien plant species that are found on the SOIs, the alien grass, *Poa annua* shows the widest distribution, followed by *Cerastium fontanum*, *Rumex acetosella*, *Stellaria media*, *Sagina procumbens*, and *Poa pratensis* (Frenot *et al.* 2005).

Table 1. Indigenous vascular flora (a) and alien vascular flora (b) of Marion Island.

(Sources: Smith 1978; Gremmen 1981; Gremmen 1997; Gremmen &amp; Smith 1999)

<b>A Indigenous vascular flora of Marion Island</b>		
<b>Species</b>	<b>Confirmed indigenous</b>	<b>Unknown but likely to be indigenous</b>
<i>Acaena magellanica</i>	x	
<i>Agrostis magellanica</i>	x	
<i>Azorella selago</i>	x	
<i>Blechnum penna-marina</i>	x	
<i>Callitriche antarctica</i>	x	
<i>Colobanthus kerguelensis</i>	x	
<i>Cotula plumosa</i>	x	
<i>Crassula moschata</i>	x	
<i>Elaphoglossum randii</i>	x	
<i>Grammitis kerguelensis</i>	x	
<i>Hymenophyllum peltatum</i>	x	
<i>Juncus scheuchzerioides</i>	x	
<i>Juncus cf. effusus</i>		x
<i>Lycopodium saururus</i>	x	
<i>Lycopodium magellanica</i>	x	
<i>Montia fontana</i>	x	
<i>Poa cookii</i>	x	
<i>Polystichum marionense</i>	x	
<i>Potamogeton sp.</i>		x
<i>Ranunculus moseleyi</i>	x	
<i>Ranunculus biternatus</i>	x	
<i>Uncinia compacta</i>	x	

**B Alien vascular flora of Marion Island**

<b>Species</b>	<b>Transient alien</b>	<b>Naturalised alien</b>
<i>Agropyron repens</i>		X
<i>Agrostis castellana</i>		X
<i>Agrostis gigantea</i>		X
<i>Agrostis stolonifera</i>		X
<i>Alopecurus australis</i>		X
<i>Avena sativa</i>	X	
<i>Cerastium fontanum</i>		X
<i>Festuca rubra</i>		X
<i>Holcus lanatus</i>	X	
<i>Hypochoeris radicata</i>	X	
<i>Plantago lanceolata</i>	X	
<i>Poa annua</i>		X
<i>Poa pratensis</i>		X
<i>Rumex acetocella</i>		X
<i>Sagina procumbens</i>		X
<i>Senecio</i> sp.	X	
<i>Sonchus</i> sp.	X	
<i>Stellaria media</i>		X

### *Vertebrates*

Additions to the indigenous vertebrate and invertebrate faunas have also occurred. The indigenous vertebrate fauna of Marion Island (Table 2) comprises three terrestrial breeding seal species, killer whales and a rich species assemblage of birds that depend partially or wholly on the ocean for non-breeding resources. These include four species of penguin, four species of albatross, five scavenging species, 12 burrowing petrel species, two terns, and one cormorant species (Cooper & Brown 1990). These species contribute nutrients to the system in the form of guano, scats and urine, which are essential for the Islands' terrestrial ecosystems (Smith 1978).

The SOIs and the Antarctic continent have no indigenous fish, amphibians, or reptiles (Frenot *et al.* 2005), but several alien vertebrates have been introduced to Marion Island (Table 3). However, mice, cats and sheep were the only mammals ever to become feral. Sheep never became very abundant (abundance did not exceed 60 individuals) and have been removed from Marion Island completely (Gremmen 1981; Watkins & Cooper 1986). Cats (*Felis catus*), which were eradicated from Marion Island, however, did reach problem proportions. The problems caused by cats on SOIs (and elsewhere, e.g. Pimentel 2002) are well documented and discussed in detail by several authors (e.g. Bloomer & Bester 1992; Chapuis *et al.* 1994; Bester *et al.* 2000). Cats were originally taken to Marion Island in an attempt to control mice at the Meteorological station, but soon became feral (Crafford & Scholtz 1987). By 1977 an estimated 3400 cats threatened local burrowing petrel species across the entire island (Van Aarde 1979; 1980). The process to rid Marion Island of its



feral cat population commenced in 1977 (Bester *et al.* 2000) and by July 1991 the cat population had been successfully eradicated (Bester *et al.* 2000).

Mice (*Mus musculus*) have been present on Marion Island since at least 1818, and are thought to have been introduced through sealing activities early in the 1800s (Watkins & Cooper 1986). Prince Edward Island is one of only a few mouse-free (and thus alien vertebrate-free) SOIs and as a result, striking differences exist between the invertebrate fauna of Prince Edward Island and nearby Marion Island in terms of the size, structure and species composition of their macro-invertebrate populations, and maximum body sizes of macro-invertebrate species (Crafford & Scholtz 1987). The mice are mostly carnivorous (their diet comprises *c.* 70 to 90 % invertebrates) (Gleeson & van Rensburg 1982; Smith *et al.* 2002) and as such mice are having profound influences on invertebrate populations on Marion Island (Crafford 1990). In 1987, mice consumed an estimated 23.7 kg ha<sup>-1</sup> y<sup>-1</sup> (dry weight) larvae of the Marion flightless moth (*Pringleophaga marioni*) (Rowe-Rowe *et al.* 1989), whereas Crafford (1990) estimated that mice reduce the amount of plant litter processed per hectare by at least 1000 kg per annum as a consequence of the removal of *Pringleophaga* larvae, which comprise an important component in the detritus chain. Mice also directly compete with birds (the indigenous lesser sheathbill (*Chionis minor*) and the kelp gull (*Larus dominicanus*)) for dietary resources (Rowe-Rowe *et al.* 1989; Huyser *et al.* 2000). On Guillou Island (Kerguelen archipelago) mice also display opportunistic and generalist feeding behaviour, where earthworms, aphids, weevils and seed of *Acaena magellanica* and floral parts of dandelion represent the bulk of the diet of mice (Le Roux *et al.* 2002).

Table 2. The terrestrially breeding indigenous vertebrates of Marion Island

<b>Group</b>	<b>Common name</b>	<b>Scientific name</b>
<b>Breeding birds</b>		
<b>Penguins</b>	King penguin	<i>Aptenodytes patagonicus</i>
	Gentoo penguin	<i>Pygoscelis papua</i>
	Macaroni penguin	<i>Eudyptes chrysolophus</i>
	Rockhopper penguin	<i>E. chrysocome</i>
<b>Albatrosses</b>	Wandering albatross	<i>Diomedea exulans</i>
	Grey-headed albatross	<i>Thalassarche chrysotoma</i>
	Sooty albatross	<i>Phoebetria fusca</i>
	Light-mantled albatross	<i>P. palpebrata</i>
<b>Petrels</b>	Northern giant petrel	<i>Macronectes halli</i>
	Southern giant petrel	<i>M. giganteus</i>
	Fairy prion	<i>Pachyptila turtur</i>
	Salvin's prion	<i>P. salvini</i>
	Blue petrel	<i>Halobaena caerulea</i>
	Greatwinged petrel	<i>Pterodroma macroptera</i>
	Softplumaged petrel	<i>P. mollis</i>
	Kerguelen petrel	<i>Lugensa brevirostris</i>
	Grey petrel	<i>Procellaria cinerea</i>
	Whitechinned petrel	<i>P. aequinoctialis</i>
	Black-bellied storm-petrel	<i>Fregetta tropica</i>
	Grey-backed storm-petrel	<i>Garrodia nereis</i>
	South Georgian diving petrel	<i>Pelecanoides georgicus</i>
	Common diving petrel	<i>P. urinatrix</i>
<b>Miscellaneous</b>	Imperial cormorant	<i>Phalacrocorax atriceps</i>
	Sub-Antarctic skua	<i>Catharacta antarctica</i>
	Kelp gull	<i>Larus dominicanus</i>
	Antarctic tern	<i>Sterna vittata</i>
	Kerguelen tern	<i>S. virgata</i>
	Lesser sheathbill	<i>Chionis minor marionensis</i>
<b>Mammals</b>		
<b>Seals</b>	Southern elephant seal	<i>Mirounga leonina</i>
	Sub-Antarctic fur seal	<i>Arctocephalus tropicalis</i>
	Antarctic fur seal	<i>Arctocephalus gazella</i>

Table 3. Deliberate and accidental vertebrate introductions to Marion Island. (Sources: Grobbelaar 1974; Gremmen 1981; Watkins & Cooper 1986; Bloomer & Bester 1992)

<b>Species name</b>	<b>Common name</b>	<b>Current status on Marion Island</b>
<b>Birds</b>		
<i>Amazona</i> sp.	Amazon parrot	Absent
<i>Anser anser</i>	Domestic goose	Absent
<i>Gallus gallus</i> var. <i>domesticus</i>	Domestic fowl	Absent
<i>Psittacus erithacus</i>	African grey parrot	Absent
<b>Fish</b>		
<i>Salmo gairdneri</i>	Rainbow trout	Absent
<i>S. trutta</i>	Brown trout	Absent
<b>Mammals</b>		
<i>Canis familiaris</i>	Domestic dog	Absent
<i>Capra hircus</i>	Domestic goat	Absent
<i>Equus asinus</i>	Donkey	Absent
<i>Felis catus</i> <sup>#</sup>	Domestic cat	Absent
<i>Mus musculus</i> <sup>*#</sup>	House mouse	Naturalised
<i>Ovis aries</i> <sup>#</sup>	Merino sheep	Absent
<i>Sus scrofa</i>	Domestic pig	Absent

\*The only accidental vertebrate introduction

<sup>#</sup>Species which, at some stage, became feral on Marion Island

*Invertebrates*

As with many other SOIs (see Frenot *et al.* 2005 for a detailed review), Marion Island has a large number of indigenous and alien invertebrates (Hänel *et al.* 1998; Chown *et al.* 2002) (Table 4). The Island's insect species diversity consists of 41 % indigenous species, 34 % transient alien species and 25 % naturalised alien species (18, 15 and 11 species out of a total of 44 described species, respectively) (Hänel *et al.* 1998; Chown *et al.* 2002), but micro-arthropods (mites and springtails) dominate invertebrate numbers on Marion Island (Barendse & Chown 2001). Sixteen springtail species, of which ten are indigenous (Gabriel *et al.* 2001; Chown *et al.* 2002), four spider species, more than 70 indigenous mite species, at least three species of earthworms, a snail, a slug, many species of nematodes (Crafford *et al.* 1986; Smith 1987; Hänel & Chown 1999), a recently introduced terrestrial isopod (*Porcellio scaber*) (Slabber & Chown 2002), an *Aphidius* sp. and *Agrotis* sp. comprise other elements of the invertebrate fauna of Marion Island (see Table 4).

Introduced invertebrates are causing great conservation concern worldwide (Pimentel 2002), as well as at the SOIs (Ernsting *et al.* 1995; Hänel & Chown 1998; Ernsting *et al.* 1999; Chown *et al.* 2002; Greenslade 2002). One well-documented example is that of the diamondback moth (*Plutella xylostella*), which was probably introduced to Marion Island with supplies of fresh vegetables taken to the island for human consumption (Crafford & Chown 1990; Chown & Avenant 1992). The diamondback moth is a very destructive cosmopolitan pest species, and has been present on Marion Island since at least 1986, where it infests individuals of the indigenous brassica, *Pringlea antiscorbutica*. Indeed, *P.*

*xylostella* poses a severe conservation threat to this unique plant species, which is experiencing herbivore grazing pressure on other SOIs (e.g. on the French sub-Antarctic islands - Chapuis *et al.* 1994).

Introduced invertebrates are impacting severely on invaded landscapes at a global scale (Pimentel 2002). *Plutella xylostella*, which is one of the most notorious pest species (Talekar & Shelton 1993), also occurs on several SOIs (Crafford & Chown 1987; Chown & Avenant 1992). The success of *P. xylostella* as an invasive species is attributable to their high reproductive potential, high generation turnover and good long-distance migratory ability (Crafford & Chown 1990; Coulson *et al.* 2002), which include some of the most important factors that increase the ability of organisms to become successful invaders (Prinzing *et al.* 2002). Climate is probably the main factor that has been limiting *P. xylostella* on Marion Island, but an ameliorating climate may change this situation. Indigenous species, on the other hand, may not be able to show such rapid responses to climate change (Gabriel *et al.* 2001). *Limnophyes minimus* is another species that has successfully invaded several vegetation communities on Marion Island (Hänel & Chown 1998). Despite their small size, larvae of these chironomid midges are capable to consume litter to an order of magnitude greater per unit area in some vegetation communities than that of indigenous *Pringleophaga marioni* larvae (Hänel & Chown 1998).

Table 4. The terrestrial arthropods of Marion Island (excluding mites, ticks, nematodes, tardigrades and freshwater Crustacea). Categories are: Indigenous invertebrates (a), naturalised alien invertebrates (b), transient alien invertebrates (c), indigenous Collembola (d), and alien Collembola (e). The Collembola were grouped separately because they form such an integral part of this thesis. (Sources: Crafford *et al.* 1986; Crafford & Chown 1990; Hänel *et al.* 1998; Gabriel *et al.* 2001; Chown *et al.* 2002; Slabber & Chown 2002; Frenot *et al.* 2005). Species names that are in bold formed part of investigations in this thesis.

Species		Family	Order	Class
<b>A Indigenous invertebrates (22 species)</b>				
<i>Meropathus chuni</i>	Enderlein, 1901	Hydraenidae	Coleoptera	Insecta
<b><i>Halmaeusa atriceps</i></b>	<b>C.O. Waterhouse</b>	<b>Staphylinidae</b>	<b>Coleoptera</b>	<b>Insecta</b>
<i>B. parvulus</i>	C.O. Waterhouse	Curculionidae	Coleoptera	Insecta
<i>B. randi</i>	Jeannel	Curculionidae	Coleoptera	Insecta
<i>Bothrometopus elongatus</i>	Jeannel	Curculionidae	Coleoptera	Insecta
<i>Ectemnorhinus similis</i>	C.O. Waterhouse	Curculionidae	Coleoptera	Insecta
<i>E. marioni</i>	Jeannel	Curculionidae	Coleoptera	Insecta
<i>Palirhoeus eatoni</i>	C.O. Waterhouse	Curculionidae	Coleoptera	Insecta
<i>Telmatogeton amphibius</i>	Eaton	Chironomidae	Diptera	Insecta
<i>Apetaenus litoralis</i>	Eaton	Tethinidae	Diptera	Insecta
<i>Listriomastax litorea</i>	Enderlein	Tethinidae	Diptera	Insecta
<i>Paractora dreuxi mirabilis</i>	Seguy	Helcomyzidae	Diptera	Insecta
<i>Kleidotoma icarus</i>	Quinlan	Eucoilidae	Hymenoptera	Insecta
<i>Pringleophaga marioni</i>	Viette	Tineidae	Lepidoptera	Insecta
<i>Embryonopsis halticella</i>	Eaton	Yponomeutidae	Lepidoptera	Insecta

Species		Family	Order	Class
<i>(continued from previous page)</i>				
<i>Antarctopsocus jeanneli</i>	Badonnel	Elipsocidae	Psocoptera	Insecta
<i>Notodiscus hookeri</i>	Reeve	Endodontidae	Stylommatophora	Mollusca
<i>Microscolex kerguelarum</i>	Grube	Acanthodrilidae	Haplotaxidae	Oligochaeta
<i>Myro kerguelensis</i>	Cambridge	Agelenidae	Araneida	Arachnida
<i>Myro paucispinosus</i>	Berland	Agelenidae	Araneida	Arachnida
<i>Porrhomma antarctica</i>	Hickman	Linyphiidae	Araneida	Arachnida
<i>Erigone vagans</i>	Audouin	Linyphiidae	Araneida	Arachnida
<b>B Naturalised alien invertebrates (15 species)</b>				
<i>Calliphora vicina</i>	Robineau-Desvoidy	Calliphoridae	Diptera	Insecta
<i>Lycoriella aubertii</i>	Séguy	Sciaridae	Diptera	Insecta
<i>Fannia canicularis</i>	Linné	Fanniidae	Diptera	Insecta
<i>Limnophyes minimus</i>	Meigen	Chironomidae	Diptera	Insecta
<i>Psychoda parthenogenetica</i>	Tonnoir	Psychodidae	Diptera	Insecta
<i>Scaptomyza oxyphallus</i>	Tsacas	Drosophilidae	Diptera	Insecta
<i>Myzus ascalonicus</i>	Doncaster	Aphididae	Hemiptera	Insecta
<i>Rhopalosiphum padi</i>	Linnaeus	Aphididae	Hemiptera	Insecta
<i>Macrosiphum euphorbiae</i>	Thomas	Aphididae	Hemiptera	Insecta
<i>Plutella xylostella</i>	Dugdale	Plutellidae	Lepidoptera	Insecta
<i>Vanessa cardui</i>	Linnaeus	Nymphalidae	Lepidoptera	Insecta
<i>Apterothrips apteris</i>	Daniel	Thripidae	Thysanoptera	Insecta
<i>Porcellio scaber</i>	Latreille	Porcellionidae	Isopoda	Crustacea
<i>Deroceras panormitanum</i>	Lessona & Pollonera	Limacidae	Eupulmonata	Mollusca
<i>Dendrodrilus rubidus tenuis</i>	Eisen	Acanthodrilidae	Haplotaxidae	Oligochaeta

Species		Family	Order	Class
<b>C Transient alien invertebrates (16 species)</b>				
<i>Blatella germanica</i>	L.	Blattidae	Blattodea	Insecta
<i>Anobiidae sp.</i>		Staphylinidae	Coleoptera	Insecta
<i>Dermestidae sp.</i>		Staphylinidae	Coleoptera	Insecta
<i>Chrysomelidae sp.</i>		Staphylinidae	Coleoptera	Insecta
<i>Lamphrolonchaea smaragdi</i>	Walker	Lonchaeidae	Diptera	Insecta
<i>Anthomyiidae sp.</i>		Tethinidae	Diptera	Insecta
<i>Cryptacrus comes</i>	Fabricius	Scutelleridae	Hemiptera	Insecta
<i>Lepisiota capensis</i>	Mayr	Formicidae	Hymenoptera	Insecta
<i>Agrotis ipsilon</i>	Walker	Noctuidae	Lepidoptera	Insecta
<i>Agrotis segetum</i>	Denis & Schiffermuller	Noctuidae	Lepidoptera	Insecta
<i>Chrysodeixis acuta</i>	Walker	Noctuidae	Lepidoptera	Insecta
<i>Cosmophila sabulifera</i>	Guenee	Noctuidae	Lepidoptera	Insecta
<i>Helicoverpa armigera</i>	Hubner	Noctuidae	Lepidoptera	Insecta
<i>Nomophila sp.</i>		Pyralidae	Lepidoptera	Insecta
<i>Spodoptera exigua</i>	Hubner	Noctuidae	Lepidoptera	Insecta
<i>Trichoplusia orichalcea</i>	Fabricius	Noctuidae	Lepidoptera	Insecta



Species		Family	Order	Class
<b>D Indigenous Collembola (10 species)</b>				
<i>Cryptopygus antarcticus</i>	Déharveng	Isotomidae	Collembola	Insecta <sup>#</sup>
<i>Cryptopygus caecus</i>	Wahlgren	Isotomidae	Collembola	Insecta
<i>Cryptopygus dubius</i>	Déharveng	Isotomidae	Collembola	Insecta
<i>Cryptopygus tricuspis</i>	Enderlein	Isotomidae	Collembola	Insecta
<i>Folsomotoma marionensis</i>	Déharveng	Isotomidae	Collembola	Insecta
<i>Friesea tilbrooki</i>	Wise	Neanuridae	Collembola	Insecta
<i>Tullbergia bisetosa</i>	Börner	Onychiuridae	Collembola	Insecta
<i>Katianna sp.</i>		Sminthuridae	Collembola	Insecta
<i>Sminthurinus tuberculatus</i>	Delamarre Deboutteville and Massoud	Sminthuridae	Collembola	Insecta
<i>Sminthurinus granulatus</i>	Enderlein	Sminthuridae	Collembola	Insecta
<b>E Alien Collembola (6 species)</b>				
<i>Ceratophysella denticulata</i>	Bagnall	Hypogastruridae	Collembola	Insecta
<i>Hypogastrura viatica</i>	Tullberg	Hypogastruridae	Collembola	Insecta
<i>Parisotoma notabilis</i>	Schäffer	Isotomidae	Collembola	Insecta
<i>Isotomurus cf. palustris</i>	Müller	Isotomidae	Collembola	Insecta
<i>Megalothorax sp.</i>		Neelidae	Collembola	Insecta
<i>Pogonognathellus flavescens</i>	Tullberg	Tomoceridae	Collembola	Insecta

<sup>#</sup>According to Hopkin (1997)

Because of the known deleterious effects of invasive species on native landscapes, control systems are in place in attempt to reduce the introduction of non-indigenous species to the SOIs. The Prince Edward Islands Management Plan (Anonymous 1996) requires that strict precautions be taken to avoid future introductions of non-native species to the Prince Edward Islands. Only if these precautions and guidelines are adhered to, will the number of species introductions as a direct result of human occupancy on SOIs decline. Constant vigilance, and a firm knowledge of the fauna and flora (and microbes) found on these island ecosystems will aid considerably in the prevention of introductions, and control of alien species. The following section documents the most recent accounts of invertebrate introductions to Marion Island.

**The first record of a terrestrial crustacean, *Porcellio scaber* (Isopoda, Porcellionidae)<sup>1</sup>, and an *Aphidius* species from Marion Island**

As has already been demonstrated above, the most significant conservation problems facing Southern Ocean Islands (SOIs) are invasive species, climate change, and their interaction (Smith & Lewis Smith 1987; Bergstrom & Chown 1999). Invasive species are already having significant direct and indirect effects on the biotas and ecosystems of these islands, and the extent and intensity of the impacts is expected to increase as climates become warmer and drier on many of the islands (Smith & Steenkamp 1990). Both Chown & Language (1994) and Kennedy (1995) have argued that the intensity of the impact of invasive species will increase owing to a rise in the number of successful human-mediated

---

<sup>1</sup> Published as: **Slabber S. & Chown S. L.** (2002) The first record of a terrestrial crustacean, *Porcellio scaber* (Isopoda, Porcellionidae), from sub-Antarctic Marion Island. *Polar Biology* **25**: 855-858

introductions, in turn a result of ameliorating climates in the region, and increasing numbers of visitors to the islands (Chown *et al.* 1998; Chown & Gaston 2000). There is now widespread concern regarding the extent to which climate change will interact with introduced species to affect local ecosystems in the sub-Antarctic (Bergstrom & Chown 1999; Walther *et al.* 2002). In consequence, strict quarantine procedures have been implemented for many of the islands.

The Prince Edward Islands Management Plan (Anonymous 1996), is particularly rigorous in this regard. No fresh produce is allowed ashore to the permanently occupied scientific station, and there are inspections of clothing, footwear and hiking gear before personnel are allowed ashore. Nonetheless, over the past decade several new species, including a grass and a rush, at least two moth species, and a blowfly, have been recorded at the island, all of which are thought to be recent introductions (Hänel *et al.* 1998; Gremmen & Smith 1999). In this section, the two most recent introductions to the island are reported.

## **Materials and methods**

The scientific station at Marion Island is permanently occupied (and has been since 1947) and visited generally only once a year during the April relief. During the relief, and when occasional visits by other vessels are made, reasonably strict quarantine procedures are enforced. In consequence, monitoring for alien species has been ongoing for at least two decades. Indeed, since the early 1980s, a watching brief for vagrant and potentially resident alien invertebrate and plant species has been kept at sub-Antarctic Marion Island (46° 54'

S, 37° 45' E), with occasionally reconnaissance visits being made to nearby Prince Edward Island (see e.g. Crafford *et al.* 1986; Crafford & Chown 1987; Chown & Avenant 1992; Chown & Language 1994; Hänel *et al.* 1998; Gremmen & Smith 1999). The most recent set of surveys, during which a variety of habitats were hand-searched for fixed time periods (15-30 minutes), and undertaken on several occasions in the April 2001 – April 2002 season, included regular searches at the scientific station and two searches around the island. These were supplemented by searches on the east coast of the island in April 2003 and April 2004. During the former searches, terrestrial isopods were discovered on Boulder Beach, immediately below the scientific station, and 10 m to the north east of the “Brown Store” at the scientific station. From this date on, the area around the scientific station was searched on a fortnightly basis for isopods (mostly under rocks and loose building timber), and a second search around the island was undertaken in April 2002 as part of a larger survey of the introduced slug *Deroceras caruanae* (now *D. panormitanum*) Pollonera (see Smith 1992b). During this survey, the abundance of slugs was quantified in more than 100, 10 x 10 cm quadrats, and records were kept of the presence/absence of isopods.

All of the isopods that were collected were examined for reproductive state (gravid or non-gravid), approximately 100 individuals collected in mid-summer (December to February) were measured (from the anterior margin of the head to the posterior margin of the abdomen, excluding the telson) using an ocular micrometer on a Wild microscope, and ten specimens collected in April 2002 were dissected and their gut contents examined under a compound microscope.

To determine whether potential vertebrate predators would consume isopods, five individuals (two live and three dead) were offered to five individuals of the indigenous lesser sheathbill at the scientific station. Five live and three dead isopods were also offered to a single individual of the alien house mouse. The individual had been trapped at the scientific station and starved overnight.

#### *Aphidius species*

Several individuals of an *Aphidius* wasp species were collected from among *Poa cookii* tussocks and the alien grass, *Poa annua*, growing underneath rock overhangs along the eastern seaboard of Marion Island during sampling surveys in April 2003. This species is very different in appearance to the indigenous, flightless wasp (*Kleidotoma icarus*), which parasitizes the indigenous flightless kelp fly (*Apetaenus litoralis*) on Marion Island (Crafford *et al.* 1986).

### **Results and discussion**

#### *Porcellio scaber*

As part of the former set of surveys, *Porcellio scaber* Latreille, 1804 was discovered at the eastern section of the Marion Island scientific station, immediately (10 m) to the north-east of what is known as the “Brown Store”, and in patches down to Boulder Beach (Transvaal Cove) (Fig. 1). These areas are all close to sites where building materials and containers are landed. Between 11th September 2001, when the species was first discovered, and 17th

April 2002, a total of 391 specimens was collected at the scientific station. Following the initial report in September 2001, the intention was to eradicate the species. Therefore, the individuals that were collected represent all of those that could be found in and around the station, and hence the total, detectable population. Subsequent searches at many localities around the scientific station failed to reveal any other individuals of this species. In addition, an intensive search for *P. scaber*, conducted while quantifying the abundance of the alien slug, failed to reveal the presence of this species elsewhere on Marion Island. Therefore, we can state with confidence that at present, the isopod is restricted to the immediate vicinity of the scientific station, and seems likely to have been introduced during the April 2001 relief voyage to the island.

Of the 391 individuals examined, 381 were undamaged and could be assessed for reproductive status. Many of the females were gravid (Fig. 2), and this was indicated by the presence of eggs and juveniles (carried ventrally by the females, a characteristic of this species (Carefoot 1993)). There is therefore little doubt that the species reproduces on the island and the size variation of the individuals collected also indicates that a variety of age classes is present (Fig. 3). Indeed, the stable, moist, cool conditions at Marion Island, with the absence of a very harsh winter (Stevenson Screen winter mean temperatures are approximately 2°C, Smith & Steenkamp 1990), mean that this species is unlikely to be restricted by winter temperatures. Several studies have investigated the low temperature tolerance of *P. scaber*. In the Palearctic this species has a lower lethal temperature of approximately -4.6°C (Tanaka & Udagawa 1993), and can survive for at least one week at -2°C, so long as individuals have access to food and have been previously exposed to

relatively low, but not subzero, temperatures (Lavy *et al.* 1997). Thus, low temperatures are unlikely to limit this species on Marion Island, especially given climate warming at the island (Smith 2002). However, day length variation and low temperatures might still be responsible for inducing seasonal reproduction (Fig. 2).

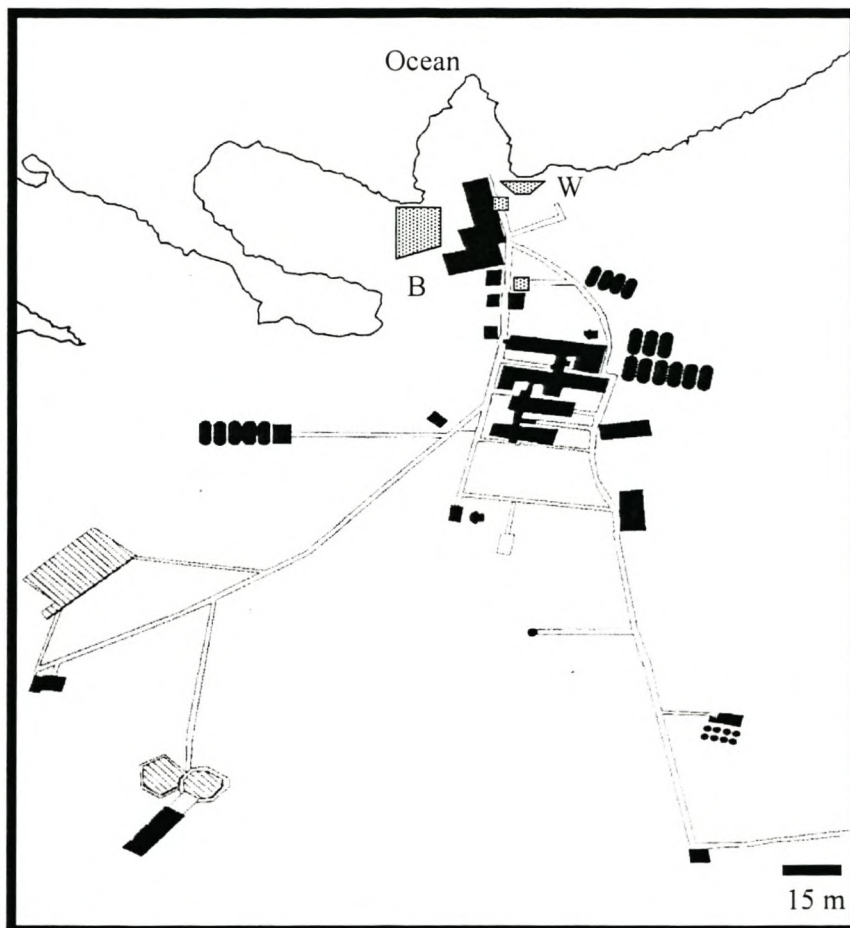


Figure 1. Areas around the scientific station at Marion Island where *Porcellio scaber* has been found (stippled). The building to the right of the letter "B" is the Brown Store, and the letter "W" represents Boulder Beach where the wasp species was first sighted. The scale bar in the right hand lower corner represents 15 metres.

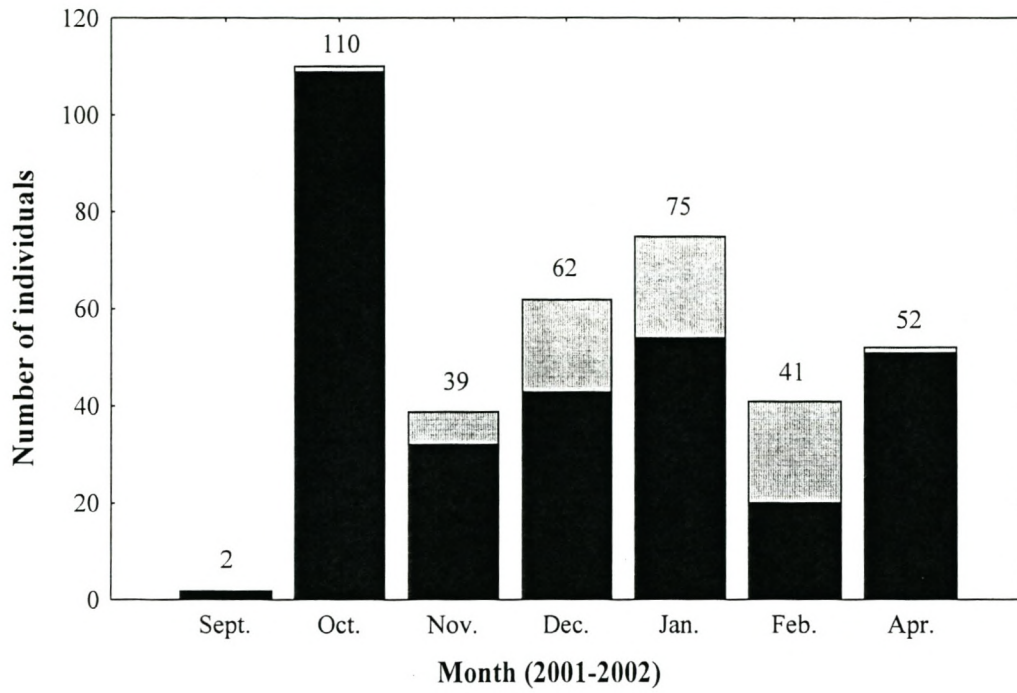


Figure 2. Numbers of non-gravid male and female (shaded bar) and gravid female (hatched bar) individuals of *Porcellio scaber* collected around the Marion Island scientific station between September 2001 and April 2002. Numbers above the bars indicate monthly totals.



There was little variation in the gut contents of the dissected individuals. At Marion Island, *P. scaber* feeds on detritus (including plant and animal remains, soil algae, and fungi), as is the case with most isopods and this species elsewhere (Barnes 1980). In turn, it appears that, at least for the moment, the two major vertebrate predators of invertebrates on Marion Island, the indigenous lesser sheathbill and the introduced house mouse (Burger 1978; Crafford 1990), will not accept this species as prey. When offered to sheathbills, the birds showed no interest in either dead or live isopods. Likewise, the house mouse handled the isopods, but rejected them. However, it immediately consumed a caterpillar of the flightless tineid moth, *Pringleophaga marioni* Viette, its favourite prey (see Crafford 1990), when this was offered. A second feeding bout involved rejection of the isopods, but acceptance of an earthworm (*Microscolex kerguelarum*). Although it appears that the only potential vertebrate predators currently either find this species distasteful or are naïve regarding the species as a prey item, this situation may change. House mice on Gough Island regularly consume *Porcellio scaber* (Jones *et al.* 2003).

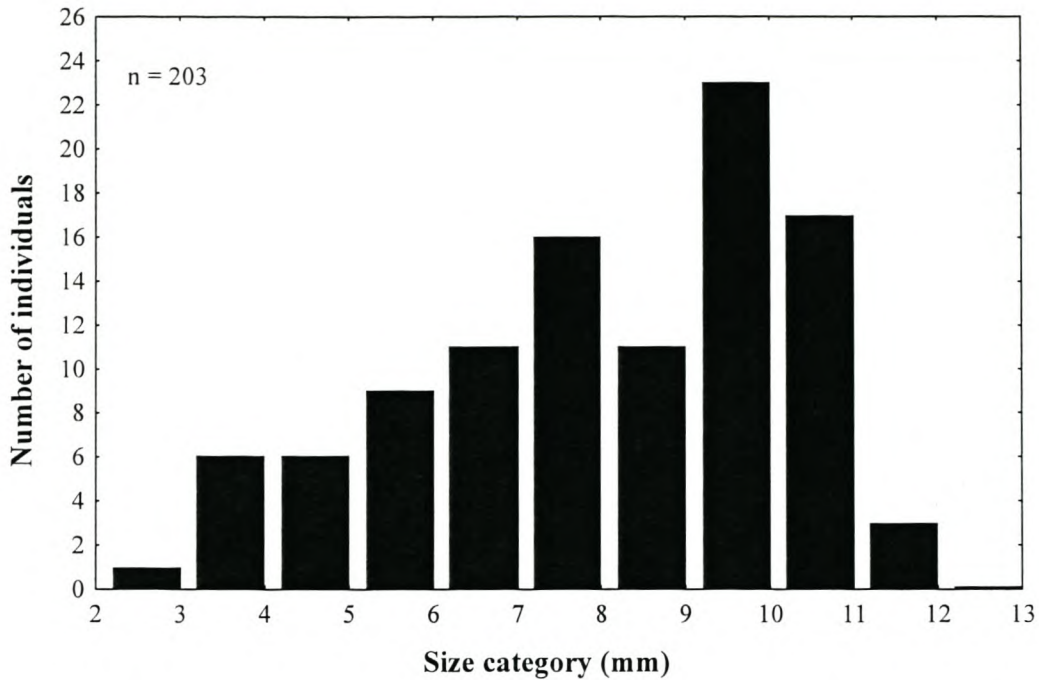


Figure 3. Frequency distribution of body lengths of *Porcellio scaber* collected between December 2001 and February 2002 at the Marion Island scientific station.

The introduction of a terrestrial crustacean to Marion Island represents one of only a few such introductions to a sub-Antarctic island (see for example Richardson & Jackson 1995 for information on introduced amphipods and isopods at Macquarie Island). *Porcellio scaber* is a southwestern European species, which has colonized the rest of Europe, North America, and many other areas, including Africa, largely as a result of passive transport by humans (Wang & Schreiber 1999). Indeed, according to Wang & Schreiber (1999), *P. scaber* is adept at making use of human-aided dispersal via ballast, rubble, agricultural products, compost and plants. It thus seems likely that the Marion Island population of this species has come either from South Africa, from where it is also known (Lawrence 1953),

or Gough Island, where it is an abundant, introduced species (Holdgate 1965). The latter island, which houses a permanently occupied meteorological station run by the South African National Antarctic Programme, is visited annually in October. Whilst there are seldom direct transfers between the two islands, building supplies and shipping containers often move between the islands, although they are housed in Cape Town during the intervening period. Thus, it seems probable that the species was introduced on building materials or on one of the containers regularly shipped between Cape Town, Marion Island and Gough Island. Although, since 1999, the majority of the containers used to ship supplies and scientific equipment to Marion Island are washed down in Cape Town, inspected prior to the voyage, and not used interchangeably with containers destined for Gough Island, other materials, such as building supplies, are not always cleaned or inspected as rigorously.

Introduced terrestrial isopods are thought to be having a large effect on terrestrial ecosystems on Gough Island (Jones *et al.* 2002), and this will undoubtedly also come to be the case on Marion Island. As a detritivore, this large-bodied, and very successful invasive species, is likely to process considerable quantities of litter (even the small midge, *Limnophyes minimus* accounts for significant litter breakdown (Hänel & Chown 1998)). In this role, it may also eventually compete with the primary detritivores on the island, *Pringleophaga marioni* and earthworms (Smith & Steenkamp 1992). However, what exactly this will mean for ecosystem functioning and for the biota is presently not clear, especially in the context of climate change, and indirect effects of mice on invertebrates (see Smith & Steenkamp 1990, and Smith 2002 for additional discussions). In keeping both

with the precautionary principle, and with the requirements of the Prince Edward Islands Management Plan (Anonymous 1996), the most appropriate conservation strategy would be complete eradication of *P. scaber*. This recommendation has been made to the Prince Edward Island Management Committee, which oversees conservation at the islands. Eradication attempts are now underway.

#### *Aphidius species*

Individuals of an *Aphidius* species were first observed on Boulder Beach situated close to the permanently occupied Meteorological station (Fig. 1). Subsequent searches led to their discovery at Trypot Beach in the east, and Goodhope Bay at Greyheaded Albatross Ridge in the south, but they have not yet invaded beaches along Swartkop Point along the west coast of Marion Island (E. Nortje, personal communication, 2004) (Fig. 4). This wasp species is an aphid parasitoid and has been found mummifying *Rhopalosiphum padi* (itself introduced) in biotically influenced areas, such as Trypot Beach on the eastern side of Marion Island (Fig. 4). The exact mechanism of introduction, and the country of origin of the *Aphidius* species is unknown, but it is possible that it could have been introduced by humans (see Crafford & Chown 1990). It is also clear that this species is reproducing at Marion Island given the large numbers of mummified aphids discovered in 2004, many of which contained parasitoid pupae. High densities at several sites, some of which are remote from the Meteorological station, also suggest that the species is now well established.

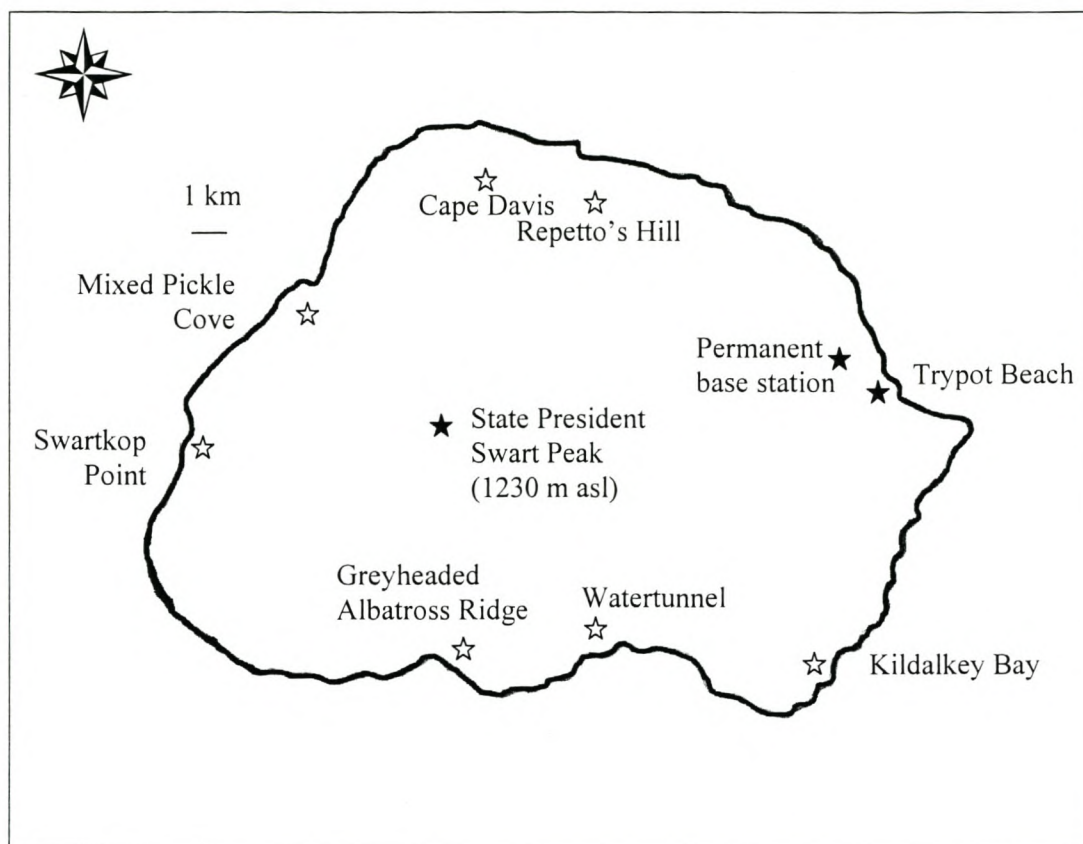


Figure 4. Marion Island. The recently discovered *Aphidius* sp. was first encountered at Boulder Beach in the east, close to the permanently occupied base station (solid symbols). The highest peak (State President Swart) rises to 1230 m asl. Field huts are indicated by blank symbols. The line in the top left corner represents 1 km.

In addition to the *Aphidius* species, another previously recorded non-indigenous moth species was found to be reproducing successfully on Marion Island. Two noctuid larvae (probably *Agrotis* sp.) were discovered halfway between the permanent base station and

Kildalkey Bay in wandering albatross nesting material (S.L. Chown, personal communication, 2004). Attempts to rear these larvae to adult stage failed, but it appears as if *Agrotis* is successfully reproducing at sites relatively far removed from the base station (S.L. Chown, personal communication, 2004). Live adult lepidopterans, belonging to the genus *Agrotis* are sporadically observed on Marion Island (personal observations), as they have been on sub-Antarctic South Georgia (*A. ipsilon*) (Convey 2005), and the species has also been recorded in the larval stage at Marion Island (Hänel *et al.* 1998).

In conclusion, these invasions highlight the need for constant vigilance for alien species irrespective of the human activity taking place on SOIs. In the context of the proposed construction of a new scientific station at Marion Island (J.A. Dreyer, personal communication 2002), which will involve much ship to shore transfer of goods and personnel, these recent introductions suggest that full-time inspection for alien species should take place during construction and for several years on completion of the new station. These introductions also illustrate the ongoing conflict between use and conservation at SOIs.

## References

- Anonymous** (1996) *Prince Edward Islands management plan*. Department of Environmental Affairs and Tourism, Pretoria
- Barendse J. & Chown S. L.** (2001) Abundance and seasonality of mid-altitude fellfield arthropods from Marion Island. *Polar Biology* **24**: 73-82
- Barnes R. D.** (1980) *Invertebrate zoology*. Holt-Saunders, Tokyo
- Beaumont L. J. & Hughes L.** (2002) Potential changes in the distributions of latitudinally restricted Australian butterfly species in response to climate change. *Global Change Biology* **8**: 954-971
- Bergstrom D. M. & Chown S. L.** (1999) Life at the front: history, ecology and change on southern ocean islands. *Trends in Ecology and Evolution* **14**: 472-477
- Bester M. N., Bloomer J. P., Bartlett P. A., Muller D. D., van Rooyen M. & Büchner H.** (2000) Final eradication of feral cats from sub-Antarctic Marion Island, southern Indian Ocean. *South African Journal of Wildlife Research* **30**: 53-57
- Blake B. J.** (1996) *Microclimate and prediction of photosynthesis at Marion Island*. M.Sc. Thesis. University of the Orange Free State
- Bloomer J. P. & Bester M. N.** (1992) Control of feral cats on sub-Antarctic Marion Island, Indian Ocean. *Biological Conservation* **60**: 211-219
- Burger A. E.** (1978) Terrestrial invertebrates: a food resource for birds at Marion Island. *South African Journal of Antarctic Research* **8**: 87-99
- Carefoot T. H.** (1993) Physiology of terrestrial isopods. *Comparative Biochemistry and Physiology A* **106**: 413-429
- Chapuis J. L., Bousses P. & Barnaud G.** (1994) Alien mammals, impact and management in the French sub-Antarctic islands. *Biological Conservation* **67**: 97-104
- Chown S. L. & Avenant N.** (1992) Status of *Plutella xylostella* at Marion Island six years after its colonization. *South African Journal of Antarctic Research* **22**: 37-40
- Chown S. L. & Crafford J. E.** (1992) Microhabitat temperatures at Marion Island. *South African Journal of Antarctic Research* **22**: 51-58
- Chown S. L. & Gaston K. J.** (2000) Island-hopping invaders hitch a ride with tourists in the southern ocean. *Nature* **408**: 637

- Chown S. L., Gremmen N. J. M. & Gaston K. J.** (1998) Ecological biogeography of southern ocean islands: species-area relationships, human impacts, and conservation. *American Naturalist* **152**: 562-575
- Chown S. L. & Language K.** (1994) Recently established Diptera and Lepidoptera on sub-Antarctic Marion Island. *African Entomology* **2**: 57-76
- Chown S. L., McGeoch M. A. & Marshall D. J.** (2002) Diversity and conservation of invertebrates on the sub-Antarctic Prince Edward Islands. *African Entomology* **10**: 67-82
- Chown S. L., Rodrigues A. S., Gremmen N. J. M. & Gaston K. J.** (2001) World Heritage status and the conservation of southern ocean islands. *Conservation Biology* **15**: 550-557
- Convey P.** (2005) Recent lepidopteran records from sub-Antarctic South Georgia. *Polar Biology* **28**: 108-110
- Cooper J. & Brown C. R.** (1990) Ornithological research at the sub-Antarctic Prince Edward Islands: a review of achievements. *South African Journal of Antarctic Research* **20**: 40-57
- Coulson S. J., Hodkinson I. D., Webb N. R., Mikkola K., Harrison J. A. & Pedgley D. E.** (2002) Aerial colonization of high Arctic islands by invertebrates: the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae) as a potential indicator species. *Diversity and Distributions* **8**: 327-334
- Crafford J. E.** (1990) The role of feral house mice in ecosystem functioning on Marion Island. *Antarctic ecosystems. Ecological change and conservation*. Ed. Kerry K. R. & Hempel G., Springer, Berlin. pp 359-364
- Crafford J. E. & Chown S. L.** (1987) *Plutella xylostella* on Marion Island. *Journal of the Entomological Society of Southern Africa* **50**: 257-260
- Crafford J. E. & Chown S. L.** (1990) The introduction and establishment of the diamondback moth (*Plutella xylostella* L., Plutellidae) on Marion Island. *Antarctic ecosystems. Ecological change and conservation*. Ed. Kerry K. R. & Hempel G. pp 354-358
- Crafford J. E. & Scholtz C. H.** (1987) Quantitative differences between the insect faunas of sub-Antarctic Marion and Prince Edward Islands: a result of human intervention? *Biological Conservation* **40**: 255-262
- Crafford J. E., Scholtz C. H. & Chown S. L.** (1986) The insects of sub-Antarctic Marion and Prince Edward Islands; with a bibliography of entomology of the Kerguelen Biogeographical Province. *South African Journal of Antarctic Research* **16**: 42-84



- Ernsting G., Block W., MacAlister H. & Todd C.** (1995) The invasion of the carnivorous carabid beetle *Trechisibus antarcticus* on South Georgia (sub-Antarctic) and its effect on the endemic herbivorous beetle *Hydromedion sparsutum*. *Oecologia* **103**: 34-42
- Ernsting G., Brandjes G. J., Block W. & Isaaks A.** (1999) Life history consequences of predation for a subantarctic beetle: evaluating the contribution of direct and indirect effects. *Journal of Animal Ecology* **68**: 741-752
- Frenot Y., Chown S. L., Whinam J., Selkirk P. M., Convey P., Skotnicki M. & Bergstrom D. M.** (2005) Biological invasions in the Antarctic: extent, impacts and implications. *Biological Reviews* **80**: 45-72
- Frenot Y., Gloaguen J. C., Massé L. & Lebouvier M.** (2001) Human activities, ecosystem disturbance and plant invasions in subantarctic Crozet, Kerguelen and Amsterdam Islands. *Biological Conservation* **101**: 33-50
- Frenot Y., Gloaguen J.-C. & Trehen P.** (1997) Climate change in Kerguelen Islands and colonization of recently deglaciated areas by *Poa kerguelensis* and *P. annua*. *Antarctic communities: species, structure and survival*. Ed. Battaglia B., Valencia J. & Walton D. W. H., Cambridge University Press, Cambridge. pp 358-366
- Gabriel A. G. A., Chown S. L., Barendse J., Marshall D. J., Mercer R. D., Pugh P. J. A. & Smith V. R.** (2001) Biological invasions of southern ocean islands: the Collembola of Marion Island as a test of generalities. *Ecography* **24**: 421-430
- Gleeson J. P. & van Rensburg P. J. J.** (1982) Feeding ecology of the house mouse, *Mus musculus* on Marion Island. *South African Journal of Antarctic Research* **12**: 34-39
- Greenslade P.** (2002) Assessing the risk of exotic Collembola invading subantarctic islands: prioritising quarantine management. *Pedobiologia* **46**: 338-344
- Gremmen N. J. M.** (1981) *The vegetation of the subantarctic islands Marion and Prince Edward*. Dr W. Junk Publishers, London
- Gremmen N. J. M.** (1997) Changes in the vegetation of sub-Antarctic Marion Island resulting from introduced vascular plants. *Antarctic communities: species, structure and survival*. Ed. Battaglia B., Valencia J. & Walton D. W. H., Cambridge University Press, Cambridge. 2003: pp 417-423
- Gremmen N. J. M. & Smith V. R.** (1999) New records of alien vascular plants from Marion and Prince Edward Islands, Sub-Antarctic. *Polar Biology* **21**: 401-409
- Grobbelaar J. U.** (1974) *A contribution to the limnology of the sub-Antarctic Island Marion*. D.Sc. Thesis. University of the Free State

- Hänel C. & Chown S. L.** (1998) The impact of a small, alien macro-invertebrate on a sub-Antarctic terrestrial ecosystem: *Limnophyes minimus* Meigen (Diptera, Chironomidae) at Marion Island. *Polar Biology* **20**: 99-106
- Hänel C. & Chown S. L.** (1999) *An introductory guide to the Marion and Prince Edward Island special nature reserves. Fifty years after annexation.* Department of Environmental Affairs & Tourism, Pretoria
- Hänel C., Chown S. L. & Davies L.** (1998) Records of alien insect species from sub-Antarctic Marion and South Georgia Islands. *African Entomology* **6**: 366-369
- Holdgate M. W.** (1965) Part III. The fauna of the Tristan da Cunha Islands. *Philosophical Transactions of the Royal Society of London B* **249**: 361-402
- Hopkin S. P.** (1997) *Biology of the springtails: Insecta: Collembola.* Oxford University Press, Oxford
- Huysen O., Ryan P. G. & Cooper J.** (2000) Changes in population size, habitat use and breeding biology of lesser sheathbills (*Chionis minor*) at Marion Island: impacts of cats, mice and climate change? *Biological Conservation* **92**: 299-310
- IPCC** (2001) *Climate change 2001: synthesis report. A contribution of working groups I, II, and III to the third assessment report of the intergovernmental panel on climate change.* Cambridge University Press, Cambridge
- Jones A. G., Chown S. L. & Gaston K. J.** (2002) Terrestrial invertebrates of Gough Island: an assemblage under threat? *African Entomology* **10**: 81-91
- Jones A. G., Chown S. L. & Gaston K. J.** (2003) Introduced house mice as a conservation concern on Gough Island. *Biodiversity and Conservation* **12**: 2107-2119
- Karban R. & Strauss S. Y.** (2004) Physiological tolerance, climate change, and a northward range shift in the spittlebug, *Philaenus spumarius*. *Ecological Entomology* **29**: 251-254
- Kennedy A. D.** (1995) Antarctic terrestrial ecosystem response to global environmental change. *Annual Review of Ecology and Systematics* **26**: 683-704
- Kritikos D. J., Sutherst R. W., Brown J. R., Adkins S. W. & Maywald G. W.** (2003) Climate change and the potential distribution of an invasive alien plant: *Acacia nilotica* ssp. *indica* in Australia. *Journal of Applied Ecology* **40**: 111-124
- Lavy D., Nedved O. & Verhoef H. A.** (1997) Effects of starvation on body composition and cold tolerance in the collembolan *Orchesella cincta* and the isopod *Porcellio scaber*. *Journal of Insect Physiology* **43**: 973-978
- Lawrence R. F.** (1953) *The biology of the cryptic fauna of forests with special reference to the indigenous forests of South Africa.* A.A. Balkema, Cape Town

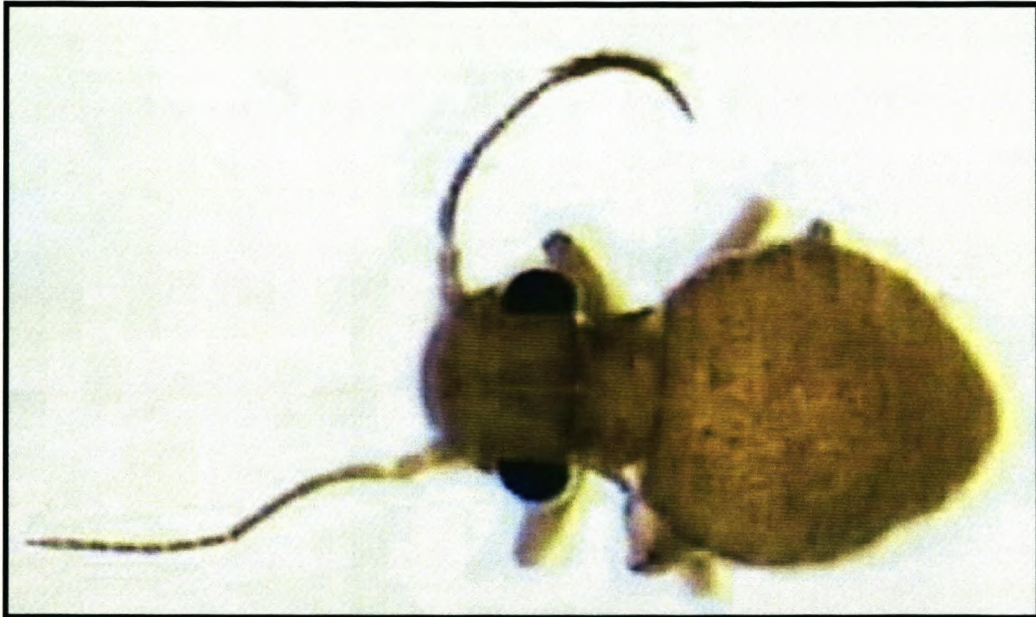
- Le Roux V., Chapuis J. L., Frenot Y. & Vernon P.** (2002) Diet of the house mouse (*Mus musculus*) on Guillou Island, Kergeulen archipelago, Subantarctic. *Polar Biology* **25**: 49-57
- McDougall I.** (1971) Geochronology. *Marion and Prince Edward Islands: Report on the South African Biological and Geological Expedition / 1965-1966*. Ed. Van Zinderen Bakker Sr E. M., Winterbottom J. M. & Dyer R. A., Balkema, Cape Town. pp 72-77
- Pakhomov E. A. & Chown S. L.** (2003) The Prince Edward Islands: southern ocean oasis. *Ocean Yearbook* **17**: 348-379
- Parmesan C., Ryrholm N., Stefanescu C., Hill J. K., Thomas C. D., Descimon H., Huntley B., Kaila L., Kullberg J., Tammaru T., Tennent W. J., Thomas J. A. & Warren M.** (1999) Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* **399**: 579-583
- Pimentel D.** (2002) *Economic and environmental threats of alien plant, animal, and microbe invasions. Biological invasions*. CRC Press LCC, Boca Raton
- Prinzing A., Durka W., Klotz S. & Brandl R.** (2002) Which species become aliens? *Evolutionary Ecology Research* **4**: 385-405
- Richardson A. M. M. & Jackson J. E.** (1995) The first record of a terrestrial landhopper (Crustacea, Amphipoda, Tallitridae) from Macquarie Island. *Polar Biology* **15**: 419-422
- Rowe-Rowe D. T., Green B. & Crafford J. E.** (1989) Estimated impact of feral house mice on sub-Antarctic invertebrates at Marion Island. *Polar Biology* **9**: 457-460
- Schulze B. R.** (1971) The climate of Marion Island. *Marion and Prince Edward Islands: Report on the South African Biological and Geological Expedition / 1965-1966*. Ed. Van Zinderen Bakker Sr E. M., Winterbottom J. M. & Dyer R. A., Balkema, Cape Town. pp 16-31
- Slabber S. & Chown S. L.** (2002) The first record of a terrestrial crustacean, *Porcellio scaber* (Isopoda, Porcellionidae), from sub-Antarctic Marion Island. *Polar Biology* **25**: 855-858
- Smith V. R.** (1978) Animal-plant-soil nutrient relationships on Marion Island (Subantarctic). *Oecologia* **32**: 239-253
- Smith V. R.** (1987) The environment and biota of Marion Island. *South African Journal of Science* **83**: 211-220
- Smith V. R.** (1992a) Surface air temperatures at Marion Island, sub-Antarctic. *South African Journal of Science* **88**: 575-578

- Smith V. R.** (1992b) Terrestrial slug recorded from Marion Island. *Journal of Molluscan Studies* **58**: 50-51
- Smith V. R.** (2002) Climate change in the sub-Antarctic: An illustration from Marion Island. *Climatic Change* **52**: 345-357
- Smith V. R., Avenant N. L. & Chown S. L.** (2002) The diet of house mice on a sub-Antarctic island. *Polar Biology* **25**: 703-715
- Smith V. R. & Lewis Smith R. I.** (1987) The biota and conservation status of sub-Antarctic islands. *Environment International* **13**: 95-104
- Smith V. R. & Steenkamp M.** (1990) Climatic change and its ecological implications at a sub-Antarctic island. *Oecologia* **85**: 14-24
- Smith V. R. & Steenkamp M.** (1992) Soil macrofauna and nitrogen on a sub-Antarctic island. *Oecologia* **92**: 201-206
- Talekar N. S. & Shelton A. M.** (1993) Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology* **38**: 275-301
- Tanaka K. & Udagawa T.** (1993) Cold adaptation of the terrestrial isopod, *Porcellio scaber*, to subnivean environments. *Journal of Comparative Physiology B* **163**: 439-444
- Van Aarde R. J.** (1979) Distribution and density of the feral house cat *Felis catus* on Marion Island. *South African Journal of Antarctic Research* **9**: 14-19
- Van Aarde R. J.** (1980) The diet and feeding behaviour of feral cats, *Felis catus* at Marion Island. *South African Journal of Antarctic Research* **10**: 123-128
- Vitousek P. M., D'Antonio C. M., Loope L. L., Rejmánek M. & Westbrooks R.** (1997) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology* **21**: 1-16
- Walther G.-R., Post E., Convey P., Menzel A., Parmesan C., Beebee T. J. C., Fromentin J. M., Hoegh-Guldberg O. & Bairlein F.** (2002) Ecological responses to recent climate change. *Nature* **416**: 389-395
- Wang M. & Schreiber A.** (1999) Population genetics of the woodlouse *Porcellio scaber* Latr. (Isopoda: Oniscoidea) in central Europe: passive dispersal and postglacial range expansion. *Canadian Journal of Zoology* **77**: 1337-1347
- Watkins B. P. & Cooper J.** (1986) Introduction, present status and control of alien species at the Prince Edward Islands, sub-Antarctic. *South African Journal of Antarctic Research* **16**: 86-94

## Chapter 3<sup>1</sup>

### Thermal tolerance and cold hardiness strategy of the sub-Antarctic psocid *Antarctopsocus jeanneli* Badonnel

*Happy is he who gets to know the reasons for things.*  
- Virgil -



*Antarctopsocus jeanneli*

---

<sup>1</sup> Published as: **Slabber S. & Chown S.L.** (2004) Thermal tolerance and cold hardiness strategy of the sub-Antarctic psocid *Antarctopsocus jeanneli* Badonnel. *Polar Biology* **28**: 56-61

## Introduction

Patterns of variation in the thermal tolerances of invertebrates, over both small and large temporal scales, have seen growing interest over the last decade. In part, this is a consequence of the growing realization that changing climates are likely to have significant effects on insects (Parmesan *et al.* 1999; Bale *et al.* 2002). To understand these effects not only requires insight into the interactions between insect populations and their environments (Cannon 1998), but also how these are likely to vary through space and time (Gilchrist *et al.* 1997; Chown 2001; David *et al.* 2003), and how they are likely to evolve (Feder 1999; Pörtner 2001; Hoffmann *et al.* 2003). In consequence, an increasingly wide array of techniques is being used to examine spatial and temporal variation in the thermal tolerances of invertebrates. These range from cellular level molecular tools (Feder & Mitchell-Olds 2003) to broad scale comparative analyses (Chown & Nicolson 2004).

Comparative analyses of the thermal tolerances of insects have revealed several key patterns that seem to be consistent across multiple scales: Upper thermal tolerances vary much less through space, and as a consequence of acclimation, than do lower thermal tolerances (Chown 2001; Klok & Chown 2003); lower thermal tolerances show considerable variation, but minimum levels correspond closely with likely environmental temperatures (Addo-Bediako *et al.* 2000; but see Hodkinson 2003); cold hardiness strategies appear to show consistent variation associated with environmental conditions and with phylogeny (Zachariassen 1985; Sinclair *et al.* 2003a). In the latter case, it has been argued that freeze intolerant insects tend to occur in environments which show predictable

cold winter spells that last for a full season, such as those characteristic of the continental northern hemisphere, whilst moderately freeze tolerant insects are more common in the southern hemisphere. Here, environmental conditions tend to cross the freezing point unpredictably, so making a strategy of moderate freeze tolerance least costly from an evolutionary perspective (Sinclair *et al.* 2003a). Such a strategy of moderate freeze-tolerance has also been found in other environments where temperatures regularly vary across the 0 °C threshold (Sømme & Zachariassen 1981; Zachariassen 1985; Sinclair *et al.* 2003b).

Recent work has also demonstrated that cold hardiness strategies are not randomly distributed across higher taxa in the invertebrates or arthropods. Whilst it has long been appreciated that all springtails and mites are freeze intolerant (Sømme & Block 1982; Cannon & Block 1988; Block 1990), it has also recently been shown that virtually all molluscs are freeze-tolerant (Ansart & Vernon 2003), and that the distribution of cold tolerance strategies across the higher taxa of the arthropods and insects is non-random (Sinclair *et al.* 2003a). Within the insects it is clear that several orders include both freeze-tolerant and freeze-intolerant species (Diptera, Coleoptera), and some species that appear to have a mixed strategy or one that can be altered between years (Kukal & Duman 1989; Bale *et al.* 2001), others have individuals that are only freeze-intolerant (Hemiptera), and others (Blattaria, Orthoptera) include species that are only freeze-tolerant.

Although these generalities regarding the geographic and taxonomic distribution of cold hardiness strategies appear to be reasonably well supported, both of the investigations that

have sought to elucidate these patterns have shown that there is a paucity of data for some taxonomic groups, and for the southern hemisphere (Addo-Bediako *et al.* 2000; Sinclair *et al.* 2003a). Indeed, this is a recurrent theme in the macrophysiological literature on insects (Chown *et al.* 2002a). Here we address a significant gap in the knowledge of cold hardiness strategies of insects namely the absence of information on adult Psocoptera (Sinclair *et al.* 2003a), by providing information on *Antarctopsocus jeanneli* Badonnel from sub-Antarctic Marion Island. In doing so, we also contribute both lower and upper thermal tolerance data for another species from the southern hemisphere, which has been the subject of comparatively few investigations (see global coverage maps in Chown *et al.* 2002a).

## Materials and methods

This work was undertaken on the east coast of Marion Island (46°54'S, 37°45'E) (see Smith 1987; Hänel & Chown 1999; Chown *et al.* 2002b for a summary of the environment and biota of the island). The island has an extremely oceanic climate with little fluctuation in daily and seasonal temperatures (mean annual temperature is c. 5.7 °C, but increasing - Smith 2002). Microclimate (just below soil surface) temperatures vary considerably with altitude (Fig. 1), and regularly decline below 0 °C (Chown & Crafford 1992; J. Deere & S.L. Chown unpublished data). The Psocoptera is represented on Marion Island by a single species, *Antarctopsocus jeanneli* Badonnel, which also occurs on Ile aux Cochons in the the Crozet Archipelago (Crafford *et al.* 1986). A second species in the genus, *A. daviesi* Badonnel, is known from Ile de la Possession and Ile de l'Est, also in the Crozet Archipelago (Dreux 1971). These species differ substantially in size and in several other



morphological features. On Marion Island, *A. jeanneli* is locally abundant and occurs from the supralittoral zone to about 300 m elevation (Crafford *et al.* 1986). Individuals are found on rock faces covered with crustose lichens, and at higher elevations, under boulders or freeze-shattered rocks. Adults and nymphs co-occur, and are thought to feed on algae, lichens, fungi and fungal spores (Crafford *et al.* 1986).

For the present study, adult *A. jeanneli* (mean mass  $\pm$  SE = 435  $\pm$  67  $\mu$ g, n = 5) were collected with an aspirator from areas below 25 m above sea level (asl) close to the Scientific Station. Field collected specimens were taken to the laboratory within an hour of collection. In the laboratory, the animals were either used in experiments within 48 h (field-fresh), or were kept in temperature-controlled incubators (0 and 5 °C with a 9L:15D photoperiod, and 15 °C with a 14L:10D photoperiod). Animals were maintained in plastic containers on a substrate of moist Plaster of Paris and supplied with pieces of the crustose lichens with which they were associated in the field. The acclimation treatments lasted for seven days (see Klok & Chown 2003 for rationale).

Supercooling points (SCP, crystallization temperatures) of 20 individual *A. jeanneli*, for each treatment, were determined by lightly securing an individual against a thermocouple (Type T copper-constantan 40-gauge) inside a pipette tip. Each pipette tip was placed in an air-filled vacutest container, and the latter submerged in a Grant LTC 12 water bath: at 0 °C for those animals acclimated at 0 °C; at 5 °C for field fresh animals and those acclimated at 5, and 15 °C. After a 15-min equilibration period at 0 °C for all treatments, water bath temperature was lowered by 0.1 °C.min<sup>-1</sup> (Van der Merwe *et al.* 1997). Thermocouples

were connected to a Campbell Scientific CR10 data logger that logged temperatures every second and calculated a mean every 10 s. The lowest temperature reached before the onset of a freezing exotherm was taken as the SCP. Treatment effects on SCPs were investigated using a generalized linear model because of departures from normality (Quinn & Keough 2002), such as those commonly found for freezing point distributions (Rothery & Block 1992). Because the frequency distributions were bimodal, data were also divided into high and low groups based on the distributions. These groups were separately compared between treatments (GLZ), and the distribution of individuals in the high and low SCP groups was expressed as  $R = LG/(HG+LG)$ , where  $R$  is a ratio and LG and HG are the number of individuals in each group, respectively (Block 1982).

Upper and lower lethal temperatures were also determined using static methods (Chown & Nicolson 2004). Batches of ten animals were placed, together with a standardized piece of dry (or wet, upper) filter paper, in 8 x 1 cm cylindrical airtight plastic screw top vials, which were submerged in a Grant LTD 20 water bath at a set temperature for 1 h. An identical airtight plastic vial containing a 40-gauge copper-constantan thermocouple connected to a handheld thermometer (CHY 507 Thermometer, Taiwan) was submerged with these vials to monitor temperature. Vials were removed from the water bath and transferred to an incubator after 1 h (at the original acclimation temperature, except for field fresh batches that were transferred to a 10 °C incubator). Pieces of dry filter paper in the vials of LLT experiments were replaced with wet filter paper for the 24 h recovery period. Survival of individuals was scored after 24 h. Individuals that were active were classified as alive, and those showing limited or no activity were classified as dead. The

procedure was repeated for a new batch of animals following a 1 °C change in the water bath temperature. These trials were continued until the temperature range encompassed less than 5 % to more than 95 % survival at either end of the temperature spectrum. The entire procedure was replicated 5 times at each experimental temperature for field fresh organisms, and those acclimated at 0 °C and 5 °C. Logistic regression was used to model the mortality data and to calculate LT50 values (only for data for which a significant fit was obtained using the Wald statistic). The LT100 was calculated as the temperature at which more than 90 % of a specific sample died. LT50s and LT100s for the different treatments were compared using analyses of variance.

## **Results and discussion**

Field collected *A. jeanneli* had a mean supercooling point of  $-11.1$  °C (Table 1) and none of the individuals survived freezing in these experiments. The lower lethal temperature experiments (Table 2) also indicated that no individuals survived the lowest SCP recorded for an individual, and suggested that there might be some pre-freeze mortality (LT50 =  $-7.7$  °C). However, the strongly bimodal SCP frequency distributions, and the mean SCP values for the high group individuals, suggest that pre-freeze mortality was probably limited. These data therefore suggest that *A. jeanneli* is freeze intolerant and, more specifically, moderately chill tolerant (Bale 1993). This moderate level of chill tolerance is in keeping with that found for several other species on Marion Island, such as the moderate chill tolerance of adult kelp flies, *Paractora dreuxi* (Klok & Chown 2001), and the moderately chill tolerant weevil, *Palirhoeus eatoni* (Van der Merwe *et al.* 1997). However, it differs

substantially from the more pronounced chill tolerance of the larvae of the moth *Embryonopsis halticella* (Klok & Chown 1998) and the moderate freeze tolerance of several of the other species on the island (Klok & Chown 1997; Van der Merwe *et al.* 1997). Because *A. jeanneli* is freeze intolerant it is unlike the majority of the insects investigated to date in the southern hemisphere (e.g. Sinclair *et al.* 2003a). Nonetheless, this moderate chill tolerance is in keeping with the microclimate temperatures *A. jeanneli* is likely to encounter (Fig. 1 and Chown & Crafford 1992), and may also reflect the fact that it tends to avoid wet environments (preferring dry rock faces and overhangs). It has been suggested that freeze intolerance is a likely strategy where external inoculation is uncommon or can be avoided (Duman *et al.* 1991; Klok & Chown 1997; Sinclair *et al.* 2003a).

Table 1. Summary statistics of mean supercooling points ( $^{\circ}\text{C} \pm \text{SE}$ ) of field fresh *Antarctopsocus jeanneli* and those acclimated at 0, 5 and 15  $^{\circ}\text{C}$ . A summary of all data combined and low group and high group data are given. Differences between groups as indicated by different letters are based on 95 % confidence intervals.

		<i>n</i>	Mean $\pm$ SE	Median	Minimum	Maximum	Range
<b>All</b>							
Field fresh	ab	20	-11.1 $\pm$ 1.45	-9.1	-22.9	-4.6	18.3
0 $^{\circ}\text{C}$	a	20	-15.8 $\pm$ 1.52	-17.8	-24.0	-4.8	19.2
5 $^{\circ}\text{C}$	b	20	-6.7 $\pm$ 1.50	-3.4	-24.6	-3.0	21.6
15 $^{\circ}\text{C}$	b	20	-7.3 $\pm$ 0.80	-6.6	-16.5	-3.4	13.1
Wald $X^2_{(3)} = 30.195$ , $p < 0.0001$							
<b>Low group</b>							
Field fresh	a	7	-19.0 $\pm$ 1.01	-18.1	-22.9	-16.1	6.8
0 $^{\circ}\text{C}$	a	11	-21.4 $\pm$ 0.65	-21.1	-24.0	-17.8	6.2
5 $^{\circ}\text{C}$	a	3	-21.7 $\pm$ 2.36	-23.4	-24.6	-17.0	7.6
Wald $X^2_{(2)} = 4.844$ , $p > 0.05$							
<b>High group</b>							
Field fresh	a	13	-6.8 $\pm$ 0.70	-5.5	-12.0	-4.6	7.4
0 $^{\circ}\text{C}$	a	9	-9.0 $\pm$ 1.06	-8.5	-13.8	-4.8	9.0
5 $^{\circ}\text{C}$	b	17	-4.1 $\pm$ 0.39	-3.5	-9.7	-3.0	6.7
15 $^{\circ}\text{C}$	a	19	-6.8 $\pm$ 0.68	-5.1	-12.2	-3.4	8.8
Wald $X^2_{(3)} = 25.032$ , $p < 0.0001$							

Table 2. Summary statistics for upper and lower lethal limits ( $^{\circ}\text{C} \pm \text{SE}$ ) for field fresh *Antarctopsocus jeanneli* and lower lethal limits for those acclimated at  $0^{\circ}\text{C}$ .

<b>Treatment</b>	<b>LLT LT50 <math>\pm</math> SE</b>	<b>LLT LT100</b>
Field fresh	-7.7 $\pm$ 0.12	-12
$0^{\circ}\text{C}$	-13 $\pm$ 0.11	-15
	$F_{(1,8)} = 330.31, p < 0.0001$	$F_{(1,8)} = 256.00, p < 0.0001$
	<b>ULT LT50 <math>\pm</math> SE</b>	<b>ULT LT100</b>
Field fresh	29.5 $\pm$ 0.21	31.6

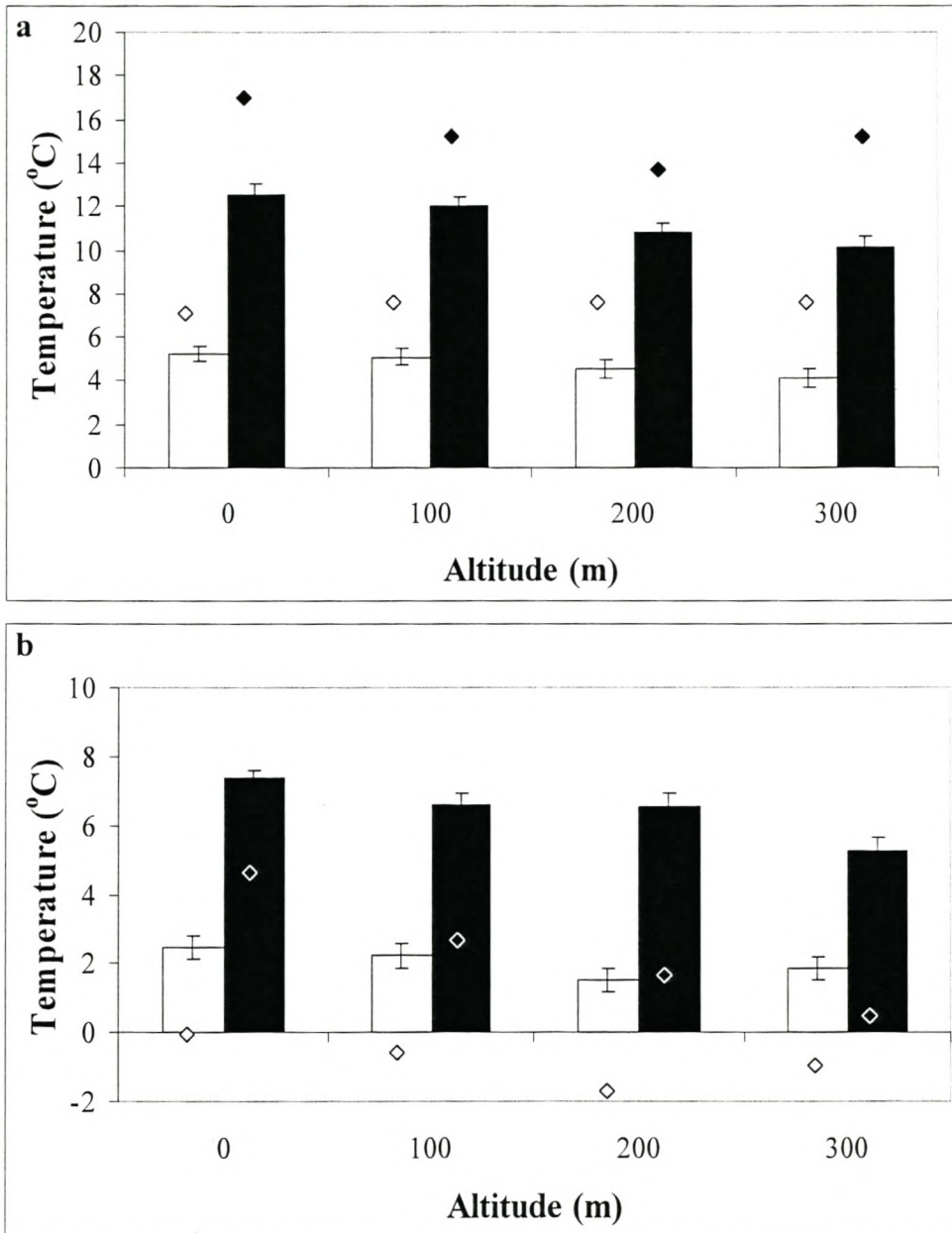


Figure 1. Mean maximum (a) and mean minimum (b) daily temperatures for July (clear bar) and February (shaded bar) 2003 across an eastern altitudinal gradient on Marion Island. Absolute minimum and maximum temperatures are shown for July (clear diamonds) and February (shaded diamonds).

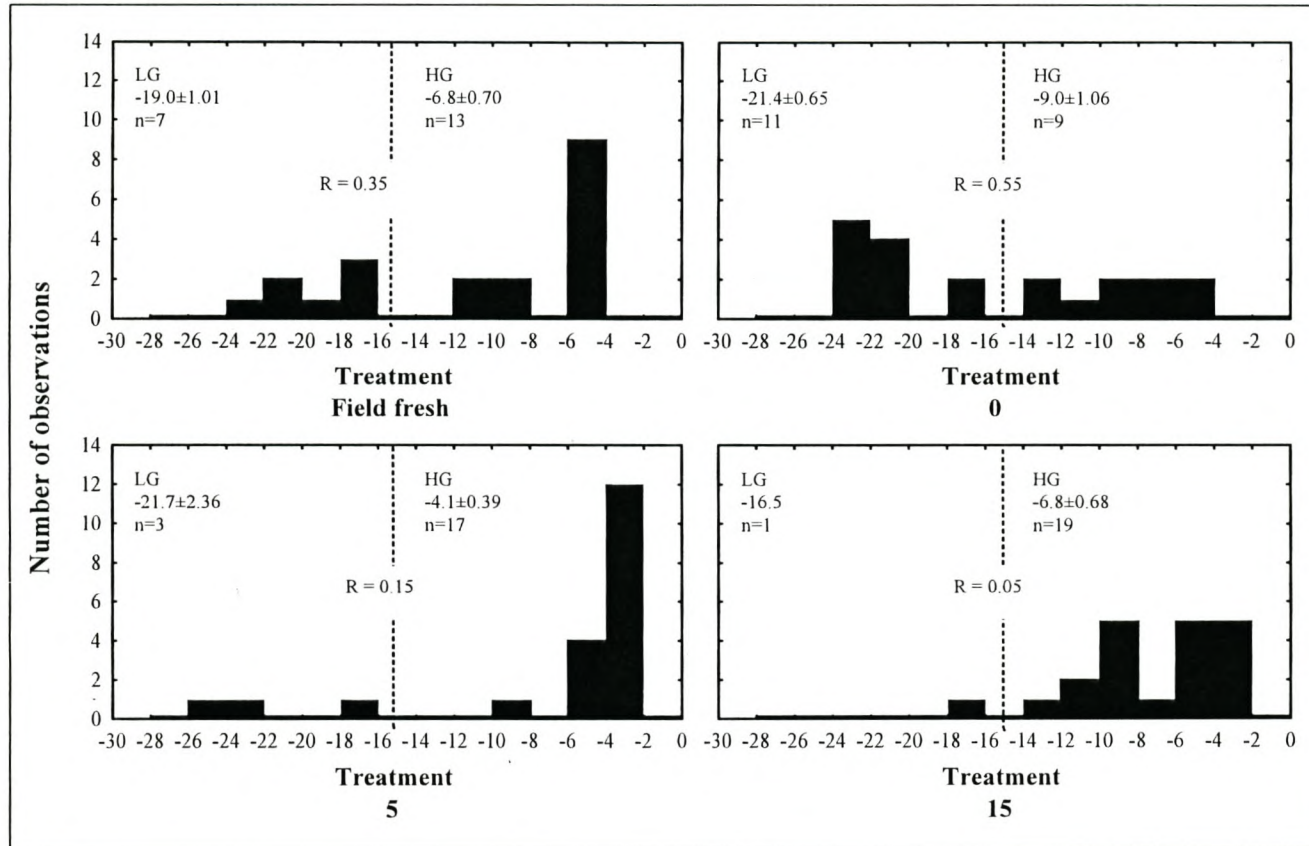


Figure 2. Frequency distributions of supercooling points for field fresh *Antarctopsocus jeanneli* and those acclimated at 0, 5 and 15 °C with the mean  $\pm$  SE and sample size ( $n$ ) for the high group (HG) and low group (LG) values and the relationship between these ( $R = \text{LG}/(\text{LG} + \text{HG})$ ) indicated.



Despite showing only moderate chill tolerance, *A. jeanneli* was clearly capable of mounting a strong response to acclimation. A decrease in treatment temperature was accompanied by a pronounced decrease in the SCP, and this was particularly marked in the transition between 0 and 5 °C, although there was little change between 5 and 15 °C (Table 1). A pronounced change in thermal tolerance with the transition between 0 and 5 °C has been found in other insects on Marion Island and elsewhere in the sub-Antarctic (Klok & Chown 1998; 2003). This transition undoubtedly marks a biologically significant one for these insects, particularly given changes in summer microclimate temperatures from approximately 15 °C to those in winter of approximately 0 °C (Fig. 1), and a similar response in the lower lethal temperatures to a 0 °C treatment (limited numbers of individuals precluded completion of trials for 5 and 15 °C).

The dynamics of the SCP response to the treatment temperatures involved a change in the proportion of individuals in the high and low groups (Fig. 2), but little difference in the SCPs of individuals within these groups (Table 1). Such a response is not uncommon either in insects (Klok & Chown 1998) or in springtails (Sømme & Block 1982; Sinclair *et al.* 2003c), irrespective of whether it takes place over a time scale of months, weeks or days. Indeed, it might represent a bet-hedging strategy that is common to many freeze intolerant species, and illustrates the flexibility that is being increasingly recognized as a major feature of insect responses to low temperature (Lee *et al.* 1987; Bale *et al.* 2000; Bale *et al.* 2001; Kelty & Lee 2001; Worland & Convey 2001). Alternatively, the change in the proportion of individuals in each group might be a consequence of an absence of feeding

and therefore nucleators in the gut. However, this idea remains controversial (Baust & Rojas 1985; Cannon & Block 1988).

The upper lethal temperatures of *A. jeanneli* (Table 2) are similar to those of a range of other species on Marion Island, but, not unexpectedly, are lower than the dynamic, CTmax values recorded for many of the species. For example, the CTmax values of *Embryonopsis halticella*, *Pringleophaga marioni* and *Paractora dreuxi* were 39.7, 38.7 and 35.5 °C, respectively (Klok & Chown 1997; 1998; 2001). Likewise, the ULT LT50 of six weevil species found on the island varied between 31 and 34 °C (Van der Merwe *et al.* 1997). By contrast, the LT50 and LT100 values for upper thermal tolerance found for *A. jeanneli* are substantially lower than those found for the adults of three stored-product *Liposcelis* pest booklice species. Beckett & Morton (2003) found that the least thermally tolerant of these species, *L. bostrychophila*, had a LT90 of 6.7 h at 45 °C, whilst the hardiest one, *L. paeta*, had a LT90 of 95.8 h at 45 °C. These values are substantially higher than the approximately 29 °C that *A. jeanneli* could survive with 50 % mortality. Nonetheless, this variation between species is within the range documented by Addo-Bediako *et al.* (2000) for insects across the globe.

In conclusion, this study has demonstrated that *A. jeanneli* is freeze intolerant, with a strategy in keeping with what might be expected from microclimates at Marion Island. Indeed, its thermal tolerance range is quite predictable from the range of temperatures encountered at low altitudes on the island. Whether such freeze intolerance is characteristic of all Psocoptera cannot be determined at present. However, the tendency of these small

insects to prefer dry areas and to survive them by making use of atmospheric water uptake (Rudolph 1982) suggests that this might well be the case.

## References

- Addo-Bediako A., Chown S. L. & Gaston K. J.** (2000) Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B* **267**: 739-745
- Ansart A. & Vernon P.** (2003) Cold hardiness in molluscs. *Acta Oecologica* **24**: 95-102
- Bale J. S.** (1993) Classes of insect cold hardiness. *Functional Ecology* **7**: 751-753
- Bale J. S., Block W. & Worland M. R.** (2000) Thermal tolerance and acclimation response of larvae of the sub-Antarctic beetle *Hydromedion sparsutum* (Coleoptera: Perimylopidae). *Polar Biology* **23**: 77-84
- Bale J. S., Masters G. J., Hodkinson I. D., Awmack C., Bezemer T. M., Brown V. K., Butterfield J., Buse A., Coulson J. C., Farrar J., Good J. E. G., Harrington R., Hartley S., Jones T. H., Lindroth R. L., Press M. C., Symrnioudis I., Watt A. D. & Whittaker J. B.** (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology* **8**: 1-16
- Bale J. S., Worland M. R. & Block W.** (2001) Effects of summer frost exposures on the cold tolerance strategy of a sub-Antarctic beetle. *Journal of Insect Physiology* **47**: 1161-1167
- Baust J. G. & Rojas R. R.** (1985) Insect cold hardiness: Facts and fancy. *Journal of Insect Physiology* **31**: 755-759
- Beckett S. J. & Morton R.** (2003) The mortality of three species of Psocoptera, *Liposcelis bostrychophila* Badonnel, *Liposcelis decolor* Pearman and *Liposcelis paeta* Pearman, at moderately elevated temperatures. *Journal of Stored Product Research* **39**: 103-115
- Block W.** (1982) Supercooling points of insects and mites on the Antarctic Peninsula. *Ecological Entomology* **7**: 1-8
- Block W.** (1990) Cold tolerance of insects and other arthropods. *Philosophical Transactions of the Royal Society of London B* **326**: 613-633
- Cannon R. J. C.** (1998) The implications of predicted climate change for insect pests in the UK, with emphasis on non-indigenous species. *Global Change Biology* **4**: 785-796
- Cannon R. J. C. & Block W.** (1988) Cold tolerance of microarthropods. *Biological Reviews* **63**: 23-77
- Chown S. L.** (2001) Physiological variation in insects: hierarchical levels and implications. *Journal of Insect Physiology* **47**: 649-660

- Chown S. L., Addo-Bediako A. & Gaston K. J.** (2002a) Physiological variation in insects: large-scale patterns and their implications. *Comparative Biochemistry and Physiology B* **131**: 587-602
- Chown S. L. & Crafford J. E.** (1992) Microhabitat temperatures at Marion Island. *South African Journal of Antarctic Research* **22**: 51-58
- Chown S. L., McGeoch M. A. & Marshall D. J.** (2002b) Diversity and conservation of invertebrates on the sub-Antarctic Prince Edward Islands. *African Entomology* **10**: 67-82
- Chown S. L. & Nicolson S. W.** (2004) *Insect physiological ecology. Mechanisms and patterns*. Oxford University Press, Oxford
- Crafford J. E., Scholtz C. H. & Chown S. L.** (1986) The insects of sub-Antarctic Marion and Prince Edward Islands; with a bibliography of entomology of the Kerguelen Biogeographical Province. *South African Journal of Antarctic Research* **16**: 42-84
- David J. R., Gibert P., Moreteau B., Gilchrist G. W. & Huey R. B.** (2003) The fly that came in from the cold: geographic variation of recovery time from low-temperature exposure in *Drosophila subobscura*. *Functional Ecology* **17**: 425-430
- Dreux P.** (1971) Premières données sur la faune entomologique de l'île de l'est (Archipel Crozet). *Terres Australes Antarctiques Françaises* **55-56**: 45-54
- Duman J. G., Wu D. W., Xu L., Tursman D. & Olsen T. M.** (1991) Adaptations of insects to subzero temperatures. *Quarterly Review of Biology* **66**: 387-410
- Feder M. E.** (1999) Engineering candidate genes in studies of adaptation: the heat-shock protein Hsp70 in *Drosophila melanogaster*. *American Naturalist Supplement* **154**: s55-s66
- Feder M. E. & Mitchell-Olds T.** (2003) Evolutionary and ecological functional genomics. *Nature Reviews* **4**: 649-655
- Gilchrist G. W., Huey R. B. & Partridge L.** (1997) Thermal sensitivity of *Drosophila melanogaster*: evolutionary responses of adults and eggs to laboratory natural selection at different temperatures. *Physiological Zoology* **70**: 403-414
- Hänel C. & Chown S. L.** (1999) *An introductory guide to the Marion and Prince Edward Island Special Nature Reserves. Fifty years after annexation*. Department of Environmental Affairs & Tourism, Pretoria
- Hodkinson I. D.** (2003) Metabolic cold adaptation in arthropods: a smaller-scale perspective. *Functional Ecology* **17**: 562-567
- Hoffmann A. A., Hallas R. J., Dean J. A. & Schiffer M.** (2003) Low potential for climatic stress adaptation in a rainforest *Drosophila* species. *Science* **301**: 100-102

- Kelty J. D. & Lee R. E.** (2001) Rapid cold-hardening of *Drosophila melanogaster* (Diptera : Drosophilidae) during ecologically based thermoperiodic cycles. *Journal of Experimental Biology* **204**: 1659-1666
- Klok C. J. & Chown S. L.** (1997) Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic caterpillar, *Pringleophaga marioni* Viette (Lepidoptera: Tineidae). *Journal of Insect Physiology* **43**: 685-694
- Klok C. J. & Chown S. L.** (1998) Interactions between desiccation resistance, host-plant contact and the thermal biology of a leaf-dwelling sub-Antarctic caterpillar, *Embryonopsis halticella* (Lepidoptera: Yponomeutidae). *Journal of Insect Physiology* **44**: 615-628
- Klok C. J. & Chown S. L.** (2001) Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic kelp fly, *Paractora dreuxi* (Diptera: Helcomyzidae). *Journal of Insect Physiology* **47**: 95-109
- Klok C. J. & Chown S. L.** (2003) Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. *Biological Journal of the Linnean Society* **78**: 401-414
- Kukal O. & Duman J. G.** (1989) Switch in the overwintering strategy of two insect species and latitudinal differences in cold hardiness. *Canadian Journal of Zoology* **67**: 825-827
- Lee R. E., Jr., Chen C.-P. & Denlinger D. L.** (1987) A rapid cold-hardening process in insects. *Science* **238**: 1415-1417
- Parmesan C., Ryrholm N., Stefanescu C., Hill J. K., Thomas C. D., Descimon H., Huntley B., Kaila L., Kullberg J., Tammaru T., Tennent W. J., Thomas J. A. & Warren M.** (1999) Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* **399**: 579-583
- Pörtner H. O.** (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**: 137-146
- Quinn G. P. & Keough M. J.** (2002) *Experimental design and data analysis for ecologists*. Cambridge University Press, Cambridge
- Rothery P. & Block W.** (1992) Characterizing supercooling point distributions. *Cryoletters* **13**: 193-198
- Rudolph D.** (1982) Site, process and mechanism of active uptake of water-vapor from the atmosphere in the Psocoptera. *Journal of Insect Physiology* **28**: 205-212
- Sinclair B. J., Addo-Bediako A. & Chown S. L.** (2003a) Climatic variability and the evolution of insect freeze tolerance. *Biological Reviews* **78**: 181-195

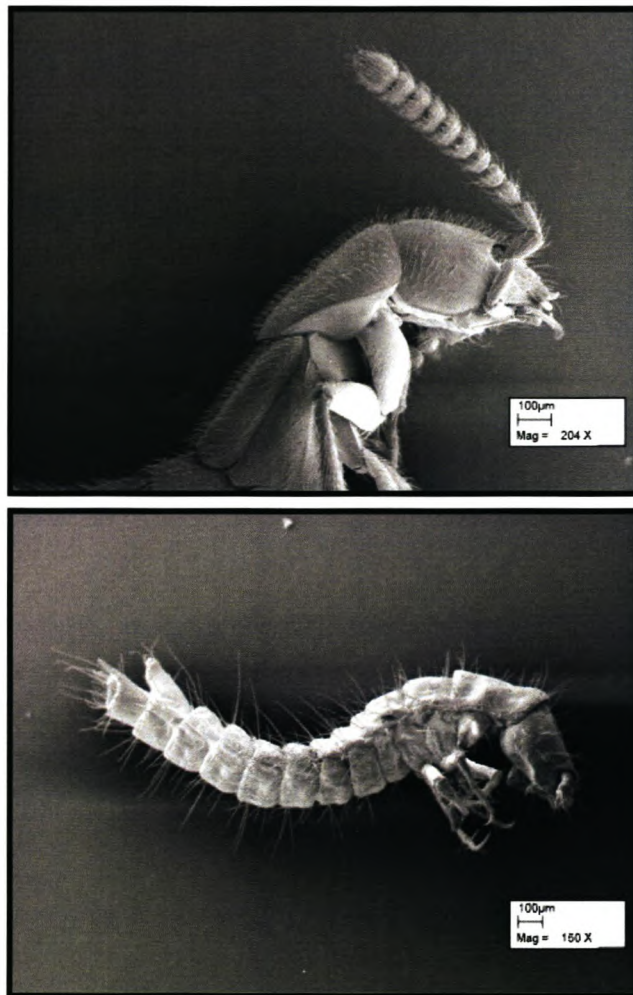
- Sinclair B. J., Klok C. J., Scott M. B., Terblanche J. S. & Chown S. L.** (2003c) Diurnal variation in supercooling points of three species of Collembola from Cape Hallett, Antarctica. *Journal of Insect Physiology* **49**: 1049-1061
- Sinclair B. J., Vernon P., Klok C. J. & Chown S. L.** (2003b) Insects at low temperatures: an ecological perspective. *Trends in Ecology and Evolution* **18**: 257-262
- Smith V. R.** (1987) The environment and biota of Marion Island. *South African Journal of Science* **83**: 211-220
- Smith V. R.** (2002) Climate change in the sub-Antarctic: An illustration from Marion Island. *Climatic Change* **52**: 345-357
- Sømme L. & Block W.** (1982) Cold hardiness of Collembola at Signy Island, maritime Antarctic. *Oikos* **38**: 168-176
- Sømme L. & Zachariassen K. E.** (1981) Adaptations to low temperature in high altitude insects from Mount Kenya. *Ecological Entomology* **6**: 199-204
- Van der Merwe M., Chown S. L. & Smith V. R.** (1997) Thermal tolerance limits in six weevil species (Coleoptera, Curculionidae) from sub-Antarctic Marion Island. *Polar Biology* **18**: 331-336
- Worland M. R. & Convey P.** (2001) Rapid cold hardening in Antarctic microarthropods. *Functional Ecology* **15**: 515-524
- Zachariassen K. E.** (1985) Physiology of cold tolerance in insects. *Physiological Reviews* **65**: 799-832

## Chapter 4<sup>1</sup>

### Differential responses of thermal tolerance to acclimation in the sub-Antarctic rove beetle *Halmaeus atriceps*

*Science is facts; just as houses are made of stones, so is science made of facts; but a pile of stones is not a house and a collection of facts is not necessarily science.*

- Jules Henri Poincaré -



*Halmaeus atriceps* adult (top) and larva (bottom)

<sup>1</sup> Published as: **Slabber S. & Chown S.L.** (2005) Thermal tolerance responses to acclimation in the sub-Antarctic rove beetle *Halmaeus atriceps* (C.O. Waterhouse). *Physiological Entomology*: In Press



## Introduction

The responses of ectotherms to their surrounding thermal environments have long fascinated environmental physiologists. In particular, the ways in which these responses are modified over time, the ways in which they vary spatially, and the extent to which upper and lower temperature limits are coupled, are enjoying renewed attention (Chen *et al.* 1991; Kingsolver & Huey 1998; Hercus *et al.* 2000; Klok & Chown 2003). Most notable amongst the several reasons for this recent vigour in the field, at least from an ecological perspective, are the realization that spatial variation (or the lack thereof) in thermal tolerance limits might determine both species abundances and their range limits (Chown & Gaston 1999), and that an understanding of the rate at which tolerance limits might evolve, and the extent to which change is adaptive or a consequence of phenotypic plasticity (Huey & Berrigan 1996; Berrigan & Hoffmann 1998), are essential for understanding the likely future impacts of rapid climate change (Walther *et al.* 2002; Hoffmann *et al.* 2003a).

Major concerns of recent work have therefore been twofold. First, the extent to which acclimation (or phenotypic plasticity) might account for differences in thermal tolerances among populations and among species, and whether this acclimation response is beneficial (Berrigan & Hoffmann 1998; Huey *et al.* 1999; Woods & Harrison 2002). Although much of the variation between species in thermal tolerances is probably adaptive in the strict sense (reviews in Spicer & Gaston 1999; Chown & Nicolson 2004), it is becoming clear that at the population level much of the geographic variation is a consequence of phenotypic plasticity (Klok & Chown 2003; Ayrinhac *et al.* 2004). The second major

concern is the form of the relationship between upper and lower lethal limits and the way in which this relationship might change in response to acclimation (Chown 2001). Essentially it appears that in insects responses to high and low temperatures are decoupled, such that a change in lower lethal limits is not necessarily accompanied by a change in upper lethal limits, either when populations, species, or populations in selection experiments are compared (Gilchrist *et al.* 1997; Addo-Bediako *et al.* 2000; Hercus *et al.* 2000). In addition, acclimation responses to low temperatures typically are stronger than those to high temperatures (Chown 2001). The likely mechanistic reasons for this decoupling are now being explored (Klok *et al.* 2004; Košťál *et al.* 2004; Sinclair *et al.* 2004).

Nonetheless, these responses might not be common to all insect species. For example, Hoffmann *et al.* (2002) found that there is substantial variation in both high and low temperature responses in *Drosophila melanogaster* such that there are opposing clines for upper and lower thermal tolerance. Moreover, Klok & Chown (2003) noted that exploration of these kinds of thermal responses is limited. They argued that much of the work on the relationships between high and low temperature tolerance, and the responses of these tolerances to acclimation has been undertaken on *Drosophila*, and that coverage in other groups, which might have considerably different responses to temperature, is relatively poor. Based on the fact that a substantial amount of the variance in high and low temperature tolerance is partitioned above the genus level (Addo-Bediako *et al.* 2000), Klok & Chown (2003) argued that investigations of thermal tolerances and their responses to acclimation should be broadened to include a wider range of taxa than simply a few model organisms. This call echoes several recent reviews that have shown not only that

comparisons of laboratory selection experiments and comparative studies can substantially inform evolutionary physiology (Kingsolver & Huey 1998; Feder *et al.* 2000), but also that broadening the phylogenetic and geographic extent of such work can contribute substantially to the developing field of macrophysiology (Chown & Nicolson 2004).

Here, this call is taken up by examining the response of upper and lower lethal temperature limits to acclimation in *Halmaeus atriceps* (C.O. Waterhouse), a beetle of the family Staphylinidae found on sub-Antarctic Marion Island. The Staphylinidae is one of the largest beetle families, containing several thousand genera, belonging to 31 subfamilies (Lawrence & Newton 1995). *Halmaeus atriceps* is wingless, occurs on the Prince Edward, Crozet, Kerguelen and Falkland Islands, and South Georgia, and is closely related to the sub-Antarctic *Leptusa* species that inhabit terrestrial habitats on Campbell and Auckland Islands (Leschen *et al.* 2002). On Marion Island, *H. atriceps* adults and larvae inhabit a wide range of habitats on the coastal plain. They are associated with nitrogenous decomposing plant material, on which they apparently feed, although they also consume nematodes (Crafford *et al.* 1986) and springtails (SS, personal observation). Both larvae and adults are active foragers on Marion Island and appear to inhabit the same niche throughout the year. Although the Staphylinidae is represented in the cold hardiness literature (e.g. Luff 1966; Petersen *et al.* 1996), as is *H. atriceps* (on South Georgia - (Block & Sømme 1983), neither the critical thermal limits, upper thermal tolerance, response to acclimation, or the relationships between these variables have been investigated for either the family or the species.

## Materials and methods

### *Study site and microclimate measurements*

This study was undertaken at Marion Island (46°54'S, 37°45'E) between April 2001 and May 2002, with additional data on winter supercooling points (see below) obtained in 2004. Marion Island, the larger of two volcanic islands forming the Prince Edward Island Group in the sub-Antarctic, lies to the north of the Antarctic Polar Frontal Zone. It has an oceanic climate, with much reduced seasonal and daily fluctuations in air temperature. Mean annual air temperature (1.2 m above-ground) is 5.7 °C, whereas mean annual soil temperature (2 cm below ground) at 0 m above sea level (asl) is 6.7 °C (hourly measurements between May 2002 and April 2003). Despite the climate of Marion Island being oceanic and hence rather stable, climatic changes have been shown to be rapid, and during the past 50 years, mean annual air temperatures have increased by 1.2 °C and mean annual precipitation has declined by *c.* 500 mm (almost 20 % of the pre-decline total) (Smith 2002).

Microhabitat temperatures were recorded at 60-min intervals 2 cm below ground (where invertebrates are typically highly abundant) at 100 m altitude increments, between sea level and 300 m asl, which is considered to be the upper altitudinal distribution limit of *H. atriceps*, on the eastern side of Marion Island using calibrated Thermochron I-Buttons (Dallas Semi-conductors) between May 2002 and April 2003. I-Buttons were collected and replaced every three months. Microclimate data were divided into 3-month periods (May to July, July to September, September to November, November to January, and January to

March), and data from each period analysed separately. Mean, mean minimum and mean maximum daily temperatures were determined for each period, as were absolute minimum and maximum temperatures.

### *Study animals*

Individuals were collected from the field (from below 25 m asl) and transported to the laboratory within 3 h of collection. Specimens were kept in vials with a moist Plaster of Paris substrate and transferred to temperature-controlled incubators (0, 5 °C with a LD 9:15 photoperiod, and 15 and 20 °C with a LD 14:10 photoperiod). Acclimation treatments lasted seven days (see Klok & Chown 2003 for rationale). Insects were provided with detritus from their sites of collection (including microarthropods), and were thus not intentionally starved. All experiments were also performed on field-fresh (unacclimated) individuals. In this study, field-fresh refers to individuals kept in plastic containers, as described above, at ambient L:D cycles and temperatures for no longer than seven days. The order of experimentation on acclimated and field-fresh individuals was randomized to avoid any order effects.

### *Supercooling point (crystallisation temperature) determination*

For the determination of supercooling points the methods of Klok & Chown (1997) were adopted. The supercooling points (SCP) of individual specimens were determined by securing each animal against a thermocouple inside a pipette tip using paper towel. Thermocouples (Type T copper-constantan 40-gauge) were connected to a Campbell CR10

datalogger (Campbell Scientific, Inc., England) that logged temperatures every second and calculated a mean every 10 s. The pipette tips (10) containing the specimens were placed into air-filled Vacutainers (BD Vacutainer Systems, UK) that were in turn submerged into a Grant LTC 12 waterbath (or a custom-built Peltier cooling device in 2004) at 0 °C for those animals acclimated at 0 °C, and 5 °C for field fresh animals and those acclimated at 5, 15 and 20 °C, respectively. After a 15 min equilibration period, the temperature was lowered by 0.1 °C.min<sup>-1</sup>. The lowest temperature reached before the onset of an exotherm was taken as the SCP for that individual. Sample sizes were typically 20-30 individuals for each treatment and stage (adults and larvae were examined), though in one instance the sample size was eight. Because distributions were not normal, SCPs were compared using a generalized linear model with normal errors and an identity link function, and the 95 % confidence intervals of the weighted marginal means were used for comparisons among groups.

#### *Upper and lower thermal tolerances*

Lethal temperature experiments provide information on the thermal tolerance ranges of species and were employed here to investigate the responses of two different life stages to low and high temperature exposure following different acclimation treatments. Lower lethal temperature (LLT) results also provide a means of determining whether or not there is significant pre-freeze mortality (Bale 1993), information that could not be obtained from the SCP trials. Upper and lower thermal limit investigations are more animal intensive than the SCP or the critical thermal limit methods described below. These experiments were therefore conducted on a subset of the treatment (acclimation) temperatures.

Batches of 10 animals were placed into 8 x 1 cm cylindrical screw-top plastic vials, which were submerged into a Grant LTD 20 water bath at a set temperature (high or low) for 1 h. An identical airtight plastic vial containing a 40-gauge copper-constantan thermocouple was submerged with the vials containing insects to monitor temperature, using a handheld thermometer (CHY 507 Thermometer, Taiwan). Pieces of wet or dry filter paper were inserted into the plastic vials containing insects for upper and lower lethal thermal tolerance experiments, respectively, but otherwise the procedure undertaken was the same for both. Conducting upper thermal tolerances in a saturated environment removes the confounding effects of desiccation on survival when assessing tolerance to high temperatures (Hoffmann *et al.* 2003b). Vials were removed from the water bath and transferred to an incubator (at the original acclimation temperature, except for field fresh batches that were transferred to a 10 °C incubator). Survival of individuals was scored after 24 h (see Chown & Nicolson 2004 for rationale). Individuals that showed normal locomotor activity were classified as alive. Those that were active but not walking normally were classified as uncoordinated and those that showed no motion at all were classified as dead. Total mortality per sample was calculated by adding the number of uncoordinated individuals to the number that died and expressing this as a proportion of all individuals. The temperature was altered by 1 °C and the experiment repeated with new individuals until the temperature range encompassed 5 to 95 % survival. The procedure was replicated at least four times at each experimental temperature for each of the treatment temperatures.

*Critical thermal limits*

Critical thermal minima (CT<sub>min</sub>) and maxima (CT<sub>max</sub>) techniques were used to investigate chill coma and heat stupor of *H. atriceps* adults and larvae. This dynamic method (see Lutterschmidt & Hutchison 1997) employs a relatively rapid ramped temperature protocol up to or down to that temperature where the last individual in a batch can no longer right itself or at which it shows spasms. Ten insects were placed individually into chambers (1 x 1 cm) situated on a small (10 x 5 x 1 cm) aluminium stage, which was connected to a Grant LTC 12 water bath (0.1 °C accuracy). A 40-gauge copper-constantan thermocouple was connected to the stage and the temperature monitored by a handheld thermometer. The temperature recorded on the stage was assumed to be representative of the body temperatures ( $T_b$ ) of the 10 experimental animals due to their small size (adult and larval live masses were equal to, or less than 1 mg). Specimens were observed through a dissecting microscope for the duration of an experiment. Before an experiment commenced, several dry silica gel crystals were placed on the stage out of reach of the insects to prevent condensation inside the chambers and thus to rule out any chance of inoculative freezing during CT<sub>min</sub> determinations.

For CT<sub>min</sub>, water bath temperature was set at 5 °C for 5 min and lowered at 0.5 °C.min<sup>-1</sup> until the onset of cold stupor was observed in all specimens. The onset of chill coma (CT<sub>min0</sub>) in the adults and larvae was considered as the temperature at which a particular insect lost motor function. Water bath temperature was allowed to decline to 1 °C below the temperature at which the last CT<sub>min0</sub> value was recorded and remained there for 5 min. Temperature was then increased by 0.5 °C.min<sup>-1</sup>. The temperature at which each individual



regained complete motor function was noted as its recovery temperature ( $CT_{minR}$ ). Essentially the same protocol was used for the  $CT_{max}$  experiments, except that the temperature was ramped upwards at  $0.5\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ , and only the temperature at which an individual lost motor control due to heat stupor ( $CT_{max}$ ) was recorded (animals often did not survive this treatment). Analyses of variance were used to test for critical thermal limit differences between the experimental groups for  $CT_{minO}$ ,  $CT_{minR}$  and  $CT_{max}$ .

## Results

Mean microhabitat temperatures varied between  $3\text{ }^{\circ}\text{C}$  and  $9\text{ }^{\circ}\text{C}$  depending on the altitude of the site (Table 1, Fig. 1). The absolute minimum temperature recorded was  $-6.0\text{ }^{\circ}\text{C}$  at 200 m asl (September 2002) and the absolute maximum recorded was  $22.5\text{ }^{\circ}\text{C}$  at sea level (December 2002) (Table 1). At the most extreme site (200 m), there were 29 freeze-thaw cycles and temperature remained below  $0\text{ }^{\circ}\text{C}$  for at least 176 h over the year. At other altitudes microhabitat temperatures were warmer, perhaps owing to the heavy snowfalls that were encountered during the winter.

Table 1. Absolute minimum and maximum temperatures, and the range in temperature (°C) measured at 0, 100, 200 and 300 m asl on Marion Island between May 2002 and March 2003.

Altitude	May – Jul			Jul - Sep			Sep - Nov			Nov - Jan			Jan - Mar		
	Min	Max	Range	Min	Max	Range	Min	Max	Range	Min	Max	Range	Min	Max	Range
0	0.5	11.0	10.5	0.5	10.0	9.5	0.5	11.5	11.0	3.5	22.5	19.0	5.0	17.5	12.5
100	0.0	11.0	11.0	0.5	13.0	12.5	0.5	15.5	15.0	2.5	16.0	13.5	3.0	15.5	12.5
200	0.5	9.0	8.5	-2.5	18.5	21.0	-1.0	13.0	14.0	-6.0	16.0	22.0	1.5	14.5	13.0
300	0.5	9.5	9.0	0.0	11.5	11.5	0.5	13.0	12.5	1.5	15.0	13.5	1.0	15.5	14.5

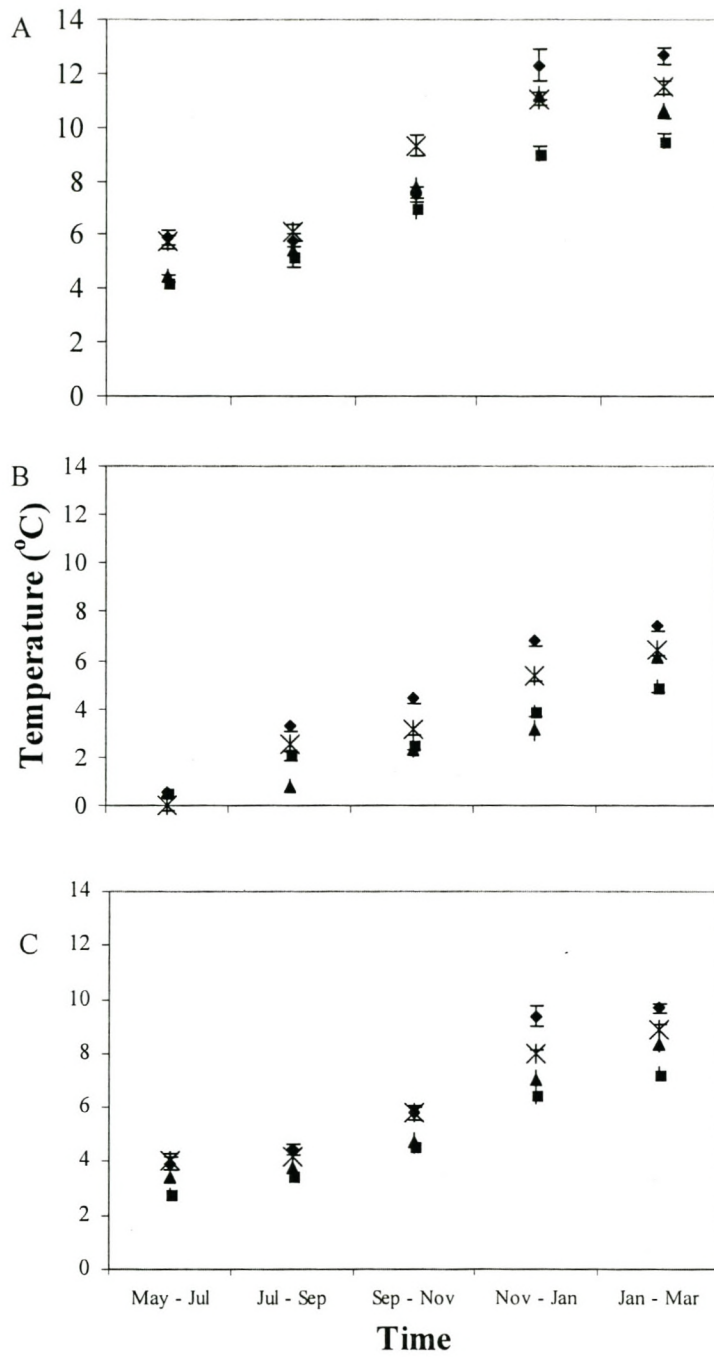


Figure 1. Mean daily maximum (a), mean daily minimum (b), and overall mean microhabitat temperatures (c) ( $^{\circ}\text{C} \pm \text{SE}$ ) for Marion Island for each of the periods investigated at 0 (◆), 100 (\*), 200 (▲) and 300 (■) m asl

Supercooling points in field-fresh summer acclimatized individuals were low, and there were no differences between larvae and adults (Table 2,  $\chi^2_{(1)} = 0.002$ ,  $P = 0.97$ ). By contrast, although there were no significant differences in SCPs between adults and larvae in winter-acclimatized individuals ( $\chi^2_{(1)} = 0.97$ ,  $P = 0.33$ ), there was a strong elevation in SCPs such that winter-acclimatized individuals had much higher SCPs than summer-acclimatized individuals (Table 2, Adults:  $\chi^2_{(1)} = 136.7$ ,  $P < 0.0001$ ); Table 3, Larvae:  $\chi^2_{(1)} = 215.8$ ,  $P < 0.0001$ ). In both adults and larvae, this change was not effected in all members of the population. Rather, a few individuals in the group retained SCPs below  $-12$  °C, as is also reflected by the differences between the mean and median SCPs (Tables 2 and 3). The summer-acclimatized larvae, but not adults, showed a reverse trend, with just a few individuals having relatively high SCPs, but the majority showing low SCPs (Fig. 2d). In the laboratory acclimation experiments, there were significant differences between the summer-acclimated individuals, but these differences were generally small and somewhat idiosyncratic in both adults and larvae, although the strongest depression in the SCP was found in animals acclimated to  $20$  °C (Tables 2 and 3). However, there were significant differences between the animals acclimated to the  $0$  °C conditions in autumn and in summer, although this difference was not pronounced in the adults, owing possibly to the small sample size. Nonetheless, it is clear that in individuals collected in the summer there is no strong response to a  $0$  °C acclimation treatment, but that the response in autumn is much more pronounced. Thus, it appears that in summer, irrespective of the acclimation treatment, SCPs are low in the laboratory, as they are in the field (Tables 2 and 3), but as winter approaches, SCPs increase considerably. By contrast, lower lethal temperatures appear to vary by only a few degrees, between  $-7.6$  °C and  $-10.8$  °C in adults and between -

11.1 °C and -13.4 °C in larvae (Tables 2 and 3). In winter, these temperatures lie well below the SCP, whereas in summer they lie above it.

Upper lethal limits showed a similar small range in variation (Fig. 3), and although significant, differences among acclimation treatments were small. There also seemed to be little difference between individuals that were summer or winter acclimated or acclimatized.

Table 2. Summary statistics for supercooling points (°C) of field-fresh (FF) *Halmaeus atriceps* adults, and adults acclimated at 0, 5, 15 and 20 °C. Different letters for SCPs denote significantly different means based on at the 95 % confidence intervals of the weighted marginal means obtained from fitting a generalized model with normal errors and an identity link function to the data. The  $\chi^2$  statistics and significances of these models are shown. In the case of the lower lethal temperature data, an ANOVA approach was used and different letters denote differences based on Tukey's Honest Significant Difference.

Life stage and treatment	<i>n</i>	Mean ± S.E.	Median	Minimum	Maximum
<b>Adults</b>					
<b>Supercooling points</b>					
FF adults (Late summer)	20	-18.9 ± 0.7	-19.9	-22.7	-13.4
FF adults (Winter, whole group)	28	-6.3 ± 0.8	-3.8	-17.0	-3.0
FF adults (Winter, high group)	23	-4.5 ± 0.4	-3.6	-8.6	-3.0
0 °C adults (Autumn)	20	-9.8 ± 0.5 <sup>a</sup>	-9.2	-16.7	-7.2
0 °C adults (Summer)	8	-14.1 ± 1.95 <sup>ab</sup>	-15.8	-20.6	-3.8
5 °C adults (Summer)	20	-13.6 ± 1.2 <sup>b</sup>	-15.5	-21.3	-4.2
15 °C adults (Summer)	20	-14.4 ± 1.1 <sup>b</sup>	-14.8	-23.1	-5.0
20 °C adults (Summer)	20	-19.0 ± 0.4 <sup>c</sup>	-19.5	-21.4	-12.5
$\chi^2_{(4)} = 50.8, P < 0.0001$					
<b>Lower lethal temperature</b>		<b>LT50</b>			
FF adults (Winter)		-7.6 ± 0.03			
0 °C adults (Autumn)		-10.8 ± 0.05			
20 °C adults (Spring)		-9.7 ± 0.12			
		ANOVA	$F_{(2,7)} = 4.09$	$P > 0.05$	

Table 3. Summary statistics for supercooling points (°C) of field-fresh (FF) *Halmaeus atriceps* larvae, and larvae acclimated at 0, 5, 15 and 20 °C. Different letters for SCPs denote significantly different means based on at the 95 % confidence intervals of the weighted marginal means obtained from fitting a generalized model with normal errors and an identity link function to the data. The  $\chi^2$  statistics and significances of these models are shown. In the case of the lower lethal temperature data, an ANOVA approach was used and different letters denote differences based on Tukey's Honest Significant Difference.

Life stage and treatment	<i>n</i>	Mean ± S.E.	Median	Minimum	Maximum
<b>Larvae</b>					
<b>Supercooling points</b>					
FF larvae (Late Summer)	20	-18.9 ± 1.0	-18.3	-25.3	-7.7
FF larvae (Winter, whole group)	29	-5.4 ± 0.4	-4.4	-14.2	-3.2
FF larvae (Winter, high group)	28	-5.1 ± 0.3	-4.4	-8.0	-3.2
0 °C larvae (Autumn)	20	-5.4 ± 0.4 <sup>a</sup>	-4.7	-9.4	-3.8
0 °C larvae (Summer)	5	-20.5 ± 1.1 <sup>d</sup>	-21.4	-21.8	-16.0
5 °C larvae (Summer)	20	-14.0 ± 1.2 <sup>b</sup>	-15.4	-21.1	-5.0
15 °C larvae (Summer)	20	-16.7 ± 1.1 <sup>bc</sup>	-18.5	-22.7	-4.3
20 °C larvae (Summer)	20	-19.6 ± 0.9 <sup>cd</sup>	-20.9	-25.3	-10.7
$\chi^2_{(4)} = 142.4, P < 0.0001$					
<b>Lower lethal temperatures</b>					
FF larvae (Winter)		-11.1 ± 0.06			
0 °C larvae (Late Winter)		-13.4 ± 0.08			
ANOVA $F_{(1,4)} = 27.06 P < 0.05$					

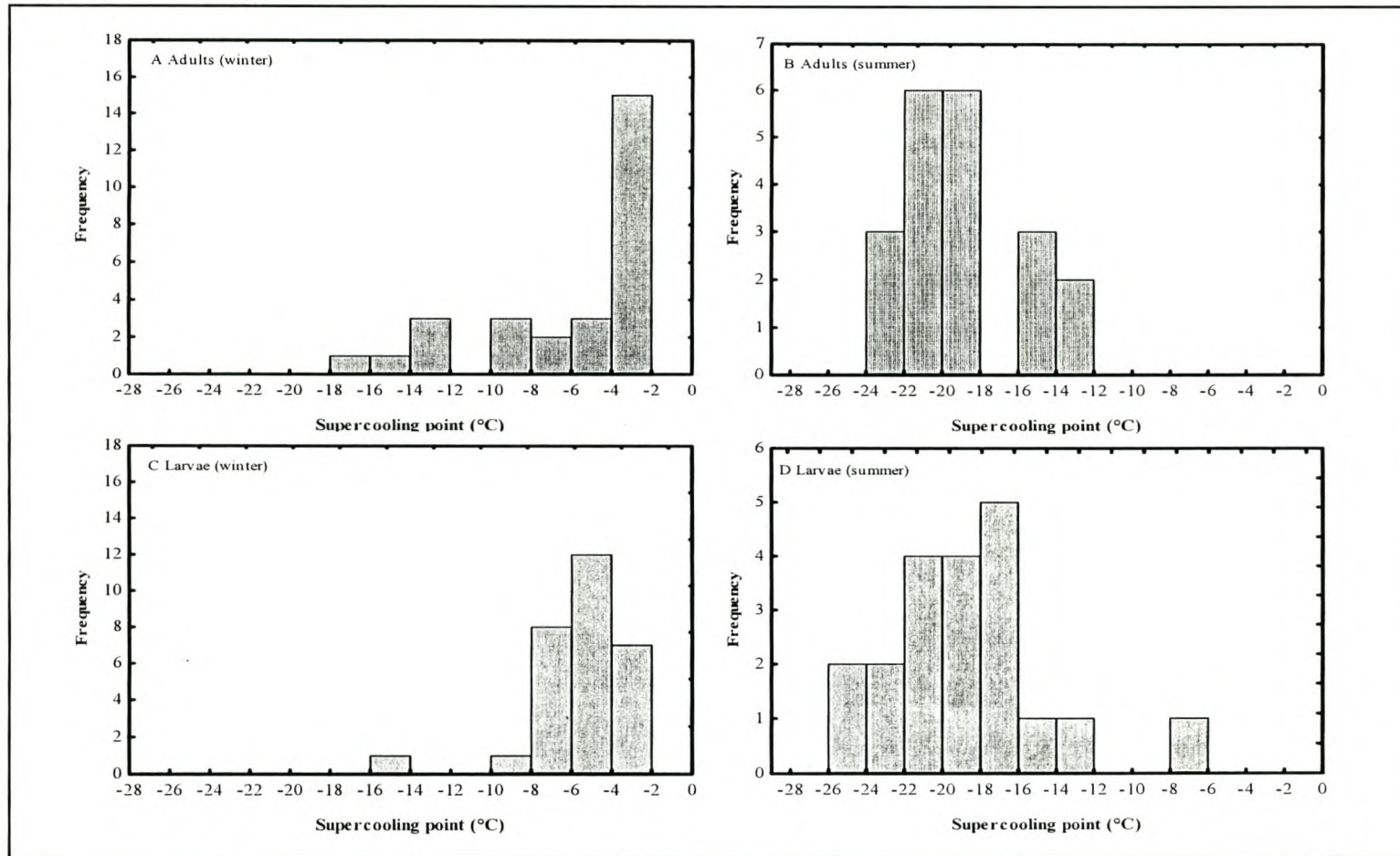


Figure 2. Frequency distributions of supercooling points (°C) for field-fresh (FF) *Halmaeus atriceps* A. winter-acclimatized adults; B. summer-acclimatized adults; C. winter-acclimatized larvae; D. summer-acclimatized larvae.



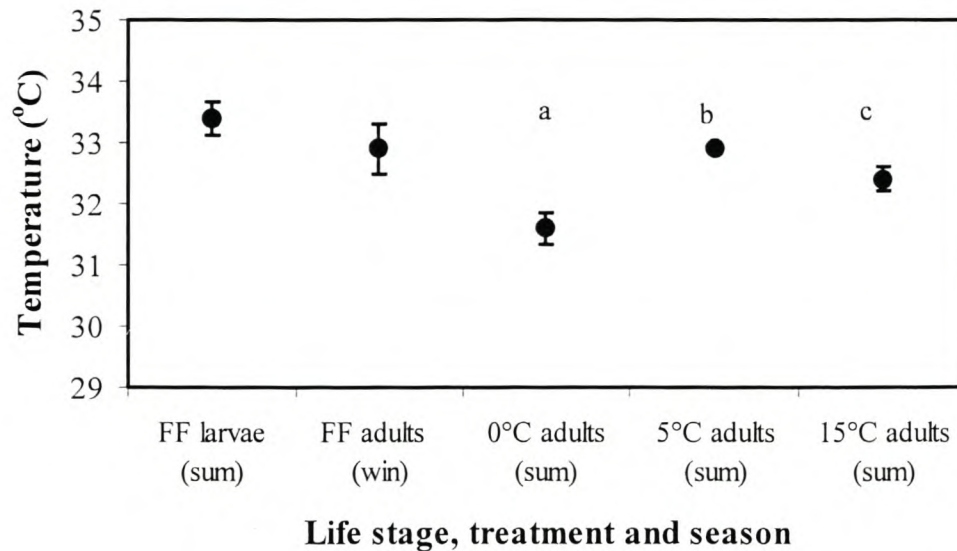


Figure 3. Upper lethal temperatures (LT<sub>50</sub>) for field-fresh (FF), and acclimated adults and larvae of *Halmaeus atriceps* (sum = summer, win = winter). Different letters above each treatment denote significant differences in the means at the 5 % level based on Tukey's Honest Significant Difference test (overall ANOVA  $F_{(4, 19)} = 19.91$ ,  $P < 0.0001$ ).

Critical thermal limits did not differ significantly between field-fresh adults and larvae, except in the case of CT<sub>max</sub>, but even here the difference was small (1.2 °C) (Table 4). Acclimation had a pronounced and significant effect on both CT<sub>min<sub>O</sub></sub> and CT<sub>min<sub>R</sub></sub>, with low temperature treatments causing a decline in both parameters. Overall, mean CT<sub>min<sub>O</sub></sub> varied by 3.3 °C and mean CT<sub>min<sub>R</sub></sub> by 6.6 °C in adults and by 3.0 °C and 4.2 °C, respectively, in larvae across the treatment temperatures (Table 4). In adults, differences in CT<sub>max</sub> across treatments were smaller (2.3 °C), though significant, whilst in the larvae there were no significant differences between treatments. CT<sub>min<sub>O</sub></sub> and CT<sub>min<sub>R</sub></sub> values in

field-fresh individuals were most similar to those in experimental individuals acclimated at either 0 °C or 5 °C (Table 4).

Table 4. Mean  $\pm$  S.E. (°C) of critical thermal minimum onset (CT<sub>minO</sub>), critical thermal minimum recovery (CT<sub>minR</sub>) and critical thermal maximum (CT<sub>max</sub>) in field-fresh (FF) *Halmaeus atriceps* adults and larvae, and adults and larvae acclimated at 0, 5, 15 and 20 °C. Different letters denote significantly different means at the 5 % level based on Tukey's Honest Significant Difference ( $n = 10$  for each treatment).

Life stage	Mean CT <sub>minO</sub>	Mean CT <sub>minR</sub>	Mean CT <sub>max</sub>
FF adults	-2.7 $\pm$ 0.2 <sup>a</sup>	1.9 $\pm$ 0.9 <sup>a</sup>	33.2 $\pm$ 0.3 <sup>a</sup>
FF larvae	-3.2 $\pm$ 0.1 <sup>a</sup>	2.9 $\pm$ 0.6 <sup>a</sup>	32.0 $\pm$ 0.3 <sup>b</sup>
ANOVA	$F_{(1,18)} = 2.82, P = 0.11$	$F_{(1,18)} = 0.84, P = 0.37$	$F_{(1,18)} = 6.98, P < 0.05$
<b>Treatment</b>			
0 °C adults	-4.1 $\pm$ 0.1 <sup>a</sup>	0.3 $\pm$ 0.7 <sup>a</sup>	30.1 $\pm$ 0.2 <sup>a</sup>
5 °C adults	-2.9 $\pm$ 0.3 <sup>b</sup>	3.8 $\pm$ 0.3 <sup>b</sup>	-
15 °C adults	-1.9 $\pm$ 0.2 <sup>c</sup>	6.2 $\pm$ 0.1 <sup>c</sup>	32.4 $\pm$ 0.3 <sup>b</sup>
20 °C adults	-0.8 $\pm$ 0.2 <sup>d</sup>	7.9 $\pm$ 0.2 <sup>d</sup>	32.3 $\pm$ 0.3 <sup>b</sup>
ANOVA	$F_{(3,36)} = 51.96, P < 0.0001$	$F_{(3,36)} = 64.06, P < 0.0001$	$F_{(2,27)} = 20.24, P < 0.0001$
0 °C larvae	-3.6 $\pm$ 0.2 <sup>a</sup>	2.7 $\pm$ 0.8 <sup>a</sup>	27.4 $\pm$ 0.3 <sup>a</sup>
5 °C larvae	-2.3 $\pm$ 0.2 <sup>b</sup>	4.4 $\pm$ 1.0 <sup>ab</sup>	27.6 $\pm$ 0.1 <sup>a</sup>
15 °C larvae	-1.6 $\pm$ 0.2 <sup>b</sup>	6.4 $\pm$ 0.3 <sup>bc</sup>	28.3 $\pm$ 0.2 <sup>a</sup>
20 °C larvae	-0.6 $\pm$ 0.2 <sup>c</sup>	6.9 $\pm$ 0.4 <sup>c</sup>	28.1 $\pm$ 0.2 <sup>a</sup>
ANOVA	$F_{(3,42)} = 27.85, P < 0.0001$	$F_{(3,42)} = 9.48, P < 0.0001$	$F_{(3,42)} = 3.01, P < 0.05$

## Discussion

In summer-acclimated and acclimatized adults and larvae of *Halmaeus atriceps* the supercooling point (or crystallization temperature) is not equivalent to the lower lethal temperature. Rather, there is substantial pre-freeze mortality as has been recorded in several species of insects and other arthropods (Bale 1993). In Bale's (1993) terminology, summer-acclimatized (and high temperature acclimated) individuals of this species are chill susceptible. Plotting SCPs and haemolymph osmolality values (c. 400-565 mOsm, Sinclair & Chown 2002) on the scheme developed by Zachariassen (1985) supports the notion that this species is chill susceptible in the summer, although both the SCP and lower lethal temperature are somewhat lower than those that are characteristic of summer-acclimatized individuals (see Zachariassen 1985: Fig. 1). The strong response of the SCP in both adults and larvae to acclimation at 20 °C is surprising in this regard. However, it may be a consequence of cross-tolerance to heat shock, which is known to alter the cold hardiness response in several species (see Chown & Nicolson 2004). Perhaps of more significance is that, at least in the adults of *H. atriceps*, a high temperature acclimation treatment has little influence on survival, despite the change in SCP.

In winter-acclimatized individuals there seems to be a wholly different response to low temperatures. Indeed, the SCP increases to a point where it is several degrees above the LT50 (Tables 2 and 3), so suggesting that both adults and larvae switch to freeze tolerance under winter conditions. The study suggests that not all individuals make this change, and indeed they may show a different response if subjected to repeated freeze-thaw cycles (Bale

*et al.* 2001; see also Brown *et al.* 2004). Such bimodality in invertebrate SCPs has been well documented previously in other groups such as the springtails (Cannon & Block 1988). It is clear that moderate freeze tolerance (see Chown & Nicolson (2004) for a discussion of freeze tolerance categories) is used as a strategy to survive winter conditions, which may also explain the findings of Sinclair & Chown (2002) that there is a change in haemolymph osmolality of more than 100 mOsm in response to low temperature acclimation in the autumn. What is curious, though, is the fact that the alteration in strategy cannot solely be effected by a change in temperature and light regimes under laboratory conditions. Rather, some other signal appears to be involved, which would make this species dissimilar to most other insects where there is a strong response to temperature and photoperiod in preparation for winter (Chown & Nicolson 2004).

The winter, freeze tolerant response of this species makes it similar to the moderate freeze tolerance that is so typical of most of the beetle species and several of the other insects on Marion Island (Klok & Chown 1997; van der Merwe *et al.* 1997; Klok & Chown 2001). Indeed, in the weevils *Bothrometopus parvulus* and *Ectemnorhinus similis* there is a significant increase in the SCP of summer acclimatized individuals, as there appears to be in *H. atriceps*. By contrast, freeze tolerance is not characteristic of the northern hemisphere rove beetles that have been investigated to date. They generally show moderate to considerable chill tolerance (Luff 1966; Petersen *et al.* 1996), although this is perhaps not surprising given the preponderance of freeze intolerance in the northern hemisphere (Zachariassen 1985; Sinclair *et al.* 2003). Nonetheless, freeze tolerance over winter makes *H. atriceps* on Marion Island very different to conspecific individuals on South Georgia,

which always froze and died at -3 to -4 °C (Block & Sømme 1983). However, *H. atriceps* specimens in the study by Block & Somme (1983) were starved and kept in zero light conditions, and were exposed to cooling rates 10 to 20 times greater than those in the present study. In addition, substantial variation in the thermal tolerances of beetles across the sub-Antarctic has been documented previously (Klok & Chown 2003).

Irrespective of the SCP values, the lower lethal temperatures of *H. atriceps* larvae and adults are well beyond the range likely to be encountered in the field. During the year that this study was undertaken, absolute minimum microhabitat temperatures never declined below -6 °C, and freezing was quite uncommon, probably as a consequence of unusually heavy and prolonged snow cover. Although previous studies have recorded more frequent freeze-thaw events and much lower absolute minimum temperatures both at sea level and at higher elevations (e.g. Chown & Crafford 1992; Klok & Chown 1998), these remain well within the range of the lower lethal temperatures of *H. atriceps* by at least a few degrees. Similarly, whilst microhabitat temperatures in the 2002/2003 year never exceeded 22.5 °C, other studies have occasionally recorded temperatures in the habitats of *H. atriceps* of greater than 30 °C (Chown & Crafford 1992). However, mean maxima are generally well below this value (Fig. 1), suggesting that the upper thermal limits of *H. atriceps* are sufficient to allow survival of most conditions on Marion Island, at least over the short term. Nonetheless, it should be noted that these upper lethal temperature limits are generally 8-10 °C lower than those found for many of the other insects on Marion Island (e.g. Klok & Chown 1997; 1998; 2001). What the longer-term population level effects of high temperature are likely to be is not known, but deserves further investigation.

Short-term limits to activity, i.e. the critical thermal limits to activity, lay within, but in the adults surprisingly close to, the lethal temperature limits. These limits showed a narrower range than is typical of most of the other insects from Marion Island, which generally span more than 40 °C (Klok & Chown 1997; 1998; 2001; 2003), whilst in *H. atriceps* the range was 30-35 °C. Moreover, comparisons of the CT<sub>min<sub>O</sub></sub> of the field-fresh individuals with those of the acclimation treatments provide a rough form of thermal monitoring for the latter. At least in autumn, field-fresh individuals have CT<sub>min<sub>O</sub></sub> temperatures of *c.* -3 °C, which is midway between CT<sub>min<sub>O</sub></sub> temperatures found for individuals acclimated at 0 °C and those acclimated at 5 °C. These temperatures are remarkably close to those recorded in the beetles' microhabitats at sea level during the same period (Fig. 1).

Perhaps more significantly, it was clear that whilst critical thermal minima (onset or recovery) were highly sensitive to acclimation, changing by as much as 7.4 °C across the acclimation temperatures, critical thermal maximum was not nearly as sensitive to acclimation temperature. The largest change found was 2.1 °C between acclimation of adults at 0 °C and at 20 °C. This large acclimation response of CT<sub>min</sub>, but a smaller response of CT<sub>max</sub> is in keeping with the responses of critical thermal limits of sub-Antarctic weevils to acclimation (Klok & Chown 2003), but differs from *Embryonopsis haiticella* caterpillars on Marion Island that showed a weak, but significant decline in CT<sub>max</sub> with declining temperatures, but no response in CT<sub>min</sub> (Klok & Chown 1998). Nonetheless, a strong response in low temperature limits to either experimentally induced or environmental variation in temperature, but a weaker one in high temperature limits does

appear to be typical of insects in general (Gilchrist *et al.* 1997; Addo-Bediako *et al.* 2000; Hercus *et al.* 2000; Chown 2001). However, as was noted in the Introduction, this generalization has, until recently, been based mostly on investigations of flies in the genus *Drosophila*. Hence, the present results substantiate this finding in another species, and one that belongs to a higher taxon, the Staphylinidae, that has to date not been investigated in this regard, so lending support to the broader generalizations.

In conclusion, the responses to temperature of *H. atriceps* adults and larvae are similar, and probably constitute chill susceptibility in the summer, and moderate freeze tolerance in the winter. Moreover, they are well within the range of environmental temperatures at Marion Island, and in some instances can be used as a monitor of them. Most significantly, as has been found in most of the other species investigated to date, lower temperature limits are far more labile than upper ones.

## References

- Addo-Bediako A., Chown S. L. & Gaston K. J.** (2000) Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B* **267**: 739-745
- Ayrinhac A., Debat V., Gibert P., Kister A.-G., Legout H., Moreteau B., Vergilino R. & David J. R.** (2004) Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Functional Ecology* **18**: 700-706
- Bale J. S.** (1993) Classes of insect cold hardiness. *Functional Ecology* **7**: 751-753
- Bale J. S., Worland M. R. & Block W.** (2001) Effects of summer frost exposures on the cold tolerance strategy of a sub-Antarctic beetle. *Journal of Insect Physiology* **47**: 1161-1167
- Berrigan D. & Hoffmann A. A.** (1998) Correlations between measures of heat resistance and acclimation in two species of *Drosophila* and their hybrids. *Biological Journal of the Linnean Society* **64**: 449-462
- Block W. & Sømme L.** (1983) Low temperature adaptations in beetles from the sub-Antarctic island of South Georgia. *Polar Biology* **2**: 109-114
- Brown C. L., Bale J. S. & Walters K. F. A.** (2004) Freezing induces a loss of freeze tolerance in an overwintering insect. *Proceedings of the Royal Society of London B* **271**: 1507-1511
- Cannon R. J. C. & Block W.** (1988) Cold tolerance of microarthropods. *Biological Reviews* **63**: 23-77
- Chen C.-P., Denlinger D. L. & Lee R. E.** (1991) Seasonal variation in generation time, diapause and cold hardiness in a central Ohio population of the flesh fly, *Sarcophaga bullata*. *Ecological Entomology* **16**: 155-162
- Chown S. L.** (2001) Physiological variation in insects: hierarchical levels and implications. *Journal of Insect Physiology* **47**: 649-660
- Chown S. L. & Crafford J. E.** (1992) Microhabitat temperatures at Marion Island (46°54'S 37°45'E). *South African Journal of Antarctic Research* **22**: 51-58
- Chown S. L. & Gaston K. J.** (1999) Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Reviews* **74**: 87-120
- Chown S. L. & Nicolson S. W.** (2004) *Insect physiological ecology. Mechanisms and patterns*. Oxford University Press, Oxford



- Crafford J. E., Scholtz C. H. & Chown S. L.** (1986) The insects of sub-Antarctic Marion and Prince Edward Islands; with a bibliography of entomology of the Kerguelen Biogeographical Province. *South African Journal of Antarctic Research* **16**: 42-84
- Feder M. E., Bennett A. F. & Huey R. B.** (2000) Evolutionary physiology. *Annual Review of Ecology and Systematics* **31**: 315-341
- Gilchrist G. W., Huey R. B. & Partridge L.** (1997) Thermal sensitivity of *Drosophila melanogaster*: evolutionary responses of adults and eggs to laboratory natural selection at different temperatures. *Physiological Zoology* **70**: 403-414
- Hercus M. J., Berrigan D., Blows M. W., Magiafoglou A. & Hoffmann A. A.** (2000) Resistance to temperature extremes between and within life cycle stages in *Drosophila serrata*, *D. birchii* and their hybrids: intraspecific and interspecific comparisons. *Biological Journal of the Linnean Society* **71**: 403-416
- Hoffmann A. A., Anderson A. & Hallas R.** (2002) Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. *Ecology Letters* **5**: 614-618
- Hoffmann A. A., Hallas R. J., Dean J. A. & Schiffer M.** (2003a) Low potential for climatic stress adaptation in a rainforest *Drosophila* species. *Science* **301**: 100-102
- Hoffmann A. A., Sørensen J. G. & Loeschcke V.** (2003b) Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *Journal of Thermal Biology* **28**: 175-216
- Huey R. B. & Berrigan D.** (1996) Testing evolutionary hypotheses of acclimation. *Animals and temperature. Phenotypic and evolutionary adaptation*. Ed. Bennett A. F., Cambridge University Press, Cambridge. pp 205-237
- Huey R. B., Berrigan D., Gilchrist G. W. & Herron J. C.** (1999) Testing the adaptive significance of acclimation: a strong inference approach. *American Zoologist* **39**: 323-336
- Kingsolver J. G. & Huey R. B.** (1998) Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *American Zoologist* **38**: 545-560
- Klok C. J. & Chown S. L.** (1997) Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic caterpillar, *Pringleophaga marioni* Viette (Lepidoptera: Tineidae). *Journal of Insect Physiology* **43**: 685-694
- Klok C. J. & Chown S. L.** (1998) Interactions between desiccation resistance, host-plant contact and the thermal biology of a leaf-dwelling sub-Antarctic caterpillar, *Embryonopsis halticella* (Lepidoptera: Yponomeutidae). *Journal of Insect Physiology* **44**: 615-628

- Klok C. J. & Chown S. L.** (2001) Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic kelp fly, *Paractora dreuxi* (Diptera: Helcomyzidae). *Journal of Insect Physiology* **47**: 95-109
- Klok C. J. & Chown S. L.** (2003) Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. *Biological Journal of the Linnean Society* **78**: 401-414
- Klok C. J., Sinclair B. J. & Chown S. L.** (2004) Upper thermal tolerance and oxygen limitation in terrestrial arthropods. *Journal of Experimental Biology* **207**: 2361-2370
- Košťál V., Vambera J. & Bastl J.** (2004) On the nature of pre-freeze mortality in insects: water balance, ion homeostasis and energy change in the adults of *Pyrrhocoris apterus*. *Journal of Experimental Biology* **207**: 1509-1521
- Lawrence J. F. & Newton A. F. J.** (1995) Families and subfamilies of Coleoptera (with selected genera, notes, references and data on family-group names). *Biology, phylogeny, and classification of Coleoptera. Papers celebrating the 80th birthday of Roy A. Crowson*. Ed. Pakaluk J. & Slipinski A. S., Muzeum i Instytut Zoologii PAN, Warsaw. pp 779-1006
- Leschen R. A. B., Bullians M. S., Michaux B. & Ahn K.** (2002) Systematics of *Baeostethus chiltoni*, a subantarctic liparocephaline (Coleoptera: Staphylinidae, Aleocharinae): a Pangean relic or a more recent immigrant? *Journal of the Royal Society of New Zealand* **32**: 189-201
- Luff M. L.** (1966) Cold hardiness of some beetles living in grass tussocks. *Entomologia Experimentalis et Applicata* **9**: 191-199
- Lutterschmidt W. I. & Hutchison V. H.** (1997) The critical thermal maximum: history and critique. *Canadian Journal of Zoology* **75**: 1561-1574
- Petersen M. K., Ekbom B. & Ravn H. P.** (1996) Temperature dependent winter survival of *Bembidion lampros* and *Tachyporus hypnorum*. *Journal of Insect Physiology* **42**: 997-1005
- Sinclair B. J., Addo-Bediako A. & Chown S. L.** (2003) Climatic variability and the evolution of insect freeze tolerance. *Biological Reviews* **78**: 181-195
- Sinclair B. J. & Chown S. L.** (2002) Haemolymph osmolality and thermal hysteresis activity in 17 species of arthropods from sub-Antarctic Marion Island. *Polar Biology* **25**: 928-933
- Sinclair B. J., Klok C. J. & Chown S. L.** (2004) Metabolism of the sub-Antarctic caterpillar *Pringleophaga marioni* during cooling, freezing and thawing. *Journal of Experimental Biology* **207**: 1287-1294

- Smith V. R.** (2002) Climate change in the sub-Antarctic: An illustration from Marion Island. *Climatic Change* **52**: 345-357
- Spicer J. I. & Gaston K. J.** (1999) *Physiological diversity and its ecological implications*. Blackwell Science, Oxford
- Van der Merwe M., Chown S. L. & Smith V. R.** (1997) Thermal tolerance limits in six weevil species from sub-Antarctic Marion Island. *Polar Biology* **18**: 331-336
- Walther G.-R., Post E., Convey P., Menzel A., Parmesan C., Beebee T. J. C., Fromentin J.-M., Hoegh-Guldberg O. & Bairlein F.** (2002) Ecological responses to recent climate change. *Nature* **416**: 389-395
- Woods H. A. & Harrison J. F.** (2002) Interpreting rejections of the beneficial acclimation hypothesis: when is physiological plasticity adaptive? *Evolution* **56**: 1863-1866
- Zachariassen K. E.** (1985) Physiology of cold tolerance in insects. *Physiological Reviews* **65**: 799-832

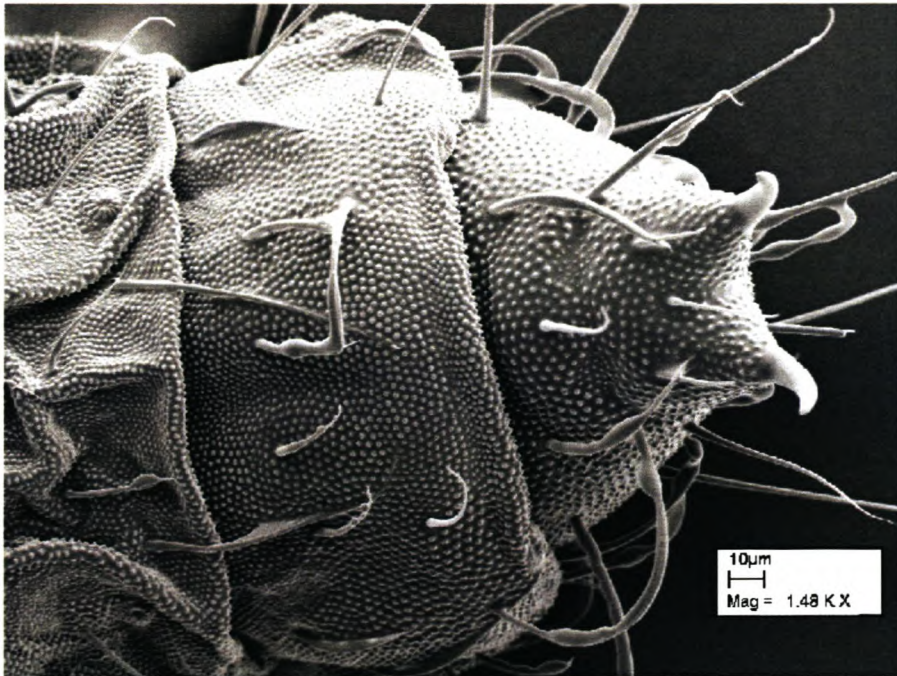
## Chapter 5

### Testing the beneficial acclimation hypothesis:

#### Indigenous and alien species

*"There is one thing even more vital to science than intelligent methods; and that is, the sincere desire to find out the truth, whatever it may be."*

- Charles Sanders Pierce -



Scanning Electron Microscope image of *Tullbergia bisetosa*

## Introduction

Humans are having unprecedented impacts on the globe at both local and regional scales (Vitousek *et al.* 1997a; Vitousek *et al.* 1997b; IPCC 2001; Pimentel 2002). These impacts, in turn, are affecting a wide variety of species both directly, through local reductions in population density or extirpation (Soulé 1991; Brooks *et al.* 2002), and indirectly via changes both to climate and to the distributions of other species. The latter, in many cases, are invasive species which can have considerable effects on indigenous species (Vitousek *et al.* 1997b; Blackburn *et al.* 2004). Moreover, there are substantial interactions between local and regional impacts, making prediction of their effects on the abundance and distribution of species problematic (e.g. Parmesan *et al.* 2000; Warren *et al.* 2001). Indeed, generalisations regarding the responses of species to changing environments are difficult to make both for this reason and because responses might involve migration, local adaptation or a combination of the two (Pitelka 1997; Parmesan *et al.* 2000; IPCC 2001; Bale *et al.* 2002; Coulson *et al.* 2002; Walther *et al.* 2002; Hill & Fox 2003).

Nonetheless, it has been suggested that there might be substantial differences in the way that indigenous as opposed to invasive alien species in a given area respond to change. The latter are thought to be more capable of responding positively to elevated environmental temperatures, especially in temperate areas, than the former, owing to their ruderal, weedy nature (Prinzing *et al.* 2002; Stachowicz *et al.* 2002; Walther *et al.* 2002; Bohn *et al.* 2004). It has also been demonstrated that weedy, introduced species show greater phenotypic plasticity than do indigenous ones in the same area. In plants, invaders are able to take advantage of a wide range of conditions, which is particularly

important in highly variable disturbed environments (Daehler 2003). In birds, generalist behaviour and behavioural flexibility are thought to enhance survival, reproduction and establishment of alien species in new environments (Duncan *et al.* 2003).

These suggestions for differential responses to the environment by invasive vs. indigenous species can readily be recast in terms of the range of hypotheses that have been developed recently in the context of acclimation (see Huey & Berrigan 1996; Huey *et al.* 1999; Wilson & Franklin 2002 for review). The prediction of greater phenotypic plasticity in invasive than in indigenous species is clearly consistent with the beneficial acclimation hypothesis (BAH), and in particular greater phenotypic plasticity in the former group. In other words, "...acclimation to a particular environment gives an organism a performance advantage in that environment over other organisms that did not have the opportunity to acclimate to that particular environment" (Leroi *et al.* 1994). By contrast, improved performance of invasive species relative to indigenous species in warm environments, either suggests that for invasive species warmer conditions enhance performance (warmer is better), or that for indigenous species colder conditions are more suitable (colder is better), or both (see Huey *et al.* (1999) for a complete discussion of these hypotheses). Thus, the beneficial acclimation hypothesis and its alternatives ("warmer is better", "colder is better", *inter alia*) are general statements regarding performance expectations that can be applied specifically to predictions concerning differences in the performance of indigenous and invasive species under changing environmental conditions. To date, however, few investigations have taken this acclimation perspective, especially not for animals, despite the obvious benefits of applying the insights gained from the latter, theoretically well-developed area of

investigation (Huey *et al.* 1999; Wilson & Franklin 2002; see also Scheiner 1993), to the former.

In this study, such an approach is taken, and the differential responses of indigenous and invasive species to temperature are explicitly examined in the context of the beneficial acclimation hypothesis and its alternatives. In particular the predictions that the beneficial application hypothesis and its alternatives apply differentially to alien invasive vs. indigenous species are tested. To do so, the thermal acclimation responses of desiccation tolerance, supercooling ability, lower and upper thermal limits are compared in two indigenous and three alien invasive species of springtails from sub-Antarctic Marion Island. These species and this system were chosen because the alien species are highly invasive (see Gabriel *et al.* 2001), explicit predictions regarding the responses of the species to rapidly changing temperature and rainfall at the island (Smith 2002) have been made based on ecological information. Thus, using data on springtail abundance and distribution on Marion Island, Gabriel *et al.* (2001) argued that the invasive species are more desiccation tolerant and less sensitive to temperature extremes than are indigenous species, and they predicted that under climate warming the indigenous species should respond poorly by comparison with the invasive species. This theme was echoed by Barendse & Chown (2001) based on an analysis of the autecology of both indigenous and alien arthropods on the island. They argued that with warming, indigenous species would be unable to respond positively to high temperatures and would be substantially compromised by comparison with alien species (see also Walther *et al.* 2002; Frenot *et al.* 2005).

In testing these predictions, the effects of acclimation, rather than developmental plasticity are examined, bearing in mind the caveats of Wilson & Franklin (2002) and Woods & Harrison (2002). That is: 1. the focus is on individual traits rather than entire suites of traits encompassing fitness; 2. the organisms used are not exposed to extreme conditions when undergoing acclimation because stressful acclimation environments may be detrimental to species' fitness even before experimentation commences; 3. a suite of competing hypotheses are examined simultaneously in a strong inference approach.

## **Materials and Methods**

This study took place on the volcanic sub-Antarctic Marion Island (46°54'S, 37°45'E), which forms part of the Prince Edward Islands Group in the southern Indian Ocean (Smith 1987; Hänel & Chown 1999). The climate of Marion Island is characterised by stable, cool temperatures (annual mean *c.* 5.7 °C) showing little variation, strong winds, high mean relative humidity (83 %), and relatively high precipitation (Smith & Steenkamp 1990; Smith 2002). Despite the stable, oceanic nature of the climate at Marion Island, recent climatic changes have been rapid (Smith & Steenkamp 1990; Smith 2002). These changes are a consequence of altered atmospheric circulation patterns (Smith & Steenkamp 1990) and changes in the position of oceanic frontal systems (Pakhomov & Chown 2003). The invertebrate fauna of Marion Island has been extensively studied and includes both indigenous and invasive species (Crafford *et al.* 1986; Hänel *et al.* 1998; Chown *et al.* 2002), although the Collembola have thus far escaped physiological investigations. The majority of physiological work on springtail species from this region have concerned those inhabiting the Antarctic continent (Block



*et al.* 1978; Block 1985; Davidson & Broady 1996; Sinclair & Sjørnsen 2001). Therefore, this study also provides the first physiological information on sub-Antarctic springtail species.

#### *Collection and acclimation*

Specimens were collected from the field (below 25 m asl) with an aspirator and placed into plastic vials with moist Plaster-of-Paris substrates and clumps of detritus as a food source and for shelter. Samples were transported to the laboratory within five hours of collection, where batches of springtails were kept in 30 ml plastic vials on moist Plaster-of-Paris in temperature-controlled incubators. The four constant acclimation temperatures used were 0, 5, 15 and 20 °C, with a 9L:15D photoperiod for the 0 and 5 °C incubators and a 14L:10D photoperiod for the 15 and 20 °C incubators to simulate winter and summer temperatures and photoperiods. All sample bottles were supplied with detritus from which specimens were collected. Acclimations lasted at least seven but not more than ten days, which has been shown previously to be sufficient to allow for physiologically plastic changes (Hoffmann & Watson 1993). All experiments were also performed on field fresh individuals that were kept at ambient L:D cycles and temperatures for no longer than ten days. The species included in this study were the alien species *Pogonognathellus flavescens* Tullberg (Tomoceridae), *Isotomurus cf. palustris* Müller (Isotomidae) and *Ceratophysella denticulata* Bagnall (Hypogastruridae), and the indigenous species *Cryptopygus antarcticus* Déharveng (Isotomidae) and *Tullbergia bisetosa* Börner (Onychiuridae). Mean ( $\pm$  SE) masses ( $\mu$ g) for these species were  $2127.53 \pm 104.49$ ,  $564.65 \pm 36.58$ ,  $66.19 \pm 4.99$ ,  $71.48 \pm 4.76$  and  $53.57 \pm 3.73$   $\mu$ g, respectively.

*Desiccation tolerance*

The desiccation tolerance of field fresh springtails was determined using a 2 x 2 experimental design (see Woods & Harrison 2001) as described below. A saturated NaCl solution was chosen as a desiccant because it provides consistent relative humidity (76 %) at temperatures varying between 0 and 20 °C (Winston & Bates 1960). This relative humidity, whilst low (see Kærsgaard *et al.* 2004), is more realistic for springtail species than the dry atmosphere used in many studies of insects (Worland & Block 1986; Block *et al.* 1990). At each treatment temperature (5 or 15 °C), springtails (n = 10) from each acclimation treatment (5 or 15 °C) were placed into one of two dry plastic containers (50 ml) attached to the inside of larger plastic bottles (300 ml), the latter containing 70 ml saturated salt solution. Plastic containers were covered with 125-micron mesh to prevent specimens from escaping. Petri dishes were secured onto the large plastic containers to allow the air inside the sample bottles to equilibrate to 76 % RH.

Survival was assessed visually every five minutes, with individuals that showed no signs of movement being scored as dead. Survival time was compared amongst species using the field fresh data and a generalized linear model assuming Poisson error structures and using a logarithmic link function. Within each species, the effects of each treatment and interactions between them were also examined using generalized linear models assuming Poisson error structures and using logarithmic link functions. The 95 % confidence intervals of the weighted marginal means were used for comparisons

among groups. Goodness of fit was assessed using the deviance statistic and the models were corrected for overdispersion.

#### *Cooling rate and crystallization temperature*

Springtails are known for their ability to supercool to increase winter survival, and are all freeze intolerant, a strategy to which they are phylogenetically constrained (Cannon & Block 1988). Thus, crystallization temperature (or supercooling point) is an important parameter describing springtail cold hardiness (Cannon & Block 1988), though perhaps not sufficient to entirely characterize the cold hardiness response (see Chown & Nicolson 2004 and below). Because cooling rate can have a pronounced effect on crystallization temperature ( $T_c$ ) (see Salt 1966 for rationale), the effect of cooling rate on  $T_c$  was investigated. For these trials, three cooling rates were employed using field fresh springtails, as well as springtails acclimated at 5 and 15 °C. Crystallization temperatures were measured using a Mettler-Toledo Differential Scanning Calorimeter (DSC) 820 (Mettler-Toledo Ltd, Leicester, UK) incorporating a mechanical intra-cooler (Lab-Plant Ltd, Huddersfield, UK) capable of cooling to -60 °C. Data were analysed using the STARe software package (Mettler-Toledo). The system was calibrated with indium (melting point 156.6 °C, enthalpy of 28.71 J.g<sup>-1</sup>) and regularly checked by measuring the melting point of 0.5µl drops of HPLC grade water. Springtails were hermetically sealed in 40µl aluminium pans and cooled at one of three cooling rates. The first cooling rate protocol used was the standard 1 °C.min<sup>-1</sup> cooling rate (e.g. Block & Worland 2001), whereas the second protocol employed a slower cooling rate of 0.1 °C.min<sup>-1</sup>. For both of these protocols, samples were cooled from 5 to -30 °C at their respective cooling rates, following a 5 min equilibration period at 5 °C. In the third

protocol, however, a slow-cooling approach, similar to that used in assessments of rapid responses of the Tc to changing temperature (Worland & Convey 2001), was used. Here, cooling commenced at 5 °C and the temperature was lowered to -1 °C at 0.5 °C.h<sup>-1</sup>. From -1 to -30 °C a cooling rate of 0.1 °C.min<sup>-1</sup> was employed. This latter treatment also enabled a comparison of the response of Tc to what has been termed rapid cold hardening, because in springtails Tc is often equivalent to death (Lee *et al.* 1987; Worland & Convey 2001), of specimens acclimated to 5 °C and to 15 °C. The lowest temperature recorded prior to a freezing exotherm was taken as the Tc of that individual in all instances. Experiments performed in the DSC precluded investigations on *P. flavescens* due to the large size of this species. Sample sizes exceeded 20 in all instances. Crystallization temperatures following different cooling rates were compared within species for field fresh, 5 and 15 °C treatments using generalized linear models (assuming normal errors and using identity link functions).

#### *Prefreeze mortality*

Recent studies have shown that temperatures of crystallization alone are insufficient to characterize the cold hardiness strategy of a given species because injury or death might take place either before or after freezing (see review in Chown & Nicolson 2004). Prefreeze mortality is of special concern in species that are considered freeze intolerant because the crystallization temperature might not be indicative of the lower lethal temperature (Bale 1993). It is generally accepted that for springtails, the temperature at which mortality is experienced (the lower lethal temperature or LLT) is equal to the Tc (however, see Nedved *et al.* 1995).

Here, experiments were conducted to determine the extent of mortality (if any) due to chilling injury (LLT). In other words, whether LLT lay close to or at the  $T_c$  of each of the species following a 15 °C acclimation treatment, and slow cooling as described previously. To test the idea that mortality only occurs on freezing in springtails, a survival experiment was conducted. Springtails were acclimated at 15 °C for seven days, thus ensuring that physiological cold hardiness was minimised to a similar extent in all species. In the survival (LLT) experiments, batches of 10 springtails were placed in perforated Eppendorfs, which in turn were placed into 10 ml plastic tubes, each containing moist paper towel. This prevented specimens from dehydrating at temperatures above zero, whilst preventing contact with moisture, thus preventing inoculative freezing at subzero temperatures. Thirteen numbered tubes, each containing 10 specimens, were submerged in a Grant LTC 12 water bath, programmed to cool from 5 to -1 °C at 0.5 °C.h<sup>-1</sup>, and then from -1 to -25 °C at 0.1 °C.min<sup>-1</sup>. A thermocouple (Type T, 40 gauge) was placed inside a tube (as described above) and connected to a handheld thermometer. Starting at -1 °C, one numbered tube containing 10 springtails was removed from the waterbath with every 2 °C drop in waterbath temperature, as recorded by the thermocouple. Upon removal from the waterbath, each tube was placed in an incubator set at 0 °C until two hours after the last tube from that experiment was placed in the incubator, after which all tubes from that experiment were moved to an incubator set to 5 °C. After 24 hours at 5 °C, survival was scored. Individuals that showed no signs of movement were classified as dead, while those that did move were classified as alive. Survival scores ranging between 0 and 100 % were obtained using this method. Logistic regression was used to determine the temperature at which 50 % (LT50) of the sample population died for each species. LT100 was taken as the actual temperature at which the sample population suffered 100 % mortality.

If prefreeze mortality does not occur in springtails, as is regularly assumed (Sømme 1982), then the  $T_c$  must represent the point of death. Based on this assumption, a survival curve can be constructed by converting the point at freezing of each animal in a sample population to the proportion of the population that has died. The  $T_c$  data can then be converted to survival data and compared with survival experiments undertaken under either the same conditions (as above) or under a different set of conditions. This can be done either by comparison of the logistic regression curves or using the Kaplan-Meier product limit estimation technique (Hertzberg & Leinaas 1998). The latter procedure was followed here, by examining the  $T_c$ s of a sample of springtails cooled under the same conditions using the differential scanning calorimeter. Owing to instrument time constraints, repeated logistic regression curves were not constructed using the DSC approach, and only a single curve was available. Thus, the Kaplan Meier method was used to compare this curve to the summed data from the water bath experiment described above, within each species (excluding *P. flavescens* because of their large size). The two sets of logistic curves were also inspected visually and these, together with the Kaplan-Meier statistics are reported.

#### *Lower lethal temperatures (mortality assays)*

To test the responses of lower lethal temperatures to different treatment temperatures (0, 5, 15 and 20 °C), mortality assays were conducted on springtails exposed to different test temperatures. Because the slow cooling approach (as implemented in the cooling rate trials) was time consuming (17 hours per experiment), mortality assays (lower lethal temperature or LLT) were conducted to test for lethal limit responses to

acclimation treatments. For LLT experiments, batches of ten individuals were placed into plastic tubes containing dry Whatman's filter paper, which were submerged into Grant LTD20 or LTC12 water baths at a set temperature for one hour. Tubes were removed from the water bath, supplied with moist filter paper, and transferred to an incubator (at the original acclimation temperature, except for field fresh batches that were transferred to a 10 °C incubator). Survival was scored after 24 h. Individuals that showed movement were scored as alive, whereas those that were motionless were scored as dead. Waterbath temperature was altered by 1 °C and the experiment repeated with new individuals until the temperature range encompassed 5 to 95 % survival. The procedure was replicated at least four times, but generally five times at each experimental temperature for field fresh and each of the treatment temperatures for each species. Logistic regression was used to determine the temperature at which 50 % (LT50) of the sample population died for each species and acclimation temperature (0, 5, 15 and 20 °C). Only datasets for which significant Wald's statistics were obtained were used in comparisons within treatments. LT100 was taken as the actual temperature at which the sample population suffered 100 % mortality. LT50 and LT100 values were compared within species and between acclimation temperatures using ANOVA. Differences between treatments were determined based on Tukey's Honest Significant Difference.

#### *Responses of crystallization temperatures to acclimation*

The responses of springtail T<sub>c</sub> to acclimation were investigated using two indigenous and two alien springtail species. The T<sub>c</sub> of at least 20 individual specimens per species and treatment (0, 5, 15 and 20 °C) were determined with Type T copper-constantan

thermocouples (40-gauge) connected to a Campbell CR10 datalogger. Specimens ( $n = 10$ ) were placed in vacutests (BD Vacutainer Systems, UK) and submerged in a Grant LTC 12 waterbath at  $0\text{ }^{\circ}\text{C}$  for those animals acclimated at  $0\text{ }^{\circ}\text{C}$ , and  $5\text{ }^{\circ}\text{C}$  for field fresh animals and those acclimated at 5, 15 and  $20\text{ }^{\circ}\text{C}$ , respectively. Waterbath temperature was lowered by  $0.1\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$  after a 15 minute equilibration period at  $0\text{ }^{\circ}\text{C}$ . The lowest temperature recorded prior to the onset of a freezing exotherm was taken as the  $T_c$  for that individual. These experiments were also conducted on field fresh individuals for each of the species investigated to assess baseline (field) supercooling ability. Because the  $T_c$  values were not normally distributed (Rothery & Block 1992), data were analysed using generalized linear models assuming normal errors and using identity link functions. Comparisons were made within species between treatments (0, 5, 15 and  $20\text{ }^{\circ}\text{C}$  for two indigenous and two alien species, and 5 and  $15\text{ }^{\circ}\text{C}$  for the alien *C. denticulata*).

#### *Upper lethal temperatures*

For upper lethal temperature (ULT) experiments, the same experimental procedure was used as for the LLT mortality assay method, except that moist Whatman's filter paper was used in vials containing springtails. Conducting upper thermal tolerances in a saturated environment removes the confounding effects of desiccation on survival when assessing tolerance of high temperatures (Hoffmann *et al.* 2003). The data from both upper and lower lethal temperature experiments were analysed using logistic regressions, which allowed calculation of the temperature at which 50 % (LT50) of the sample population died for each species. LT50 in this study referred to the temperature at which 50 % of a sample died due to thermal stress. The highest temperature at which



more than 95 % of the sample population died was taken as the LT100 for upper thermal tolerances. For the two alien springtail species, four acclimation temperatures were used (0, 5, 15 and 20 °C), but the two indigenous springtail species showed considerable mortality at the 20 °C acclimation, hence experiments were conducted following acclimation at three temperatures only (0, 5 and 15 °C). LT50s and LT100s were compared within species, between treatments using ANOVA. Differences between treatments were determined based on Tukey's Honest Significant Difference.

## Results

*Pogonognathellus flavescens* showed significantly greater desiccation tolerance than the other species studied, followed by the indigenous *C. antarcticus*. The alien *C. denticulata* had the highest desiccation tolerance for the smaller springtails investigated in this study, and *I. palustris* and *T. bisetosa*, which were least desiccation tolerant, did not differ significantly (Table 1).

Table 1. Mean  $\pm$  SE, minimum (min) and maximum (max) desiccation survival times (minutes) for field fresh springtails. Different letters for desiccation survival times denote significantly different means based on the 95 % confidence intervals of the weighted marginal means obtained from fitting a generalized model with Poisson errors and a log link function to the data. The chi-square statistic and significance of the model are shown.

	<b>n</b>	<b>Mean <math>\pm</math> SE</b>	<b>Min</b>	<b>Max</b>	<b>Range</b>
<b>Alien</b>					
<i>Pogonognathellus flavescens</i>	20	787.5 $\pm$ 44.26 <sup>d</sup>	550	1285	735
<i>Isotomurus palustris</i>	20	44.5 $\pm$ 3.77 <sup>a</sup>	25	90	65
<i>Ceratophysella denticulata</i>	20	129.8 $\pm$ 7.17 <sup>b</sup>	65	200	135
<b>Indigenous</b>					
<i>Cryptopygus antarcticus</i>	20	397.3 $\pm$ 25.58 <sup>c</sup>	235	670	435
<i>Tullbergia bisetosa</i>	20	51.8 $\pm$ 3.74 <sup>a</sup>	25	100	75
$\chi^2_{(4)}=1388.8$	p<0.0001				

At a treatment temperature of 15 °C individuals of *Pogonognathellus flavescens* held at a 15 °C acclimation temperature survived for longer than those held at 5 °C, whereas at a treatment temperature of 5 °C no acclimation effect was visible. Thus, this species showed significant acclimation, treatment, and acclimation by treatment effects (Table 2, Fig. 1a). *Isotomurus cf. palustris* survived for longer at both 5 and 15 °C treatment temperatures following acclimation at 15 °C than after acclimation at 5 °C. In other words, there were significant treatment and acclimation effects in this species, but no acclimation by treatment interaction (Table 2, Fig.1b). The desiccation tolerance response of the alien *C. denticulata* differed from that of the other two alien species, in that no acclimation effect was found. *C. denticulata* individuals survived for longer at each treatment temperature following acclimation at that temperature (i.e. survival was greater at 5 °C following acclimation at 5 °C, and greater at 15 °C following

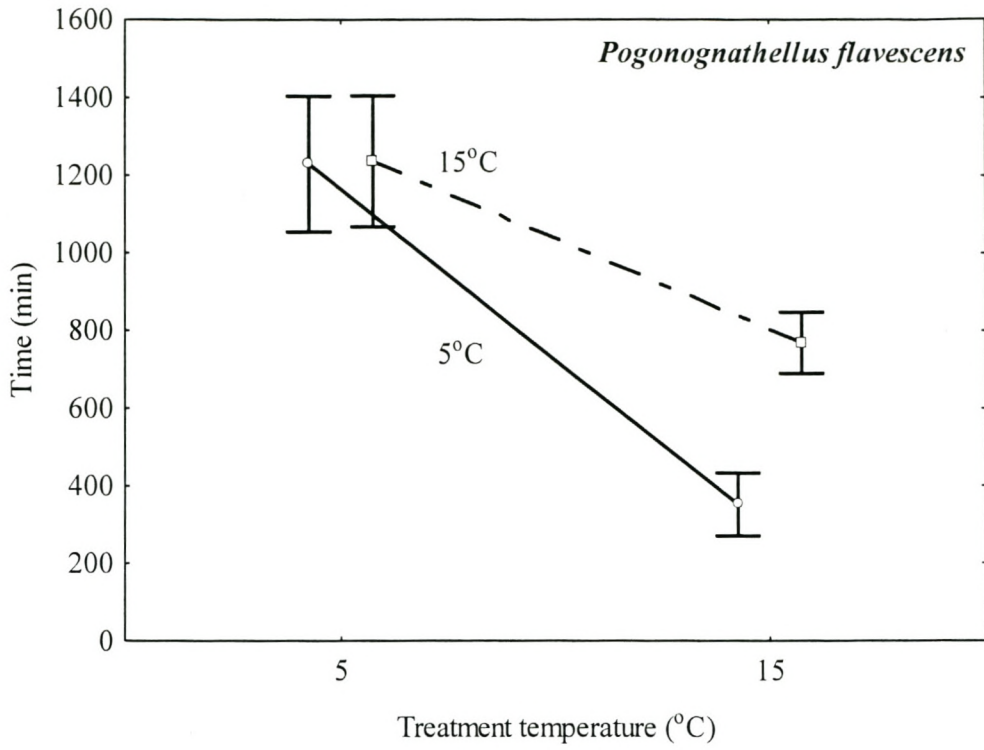
acclimation at 15 °C) (Fig. 1c), and there were significant treatment and acclimation by treatment effects (Table 2). *Cryptopygus antarcticus* survived for longer at a 5 °C treatment temperature following acclimation at 5 °C, and performed slightly better at a treatment temperature of 15 °C following acclimation at 15 °C (Fig. 1d). There were significant acclimation, treatment, and acclimation by treatment effects in *C. antarcticus* (Table 2). *T. bisetosa* performed better at both the 5 and 15 °C treatment temperatures following acclimation at 5 °C (Fig. 1e). In this species there were significant acclimation and treatment effects, but there was no acclimation by treatment interaction (Table 2).

Table 2. Outcomes of the Generalized linear models (Type III, corrected for overdispersion) for springtail desiccation. Goodness of fit (deviance/df) is given for each species.

	Alien		Indigenous	
	<i>Pogonognathellus flavescens</i>		<i>Cryptopygus antarcticus</i>	
Acclimation	$\chi^2_{(1)}=25.2$	p<0.0001	$\chi^2_{(1)}=4.95$	p<0.05
Treatment	$\chi^2_{(1)}=130.28$	p<0.0001	$\chi^2_{(1)}=169.83$	p<0.0001
Acclimation x Treatment	$\chi^2_{(1)}=24.49$	p<0.0001	$\chi^2_{(1)}=7.53$	p<0.05
Goodness of fit	6734.75/76 = 88.62*		4767.63/76 = 62.73*	
	<i>Isotomurus palustris</i>		<i>Tullbergia bisetosa</i>	
Acclimation	$\chi^2_{(1)}=4.23$	p<0.05	$\chi^2_{(1)}=16.23$	p<0.0001
Treatment	$\chi^2_{(1)}=26.45$	p<0.0001	$\chi^2_{(1)}=12.64$	p<0.001
Acclimation x Treatment	$\chi^2_{(1)}=0.12$	p>0.05	$\chi^2_{(1)}=0.33$	p>0.05
Goodness of fit	928.77/76 = 12.22*		524.75/76 = 6.90*	
	<i>Ceratophysella denticulata</i>			
Acclimation	$\chi^2_{(1)}=2.42$	p>0.05		
Treatment	$\chi^2_{(1)}=230.79$	p<0.0001		
Acclimation x Treatment	$\chi^2_{(1)}=6.30$	p<0.05		
Goodness of fit	1494.23/76 = 19.66*			

\*Scaled deviance = 1

A



B

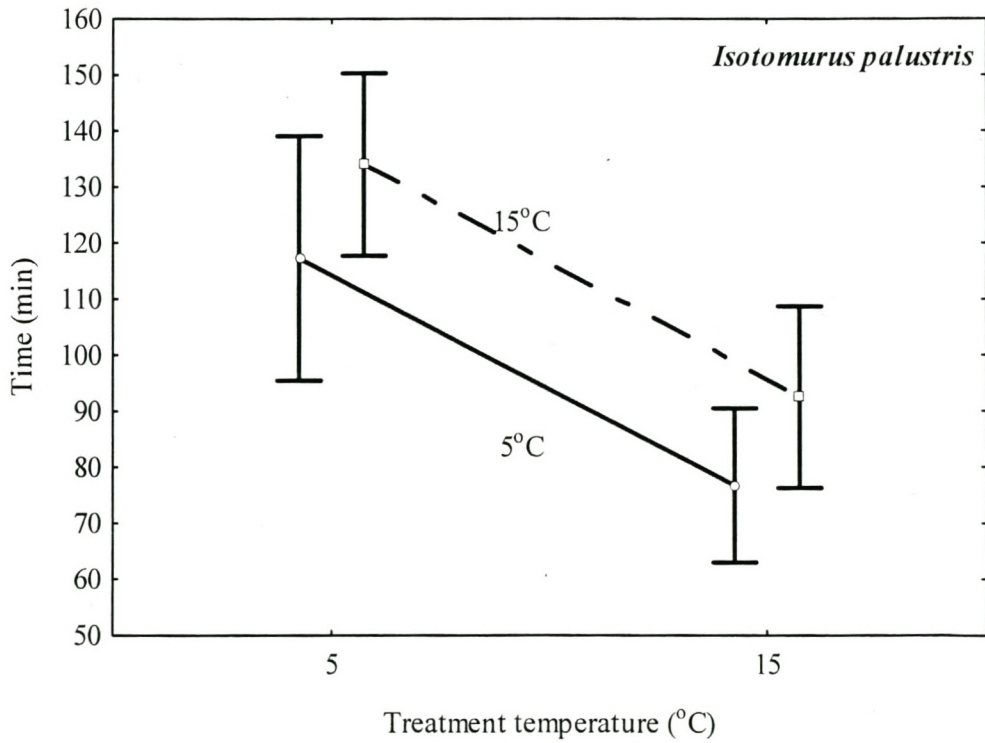
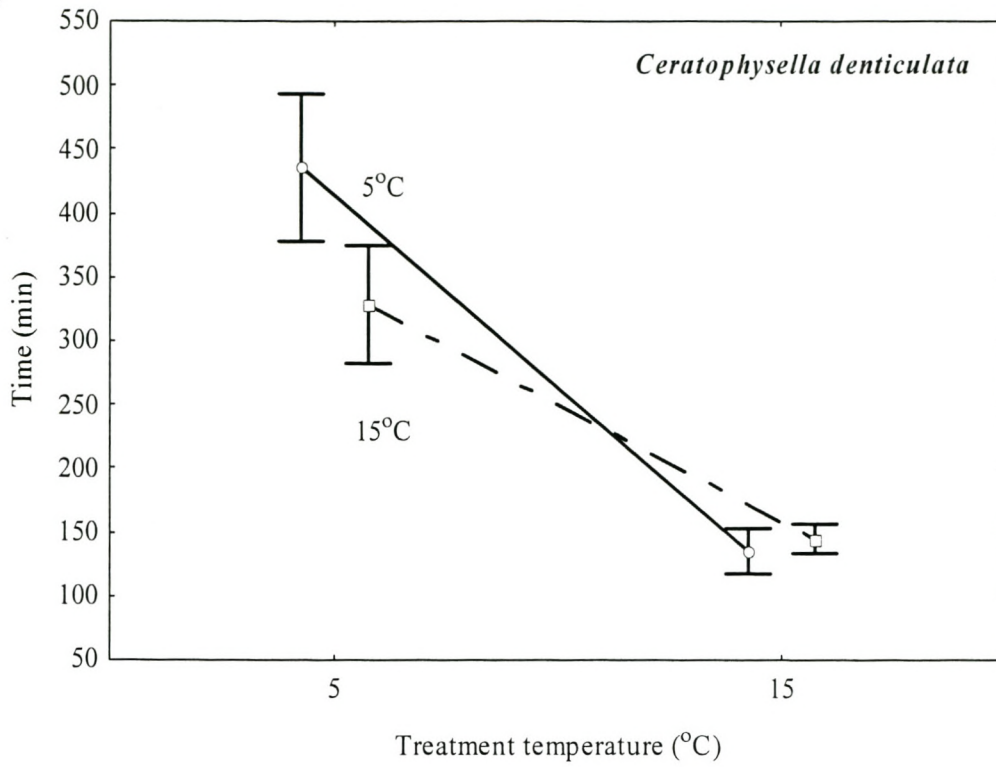


Figure 1 A and B

C



D

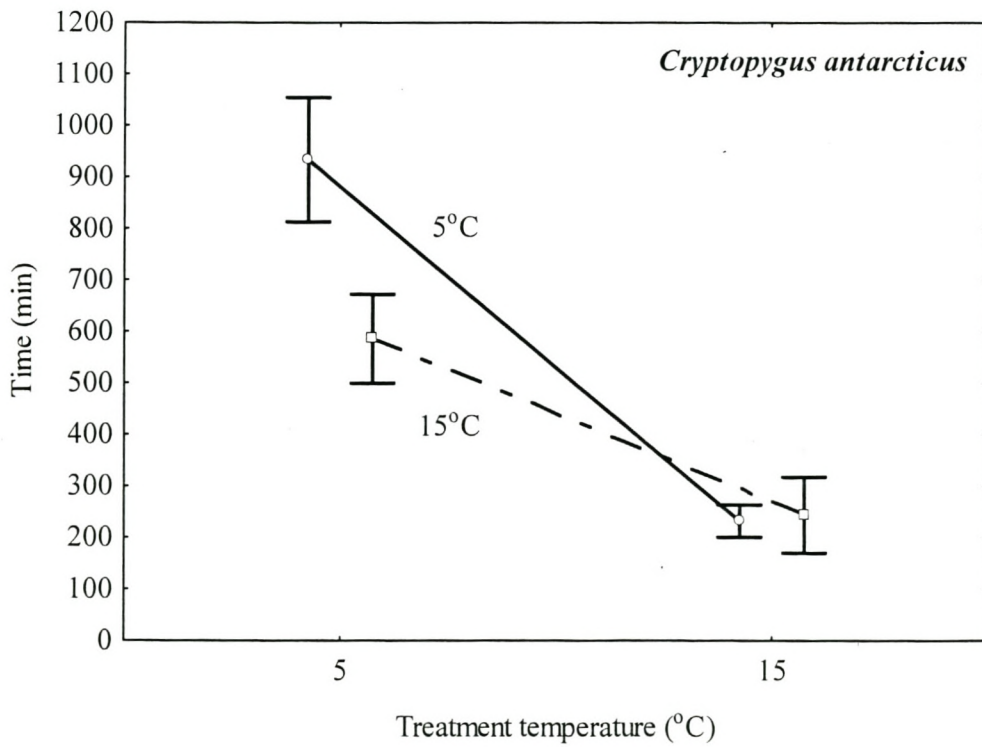


Figure 1 C and D

E

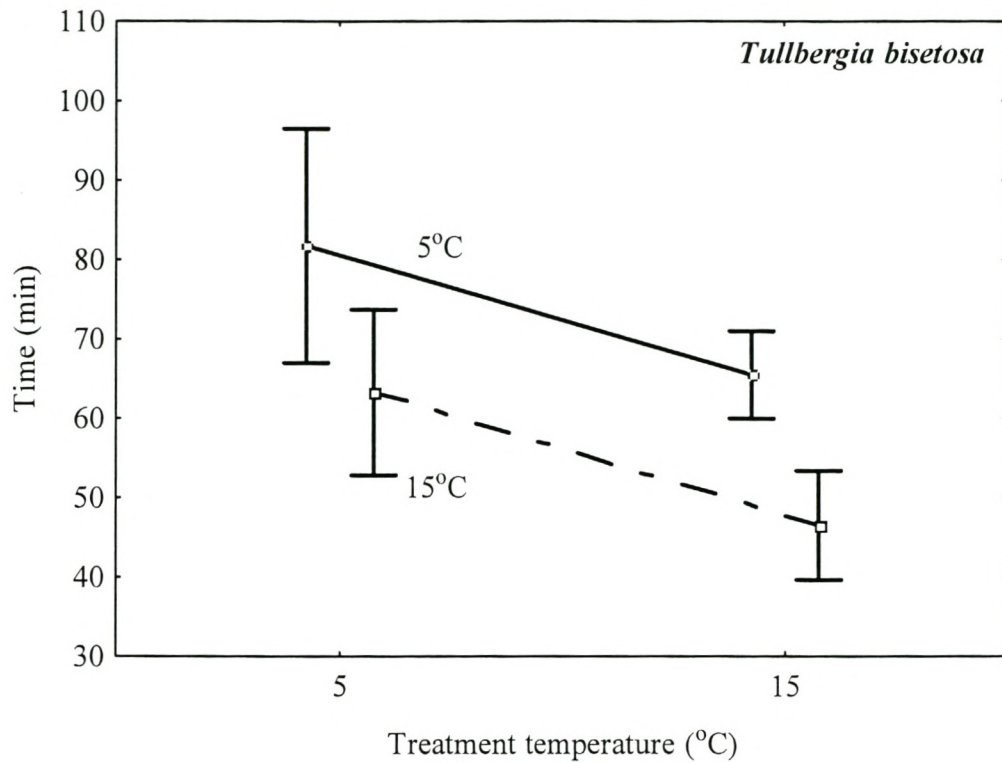


Figure 1. Springtail desiccation treatment x acclimation effects. Treatment temperatures were 5 and 15 °C, and acclimation temperatures were 5 and 15 °C. Solid lines represent springtails acclimated at 5 °C, whereas dotted lines represent springtails acclimated at 15 °C. A. *Pogonognathellus flavescens*; B. *Isotomurus palustris*; C. *Ceratophysella denticulata*; D. *Cryptopygus antarcticus*; E. *Tullbergia bisetosa*.

Field fresh individuals of the invasive alien species in this study generally had similar Tcs to the indigenous species. *I. palustris*, however, had a significantly higher mean Tc than all other species in this study, whereas the indigenous *T. bisetosa* displayed the lowest mean Tc (Table 3). Field fresh Tcs in this study ranged between -8.2 °C (*I. palustris*) and -19.4 °C (*T. bisetosa*).

Table 3. Summary statistics for crystallization temperatures (°C) of field-fresh Collembola. Different letters for crystallization temperatures denote significantly different means based on the 95 % confidence intervals of the weighted marginal means obtained from fitting a generalized model with normal errors and an identity link function to the data. The chi-square statistic and significance of the model are shown.

	<b>n</b>	<b>Mean</b>	<b>± SE</b>	<b>Min</b>	<b>Max</b>	<b>Median</b>	<b>Range</b>
<b>Alien</b>							
<i>Pogonognathellus flavescens</i>	31	-12.0	± 1.00 <sup>ab</sup>	-21.5	-3.2	-11.2	18.3
<i>Isotomurus palustris</i>	24	-8.2	± 1.24 <sup>a</sup>	-24.4	-3.5	-5.2	20.9
<i>Ceratophysella denticulata</i>	46	-14.3	± 0.93 <sup>b</sup>	-22.9	-3.1	-13.9	19.8
<b>Indigenous</b>							
<i>Cryptopygus antarcticus</i>	25	-13.5	± 0.71 <sup>b</sup>	-22.6	-8.8	-13.0	13.8
<i>Tullbergia bisetosa</i>	20	-19.4	± 0.90 <sup>c</sup>	-25.2	-12.6	-20.9	12.6
$\chi^2_{(4)}=43.93$	p<0.0001						



Cooling rate did not affect Tcs, and where cooling rate effects were found, changes in mean Tc were typically small (2 to 3 °C for *I. palustris* and *C. antarcticus*), except for field fresh *C. denticulata* (5.3 °C) (Table 4). The significantly lower mean Tc shown by field fresh *C. denticulata* cooled at a slow rate was not found following either a 5 or 15 °C acclimation (Table 4). Table 4 also summarizes the test of RCH effects on Tcs within species and treatment (0.1 °C.min<sup>-1</sup> cooling rate vs. the RCH protocol described in the Materials and Methods section). Except for *I. palustris* (following a 15 °C acclimation) and *T. bisetosa* (following a 5 °C acclimation), no RCH effects were found. Where significant effects were found, they were typically small (*c.* 3 °C).

Table 4. Summary statistics of the effect of cooling rate (fast (1 °C.min<sup>-1</sup>), medium (0.1 °C.min<sup>-1</sup>) and slow rates (cooling from 5 °C to -1 °C at 0.5 °C.h<sup>-1</sup>, then cooling from -1 °C to -30 °C at 0.1 °C.min<sup>-1</sup>, and finally warming from -30 °C to 0 °C at 0.1 °C.min<sup>-1</sup>) on crystallization temperature (°C ± SE) for unacclimated (field fresh (FF)) *Collembola*, and those acclimated at 5 and 15 °C. Statistics for a test of rapid cold hardening (RCH) effects (comparing medium and slow rates within species and treatment) are also shown. Generalized linear models with normal errors and identity link functions were fitted to the data. The chi-square statistics of these models are shown.

Species	Rate	n	Mean ± SE	Statistics		
				Cooling rate	RCH	
<b>Alien</b>						
<i>Isotomurus palustris</i>	FF	Fast	28	-9.1 ± 0.71	$\chi^2_{(2)}=2.88$ p>0.05	$\chi^2_{(1)}=0.03$ p>0.05
		Medium	32	-7.9 ± 0.41		
		Slow	20	-7.8 ± 0.83		
	5 °C	Fast	43	-5.9 ± 0.55	$\chi^2_{(2)}=0.77$ p>0.05	$\chi^2_{(1)}=0.57$ p>0.05
		Medium	40	-5.3 ± 0.68		
		Slow	25	-6.1 ± 0.72		
	15 °C	Fast	46	-6.3 ± 0.53	$\chi^2_{(2)}=14.29$ p<0.05	$\chi^2_{(1)}=14.49$ p<0.001
		Medium	42	-4.4 ± 0.32		
		Slow	20	-7.5 ± 0.92		
<i>Ceratophysella denticulata</i>	FF	Fast	40	-10.8 ± 0.95	$\chi^2_{(2)}=12.99$ p<0.05	$\chi^2_{(1)}=1.50$ p>0.05
		Medium	46	-14.3 ± 0.93		
		Slow	24	-16.1 ± 1.10		
	5 °C	Fast	49	-17.0 ± 0.96	$\chi^2_{(2)}=2.83$ p>0.05	$\chi^2_{(1)}=1.60$ p>0.05
		Medium	58	-17.7 ± 0.78		
		Slow	31	-19.3 ± 0.95		
	15 °C	Fast	65	-20.7 ± 0.53	$\chi^2_{(2)}=3.78$ p>0.05	$\chi^2_{(1)}=2.85$ p>0.05
		Medium	58	-19.4 ± 0.72		
		Slow	40	-21.2 ± 0.71		

Table 4. Continued

Species	Rate	n	Mean	±	SE	Statistics	
						Cooling rate	RCH
<b>Indigenous</b> <i>Cryptopygus antarcticus</i>	FF	Fast	86	-15.7	± 0.47	$\chi^2_{(2)}=10.40$ p<0.05	$\chi^2_{(1)}=0.00$ p>0.05
		Medium	53	-13.0	± 0.75		
		Slow	22	-13.0	± 1.56		
	5 °C	Fast	44	-13.3	± 0.87	$\chi^2_{(2)}=0.12$ p>0.05	$\chi^2_{(1)}=0.01$ p>0.05
		Medium	50	-12.9	± 0.90		
		Slow	29	-13.1	± 1.30		
	15 °C	Fast	41	-11.6	± 1.07	$\chi^2_{(2)}=1.30$ p>0.05	$\chi^2_{(1)}=1.27$ p>0.05
		Medium	43	-10.8	± 1.10		
		Slow	24	-12.9	± 1.42		
<i>Tullbergia bisetosa</i>	FF	Fast	37	-14.4	± 0.78	$\chi^2_{(2)}=2.64$ p>0.05	$\chi^2_{(1)}=0.63$ p>0.05
		Medium	41	-12.4	± 0.88		
		Slow	25	-13.5	± 1.10		
	5 °C	Fast	48	-14.8	± 0.65	$\chi^2_{(2)}=5.72$ p>0.05	$\chi^2_{(1)}=4.78$ p<0.05
		Medium	52	-14.0	± 0.76		
		Slow	30	-16.9	± 1.13		
	15 °C	Fast	45	-15.2	± 0.87	$\chi^2_{(2)}=0.93$ p>0.05	$\chi^2_{(1)}=0.87$ p>0.05
		Medium	50	-15.5	± 0.87		
		Slow	29	-14.1	± 1.20		

The logistic regressions of both the survival and T<sub>c</sub> experiments (Fig. 2a-d) indicated that prefreeze mortality (PFM) did not occur in any of the species investigated in this study (based on Kaplan-Meier statistics; Fig. 2, although the survival from T<sub>c</sub> curve appeared different from the survival curve for *T. bisetosa* (Fig. 2d)). In the mortality assay (LLT) experiment (Table 5), limited prefreeze mortality was found because LLT values were generally higher than the values found for T<sub>c</sub> (Table 3). This apparent prefreeze mortality was probably a consequence of very rapid cooling associated with the plunge technique and may well have involved freezing too. The differences between this technique and those employed for assessing T<sub>c</sub> and responses to slow cooling are clearly illustrated in Figure 3, where the three logistic regression curves are plotted against each other assuming that T<sub>c</sub> is equivalent to death of an individual (see Methods). Of all field fresh springtail species in this study, the alien *C. denticulata* had the lowest LT<sub>100</sub> (-17 °C), and *I. palustris*, the highest LT<sub>100</sub> (-9.4 °C) (Table 5). When LT<sub>50</sub>s were considered, both *C. denticulata* and the indigenous *T. bisetosa* had a mean LT<sub>50</sub> of -11.1 °C, representing the lowest LT<sub>50</sub>s (Table 5).

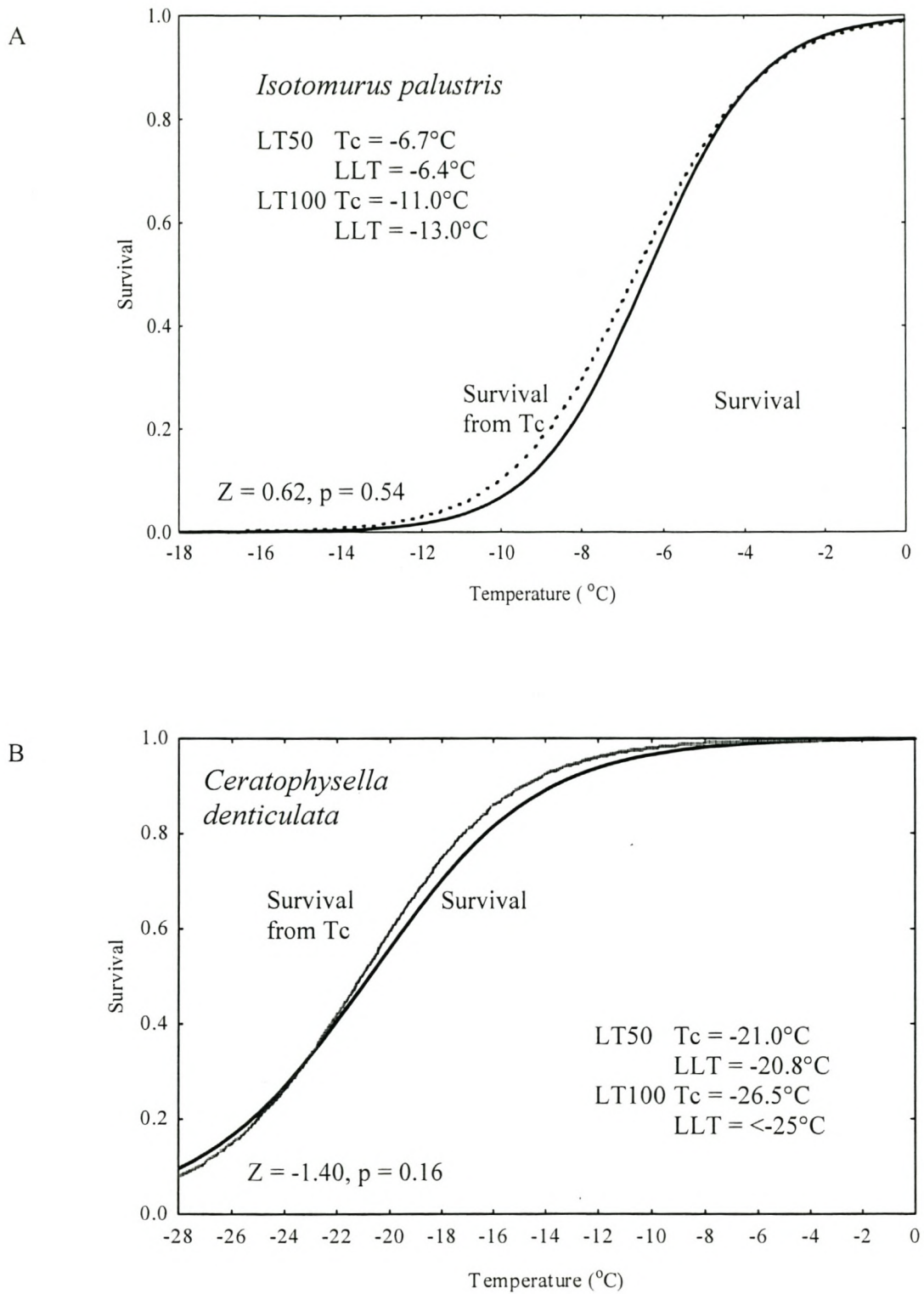


Figure 2 A and B

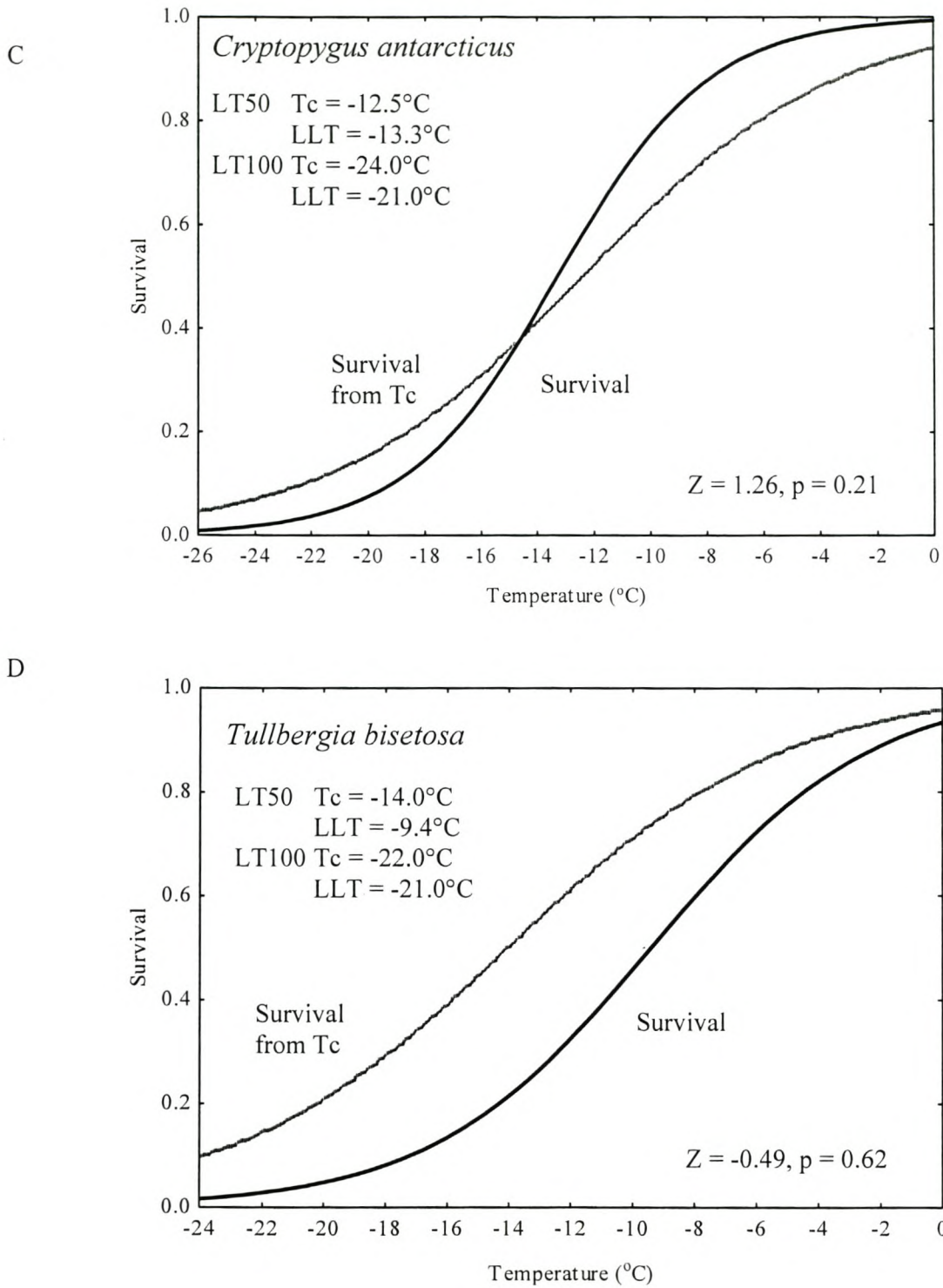


Figure 2. Survival curves of Collembola acclimated at 15 °C. Curves were constructed using logistic regression statistics, but comparisons between curves were made using the Kaplan-Meier statistic ( $Z$ ), which is reported here. A. *Isotomurus palustris*; B. *Ceratophysella denticulata*; C. *Cryptopygus antarcticus*; D. *Tullbergia bisetosa*.

Table 5. Summary statistics of lower lethal temperatures (°C) of field fresh springtails

	<b>n</b>	<b>Mean ± SE</b>	<b>Minimum</b>	<b>Maximum</b>
<b>LLT100</b>				
<b>Alien</b>				
<i>Pogonognathellus flavescens</i>	5	-12.0 ± 0.00 <sup>bc</sup>	-12.0	-12.0
<i>Isotomurus palustris</i>	5	-9.4 ± 0.24 <sup>a</sup>	-10.0	-9.0
<i>Ceratophysella denticulata</i>	5	-17.0 ± 0.00 <sup>d</sup>	-17.0	-17.0
<b>Indigenous</b>				
<i>Cryptopygus antarcticus</i>	4	-11.5 ± 0.65 <sup>b</sup>	-13.0	-10.0
<i>Tullbergia bisetosa</i>	5	-13.0 ± 0.00 <sup>c</sup>	-13.0	-13.0
ANOVA $F_{(4,19)}=119.08$		$p<0.0001$		
<b>LLT50</b>				
<b>Alien</b>				
<i>Pogonognathellus flavescens</i>	5	-6.6 ± 0.38 <sup>a</sup>	-8.1	-6.0
<i>Isotomurus palustris</i>	5	-6.3 ± 0.05 <sup>a</sup>	-6.4	-6.1
<i>Ceratophysella denticulata</i>	5	-11.1 ± 0.42 <sup>c</sup>	-12.5	-10.0
<b>Indigenous</b>				
<i>Cryptopygus antarcticus</i>	4	-9.3 ± 0.62 <sup>b</sup>	-10.7	-7.9
<i>Tullbergia bisetosa</i>	5	-11.1 ± 0.15 <sup>c</sup>	-11.4	-10.5
ANOVA $F_{(4,19)}=45.02$		$p<0.0001$		

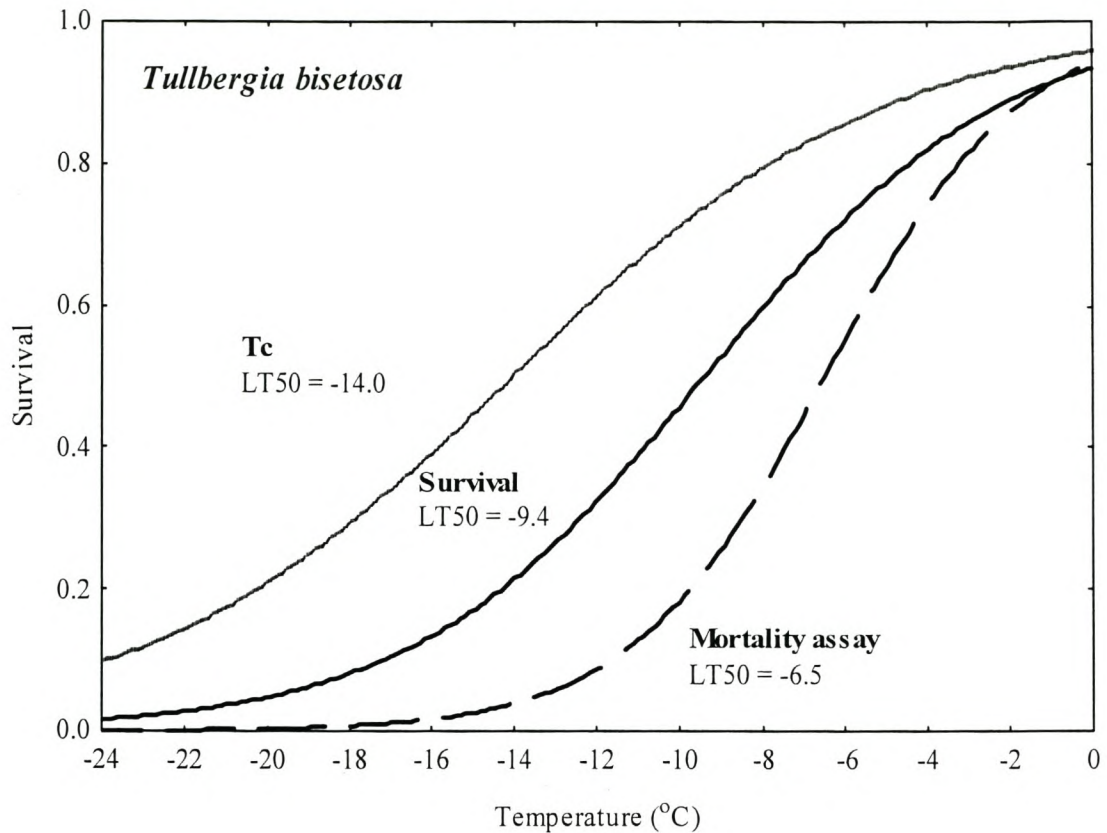


Figure 3. Survival curves of *Tullbergia bisetosa* following acclimation at 15 °C. Curves were constructed using logistic regressions and data were obtained following treatment using three different experimental protocols (Survival from Tc, survival following slow-cooling protocol in a waterbath, and the mortality assay, rapid-cooling protocol; see Methods section for details).



In *Pogonognathellus flavescens* mean Tc declined, whereas mean LLT50 increased significantly with increasing acclimation temperatures (Table 6, Fig. 4a). In *I. palustris*, both mean Tc and LLT50 showed a positive relationship with acclimation temperature (Table 6, Fig. 4b), indicating a significant decrease in cold hardiness following acclimation to high temperature. In *C. denticulata*, acclimation did not have a significant effect on Tc (Table 6, Fig. 4c). As was the case for *I. palustris*, *C. antarcticus* showed significant increases in both Tc and LLT50 with increased acclimation temperature, although the Tc response was not significant (Table 6, Fig. 4d). *T. bisetosa* also showed increased mean Tc with increased acclimation temperatures, and although the LLT50 response was significant, a 5 °C acclimation effected a lower LLT50 than either the 0 or 15 °C acclimations (Table 6, Fig. 4e).

Table 6. Summary statistics for crystallization temperatures (°C) of alien and indigenous springtails acclimated at 0, 5, 15 and 20 °C.

	<b>n</b>	<b>Mean</b>	<b>± SE</b>	<b>Median</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Range</b>
<b>Alien</b>							
<i>Pogonognathellus flavescens</i>							
0°C	22	-11.7	± 0.97 <sup>a</sup>	-11.2	-22.9	-3.8	19.1
5°C	22	-11.5	± 1.34 <sup>a</sup>	-12.5	-21.6	-3.0	18.6
15°C	25	-13.8	± 1.29 <sup>ab</sup>	-16.3	-21.9	-3.5	18.4
20°C	21	-17.8	± 1.22 <sup>b</sup>	-18.2	-25.4	-5.6	19.8
$\chi^2_{(3)}=15.73$		p<0.0001					
<i>Isotomurus palustris</i>							
0°C	20	-9.8	± 0.73 <sup>b</sup>	-8.5	-17.9	-5.1	12.8
5°C	20	-7.2	± 1.25 <sup>ab</sup>	-4.3	-20.8	-3.1	17.7
15°C	20	-5.2	± 0.52 <sup>a</sup>	-4.3	-9.8	-3.0	6.8
20°C	20	-5.4	± 0.30 <sup>a</sup>	-4.8	-8.4	-4.2	4.2
$\chi^2_{(3)}=20.65$		p<0.001					
<i>Ceratophysella denticulata</i>							
5°C	58	-17.7	± 0.78	-19.1	-24.4	-2.3	22.1
15°C	58	-19.4	± 0.72	-21.6	-25.4	-6.1	19.3
$\chi^2_{(1)}=2.54$		p>0.05					
<b>Indigenous</b>							
<i>Cryptopygus antarcticus</i>							
0°C	20	-19.6	± 1.09	-20.3	-25.4	-9.1	16.3
5°C	20	-19.1	± 1.18	-19.8	-25.6	-9.6	16.0
15°C	17	-15.7	± 1.18	-14.8	-23.2	-5.5	17.7
20°C	8	-17.8	± 1.83	-19.1	-22.6	-6.1	16.5
$\chi^2_{(3)}=6.54$		p>0.05					
<i>Tullbergia bisetosa</i>							
0°C	20	-18.6	± 0.80 <sup>b</sup>	-19.0	-22.6	-10.9	11.7
5°C	20	-17.5	± 0.81 <sup>b</sup>	-17.6	-22.4	-7.8	14.6
15°C	20	-17.6	± 1.16 <sup>b</sup>	-17.4	-24.1	-9.3	14.8
20°C	20	-12.0	± 1.05 <sup>a</sup>	-11.3	-23.1	-5.9	17.2
$\chi^2_{(3)}=25.72$		p<0.0001					

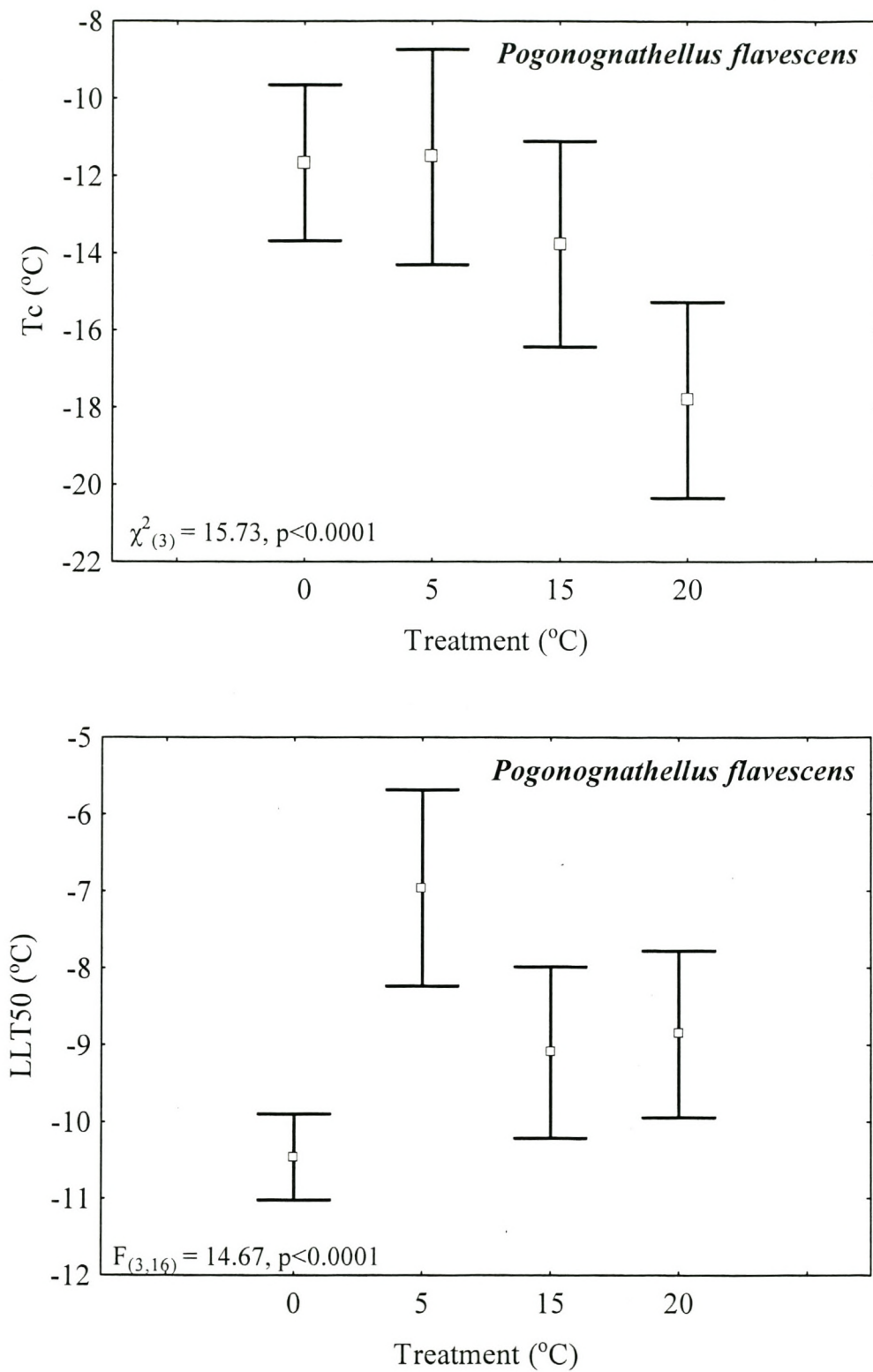


Figure 4 A

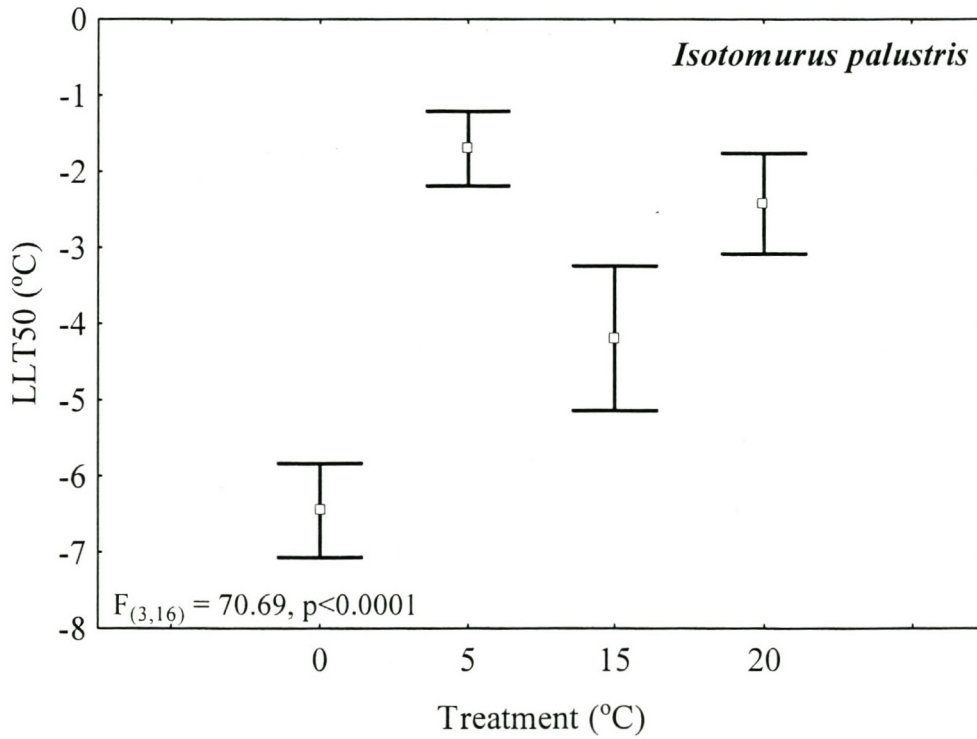
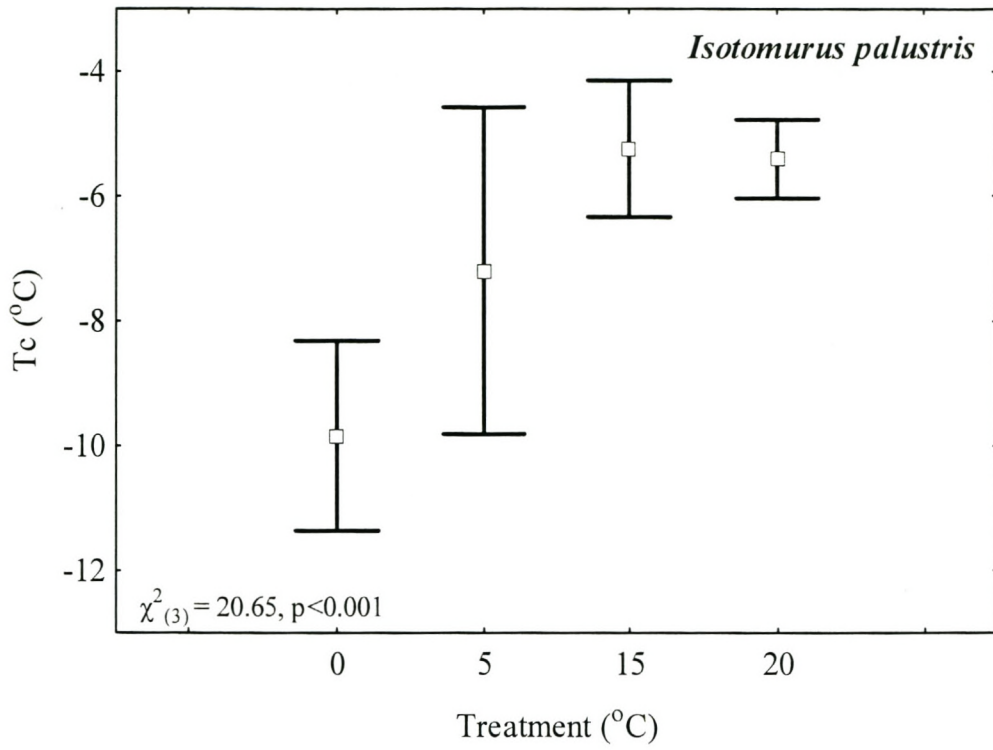


Figure 4 B

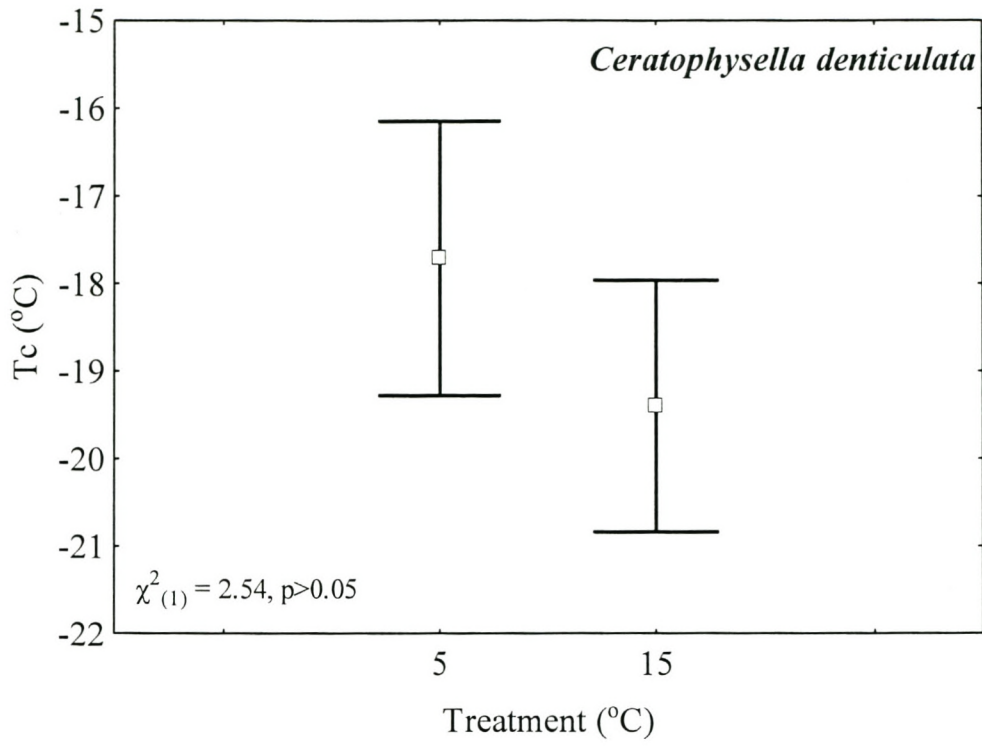


Figure 4 C

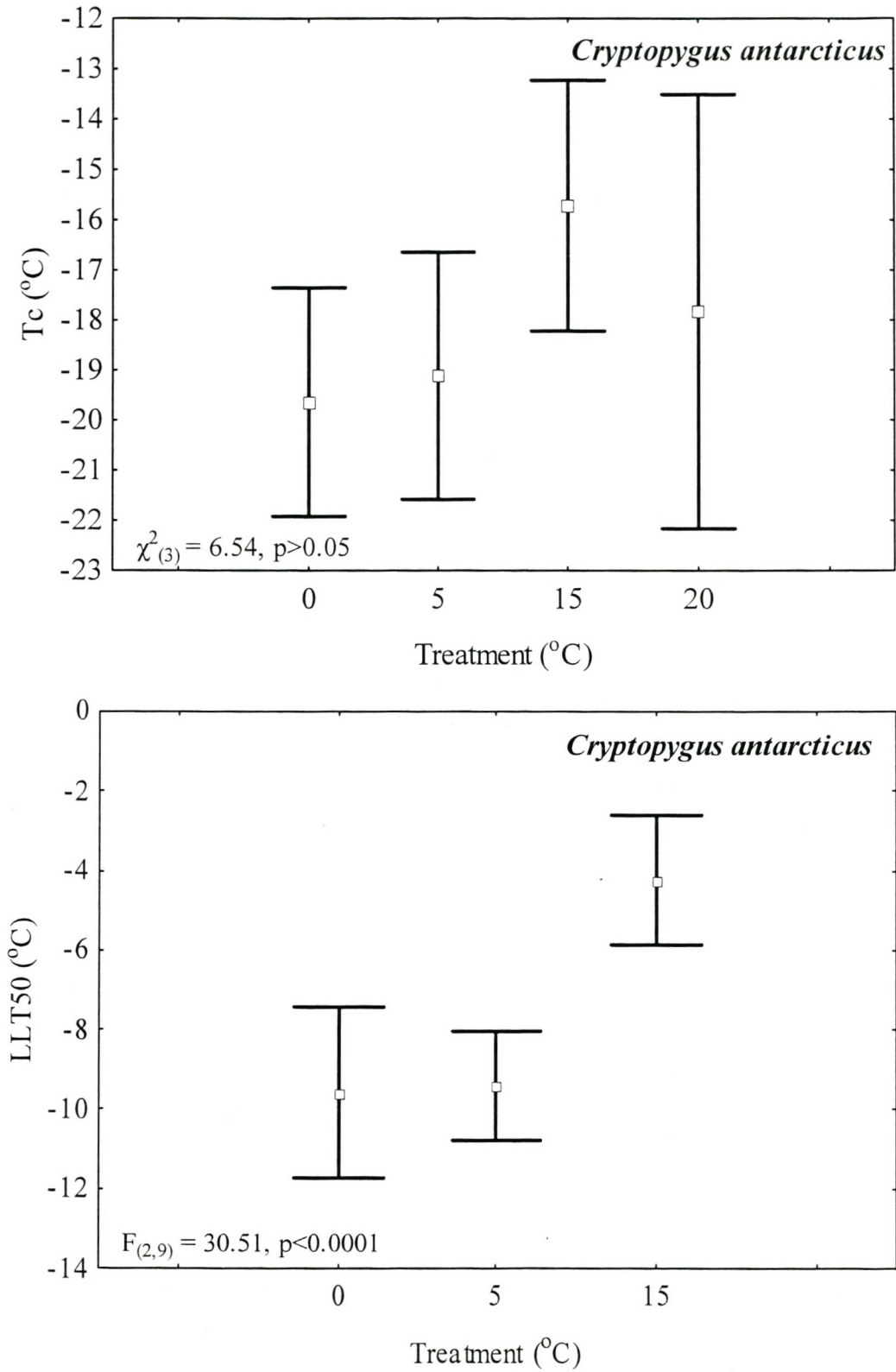


Figure 4 D

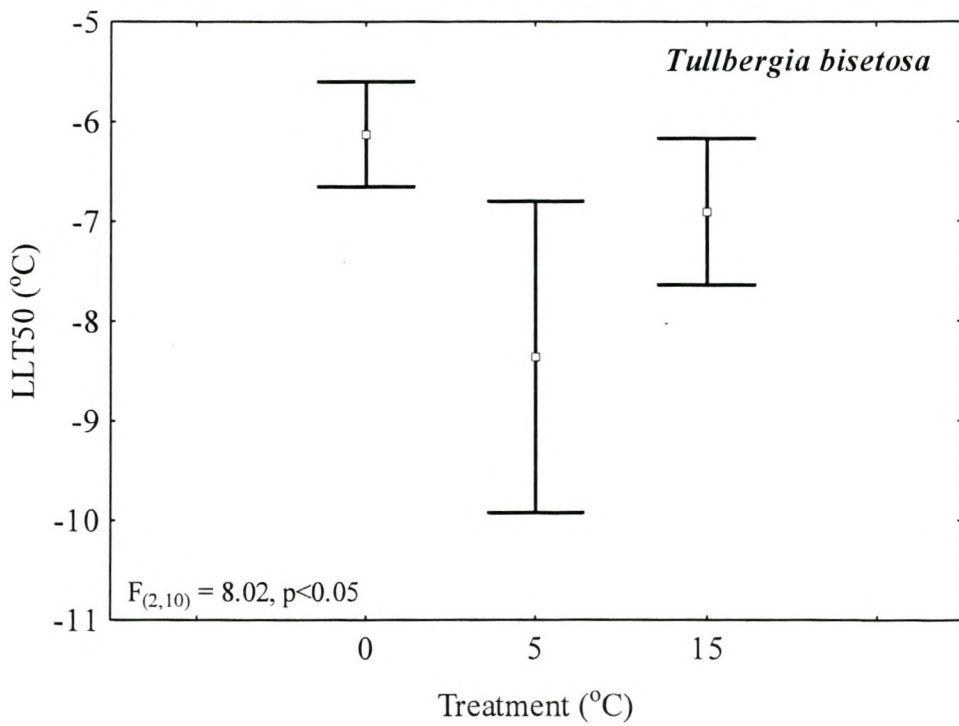
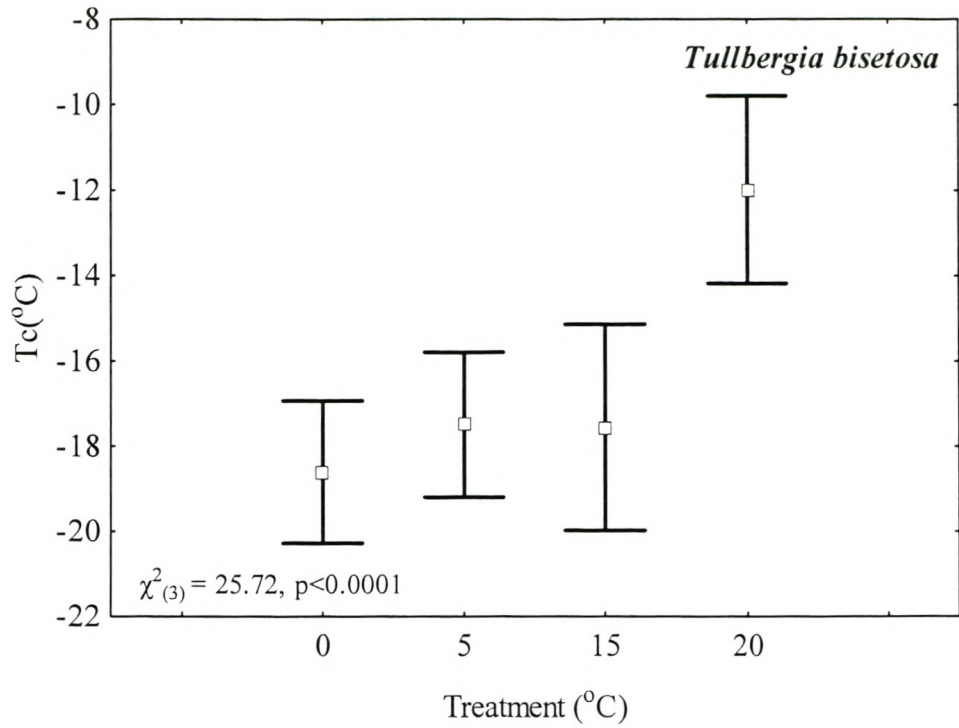


Figure 4. Crystallization temperatures ( $T_c$ ) and lower lethal temperatures (LLT50) of Collembola (mean  $\pm$  95% confidence intervals). A. *Pogonognathellus flavescens*; B. *Isotomurus palustris*; C. *Ceratophysella denticulata* ( $T_c$  only); D. *Cryptopygus antarcticus*; E. *Tullbergia bisetosa*.

The two invasive springtail species had higher ULTs than the indigenous species (Table 7), and the alien *I. palustris* had a significantly higher LT100 and LT50 than all other species (Table 7). *I. palustris* had the highest and *T. bisetosa* the lowest ULTs for field fresh springtails in this study, whereas *P. flavescens* and *C. antarcticus* showed intermediate tolerances to high temperature (ULT50s of 33.3 and 32.7 °C, respectively). *P. flavescens* showed an increase in ULT with increasing acclimation temperature (Fig. 5), and this was true also of the other two alien springtail species, *I. palustris* and *C. denticulata* (Fig. 5), although the size of the effect was smaller. These species thus performed better following acclimation at high temperatures. *C. antarcticus*, however, displayed reduced ULTs in response to high temperature acclimation, whereas *T. bisetosa* showed a small increase in high temperature tolerance following acclimation at higher temperatures.



Table 7. Summary statistics of upper lethal temperatures (°C) of field fresh springtails.

	<b>n</b>	<b>Mean</b>	<b>SE</b>	<b>Minimum</b>	<b>Maximum</b>
<b>ULT100</b>					
<b>Alien</b>					
<i>Pogonognathellus flavescens</i>	5	35.0 ±	0.00 <sup>b</sup>	35.0	35.0
<i>Isotomurus palustris</i>	5	37.0 ±	0.00 <sup>c</sup>	37.0	37.0
<b>Indigenous</b>					
<i>Cryptopygus antarcticus</i>	5	34.8 ±	0.20 <sup>b</sup>	34.0	35.0
<i>Tullbergia bisetosa</i>	5	32.8 ±	0.20 <sup>a</sup>	32.0	33.0
ANOVA $F_{(3,16)}=147.33$		p<0.0001			
<b>ULT50</b>					
<b>Alien</b>					
<i>Pogonognathellus flavescens</i>	5	33.3 ±	0.50 <sup>b</sup>	32.1	34.5
<i>Isotomurus palustris</i>	5	36.1 ±	0.13 <sup>c</sup>	35.6	36.4
<b>Indigenous</b>					
<i>Cryptopygus antarcticus</i>	5	32.7 ±	0.39 <sup>b</sup>	31.8	33.8
<i>Tullbergia bisetosa</i>	5	31.1 ±	0.16 <sup>a</sup>	30.7	31.6
ANOVA $F_{(3,16)}=38.68$		p<0.0001			

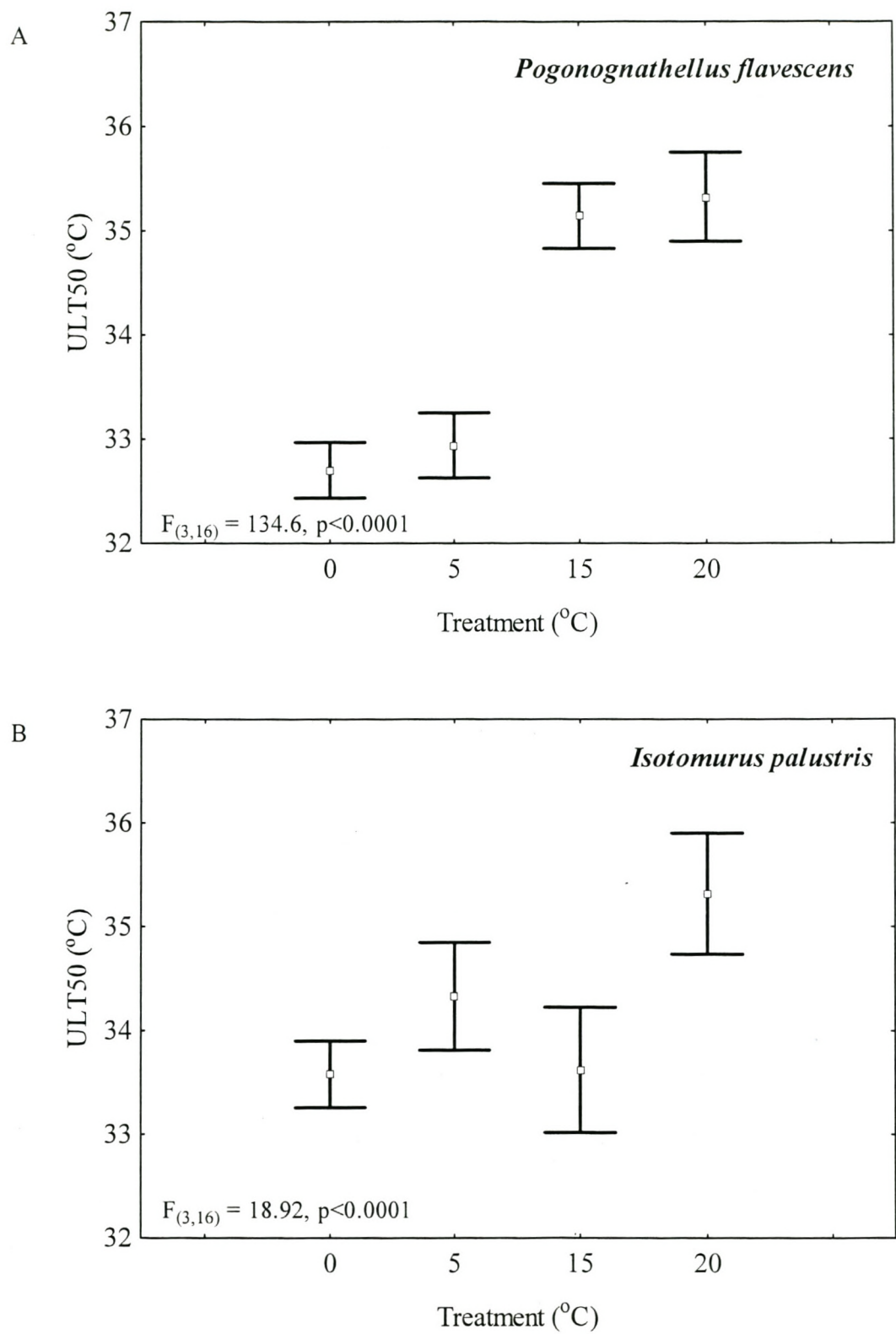


Figure 5 A and B

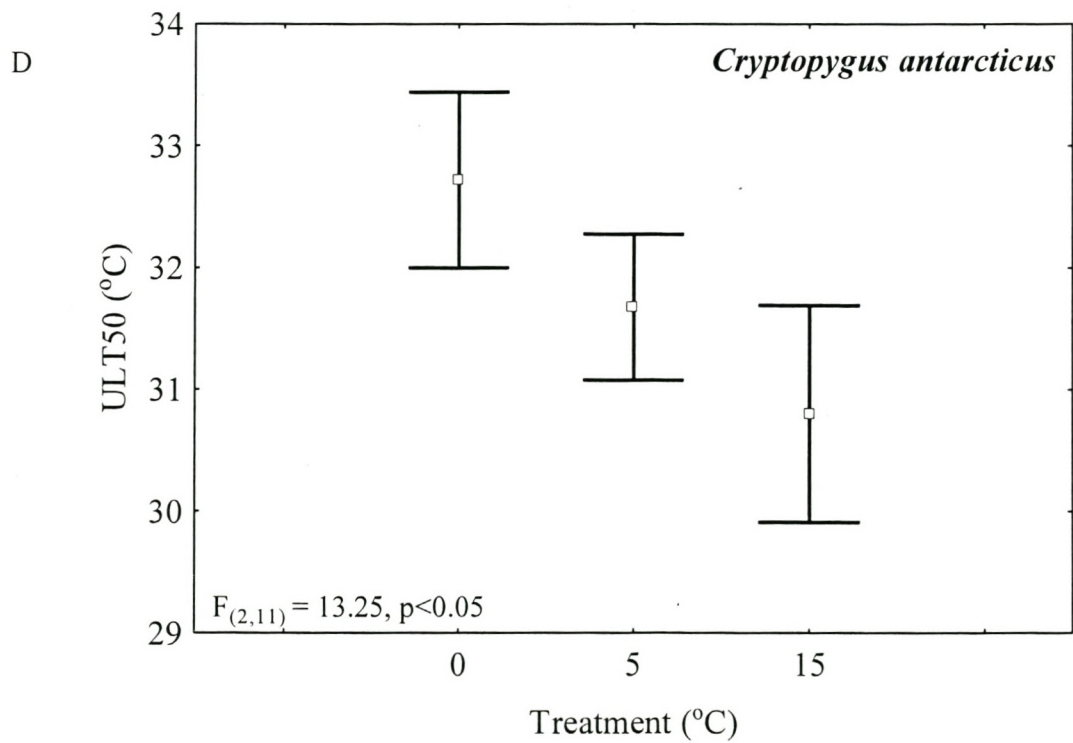
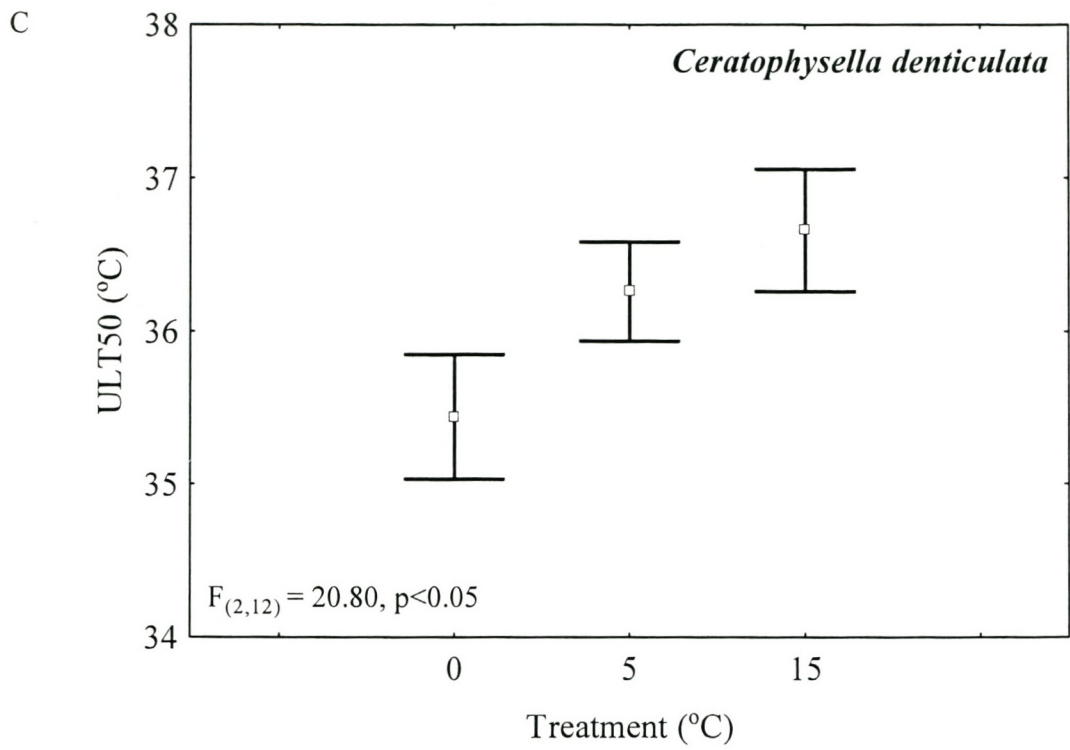


Figure 5 C and D

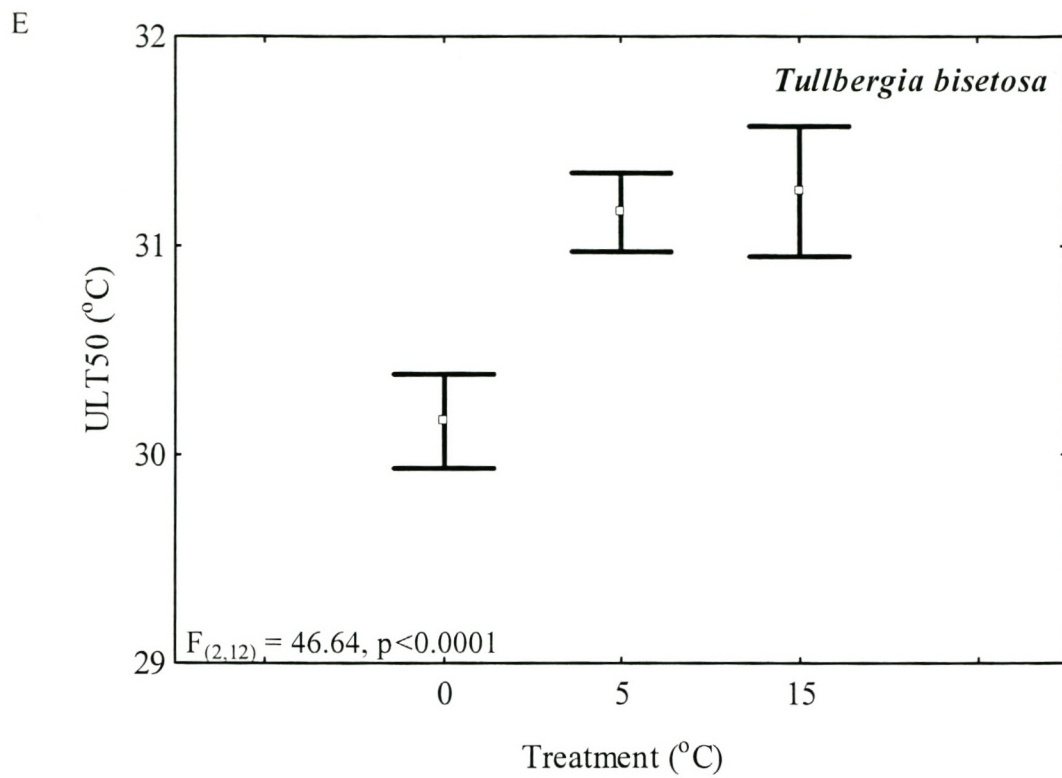


Figure 5. Upper lethal temperatures (ULT50) (mean  $\pm$  95% confidence intervals) of springtails acclimated at 0, 5 and 15 °C. For *Pogonognathellus flavescens* (A) and *Isotomurus palustris* (B) a 20°C treatment is also included. The other species included *Ceratophysella denticulata* (C), *Cryptopygus antarcticus* (D) and *Tullbergia bisetosa* (E).

## Discussion

### *Cold hardiness*

In the majority of the species examined here, cooling rate had no significant effect on crystallization temperature. This finding substantiates the early study by Salt (1966), who found no influence of cooling rate on  $T_c$  in several Canadian insect species, and later work by Cannon (1983) who found that, for *Cryptopygus antarcticus* from Signy Island, a doubling in the rate of cooling lowered  $T_c$  by only 0.53 °C. By contrast, the difference in cooling rates between the slow cooling experiment and the submergence (plunge) protocol employed in the mortality assays significantly affected lower lethal temperatures (Fig. 3). Whether the change was a consequence of an alteration of  $T_c$ , or a consequence of enhanced prefreeze mortality in the springtails as a result of rapid cooling cannot be conclusively demonstrated. The lack of an effect of cooling rate on  $T_c$ , and the absence of prefreeze mortality in the experiments that specifically tested for it certainly do not provide evidence one way or another. However, the range of cooling rates used in those trials, compared with the change likely to be associated with the submergence protocol in the mortality assays, were much narrower, and therefore the change in rate may have precipitated both a change in  $T_c$  and pre-freeze mortality. Further work will be required to establish which of the two processes are more important in contributing to the differences in mortality associated with a given temperature in the two experiments (for further discussion of the effects of measurement method on experimental outcomes see Miller 1978; Kelty & Lee 1999; Chown & Nicolson 2004). Nonetheless, these results do substantiate the fact that, at least for these springtails at ecologically relevant cooling rates, the  $T_c$  is equivalent to the point at

which the animals die. In other words, there is no pre-freeze mortality, as has long been known for springtails from elsewhere (Sømme 1982, but see also Nedved *et al.* 1995).

Recently it has also been demonstrated that Antarctic springtails respond rapidly to local sublethal changes in temperature, by altering  $T_c$ , and this has been termed rapid cold hardening (Worland & Convey 2001). However, this rapid hardening is different to the response originally defined as rapid cold hardening by Lee *et al.* (1987). In the original definition it is the lower lethal temperature that is altered, whereas in the springtail study it is  $T_c$  (Chown & Nicolson, 2004). Nonetheless, rapid changes in  $T_c$  have been demonstrated both in *Cryptopygus antarcticus* from the Antarctic Peninsula, and in *Cryptopygus cisantarcticus* and *Isotoma klovstadi* from elsewhere on the continent (Sinclair *et al.* 2003). In this study, none of the species examined showed rapid changes in  $T_c$  under experimental conditions (with the possible exception of *Tullbergia bisetosa* acclimated at 5°C). This makes the Marion Island species very different from *C. antarcticus* from the Antarctic continent, because even under experimental conditions similar to those adopted here, rapid changes in  $T_c$  were found in the latter species. By contrast, Sinclair *et al.* (2003) were unable to induce changes in  $T_c$  under experimental conditions similar to those used here, despite the fact that there was clear diurnal variation in  $T_c$  in field collected animals. Thus, it is not certain that  $T_c$  does not change over the daily cycles in the Marion species in the field, but it is obviously insensitive to the laboratory conditions that were used. That *C. antarcticus* on Marion Island and on the Antarctic Peninsula differ in this regard is not surprising given the very different conditions at the two sites (compare the microclimate data provided by Chown & Crafford (1992) and in Chapter 6, with that provided by Walton (1984)

and Worland & Convey (2001)), and the fact that population-level differentiation in physiological traits is common (Chown & Nicolson 2004).

Despite the general unresponsiveness of T<sub>c</sub> to experimental conditions (as opposed to acclimation temperatures), it is clear that there is considerable variation among the species. For field fresh animals, the indigenous species tended to have lower T<sub>c</sub>s than the invasive species, with *T. bisetosa* having the lowest mean T<sub>c</sub>. *Cryptopygus antarcticus* investigated here had a mean T<sub>c</sub> similar to that of field fresh *C. antarcticus* from Marguerite Bay, Antarctica (-14.7 °C, Hayward *et al.* 2003), and to that of individuals collected in August from Signy Island (-12.3 °C, Cannon 1986), but higher than another sample collected in August from Signy Island (-21 °C, Cannon 1986). Thus, despite differences in T<sub>c</sub> response to experimental treatments, the basal response in the species is quite similar amongst sites. The relatively high T<sub>c</sub> of the alien *I. palustris* is comparable to that of the Arctic *Onychiurus arcticus*, which has a mean T<sub>c</sub> of -6.1 (range -2 to -9 °C) (Block *et al.* 1994), indicating that T<sub>c</sub>s for Marion Island springtails fall within the range measured elsewhere.

Although there was substantial variation in LLT50 and LLT100 values both amongst species and amongst acclimation treatments, it is clear from the above discussion that the LLT values were highly sensitive to the protocol adopted. In consequence, whilst these data are reported for completeness, the inability to distinguish T<sub>c</sub> effects from prefreeze mortality means that from an acclimation perspective, the LLT data will not be considered further. Rather, the discussion will focus on the outcome of the T<sub>c</sub> acclimation trials. Here, it was clear that there is a decline in T<sub>c</sub> with declining acclimation temperature in *Tullbergia bisetosa*, *Isotomurus cf. palustris*, and

*Cryptopygus antarcticus*. This response is typical of a wide range of both springtail and other arthropod species (Sømme & Block 1982; Sømme 1986; Block 1990; Holmstrup & Sømme 1998; Klok & Chown 1998; Worland & Lukešová 2000; Worland & Block 2003; Klok & Chown 2005). In addition, it can readily be interpreted as beneficial acclimation because survival seems well matched to the conditions in which the animals find themselves, especially if the costs of reducing  $T_c$  (such as those associated with cryoprotectant production, see Storey (1997)) are kept in mind. Nonetheless, beneficial acclimation here cannot readily be distinguished from one of its alternatives, the “colder is better” hypothesis because survival of low temperature increases with declining acclimation temperature. Whilst the beneficial acclimation and other hypotheses have generally been considered alternatives to date (e.g. Leroi *et al.* 1994; Huey & Berrigan 1996; Wilson & Franklin 2002), this need not always be the case. For example, if one considers temperature of maximal speed of locomotion (see Gilchrist 1996 for definition), then a positive relationship between this variable and acclimation temperature clearly indicates that there is beneficial acclimation, but also that warmer is better and that colder is better (Deere *et al.*, submitted ms). This may well also be the case here.

By contrast with the other species, *P. flavescens* and *C. denticulata* showed the opposite response, with  $T_c$  declining, or remaining unchanged with acclimation, respectively. The former is particularly counterintuitive because there seems to be little adaptive value in an increase of  $T_c$  with a decline in temperature. However, it is well known that  $T_c$ s respond to a range of conditions, such as changes in water content, food source, and gut contents (Cannon & Block 1988; Block 1990; Duman *et al.* 1991; Sømme 1999), and this may well have influenced the outcome of these trials in a way



that was undetectable in the experimental protocol used here. That LLT50 showed the opposite response to acclimation in *P. flavescens* suggests that other factors may well have influenced Tc. Be that as it may, it is clear that the general response to acclimation of lower lethal temperatures ( $\approx$  Tc) is similar across both the invasive and the indigenous species, whilst the basal tolerance to low temperature of the indigenous species is greater than that of the invasives. This suggests that, at least in the case of cold hardiness, the indigenous species are more tolerant of cold (i.e. colder is better), but that neither group is phenotypically more flexible than the other. These results are in keeping with the ecological work of Gabriel *et al.* (2001), who found that the invasive species do not occur in the colder, higher elevation regions of the island.

#### *Upper lethal temperatures*

The ULT50 values found for the species examined here were very much in keeping with those found for other arthropod species on Marion Island, including the six weevil species examined by Van der Merwe *et al.* (1997), *Antarctopsocus jeanneli* (Slabber & Chown 2004), and the indigenous rove beetle (*Halmaeus atriceps*) (Slabber & Chown 2005), although they were slightly lower than the critical thermal maximum values of *Embryonopsis halticella*, *Pringleophaga marioni* and *Paractora dreuxi* (39.7, 38.7 and 35.5 °C, respectively) (Klok & Chown 1997; 1998; 2001) from Marion Island. However, when comparing dynamic (CTmax) and static (mortality assay) methods it is not only the experimental methods that might result in such differences, but they might also be assessing completely different traits (Hoffmann *et al.* 2003; Chown & Nicolson 2004). Nonetheless, there were clear differences between the indigenous and the invasive species, with the former generally having lower ULT50 and ULT100 values

than the latter. In addition, a clear distinction could also be drawn between the acclimation responses of the indigenous and invasive species. In the former, there was either a decline in ULT50 with increasing acclimation temperature, or a very weak increase (*c.* 1 °C). By contrast, in the latter, ULT50 increased by between 1.5 and 3 °C in response to increasing acclimation temperatures. In other words, for the invasive species it is clear that warmer conditions lead to improved survival of high temperatures and that basal tolerance is higher than in the indigenous species in the first place. This finding suggests that for the invasive species there is beneficial acclimation, although in this instance it is equivalent also to the “warmer is better” hypothesis.

Thus, in terms of the predictions made based on the ecological studies of Gabriel *et al.* (2001) and Barendse & Chown (2001) for arthropods on Marion Island, and those made based on species examined elsewhere (see Introduction) it appears that warmer conditions will favour the invasive species. Both enhanced basal tolerance and increased flexibility are likely to be responsible for the improved performance of the invasive species. Although this outcome may appear to be at odds with the beneficial acclimation vs. the “warmer is better” scenario predictions made initially, it is clear that under some circumstances these hypotheses need not be viewed as alternatives.

The acclimation responses of the springtails also reveal that they are quite different to insects in that both upper and lower lethal temperatures show similar extents of flexibility. In the insects, upper lethal temperatures (and critical thermal maxima) are much less flexible than lower lethal temperatures (and critical thermal minima) (Klok & Chown 2003; Chown & Nicolson 2004; Slabber & Chown 2005), and a similar pattern is also reflected geographically such that lower lethal temperatures tend to vary much

less through space than do upper lethal temperatures (Addo-Bediako *et al.* 2000; Chown 2001; Ayrinhac *et al.* 2004). However, the decoupling between upper and lower lethal temperatures may be a consequence of the fact that upper thermal tolerance is not a consequence of oxygen limitation in insects because of their highly efficient tracheal systems (for discussion see Pörtner 2001; Klok *et al.* 2004). The springtails examined here do not possess tracheae, which means that they may be subject to the same constraints facing marine animals in which circulation and ventilation take place by separate organ systems, hence resulting in oxygen limitation of thermal tolerance owing to a mismatch between oxygen demand and delivery capability. This issue remains to be explored, but the present data suggest that springtails are much more like marine species than terrestrial insects.

#### *Desiccation tolerance*

*Pogonognathellus flavescens* showed the greatest desiccation tolerance followed by the indigenous *C. antarcticus* and alien *C. denticulata*. *I. palustris* and *T. bisetosa* had low desiccation tolerances. Nonetheless, the range of tolerances found here are similar to those recorded for a variety of European species studies by Hertzberg & Leinaas (1998). In a recent study by Kærsgaard *et al.* (2004), *P. flavescens* was less capable of resisting desiccation than the other two epedaphic springtails species in their study (*Sinella curviseta* and *Orchesella cincta*), and accumulated glycerol in response to desiccation. In the field, however, *P. flavescens* was capable of surviving prolonged periods of drought, which Kærsgaard *et al.* (2004) contributed to the high water content in relation to surface area in this species. In both the present study, and the one undertaken by Hertzberg & Leinaas (1998), springtail desiccation tolerances were determined at *c.* 80

% RH, allowing comparison between these studies, as opposed to several studies investigating springtail desiccation at RHs of less than 5 %, making comparisons between these and the present study impossible (e.g. Worland & Block 1986; Block *et al.* 1990).

In terms of the hypotheses regarding phenotypic flexibility it is clear that both *P. flavescens* and *I. palustris* have an acclimation response that is consistent with the “warmer is better” hypothesis (Huey *et al.* 1999). That is, survival time was generally longer following acclimation at 15 °C than following acclimation at 5 °C, although in the former species this difference was only found at the higher treatment temperature. By contrast, in both of the indigenous species, *C. antarcticus* and *T. bisetosa*, performance was better following low temperature acclimation. In these instances, the “colder is better” hypothesis is supported, although in *C. antarcticus* survival at 15 °C was similar following both acclimation treatments. Thus, of the five species examined, only *C. denticulata* provided evidence, though limited, of beneficial acclimation of desiccation tolerance. In terms of the predictions that were made regarding differences between the indigenous and invasive species it is clear that the extent of acclimation ability in these groups is similar. Thus, the invasive species are not more flexible than the indigenous ones, as has been suggested for plants (Daehler 2003) and also for birds (Duncan *et al.* 2003). Rather, performance of the invasive species is better under warmer conditions and performance of the indigenous species is better under colder conditions, as was predicted for a range of indigenous and invasive species in the Antarctic and other temperate areas (Stachowicz *et al.* 2002; Walther *et al.* 2002; Frenot *et al.* 2005). Only in *C. denticulata* did it appear that the species was able to take advantage of a wide range of conditions, although the acclimation treatment was not

significant. Nonetheless, it is intriguing that this is one of the most abundant and widespread invasive species on Marion Island (Gabriel *et al.* 2001).

That there was an effect of temperature acclimation on desiccation tolerance in most of the species is itself noteworthy. Such a response has not commonly been searched for, nor found in arthropods. Hoffmann *et al.* (2005) showed that a variable temperature acclimation, which reflected seasonal temperature variation in summer and winter in the flies' natural habitat, generally improved desiccation resistance (time to death) in *Drosophila melanogaster* relative to a constant temperature regime. By contrast, although Gibbs *et al.* (1998) found increases in cuticular hydrocarbon melting points (but no changes in hydrocarbon mass) with high temperature acclimation (33 °C) in a desert-dwelling *Drosophila* species, desiccation rate remained unaffected by acclimation to either warm or cold temperature (17 °C). Similar results have been documented for woodlice (Isopoda: Oniscoidea) (Cloudsley-Thompson 1969). In the species of springtails examined here the physiological basis of this change in desiccation tolerance has not been fully elucidated, although it does appear to be associated with a decline in rate of water loss, and therefore some change either in cuticular properties or in internal solute concentration (see Kærsgaard *et al.* 2004 and Leinaas *et al.*, unpublished data).

In conclusion, it appears that, despite some overlap between indigenous and invasive species, the prediction that invasive species will have enhanced performance under warmer conditions, relative to the indigenous species is supported. In other words, the invasive species do not necessarily show greater phenotypic flexibility than the indigenous ones, but do perform better under higher temperature conditions. Moreover,

in most cases the beneficial acclimation hypothesis was either not supported (especially in the case of desiccation tolerance), or could not readily be distinguished from its alternatives. These results are very much in keeping with other studies that have found little support for the BAH (Leroi *et al.* 1994; Hoffmann 1995; Huey & Berrigan 1996; Bennett & Lenski 1997; Gibbs *et al.* 1998; Woods 1999; Woods & Harrison 2001; Wilson & Franklin 2002), even though here it was not developmental plasticity, but rather acclimation that was investigated (see Woods 1999; Wilson & Franklin 2002 for discussion). These results suggest that the prediction that invasive species in the Antarctic and other temperate regions will outperform indigenous species as climates warm (Walther *et al.* 2002) is correct. In consequence, prevention of colonization by additional alien, invasive species is of considerable importance (see also Chown & Gaston 2000; Frenot *et al.* 2005).

## References

- Addo-Bediako A., Chown S. L. & Gaston K. J.** (2000) Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B* **267**: 739-745
- Ayrinhac A., Debat V., Gibert P., Kister A.-G., Legout H., Moreteau B., Vergilino R. & David J. R.** (2004) Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Functional Ecology* **18**: 700-706
- Bale J. S.** (1993) Classes of insect cold hardiness. *Functional Ecology* **7**: 751-753
- Bale J. S., Masters G. J., Hodkinson I. D., Awmack C., Bezemer T. M., Brown V. K., Butterfield J., Buse A., Coulson J. C., Farrar J., Good J. E. G., Harrington R., Hartley S., Jones T. H., Lindroth R. L., Press M. C., Symrnioudis I., Watt A. D. & Whittaker J. B.** (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology* **8**: 1-16
- Barendse J. & Chown S. L.** (2001) Abundance and seasonality of mid-altitude fellfield arthropods from Marion Island. *Polar Biology* **24**: 73-82
- Bennett A. F. & Lenski R. E.** (1997) Evolutionary adaptation to temperature. VI. Phenotypic acclimation and its evolution in *Escherichia coli*. *Evolution* **51**: 36-44
- Blackburn T. M., Cassey P., Duncan R. P., Evans K. L. & Gaston K. J.** (2004) Avian extinction and mammalian introductions on oceanic islands. *Science* **305**: 1955-1958
- Block W.** (1985) Cold resistance of two continental Antarctic micro-arthropods. *Cryo-Letters* **6**: 391
- Block W.** (1990) Cold tolerance of insects and other arthropods. *Philosophical Transactions of the Royal Society of London B* **326**: 613-633
- Block W., Harrison P. M. & Vannier G.** (1990) A comparative study of patterns of water loss from two Antarctic springtails (Insecta, Collembola). *Journal of Insect Physiology* **36**: 181-187
- Block W., Webb N. R., Coulson J. C., Hodkinson I. D. & Worland M. R.** (1994) Thermal adaptation in the Arctic collembolan *Onychiurus arcticus* (Tullberg). *Journal of Zoology* **226**: 3455-3350
- Block W. & Worland M. R.** (2001) Experimental studies of ice nucleation in an Antarctic springtail (Collembola, Isotomidae). *Cryobiology* **42**: 170-181
- Block W., Young S. R., Conradi-Larsen E. M. & Sømme L.** (1978) Cold tolerance of two Antarctic terrestrial arthropods. *Experientia* **34**: 1166-1167

- Bohn T., Sandlund O. T., Amundsen P. A. & Primicerio R.** (2004) Rapidly changing life history during invasion. *Oikos* **106**: 138-150
- Brooks T. M., Mittermeier R. A., Mittermeier C. G., Da Fonseca G. A. B., Rylands A. B., Konstant W. R., Flick P., Pilgrim J., Oldfield S., Magin G. & Hilton-Taylor C.** (2002) Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology* **16**: 909-923
- Cannon R. J.** (1986) Diet and acclimation effects on the cold tolerance and survival of an Antarctic springtail. *British Antarctic Survey Bulletin* **71**: 19-30
- Cannon R. J. C.** (1983) Experimental studies on supercooling in two Antarctic microarthropods. *Journal of Insect Physiology* **29**: 617-624
- Cannon R. J. C. & Block W.** (1988) Cold tolerance of microarthropods. *Biological Reviews* **63**: 23-77
- Chown S. L.** (2001) Physiological variation in insects: hierarchical levels and implications. *Journal of Insect Physiology* **47**: 649-660
- Chown S. L. & Crafford J. E.** (1992) Microhabitat temperatures at Marion Island. *South African Journal of Antarctic Research* **22**: 51-58
- Chown S. L. & Gaston K. J.** (2000) Island-hopping invaders hitch a ride with tourists in the southern ocean. *Nature* **408**: 637
- Chown S. L., McGeoch M. A. & Marshall D. J.** (2002) Diversity and conservation of invertebrates on the sub-Antarctic Prince Edward Islands. *African Entomology* **10**: 67-82
- Chown S. L. & Nicolson S. W.** (2004) *Insect physiological ecology. Mechanisms and patterns*. Oxford University Press, Oxford
- Cloudsley-Thompson J. L.** (1969) Acclimation, water and temperature relations of the woodlice *Metoponorthus pruinosis* and *Periscyphis jannonei* in the Sudan. *Journal of Zoology* **158**: 267-276
- Coulson S. J., Hodkinson I. D., Webb N. R., Mikkola K., Harrison J. A. & Pedgley D. E.** (2002) Aerial colonization of high Arctic islands by invertebrates: the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae) as a potential indicator species. *Diversity and Distributions* **8**: 327-334
- Crafford J. E., Scholtz C. H. & Chown S. L.** (1986) The insects of sub-Antarctic Marion and Prince Edward Islands; with a bibliography of entomology of the Kerguelen Biogeographical Province. *South African Journal of Antarctic Research* **16**: 42-84
- Daehler C. C.** (2003) Performance comparisons of co-occurring native and alien invasive plants: Implications for conservation and restoration. *Annual Review of Ecology, Evolution and Systematics* **34**: 183-211



- Davidson M. M. & Broady P. A.** (1996) Analysis of gut contents of *Gomphiocephalus hodgsoni* Carpenter (Collembola: Hypogastruridae) at Cape Geology, Antarctica. *Polar Biology* **16**: 463-467
- Duman J. G., Wu D. W., Xu L., Tursman D. & Olsen T. M.** (1991) Adaptations of insects to subzero temperatures. *Quarterly Review of Biology* **66**: 387-410
- Duncan R. P., Blackburn T. M. & Sol D.** (2003) The ecology of bird introductions. *Annual Review of Ecology Evolution and Systematics* **34**: 71-98
- Frenot Y., Chown S. L., Whinam J., Selkirk P. M., Convey P., Skotnicki M. & Bergstrom D. M.** (2005) Biological invasions in the Antarctic: extent, impacts and implications. *Biological Reviews* **80**: 45-72
- Gabriel A. G. A., Chown S. L., Barendse J., Marshall D. J., Mercer R. D., Pugh P. J. A. & Smith V. R.** (2001) Biological invasions of southern ocean islands: the Collembola of Marion Island as a test of generalities. *Ecography* **24**: 421-430
- Gibbs A. G., Louie A. K. & Ayala J. A.** (1998) Effects of temperature on cuticular lipids and water balance in a desert *Drosophila*: Is thermal acclimation beneficial? *Journal of Experimental Biology* **201**: 71-80
- Gilchrist G. W.** (1996) A quantitative genetic analysis of thermal sensitivity in the locomotor performance curve of *Aphidius ervi*. *Evolution* **50**: 1560-1572
- Hänel C. & Chown S. L.** (1999) *An introductory guide to the Marion and Prince Edward Island Special Nature Reserves. Fifty years after annexation.* Department of Environmental Affairs & Tourism, Pretoria
- Hänel C., Chown S. L. & Davies L.** (1998) Records of alien insect species from sub-Antarctic Marion and South Georgia Islands. *African Entomology* **6**: 366-369
- Hayward S. A. L., Worland M. R., Convey P. & Bale J. S.** (2003) Temperature preferences of the mite, *Alaskozetes antarcticus*, and the collembolan, *Cryptopygus antarcticus* from the maritime Antarctic. *Physiological Entomology* **28**: 114-121
- Hertzberg K. & Leinaas H. P.** (1998) Drought stress as a mortality factor in two pairs of sympatric species of Collembola at Spitsbergen, Svalbard. *Polar Biology* **19**: 302-306
- Hill J. K. & Fox R.** (2003) Climate change and British butterfly distributions. *Biologist* **50**: 106-110
- Hoffmann A. A.** (1995) Acclimation: increasing survival at a cost. *Trends in Ecology and Evolution* **10**: 1-2
- Hoffmann A. A., Shirriffs J. & Scott M.** (2005) Relative importance of plastic versus genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. *Functional Ecology* **19**: 222-227

- Hoffmann A. A., Sørensen J. G. & Loeschcke V.** (2003) Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *Journal of Thermal Biology* **28**: 175-216
- Hoffmann A.A. & Watson M.** (1993) Geographical variation in the acclimation responses of *Drosophila* to temperature extremes. *American Naturalist* **142**: S93-S113
- Holmstrup M. & Sømme L.** (1998) Dehydration and cold hardiness in the Arctic collembolan *Onychiurus arcticus* Tullberg 1876. *Journal of Comparative Physiology B* **168**: 197-203
- Huey R. B. & Berrigan D.** (1996) Testing evolutionary hypotheses of acclimation. *Animals and temperature. Phenotypic and evolutionary adaptation*. Ed. Johnston I. A. & Bennett A. F., Cambridge University Press, Cambridge. pp 205-237
- Huey R. B., Berrigan D., Gilchrist G. W. & Herron J. C.** (1999) Testing the adaptive significance of acclimation: A strong inference approach. *American Zoologist* **39**: 323-336
- IPCC** (2001) *Climate change 2001: synthesis report. A contribution of working groups I, II, and III to the third assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge
- Kærsgaard C. W., Holmstrup M., Malte H. & Bayley M.** (2004) The importance of cuticular permeability, osmolyte production and body size for the desiccation resistance of nine species of Collembola. *Journal of Insect Physiology* **50**: 5-15
- Kelty J. D. & Lee R. E.** (1999) Induction of rapid cold hardening by ecologically relevant cooling rates in *Drosophila melanogaster*. *Journal of Insect Physiology* **45**: 719-726
- Klok C. J. & Chown S. L.** (1997) Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic caterpillar, *Pringleophaga marioni* Viette (Lepidoptera: Tineidae). *Journal of Insect Physiology* **43**: 685-694
- Klok C. J. & Chown S. L.** (1998) Interactions between desiccation resistance, host-plant contact and the thermal biology of a leaf-dwelling sub-Antarctic caterpillar, *Embryonopsis halticella* (Lepidoptera: Yponomeutidae). *Journal of Insect Physiology* **44**: 615-628
- Klok C. J. & Chown S. L.** (2001) Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic kelp fly, *Paractora dreuxi* (Diptera: Helcomyzidae). *Journal of Insect Physiology* **47**: 95-109
- Klok C. J. & Chown S. L.** (2003) Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. *Biological Journal of the Linnean Society* **78**: 401-414

- Klok C. J. & Chown S. L.** (2005) Inertia in physiological traits: *Embryonopsis halticella* caterpillars (Yponomeutidae) across the Antarctic Polar Frontal Zone. *Journal of Insect Physiology* **51**: 87-97
- Klok C. J., Sinclair B. J. & Chown S. L.** (2004) Upper thermal tolerance and oxygen limitation in terrestrial arthropods. *Journal of Experimental Biology* **207**: 2361-2370
- Lee R. E., Chen C.-P. & Denlinger D. L.** (1987) A rapid cold-hardening process in insects. *Science* **238**: 1415-1417
- Leroi A. M., Bennett A. F. & Lenski R. E.** (1994) Temperature acclimation and competitive fitness: An experimental test of the beneficial acclimation assumption. *Proceedings of the National Academy of Sciences of the USA* **91**: 1917-1921
- Miller L. K.** (1978) Freezing tolerance in relation to cooling rate in an adult insect. *Cryobiology* **15**: 345-349
- Nedved O., Hodková M., Brunnhofer V. & Hodek I.** (1995) Simultaneous measurement of low temperature survival and supercooling in a sample of insects. *Cryo-Letters* **16**: 108-113
- Pakhomov E. A. & Chown S. L.** (2003) The Prince Edward Islands: southern ocean oasis. *Ocean Yearbook* **17**: 348-379
- Parmesan C., Root T. L. & Willig M. R.** (2000) Impacts of extreme weather and climate on terrestrial biota. *Bulletin of the American Meteorological Society* **81**: 443-450
- Pimentel D.** (2002) *Economic and environmental threats of alien plant, animal, and microbe invasions. Biological invasions.* CRC Press LCC, Boca Raton
- Pitelka L. F.** (1997) Plant migration and climate change. *American Scientist* **85**: 464-473
- Pörtner H. O.** (2001) Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**: 137-146
- Prinzing A., Durka W., Klotz S. & Brandl R.** (2002) Which species become aliens? *Evolutionary Ecology Research* **4**: 385-405
- Rothery P. & Block W.** (1992) Characterizing supercooling point distributions. *Cryoletters* **13**: 193-198
- Salt R. W.** (1966) Effect of cooling rate on the freezing temperatures of supercooled insects. *Canadian Journal of Zoology* **44**: 655-659
- Scheiner S. M.** (1993) Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* **24**: 35-68

- Sinclair B. J., Klok C. J., Scott M. B., Terblanche J. S. & Chown S. L.** (2003) Diurnal variation in supercooling points of three species of Collembola from Cape Hallett, Antarctica. *Journal of Insect Physiology* **49**: 1049-1061
- Sinclair B. J. & Sjørnsen H.** (2001) Cold tolerance of the Antarctic springtail *Gomphiocephalus hodgsoni* (Collembola: Hypogastruridae). *Antarctic Science* **13**: 271-279
- Slabber S. & Chown S. L.** (2004) Thermal tolerance and cold hardiness strategy of the sub-Antarctic psocid *Antarctopsocus jeanneli* Badonnel. *Polar Biology* **28**: 56-61
- Slabber S. & Chown S. L.** (2005) Thermal tolerance responses to acclimation in the sub-Antarctic rove beetle *Halmaeus atriceps* (C.O. Waterhouse). *Physiological Entomology*: 30: 195-204
- Smith V. R.** (1987) The environment and biota of Marion Island. *South African Journal of Science* **83**: 211-220
- Smith V. R.** (2002) Climate change in the sub-Antarctic: An illustration from Marion Island. *Climatic Change* **52**: 345-357
- Smith V. R. & Steenkamp M.** (1990) Climatic change and its ecological implications at a sub-Antarctic island. *Oecologia* **85**: 14-24
- Sømme L.** (1982) Supercooling and winter survival in terrestrial arthropods. *Comparative Biochemistry and Physiology A* **73**: 519-543
- Sømme L.** (1986) Ecology of *Cryptopygus sverdrupi* (Insecta: Collembola) from Dronning Maud Land, Antarctica. *Polar Biology* **6**: 179-184
- Sømme L.** (1999) The physiology of cold hardiness in terrestrial arthropods. *European Journal of Entomology* **96**: 1-10
- Sømme L. & Block W.** (1982) Cold hardiness of Collembola at Signy Island, maritime Antarctic. *Oikos* **38**: 168-176
- Soulé M. E.** (1991) Conservation: tactics for a constant crisis. *Science* **253**: 744-750
- Stachowicz J. J., Terwin J. R., Whitlatch R. B. & Osman R. W.** (2002) Linking climate change and biological invasions: ocean warming facilitates nonindigenous species invasions. *Proceedings of the National Academy of Sciences of the USA* **99**: 15497-15500
- Storey K. B.** (1997) Organic solutes in freezing tolerance. *Comparative Biochemistry and Physiology* **117A**: 319-326
- Van der Merwe M., Chown S. L. & Smith V. R.** (1997) Thermal tolerance limits in six weevil species (Coleoptera, Curculionidae) from sub-Antarctic Marion Island. *Polar Biology* **18**: 331-336

- Vitousek P. M., D'Antonio C. M., Loope L. L., Rejmánek M. & Westbrooks R. (1997a) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology* **21**: 1-16
- Vitousek P. M., Mooney H. A., Lubchenco J. & Melillo J. M. (1997b) Human domination of earth's ecosystems. *Science* **277**: 494-499
- Walther G.-R., Post E., Convey P., Menzel A., Parmesan C., Beebee T. J. C., Fromentin J. M., Hoegh-Guldberg O. & Bairlein F. (2002) Ecological responses to recent climate change. *Nature* **416**: 389-395
- Walton D. W. H. (1984) The terrestrial environment. *Antarctic ecology*. Ed. Laws R. M., Academic Press, London. pp 1-60
- Warren M. S., Hill J. K., Thomas J. A., Asher J., Fox R., Huntley B., Roy D. B., Telfer M. G., Jeffcoate S., Harding P., Greatorex-Davies J. N., Moss D. & Thomas C. D. (2001) Rapid responses of British butterflies to opposing forces of climate and habitat change. *Nature* **414**: 65-69
- Wilson R. S. & Franklin C. E. (2002) Testing the beneficial acclimation hypothesis. *Trends in Ecology and Evolution* **17**: 66-70
- Winston P. W. & Bates D. H. (1960) Saturated solutions for the control of humidity in biological research. *Ecology* **41**: 232-237
- Woods H. A. (1999) Patterns and mechanisms of growth of fifth-instar *Manduca sexta* caterpillars following exposure to low- or high-protein food during early instars. *Physiological and Biochemical Zoology* **74**: 445-454
- Woods H. A. & Harrison J. F. (2001) The beneficial acclimation hypothesis versus acclimation of specific traits: physiological change in water-stressed *Manduca sexta* caterpillars. *Physiological and Biochemical Zoology* **74**: 32-44
- Woods H. A. & Harrison J. F. (2002) Interpreting rejections of the beneficial acclimation hypothesis: when is physiological plasticity adaptive? *Evolution* **56**: 1863-1866
- Worland M. R. & Block W. (1986) Survival and water loss in some Antarctic arthropods. *Journal of Insect Physiology* **32**: 579-584
- Worland M. R. & Block W. (2003) Desiccation stress at sub-zero temperatures in polar terrestrial arthropods. *Journal of Insect Physiology* **49**: 193-203
- Worland M. R. & Convey P. (2001) Rapid cold hardening in Antarctic microarthropods. *Functional Ecology* **15**: 515-524
- Worland M. R. & Lukešová A. (2000) The effect of feeding on specific soil algae on the cold-hardiness of two Antarctic micro-arthropods (*Alaskozetes antarcticus* and *Cryptopygus antarcticus*). *Polar Biology* **23**: 766-774

## Chapter 6

### **Microhabitat temperature variation across an altitudinal gradient at sub-Antarctic Marion Island: consequences for arthropod populations**

*The point is to provide the next generation with the tools to restore Earth and themselves... The security of humanity will not require armies of soldiers but battalions of conscious environmentalists*

- Hanne Strong -



Example of an I-button temperature logger that was used in the present study.

Thermochron DS1921G, Dallas Semi-Conductors, Texas, USA

(Photo: <http://www.maxim-ic.com/products/ibutton.cfm>)

## Introduction

Climate is one of the most important determinants of the global distribution of invertebrates (Gaston 2003), and with global climate change, the ranges occupied by invertebrates are expected to change (Parmesan *et al.* 1999; IPCC 2001; Sax 2001; Beaumont & Hughes 2002; Hill & Fox 2003; Thomas *et al.* 2004). Moreover, invertebrates interact directly with their surrounding environments, such that these environments not only shape their physiological attributes, but also substantially influence life histories and population dynamics (Andrewartha & Birch 1954; Walton 1984; Stevenson 1985; Kingsolver 1989; Unwin & Corbet 1991; Roy *et al.* 2001; Chown & Nicolson 2004). Indeed, it has been demonstrated that extreme climatic events can result in considerable mortality. For example, populations of *Euphydryas editha* were driven to extinction as a consequence of interactions between extreme weather and human landscape alteration (Parmesan *et al.* 2000). Similarly, Coulson *et al.* (2000) have shown that unseasonably warm temperatures in the Arctic can lead to surface ice-formation (rather than snow) and considerable mortality of soil-dwelling arthropods. In a similar vein, a thorough set of laboratory and field experiments has shown that the goldenrod gall fly, *Eurosta solidaginis*, is adversely affected by high winter temperatures, themselves a consequence of snow cover and soil warming in spring, owing to elevated energy consumption. Both survival and fecundity are affected (Irwin & Lee 2000; Irwin & Lee 2002; Irwin & Lee 2003). This work suggests that variability in snow cover, as well as alterations in temperature might be particularly important for soil arthropods, because the former has a direct effect on the latter. Sinclair (2001b) demonstrated just this point by examining soil temperatures over three seasons relative to potential survival (measured as physiological tolerance) of the cockroach

*Celatoblatta quinquemaculata* in the Rock and Pillar Range in New Zealand. He found that low snow cover in El Niño years resulted in much lower temperatures, and higher potential mortality, than is found in years with high snow cover.

These findings show that an understanding of microclimate variability, in the context of insect physiological responses, is of considerable significance for understanding the ultimate mechanisms underlying insect population dynamics (see also Kingsolver 1989). Moreover, they also make it clear that if the climatic information at the micro-scale relevant to insects (and other arthropods) (Willmer & Unwin 1981; Unwin & Corbet 1991), is collected over short periods only, it is unlikely to be useful because it stands a strong chance of excluding the extreme events, or extreme years, that are often of considerable importance in determining not only population fluctuations (see Gaines & Denny 1993; Parmesan *et al.* 2000), but also the physiological tolerances that might be maintained by a population (Sinclair 2001a; Irwin & Lee 2003). In this context, it has often been argued that the physiological tolerances and behavioural responses of sub-Antarctic insects are closely attuned to the abiotic environments they encounter (Vernon & Vannier 1996; Klok & Chown 1997; Todd & Block 1997; Bale *et al.* 2000; Sinclair & Chown 2003; Chown *et al.* 2004; Slabber & Chown 2004; Klok & Chown 2005, but see also Davenport & MacAlister 1996). Whilst the physiological investigations required for this statement have been thorough (see above) and are on-going, the microclimate work, and particularly information on long-term variability in microclimate (and especially temperature) variability is less common.



Microclimatic investigations (including temperature, relative humidity, solar radiation and other variables) have been undertaken in the sub-Antarctic for more than 30 years (see e.g. Huntley 1971; Chown & Crafford 1992; Pugh & MacAlister 1994; Blake 1996; Davenport & MacAlister 1996; Bale *et al.* 2000; Tweedie & Bergstrom 2000; Boelhouwers *et al.* 2003; Hugo *et al.* 2004). However, these studies have all tended to be either sporadic or short-term (with data available for a year at most), and have rarely incorporated investigations of large-scale inter-site variability (but see Boelhouwers *et al.* 2003 for a 22 month time series for one site and information on altitudinal microclimate variation at others for Marion Island, and Tweedie (2000) for an exploration of altitudinal microclimate variation on Macquarie Island). Thus, the question of the extent of temporal and spatial variability (including altitudinal variation) in microclimate remains largely unexplored for the islands of the sub-Antarctic. This is remarkable given that adaptation to the abiotic environment is thought to be a hallmark of the arthropods inhabiting these systems (for additional review see Block 1984; Crafford *et al.* 1986; Kennedy 1995; Convey 1996; Chown *et al.* 2002), and that there is increasing evidence emerging of fine scale, environment-associated spatial patterning of the distribution and abundance of the arthropods on these islands (e.g. Tréhen *et al.* 1985; Gabriel *et al.* 2001; Barendse *et al.* 2002; Hugo *et al.* 2004).

Here, a start is made at rectifying this substantial gap in the available knowledge regarding arthropod responses to the environment in the sub-Antarctic. This is done by providing microhabitat temperature data from a 24 month time series across five sites at sub-Antarctic Marion Island. These data are then interpreted in the context of the thermal tolerances that

have been recorded for a wide variety of both indigenous and alien arthropods from the island to determine whether the species all have tolerances that exceed temperatures that can be expected across the island (see e.g. Davenport & MacAlister 1996 for such a finding for the intertidal at South Georgia), or whether for some sites, during some years, temperatures are likely to exceed these thresholds, as was found for New Zealand alpine cockroaches by Sinclair (2001b). The contrast between the alien and indigenous species also provides an opportunity to determine whether they are more sensitive to low temperatures (as claimed by e.g. Crafford & Chown 1990 and Gabriel *et al.* 2001, see also Barendse & Chown 2000 and Walther *et al.* 2002).

## **Materials and methods**

### **Study site**

This study was undertaken at sub-Antarctic Marion Island (46°54'S, 37°45'E), which forms part of the Prince Edward Islands Group in the Southern Ocean. Marion Island lies about 2000 km south of South Africa, and is considered to be *c.* 450 000 years old (McDougall *et al.* 2001). Due to the small surface area of Southern Ocean Islands (SOIs) and the vast, thermally stable surrounding Southern Ocean, the climate of Marion Island is oceanic and cool (Smith 1987; Smith & French 1988; Smith 1992). It is characterised by low mean annual temperature, high precipitation (*c.* 2000 mm per annum), a high degree of cloudiness, and strong winds (Smith 1987; 2002). The mean annual Stevenson Screen temperature (25 m asl) for Marion Island is 5.7 °C, with January, February and March

being the warmest months, the coldest months being between June and September (Smith 1987; Hänel & Chown 1999). The lowest air temperature recorded for Marion Island between 1949 and 1960 at low altitude was  $-6.8\text{ }^{\circ}\text{C}$  (Smith 1987). Rainfall is distributed approximately equally throughout the year, although the winter months on Marion Island (August to October) are marginally drier (Smith 1987). The average temperature lapse rate at Marion Island ranges between 4 and  $4.5\text{ }^{\circ}\text{C}$  per 1000 m altitude in summer and winter, respectively (Smith 1987). The ocean has a moderating influence on the island's climate as it prevents the occurrence of bitterly cold weather and effects a mean air temperature difference of only  $3.7\text{ }^{\circ}\text{C}$  between the coldest and warmest months (Schulze 1971; Smith 1978).

### **Microhabitat temperature recordings**

Microhabitat (soil) temperatures were recorded at 60-minute intervals, 2 cm below ground at 200 m altitude increments, between sea level (*c.* 5 m asl) and 800 m asl on the north-eastern side of Marion Island ( $46^{\circ}54'S$ ,  $37^{\circ}45'E$ ) using calibrated I-buttons (Thermochron DS1921G, Dallas Semi-Conductors, Texas, USA;  $0.5\text{ }^{\circ}\text{C}$  resolution) between May 2002 and April 2004. Because of the large number of data points collected in this study, temperatures were summarized for each altitudinal site on a monthly basis. Mean, absolute minimum and absolute maximum temperatures were determined. The range of temperatures experienced at each altitude during the entire study period was calculated using the absolute minimum and maximum values for each altitude.

Cooling rates were calculated for each month at each altitude by displaying microhabitat temperatures for a specific month graphically. The greatest change in temperature (from a higher to a lower temperature) was selected to represent the change in temperature for that month. The date and time were noted and cooling rates were determined and are expressed in  $^{\circ}\text{C}\cdot\text{min}^{-1}$  as is normally used in physiology experiments such as supercooling point determinations in invertebrates (e.g. Salt 1966; Miller 1978). There were some months for which data were not available due to the disappearance of I-buttons, or loggers being covered by deep snow. For calculations of mean, minimum and maximum monthly temperatures, as well as cooling rates, all available data were used.

The total time (hours) spent above, below or between temperature thresholds ( $<-5$ ,  $-5$  to  $0$ ,  $0$  to  $10$ ,  $10$  to  $20$  and  $>20$   $^{\circ}\text{C}$ ) for each month and altitude was determined. Data that were missing for some altitudes were omitted for all altitudes, such that each altitude had the same number of temperature recordings for the study period (data for March, April and May 2003, as well as April 2004 were deleted from all datasets for these calculations). The number of freeze-thaw cycles across  $0$   $^{\circ}\text{C}$  for each altitude was computed for the duration of the experiment using the macro from Sinclair (2001b). Mean, absolute minimum and absolute maximum monthly temperatures were plotted per year (May 2002 until April 2003 for year one, and May 2003 until April 2004 for year two) for all months in the study period.

The lower thermal tolerances of Marion Island invertebrates were obtained from published records (Klok & Chown 1997; Van der Merwe *et al.* 1997; Klok & Chown 1998; Klok &

Chown 2000; Klok & Chown 2001; Slabber & Chown 2004; 2005) (Table 1). The temperature at which mortality occurs, rather than supercooling point/crystallization temperature (SCP) *per se* was used for the purposes of this study, hence the temperatures at which insects died were used as thresholds. For freeze intolerant species, which die when they freeze (Bale 1993), the SCP was used as the temperature at which mortality occurs because prefreeze mortality has not been widely reported from insects and other arthropods occurring at the island. For freeze tolerant species, the lower lethal temperature at which 50 % of a sample died (LLT50) was used as a threshold, because these animals die below their freezing point. Mean SCP or LLT50, as well as the maximum SCP (i.e. the temperature at which the first individual froze in an experiment) and minimum upper lethal temperature (i.e. the temperature at which the first individual experienced high temperature induced mortality in an experiment) were used as thresholds. The number of times a lethal threshold was crossed was calculated at each altitude for each species for which data were available using the macro from Sinclair (2001a).

## Results

Mean microhabitat temperatures showed relatively little variation within-altitude and month, with mean temperatures ranging between 3.4 and 10.7 °C, and 0 and 5.8 °C at 0 and 800 m asl, respectively (Fig. 1). The 0 and 800 m asl sites had the highest and lowest mean temperatures, respectively, for all months during the study period (Fig. 1). Mean monthly temperature decreased by about 1 °C per altitudinal increment, and this was seen for all months across the range of altitudes investigated. Mean monthly temperature was positive

for all months during the study period. In general, mean temperatures were lower during autumn and winter than during spring and summer.

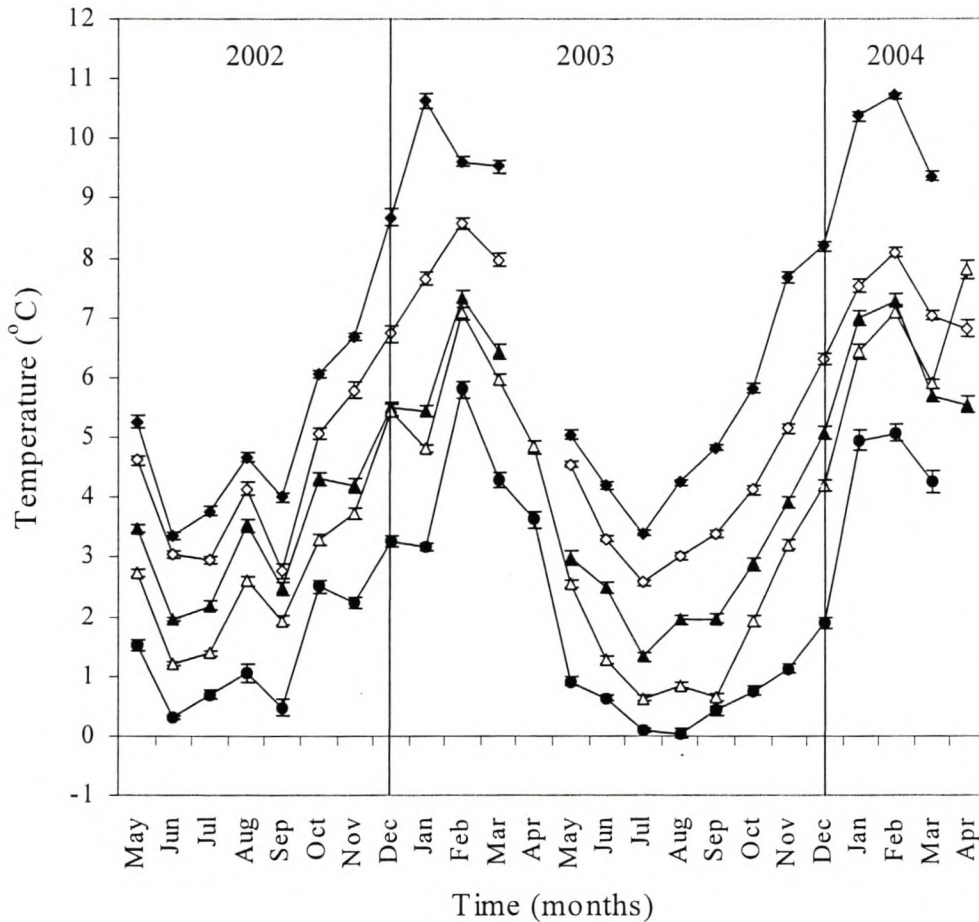


Figure 1. Mean  $\pm$  SE monthly microhabitat temperatures ( $^{\circ}$ C) for Marion Island at 0 ( $\blacklozenge$ ), 200 ( $\diamond$ ), 400 ( $\blacktriangle$ ), 600 ( $\triangle$ ) and 800 ( $\bullet$ ) m asl between May 2002 and April 2004.

Absolute minimum monthly microhabitat temperatures ranged between 7.5  $^{\circ}$ C at 0 m asl (February 2004) and -12  $^{\circ}$ C at 800 m asl (August 2002) (Fig. 2a). The 0 m asl site had the

highest minimum for most months during the study period, as could be expected, whereas the 200 m asl site showed the greatest variability in terms of minimum monthly temperatures experienced; in some cases it equalled the 0 m asl site, whereas in other instances, minimum temperatures approached those recorded at the 800 m asl site (Fig. 2a). These low minimum temperatures were most apparent during spring and summer of 2002, which probably resulted from diminishing snow depth and the resultant drop in temperature. Monthly minimum temperatures at the 400 and 600 m asl sites fluctuated around 0 °C during the entire study period, with very little difference between seasons, possibly as a consequence of frequent snowfalls at these altitudes.

Absolute maximum monthly microhabitat temperatures showed a degree of variability between altitudes (Fig. 2b). No clear trend is visible and each altitude experienced unique maximum monthly temperatures, generally independent of season. Absolute monthly maximum temperatures ranged between 3 and 23 °C, both absolute temperatures being recorded at the 800 m asl site (recorded during July 2003 and September 2002, respectively), indicating that local conditions may be more important in determining microhabitat temperature than altitude or season *per se*.

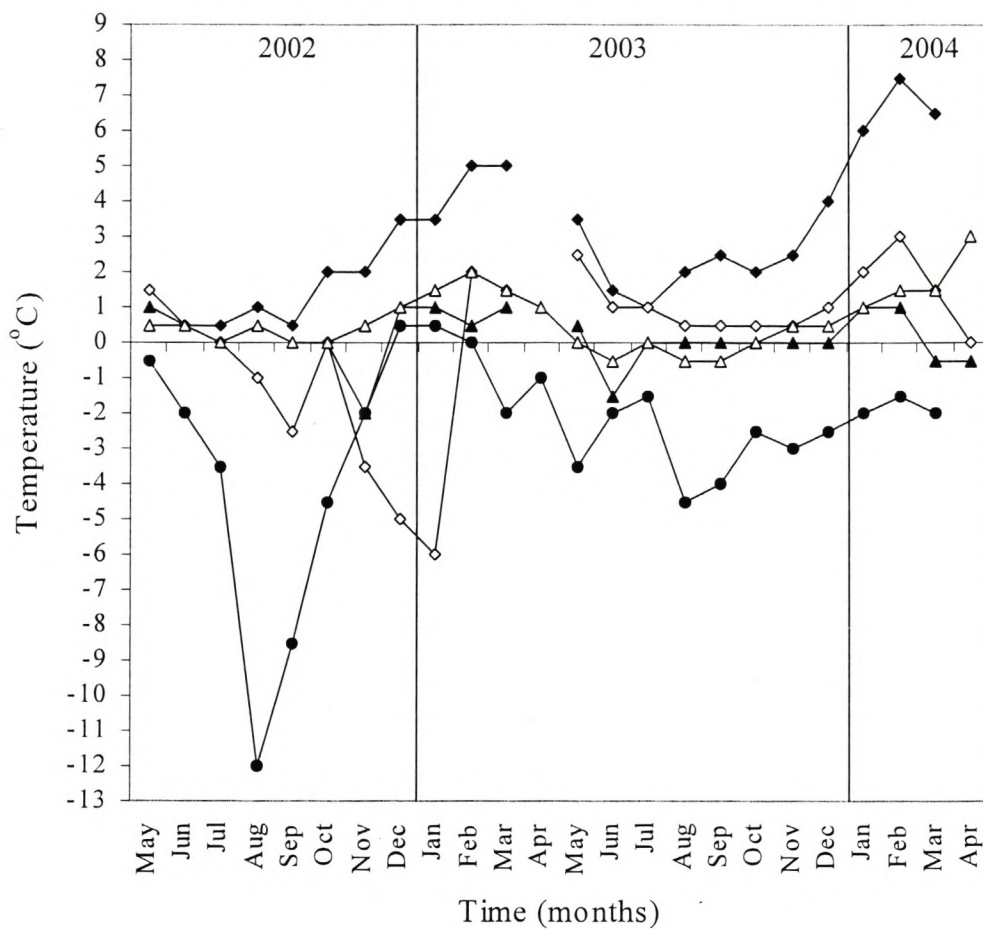


Figure 2a. Absolute minimum microhabitat temperatures (°C) for Marion Island at 0 (◆), 200 (◇), 400 (▲), 600 (△) and 800 (●) m asl between May 2002 and April 2004.



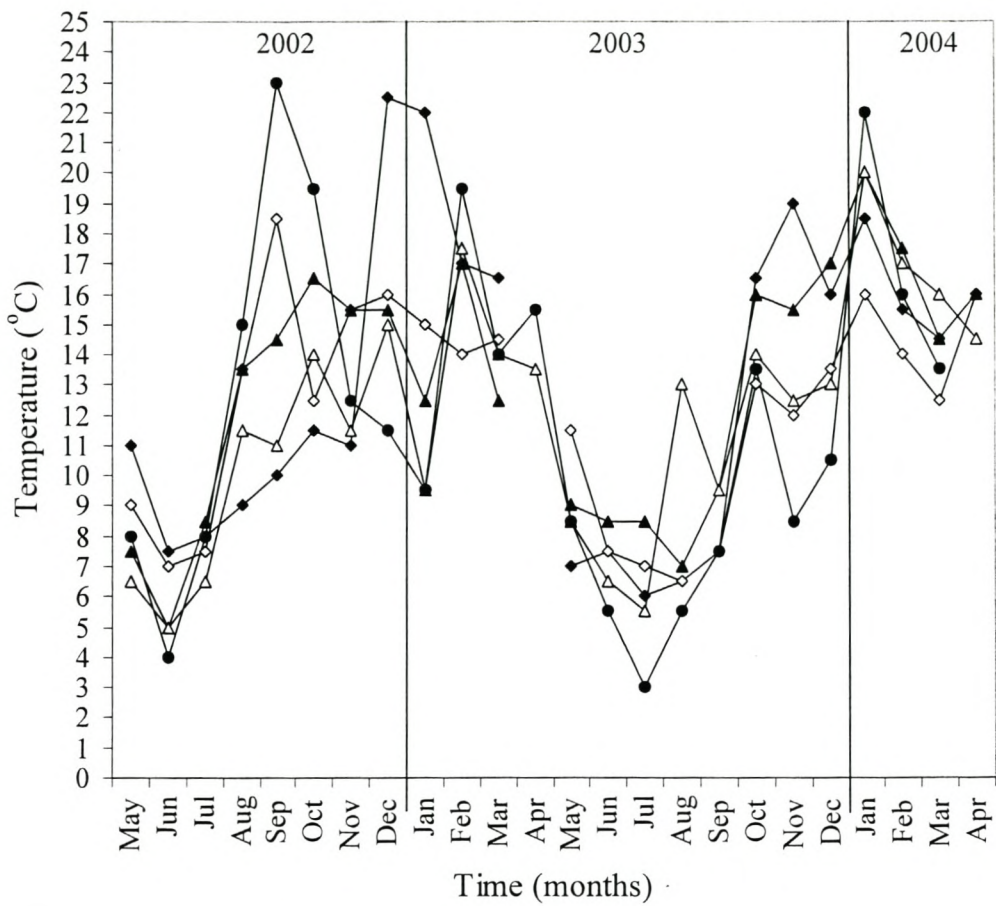


Figure 2b. Absolute maximum microhabitat temperatures (°C) for Marion Island at 0 (◆), 200 (◇), 400 (▲), 600 (△) and 800 (●) m asl between May 2002 and April 2004

Table 1. Lethal thermal limits (mean supercooling points (SCP), lower lethal temperatures (LLT50), maximum recorded SCPs and minimum recorded upper lethal temperatures (ULT) (°C)) of indigenous (I) and alien (A) Marion Island invertebrates. Values in bold indicate temperatures that were used as a microhabitat freezing-threshold for each species (i.e. LLT50 or mean SCP). The highest altitude (m asl) at which each species occurs is shown.

Species		Family	Order	I/A	Altitude	SCP	LLT50	Max SCP	Min ULT
<b>Collembola<sup>a</sup></b>									
<i>Pogonognathellus flavescens</i>	Tullberg	Tomoceridae	Collembola	A	100	<b>-12.0</b>	-6.6	-3.2	28.4
<i>Isotomurus palustris</i>	Müller	Isotomidae	Collembola	A	300	<b>-8.2</b>	-6.3	-3.5	34.6
<i>Ceratophysella denticulata</i>	Bagnall	Hypogastruridae	Collembola	A	500	<b>-14.3</b>	-11.1	-3.1	-
<i>Cryptopygus antarcticus</i>	Déharveng	Isotomidae	Collembola	I	1000	<b>-13.5</b>	-9.3	-8.8	30.8
<i>Tullbergia bisetosa</i>	Börner	Onychiuridae	Collembola	I	1000	<b>-19.4</b>	-11.1	-12.6	28.2
<b>Mites<sup>b</sup></b>									
<i>Halozetes marinus</i>	Engelbrecht	Ameronothridae	Cryptostigmata	I	<100	<b>-15.4</b>	-9.8	-11.0	35.0
<i>H. marionensis</i>	Déharveng	Ameronothridae	Cryptostigmata	I	<100	<b>-20.6</b>	-13.4	-18.2	36.0
<i>H. belgicae</i>	Michael	Ameronothridae	Cryptostigmata	I	<100	<b>-13.1</b>	-9.3	-3.6	37.0
<i>H. fulvus</i>	Engelbrecht	Ameronothridae	Cryptostigmata	I	300	<b>-14.7</b>	-13.4	-5.3	36.0
<i>Podacarus auberti</i>	Grandjean	Ameronothridae	Cryptostigmata	I	300	<b>-15.8</b>	-6.0	-2.9	36.0

a (S Slabber and SL Chown unpublished)

b (JA Deere and SL Chown unpublished)

Table 1. Continued

Species		Family	Order	I/A	Altitude	SCP	LLT50	Max SCP	Min ULT
<b>Weevils<sup>c</sup></b>									
<i>Bothrometopus elongatus</i> winter	Jeannel	Curculionidae	Coleoptera	I	1200	-6.4	<b>-11.5</b>	-	-
summer				I	1200	-5.8	<b>-9.5</b>	-	31.0
<i>B. parvulus</i> winter	C.O. Waterhouse	Curculionidae	Coleoptera	I	1200	-4.8	<b>-10.0</b>	-	-
summer				I	1200	-8.3	<b>-10.5</b>	-	32.0
<i>B. randi</i> winter	Jeannel	Curculionidae	Coleoptera	I	500	-4.8	<b>10.5</b>	-	-
summer				I	500	-5.6	<b>-9.5</b>	-	31.0
<i>Ectemnorhinus marioni</i> winter	Jeannel	Curculionidae	Coleoptera	I	650	-7.4	<b>-10.5</b>	-	-
summer				I	650	-5.5	<b>10.0</b>	-	34.0
<i>E. similis</i> winter	C.O. Waterhouse	Curculionidae	Coleoptera	I	600	-4.4	-	-	-
summer				I	600	-5.7	<b>-10.5</b>	-	34.0
<i>Palirhoeus eatoni</i> winter	C.O. Waterhouse	Curculionidae	Coleoptera	I	5	-15.5	<b>-9.5</b>	-	-
summer				I	5	-11.8	<b>-9.5</b>	-	32.0

c (Van der Merwe *et al.* 1997)

Table 1. Continued

Species		Family	Order	I/A	Altitude	SCP	LLT50	Max SCP	Min ULT
<b>Miscellaneous insects</b>									
<i>Halmaeus atriceps</i> adults <sup>d</sup>									
summer	C.O. Waterhouse	<b>Staphylinidae</b>	<b>Coleoptera</b>	I	<300	-18.9	<b>-7.6</b>	-13.4	-
winter				I	<300	-6.3	<b>-7.6</b>	-3.0	31.0
<i>H. atriceps</i> larvae				I	<300	-18.9	<b>-11.1</b>	-7.7	-
summer				I	<300	-5.4	<b>-11.1</b>	-3.2	30.0
winter				I	<300	-5.4	<b>-11.1</b>	-3.2	30.0
<i>Antarctopsocus jeanneli</i> <sup>e</sup>	Badonnel	<b>Elipsocidae</b>	<b>Psocoptera</b>	I	<300	<b>-11.1</b>	-7.7	-4.6	27.2
<i>Paractora dreuxi</i> larvae <sup>f</sup>	Seguy	Helcomyzidae	Diptera	I	<50	<b>-3.5</b>	~-3.5	-6.7	34.7
<i>P. dreuxi</i> adults				I	<50	-9.6	~-5.5	-2.8	27.9
<i>Apetaenus litoralis</i> larvae <sup>g</sup>	Eaton	Tethinidae	Diptera	I	<50	<b>-6.5</b>	-	-3.2	-
<i>A. litoralis</i> adults				I	<50	-4.0	>-3	-3.8	32.2
<i>Embryonopsis halticella</i> <sup>h</sup>	Eaton	Yponomeutidae	Lepidoptera	I	400	<b>-20.5</b>	-19.0	-11.7	39.1
<i>Pringleophaga marioni</i> <sup>i</sup>	Viette	Tineidae	Lepidoptera	I	1000	-5.0	~-9	-3.4	37.7
<i>Plutella xylostella</i> <sup>j</sup>	Dugdale	Plutellidae	Lepidoptera	A	<300	<b>-16.7</b>	-20.0	-10.6	-

d (Slabber &amp; Chown 2005)

e (Slabber &amp; Chown 2004)

f (Klok &amp; Chown 2001)

g (Klok &amp; Chown 2000)

h (Klok &amp; Chown 1998)

i (Klok &amp; Chown 1997)

j (B.J. Sinclair, unpublished)

Table 1. Continued

Species		Family	Order	I/A	Altitude	SCP	LLT50	Max SCP	Min ULT
<b>Molluscs</b>									
<i>Deroceras panormitanum</i> <sup>k</sup>	Lessona & Pollonera	Limacidae	Eupulmonata	A	<300	-3	-2.6	-	-
<b>Spiders</b>									
<i>Myro paucispinosus</i> <sup>k</sup>	Berland	Araneae	Arachnida	I	800	-6.6	-6.1	-5.6	-
<i>Erigone vagans</i> <sup>k</sup>	Audouin	Araneae	Arachnida	I	400	-7.9	-8.5	-5.4	-

k (SL Chown unpublished)

Maxima were equal to or above 20 °C at all sites, except 200 m asl where the maximum temperature recorded between May 2002 and April 2004 was 18.5 °C. The 800 m asl site experienced the highest microhabitat temperature, followed by the 0 m asl site. Absolute maximum temperatures never exceeded 23 °C at any of the sites investigated during the study period. Absolute minima calculated for each altitude for the entire study period varied more than absolute maxima between altitudes (Fig. 3). Absolute minimum temperatures ranged between 0.5 and -12 °C at 0 m and 800 m asl, respectively, showing a slight decrease in microhabitat temperature with an increase in altitude (Fig. 3). The 0 m asl site was the only one where the absolute minimum microhabitat temperature recorded never dropped below freezing. The 200 m asl site had a low absolute minimum of -6 °C when compared to the 400 and 600 m asl sites (-2.0 and -0.5 °C, respectively). The 200 m altitudinal band appears to experience the most severe cold conditions among the lower altitudes in this study. The high incidence of low temperatures is alleviated between 200 and 600 m asl, but reappears at the 800 m asl site. The 800 m asl site experienced a difference of 35 °C between the absolute maximum and minimum temperatures recorded, the next greatest difference between absolute temperatures was recorded at the 200 m asl site (24.5 °C) (Fig. 3).

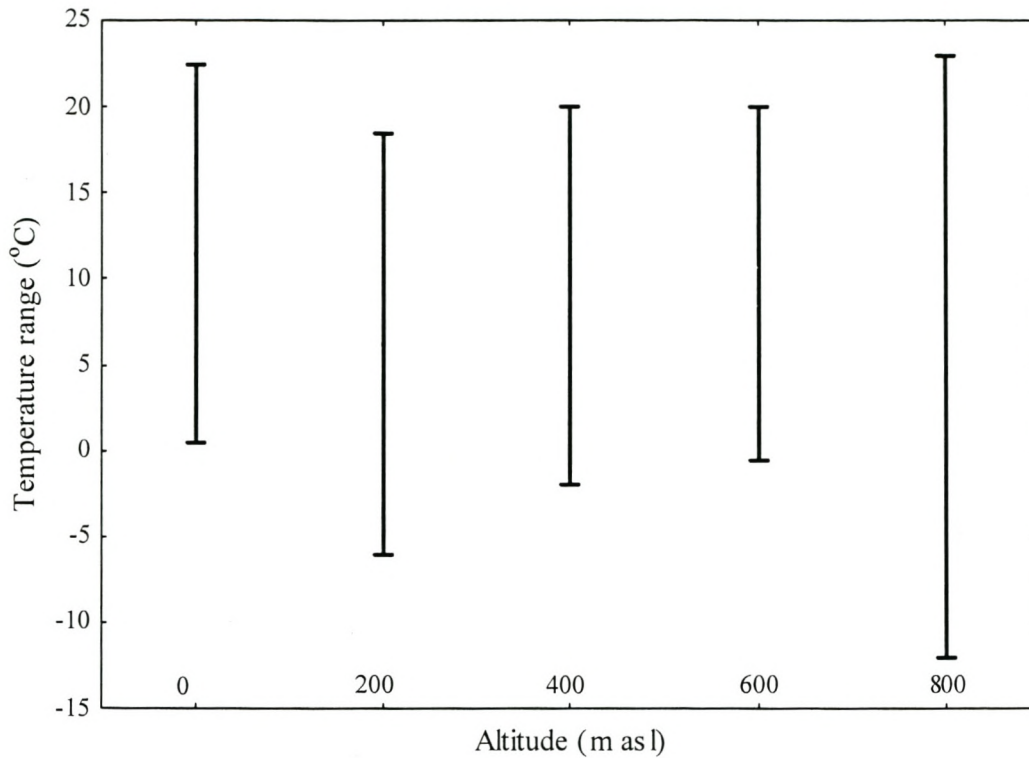


Figure 3. Range of temperatures (°C) experienced at 0 (A), 200 (B), 400 (C), 600 (D) and 800 (E) m asl between May 2002 and May 2004.

The fastest cooling rates were observed at the 800 m asl site at the end of the first year of this study (Fig. 4), but cooling rates for each month and altitude were generally slow and typically less than  $0.02 \text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ . Cooling rates tended to be slower during winter months compared to summer.

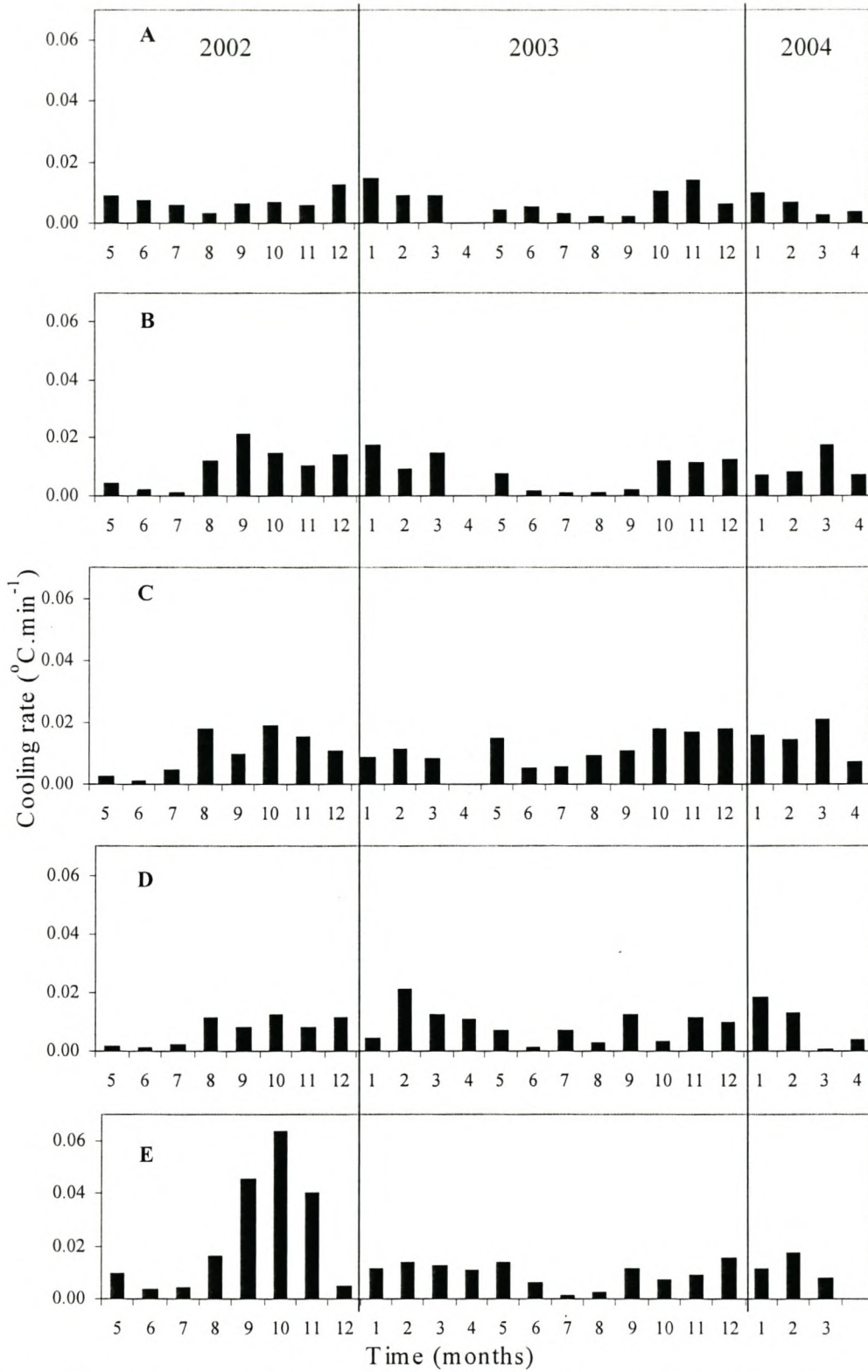


Figure 4. Cooling rates (°C.min<sup>-1</sup>) for each month during the study period for 0, 200, 400, 600 and 800 m asl



The proportions of time spent below, above or between temperature thresholds (<-5, -5 to 0, 0 to 10, 10 to 20 and >20 °C) showed reasonably clear trends with altitude (Fig. 5). At 0 m asl a large proportion of time was spent between 10 and 20 °C, but only during summer, whereas recorded temperatures in winter months increasingly fell within the 0 to 10 °C range. The same trends were visible at 200, 400, 600 and 800 m, but the proportions, as well as the threshold temperatures decreased in relation to altitudinal increase. Soil temperature ranged between 0 and 10 °C most of the time at all altitudes despite the low temperatures recorded at higher altitudes. None of the sites studied differed in terms of hours spent below -5 °C (Fig. 5), but the 800 m asl site was colder (at the -5 to 0 °C threshold) than the other sites investigated. For time spent between 0 and 10 °C, no trend is visible between altitudes; the 800 m asl site had the lowest incidence of temperatures in this threshold range, whereas the 400 m asl site had the highest incidence of temperatures between 0 and 10 °C. At the 10 to 20 °C threshold, the 0 m asl site was warmer than the 600 and 800 m asl sites, but not warmer than the 200 and 400 m asl sites. The duration spent between 10 and 20 °C at the 600 and 800 m asl sites was less than the other sites in this study. No difference is visible between sites in terms of hours spent above 20 °C. The only occurrence of microhabitat temperatures exceeding 20 °C was at the 0 and 800 m asl sites, and only for 25 and 5 h, respectively, over the two-year study period.

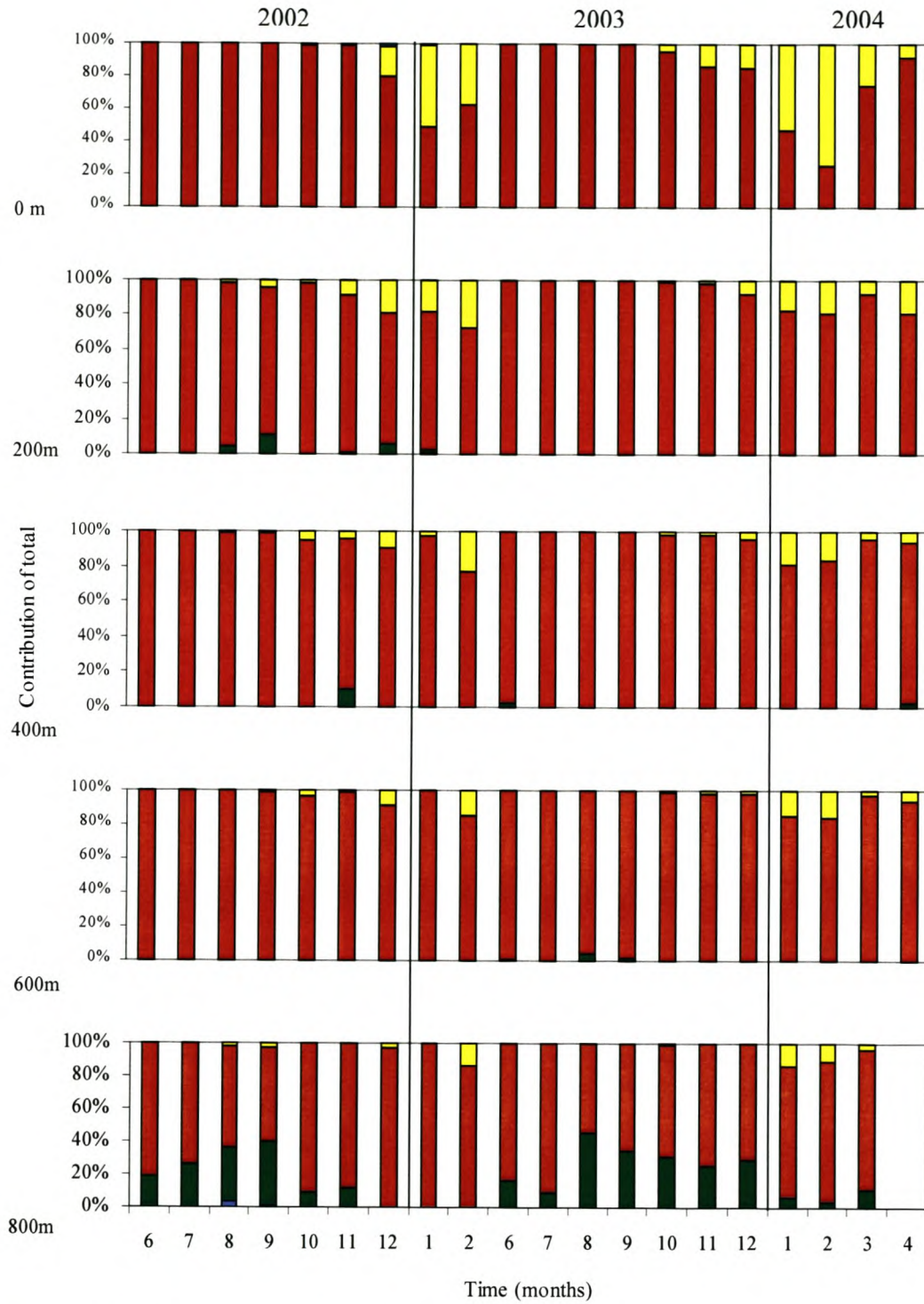


Figure 5. Proportion of time spent above, below, and between temperature thresholds at 0, 200, 400, 600 and 800 m asl on Marion Island. The thresholds are <-5 °C (blue), -5 to 0 °C (green), 0 to 10 °C (red), 10 to 20 °C (yellow) and >20 °C (turquoise)

Temperature never dropped below 0 °C at 0 m asl, and freeze-thaw cycles most often occurred at 800 m asl, followed by the 200 m asl site (Fig. 6). The high incidence of freeze-thaw events at the 200 and 800 m asl sites is in agreement with the comparatively low mean temperatures, as well as the absolute minima experienced at these sites.

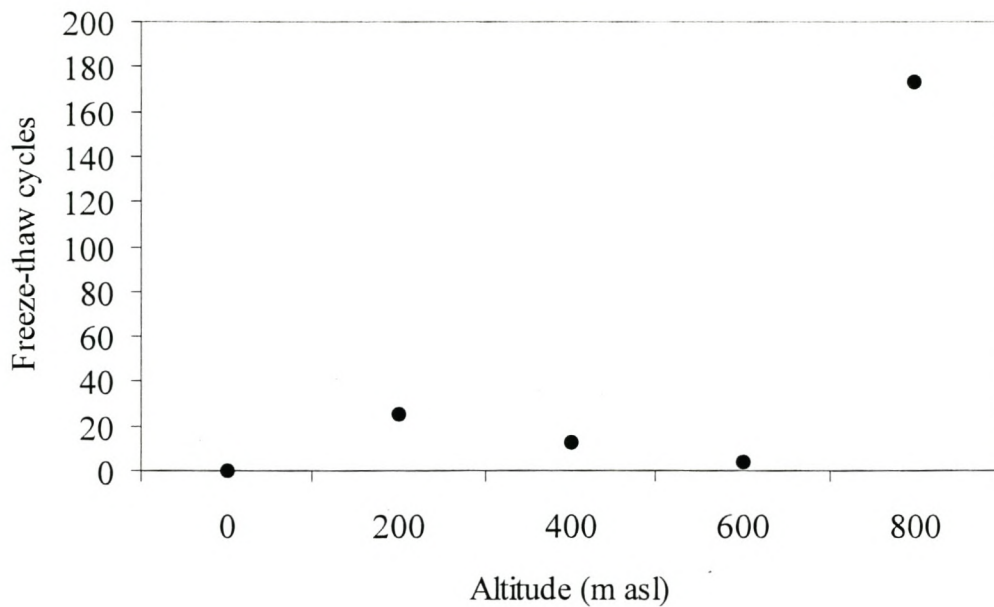


Figure 6. The number of microhabitat freeze-thaw cycles experienced at 0, 200, 400, 600 and 800 m asl at Marion Island between May 2002 and March 2004 (data for March to June 2003 were omitted)

Mean monthly microhabitat temperatures were very similar between year one (May 2002 until March 2003) and year two (May 2003 until April 2004) at the 0 and 200 m asl sites, but year one had higher microhabitat temperatures than year two at the 400, 600 and 800 m asl sites (Fig. 7 a-e). With the exception of the 400 and 600 m asl sites, year one experienced lower absolute minimum monthly temperatures, as well as higher absolute monthly temperatures. As a result of both lower absolute minimum and higher absolute maximum microhabitat temperatures at most altitudes in this study, absolute monthly temperature ranges were also greater during year one than during year two (Fig. 7 a-e). A clear seasonal trend is visible in the mean temperatures recorded at all altitudinal sites, where summers were clearly warmer than winters in both years. Year one, however, was considerably colder than year two in terms of absolute minimum temperatures experienced (Fig. 7b, c and e), whereas no clear trend is discernable for maximum monthly temperatures experienced at all study sites. At low altitudes (Fig. 7a, b and c), the temperature ranges experienced in summer exceeded those in winter, especially so for year one.

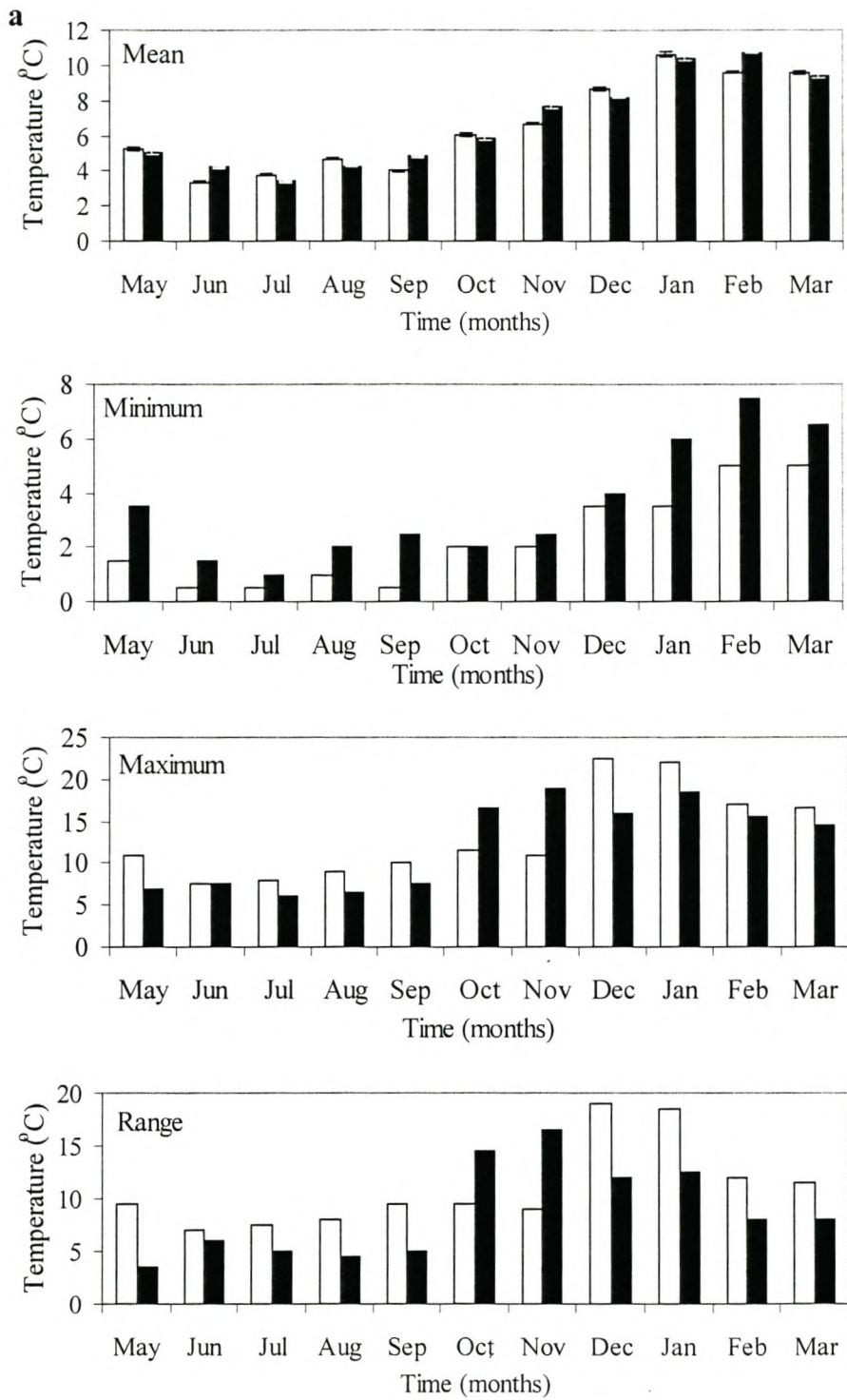


Figure 7 A

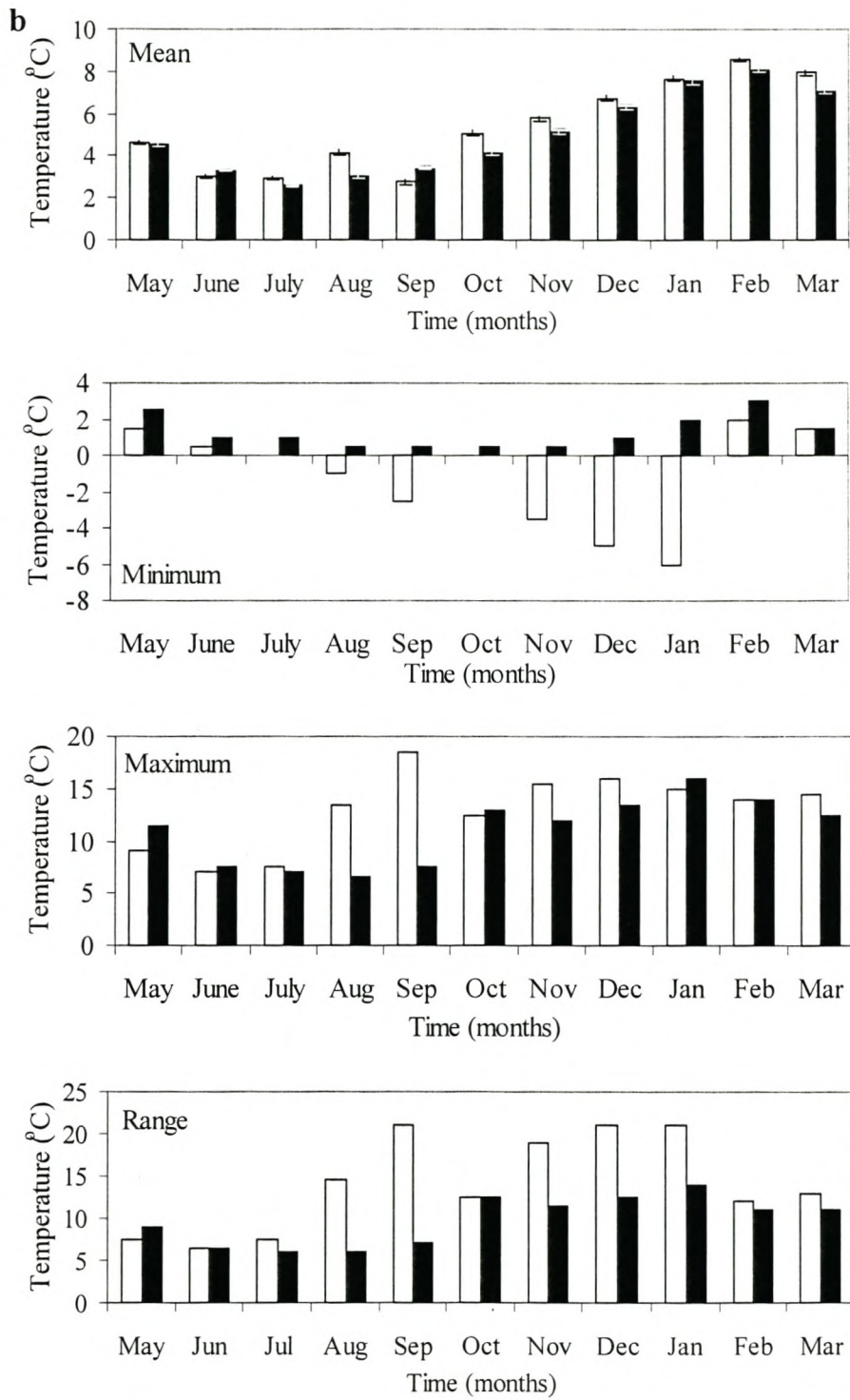


Figure 7 B

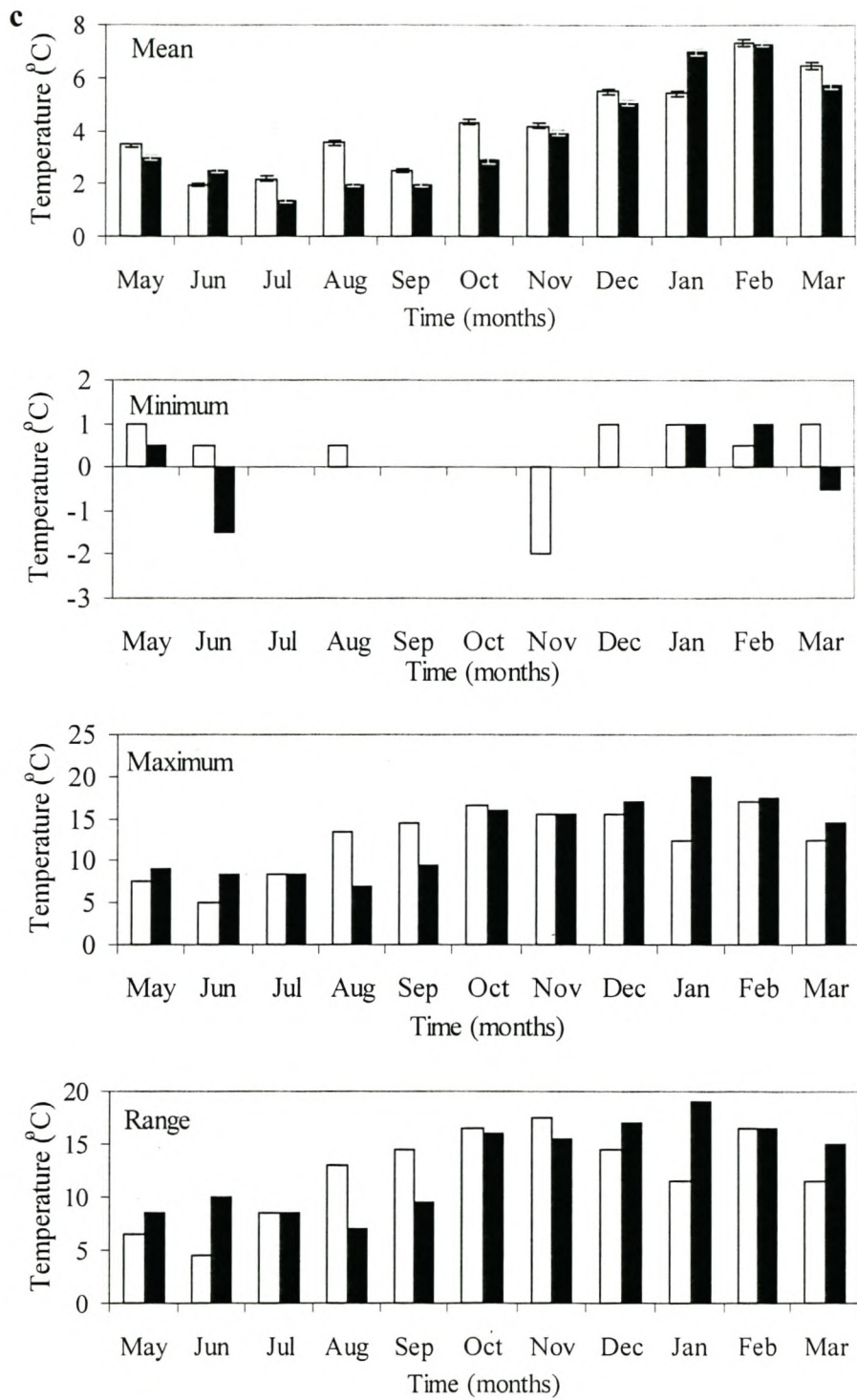


Figure 7 C

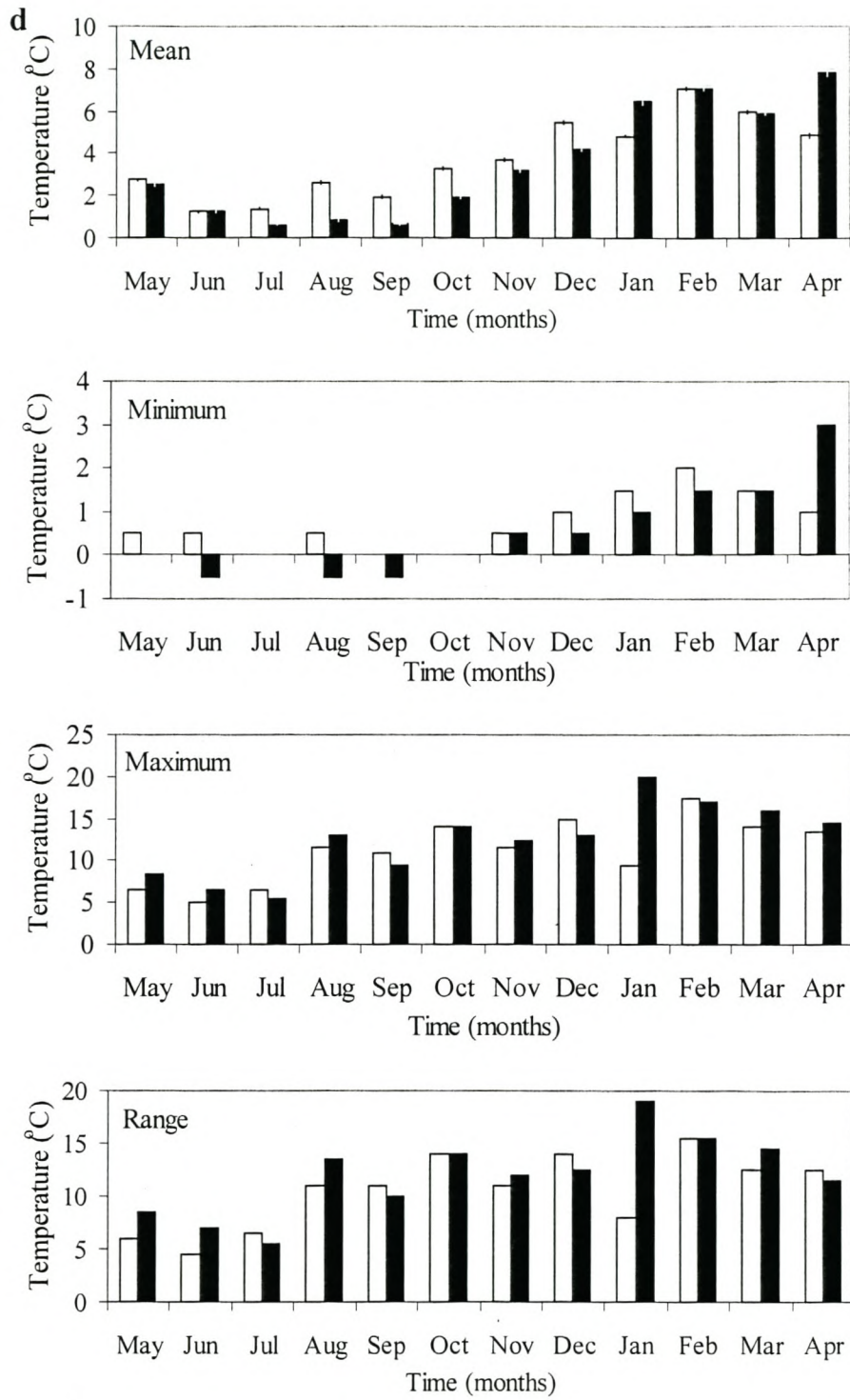


Figure 7 D



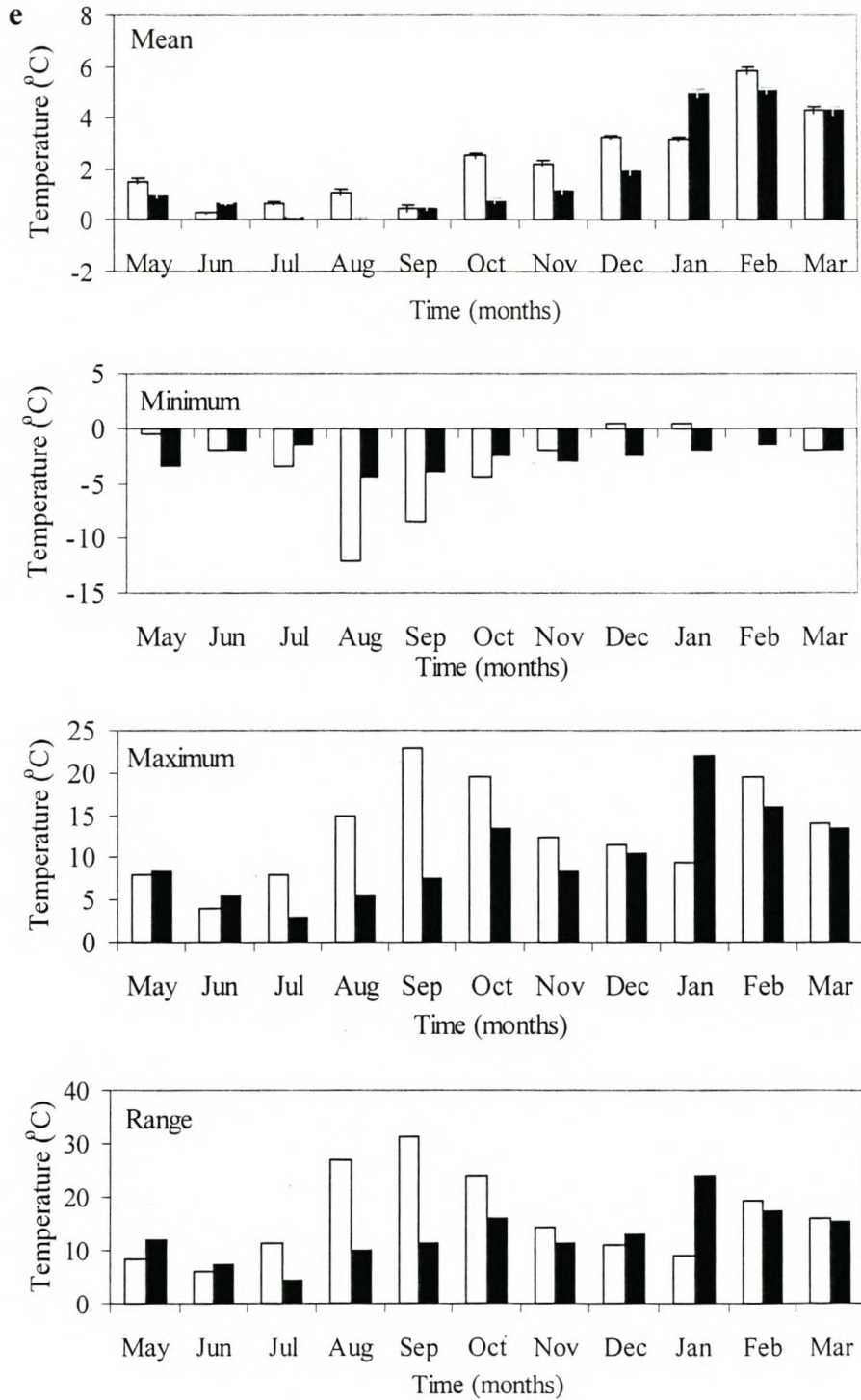


Figure 7. Mean ( $\pm$  SE), absolute minimum and absolute maximum monthly microhabitat temperatures ( $^{\circ}$ C) recorded over a two-year period on Marion Island for 0 (a), 200 (b), 400

(c), 600 (d) and 800 (e) m asl. Year one (May 2002 - March 2003) is represented by blank bars, year two (May 2003 - March 2004) by shaded bars. The monthly temperature range is also shown for each altitude.

Mortality thresholds for upper lethal temperatures were not exceeded once during this study for any of the Marion Island invertebrate species for which ULT data are available at any of the altitudes investigated (Table 1). Mortality thresholds (maximum SCPs) were however crossed for three alien springtail species at 200 m asl, where between 3 and 6 freeze-thaw events would have been experienced. Indigenous springtails would only have experienced freezing at the 800 m asl site (only 3 times across the maximum SCP for *Cryptopygus antarcticus*). The indigenous springtail, *Tullbergia bisetosa*, which does occur at 800 m asl would have experienced only one freeze-thaw event (-11.1 °C threshold (mean SCP)) during the study period. None of the mite species for which thermal tolerance data are known would have experienced mortality due to their mean freezing thresholds being crossed, but *Halozetes belgicae*, *H. fulvus* and *Podacarus auberti* would have experienced freeze-thaw cycles across their maximum recorded SCPs between 1 and 7 times at the 200 m asl site, and between 8 and 42 times at the 800 m asl site). These species mostly do not occur at altitudes exceeding 200 m asl, and some are indeed confined the coastal zone (Barendse *et al.* 2002). Maximum LLTs were not published for Marion Island weevils, hence mean SCPs were used as thresholds for these species. The cold tolerance limits of six weevil species from Marion Island are sufficiently low to ensure survival at all altitudes in this study, except at the 800 m asl site, which experienced relatively low microhabitat temperatures compared to the other altitudinal sites. As with the indigenous springtails,

mortality thresholds for weevils would not have been crossed more than three times even at 800 m asl (Table 2). Lethal thresholds would have been crossed most frequently at the 800 m asl site for both *H. atriceps* adults and larvae, and six times for adults and larvae at 200 m asl during winter-months (Table 2). *A. jeanneli* would have experienced four freezing events across its maximum SCP at 200 m asl and 11 times at 800 m asl, but only once across its mean SCP at 800 m asl. The indigenous dipterans *P. dreuxi* and *A. litoralis*, together with the alien mollusc *D. panormitanum*, were the species that experienced the highest risk of mortality at low altitudes in this study. The two spider species for which cold tolerance data are available would only have experienced 6 freeze-thaw cycles at 800 m asl, and *Myro paucispinosus* indeed does occur at this altitude. *Erigone vagans*, however, does not occur at altitudes greater than 400 m asl (K. Qwede, personal communication, 2005), and its distribution may be limited by low temperature (or its effect on moisture availability).

Table 2. The number of times mortality thresholds were crossed for each species at each altitude is shown in columns marked 0, 200, 400, 600, and 800 m above sea level. Thresholds were mean LLT50 or SCP (0), or maximum supercooling point (Max) measured for each species (refer to Table 1). Shaded cells indicate absence of species at specific altitudes (m).

Species	0m		200m		400m		600m		800m	
	0	Max	0	Max	0	Max	0	Max	0	Max
<b>Collembola</b>										
<i>Pogonognathellus flavescens</i>	0	0	0	6	0	0	0	0	1	31
<i>Isotomurus palustris</i>	0	0	0	3	0	0	0	0	4	22
<i>Ceratophysella denticulata</i>	0	0	0	6	0	0	0	0	0	31
<i>Cryptopygus antarcticus</i>	0	0	0	0	0	0	0	0	0	3
<i>Tullbergia bisetosa</i>	0	0	0	0	0	0	0	0	1	0
<b>Mites</b>										
<i>Halozetes marinus</i>	0	0	0	0	0	0	0	0	0	1
<i>H. marionensis</i>	0	0	0	0	0	0	0	0	0	0
<i>H. belgicae</i>	0	0	0	3	0	0	0	0	0	22
<i>H. fulvus</i>	0	0	0	1	0	0	0	0	0	8
<i>Podacarus auberti</i>	0	0	0	7	0	0	0	0	0	42

Table 2. Continued

<b>Species</b>	<b>0m</b>	<b>200m</b>	<b>400m</b>	<b>600m</b>	<b>800m</b>
<b>Weevils</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<i>Bothrometopus elongatus</i> winter	0	0	0	0	1
summer	0	0	0	0	3
<i>B. parvulus</i> winter	0	0	0	0	3
summer	0	0	0	0	1
<i>B. randi</i> winter	0	0	0	0	1
summer	0	0	0	0	3
<i>Ectemnorhinus marioni</i> winter	0	0	0	0	1
summer	0	0	0	0	3
<i>E. similis</i> summer	0	0	0	0	1
<i>P. eatoni</i> winter	0	0	0	0	3
summer	0	0	0	0	3

Table 2. Continued

Species	0m		200m		400m		600m		800m	
	0	Max	0	Max	0	Max	0	Max	0	Max
<i>Miscellaneous insects</i>	0	Max	0	Max	0	Max	0	Max	0	Max
<i>Halmaeus atriceps</i> adults										
summer	0	0	0	0	0	0	0	0	6	0
winter	0	0	0	6	0	0	0	0	6	31
<i>H. atriceps</i> larvae summer	0	0	0	0	0	0	0	0	1	6
winter	0	0	0	6	0	0	0	0	1	31
<i>A. jeanneli</i>	0	0	0	4	0	0	0	0	1	11
<i>Paractora dreuxi</i> larvae	0	0	6	0	0	0	0	0	31	6
<i>P. dreuxi</i> adults	0	0	1	7	0	0	0	0	8	42
<i>Apetaenus litoralis</i> larvae	0	0	0	6	0	0	0	0	7	31
<i>A. litoralis</i> adults	0	0	7	3	0	0	0	0	42	22
<i>Embryonopsis halticella</i>	0	0	0	0	0	0	0	0	0	1
<i>Pringleophaga marioni</i>	0	0	0	6	0	0	0	0	3	31
<i>Plutella xylostella</i>	0	0	0	0	0	0	0	0	0	1

Table 2. Continued

Species	0m		200m		400m		600m		800m	
	0	Max	0	Max	0	Max	0	Max	0	Max
<i>Deroceras panormitanum</i>	0	0	7	-	0	-	0	-	42	-
<b>Spiders</b>										
<i>Myro paucispinosus</i>	0	0	0	1	0	0	0	0	6	7
<i>Erigone vagans</i>	0	0	0	1	0	0	0	0	6	8

## Discussion

Because of its geographic location and small size, Marion Island has a characteristic oceanic climate, with relatively small amplitudes in daily and seasonal temperatures (Smith & Steenkamp 1990; Smith 2002). As a consequence, microhabitat temperatures on Marion Island are consistently low and monthly mean temperatures of greater than 8 °C are a rare occurrence (see also Chown & Crafford 1992; Blake 1996). Mean temperature never dropped below 0 °C at any of the sites investigated in the present study. Mean monthly temperatures were higher at low altitudes, and progressively decreased with increasing altitude, which agrees with the findings for three altitudinal sites in the study by Blake (1996) and mean annual temperatures in the study by Boelhouwers *et al.* (2003) on Marion Island. The 0 m asl site had the mildest mean monthly temperatures in the present study, whereas the 800 m asl was the most thermally severe at both the upper and lower temperature scales.

Mean annual microhabitat temperature recorded at the 0 m asl site from January 2003 until the end of December 2004 was 6.7 °C, comparable to that recorded at 35 m asl by Blake (6.3 °C, 1996), whereas the 200 m asl site had an annual mean temperature of 5.1 °C which was similar to the 5.5 °C mean recorded by Boelhouwers *et al.* (2003). As with the present study, the high altitude site (750 m asl) in the study by Blake (1996) did not have months with a mean temperature of below 0 °C, which could be ascribed to snow cover, which is known to buffer microhabitat temperatures (e.g. Merriam *et al.* 1983; Coulson *et al.* 2000; Sinclair 2001a; Sinclair *et al.* 2003a).



The adiabatic lapse rate for mean microhabitat temperatures in the present study between 0 and 1000 m asl was 5.5 °C, which is 1 °C warmer than that found for air temperature by Smith (1987). This is not surprising because mean microhabitat temperature at sea level in this study (6.7 °C) is 1 °C higher than mean air temperature (5.7 °C – Smith 2002), and because the thermal properties of soil and air are vastly different (Rosenberg *et al.* 1983; Unwin & Corbet 1991). Indeed, Chown & Crafford (1992) investigated microhabitat temperatures (at 25 m asl) over a five month period in summer and also found microhabitat temperatures to be consistently higher than Stevenson Screen temperatures.

Minimum monthly temperatures were highly variable, and for the 0 m asl site never dropped below 0 °C. The 800 m asl site had the highest incidence of absolute monthly minima below 0 °C, in agreement with the low monthly mean temperatures recorded at this altitude. The 200 m asl site, surprisingly had the second highest incidence of absolute minima below 0 °C. It is possible that thicker snow depths (see Merriam *et al.* 1983) at the 400 and 600 m asl sites buffered microhabitat temperature to a greater extent than at the 200 m asl site (which receives less snowfall than higher altitude sites) and the 800 m asl site (due to higher levels of solar radiation (see Tweedie 2000; Sinclair *et al.* 2003a)).

The absolute monthly maximum temperature for the 0 m asl site (22.5 °C) fell well below the absolute maximum (33.8 °C) recorded at sea level during the study by Chown & Crafford (1992), and the maximum temperature measured at 800 m asl (23 °C) in the present study were lower than the 30 °C rock surface temperature recorded at 1000 m asl

on Marion Island (Boelhouwers *et al.* 2003). The absolute maximum temperature (*c.* 40 °C) ever recorded for Marion Island was recorded in November during the study by Blake (1996). By contrast, extreme high microhabitat temperatures were not recorded in the present study. Low absolute high temperatures at both the 0 and 800 m asl sites in this study could not be explained by cold El Niño episodes, as both the latter half of 2002 and the first four months of 2003 were warm El Niño periods (NOAA-CIRES 2005). Sinclair (2001b) measured lower temperatures (due to less snow cover) in El Niño years in the Rock and Pillar Range (New Zealand), and although a lack of snow cover would lead to low minima with potentially lethal consequences to invertebrates (Sinclair 2001a), absolute maxima should also be higher due to increased radiation at high altitudes, especially at Marion Island, where the dark colour of the substrate at high altitudes would increase absorption of solar radiation (as was recorded by Tweedie 2000 for high altitude sites on Macquarie Island). Indeed, Sinclair (2001b) also recorded the absolute maxima during summer months in his study of microhabitat temperatures at the Rock and Pillar Range. Total monthly precipitation (recorded at 25 m asl) was also very similar between year one and two in this study (South African Weather Bureau), but the possibility that the extent of cloud cover differed between year one and two cannot be ruled out.

As was found in the study by Tweedie (2000) for Macquarie Island, the high altitude site in this study had the most variable microclimate temperature conditions (lowest absolute minimum and highest absolute maximum), and concomitantly, the largest range in temperatures (most variable microclimate). Despite the highest maximum temperature in this study being recorded at the 800 m asl site, microhabitat temperature exceeded 20 °C

for only 5 h during the entire study period, not representing potentially lethal temperatures for Marion Island invertebrates that do occur at 800 m asl (Klok & Chown 1997; Van der Merwe *et al.* 1997; Klok & Chown 1998; 2001; Slabber & Chown 2004; 2005). As mentioned above, solar radiation (associated with high air temperatures) could lead to a reduction in the amount of snow at high altitudes, resulting in low absolute minima, while the absence of snow (see Sinclair 2001a) could also lead to higher absorption of solar radiation by dark substrates, leading to higher maxima, in turn leading to larger ranges in temperature than at lower altitudes.

In the present study, the high altitude site also had the highest frequency of freeze-thaw cycles, which is in agreement with what was found for the high altitude site in the study by Tweedie (2000) for Macquarie Island. From a physiological perspective, the high altitude site (800 m asl) would pose the most severe environmental conditions that invertebrates could face (highest and lowest microhabitat temperatures, both recorded in the middle of winter, the largest range in temperatures and the highest frequency of freeze-thaw cycles). These unseasonably high (or low) temperatures could lead to considerable mortality in invertebrate populations, as was found in the study by Coulson *et al.* (2000) for High Arctic micro arthropods.

Boelhouwers *et al.* (2003) concluded that the high degree of cloudiness, relatively high precipitation and small daily temperature range experienced on Marion Island lead to a high frequency of short duration freeze-thaw cycles with shallow frost penetration. With a continued climate warming trend (IPCC 2001) reduced snow cover will lead to greater

variability in climate (more extreme absolute high and low temperatures) (Sinclair 2001a; Smith 2002), and an increase in the frequency of freeze-thaw cycles (Sinclair 2001a), further increasing the risk of mortality for invertebrates. With a reduction in cloud-cover, and increased temperatures, the number of extreme high and low temperature events are set to increase (Smith & Steenkamp 1990; Smith 2002). These extreme events (see Gaines & Denny 1993) are most likely responsible for shaping the physiological and ecological attributes of Marion Island invertebrates, thereby also influencing small and large scale distributions patterns (see Barendse & Chown 2001; Gabriel *et al.* 2001; Hugo *et al.* 2004).

Field cooling rates in the present study were slow (in the region of  $0.02\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ , comparable to the  $0.01\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$  found for the Rock and Pillar Range (Sinclair 1997)), but much faster than the  $3\text{ }^{\circ}\text{C}$  (Van der Woude 1987),  $1\text{ }^{\circ}\text{C}$  (Cannon *et al.* 1985) or  $0.1\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$  (Klok & Chown 1997) generally used in determinations of supercooling points in physiological investigations of invertebrate cold hardiness. In a study of the effect of cooling rate (ranging between  $1\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$  and *c.*  $0.5\text{ }^{\circ}\text{C}\cdot\text{h}^{-1}$ ) on the supercooling ability of springtails, no effect of cooling rate could be found (Chapter 5), indicating that the cooling rates found for Marion Island microhabitats should not present a threat to terrestrial invertebrates.

Interannual microhabitat temperature variability experienced at especially the 200 and 800 m asl sites in the present study may be explained by the findings of Sinclair (2001b), where it was shown that snow cover contributed to interannual variation in microhabitat temperature in the microhabitat of the alpine cockroach *Celatoblatta quinque maculata*.

Snow cover, in turn, depended on the effects on El Niño. In the present study, interannual variation could not be ascribed to the effects of El Niño episodes (NOAA-CIRES 2005) on microclimate, or because of differences in precipitation between years (although precipitation records are from 25 m asl and may present a different scenario to what is experienced at 800 m asl; data supplied by the South African Weather Bureau).

Microhabitat temperatures were mild when total time spent at or between temperature thresholds were considered, except for the 200 and 800 m asl sites, where temperatures below 0 °C were common. In previous studies, however, absolute maximum microhabitat temperatures far exceeded those recorded in the previous study (Blake 1996; Boelhouwers *et al.* 2003), and these extreme temperatures could lead to substantial mortality in invertebrate populations. For example, the 40 °C absolute maximum temperature recorded by Blake (1996) at 750 m asl on Marion Island would have caused substantial mortality in all invertebrate populations that have been studied to date, unless species were capable of migration deeper into the soil profile, which was the mechanism utilised by Antarctic springtails in order to avoid cold injury (Sinclair *et al.* 2003a), and the Northern Hemisphere springtail *Tomocerus minor* to avoid desiccating or cold conditions (Van der Woude 1987). Even one such an extreme event would pose major risks to invertebrate populations and could cause large fluctuations in numbers (Gaines & Denny 1993; Parmesan *et al.* 2000). So, despite the absence of extreme high microhabitat temperatures during this study, microhabitat temperatures recorded in previous studies indicate that Marion Island's invertebrate biota is occasionally exposed to physiologically severe thermal conditions, especially so for the less high temperature tolerant indigenous species

from Marion Island (e.g. *Antarctopsocus jeanneli* (Chapter 3), three indigenous springtail species (Chapter 5), and *Paractora dreuxi mirabilis* (Klok & Chown 2001)).

The high frequency of freeze-thaw events experienced at sub-Antarctic Islands (see e.g. Tweedie 2000) is exactly the reasoning behind why it is postulated that Southern Hemisphere insects tend to survive freezing (Sinclair *et al.* 2003b). Northern Hemisphere insects are exposed to climatically severe environments, but with pronounced seasonality and predictable low winter temperatures (Sinclair *et al.* 2003b). At similar latitudes in the Southern Hemisphere, however, clear seasonal trends are absent, and insects are likely to encounter freezing micro-environments at any time during the year, which has led to the evolution of freeze tolerance (see e.g. Bale 1993; Sinclair 1999) in these species, compared to freezing intolerance in Northern Hemisphere invertebrates (Sinclair *et al.* 2003b). It seems that with climate warming continuing at its current rate at the Southern Ocean Islands (Smith & Steenkamp 1990; Tweedie 2000; Smith 2002), the frequency of freeze-thaw cycles will increase (Smith 2002).

Differences that could be attributed to more severe microhabitat temperatures at high altitude were found in the altitudinal distribution of indigenous and alien invertebrates from Marion Island. Indeed, only indigenous springtails, mites, several weevil species, one of the spider species, and the flightless moth *Pringleophaga marioni* inhabit sites at higher altitudes in this study, indicating that low temperature may be limiting the altitudinal distribution and spread of alien species along the eastern side of Marion Island. It appears that the alien invertebrates from Marion Island are confined to lower (warmer) altitudinal

bands to a greater extent than any of the indigenous invertebrates (excluding species that are confined to habitat resources such as kelp wracks (*Paractora dreuxi mirabilis* and *Apetaenus litoralis* - Crafford *et al.* 1986) or marine environments (e.g. mites - Barendse *et al.* 2002), which have lower maximum SCPs and LLTs than alien species (Table 1 and Chapter 5). In addition, if climatic conditions are becoming more extreme, as has been suggested recently (Smith 2002), the benefit that alien species will incur due to warmer (mild) microclimates (see Chapter 5) may be removed by extreme cold conditions, which could cause substantial cold-induced mortality, especially at low altitudes, further shaping the physiological attributes of invertebrate populations (Sinclair 2001a; Irwin & Lee 2003). To conclude, neither indigenous nor alien species in this study would have experienced physiologically lethal high temperatures at any of the altitudes investigated, especially so because the duration spent above 20 °C was limited (25 and 5 h at 0 and 800 m asl, respectively). Absolute maxima of over 40 °C have however been recorded in a previous study (Blake 1996), which would cause substantial mortality in invasive and indigenous invertebrates from Marion Island. Indigenous species were however found to be better able to tolerate the high incidence of low temperatures at higher altitudes than alien species (Table 2), which follows the prediction by Gabriel *et al.* (2001) that indigenous species perform better at low temperatures than alien species. Interannual variation (as was seen in this study where year one was considerably colder than year two) could lead to increased mortality in alien species, whereas warming could deleteriously influence high temperature-sensitive indigenous species (Klok & Chown 2001; Slabber & Chown 2004; 2005). Continued warming would thus favour the introduction and spread of alien species at low altitudes on Marion Island (Bergstrom & Chown 1999; Chown *et al.* 2002), whereas

an increase in the number of extreme events, as predicted by Smith (2002), would prevent the spread of aliens, especially at higher altitudes on Marion Island. Nonetheless, because of the known deleterious impacts of invasive species on landscapes (Bergstrom & Chown 1999; Pimentel *et al.* 2002; Slabber & Chown 2002; Van Wilgen *et al.* 2002), it is essential that the guidelines set out by the Prince Edward Islands Management Plan (Anonymous 1996) are adhered to in order to prevent the accidental introduction of alien species.



## References

- Andrewartha H. G. & Birch L. C.** (1954) *The distribution and abundance of animals*. University of Chicago Press, Chicago
- Anonymous** (1996) *Prince Edward Islands management plan*. Department of Environmental Affairs and Tourism, Pretoria
- Bale J. S.** (1993) Classes of insect cold hardiness. *Functional Ecology* **7**: 751-753
- Bale J. S., Block W. & Worland M. R.** (2000) Thermal tolerance and acclimation response of larvae of the sub-Antarctic beetle *Hydromedion sparsutum* (Coleoptera: Perimylopidae). *Polar Biology* **23**: 77-84
- Barendse J. & Chown S. L.** (2001) Abundance and seasonality of mid-altitude fellfield arthropods from Marion Island. *Polar Biology* **24**: 73-82
- Barendse J., Mercer R. D., Marshall D. J. & Chown S. L.** (2002) Habitat specificity of mites on sub-Antarctic Marion Island. *Environmental Entomology* **31**: 612-625
- Beaumont L. J. & Hughes L.** (2002) Potential changes in the distributions of latitudinally restricted Australian butterfly species in response to climate change. *Global Change Biology* **8**: 954-971
- Bergstrom D. M. & Chown S. L.** (1999) Life at the front: history, ecology and change on southern ocean islands. *Trends in Ecology and Evolution* **14**: 472-477
- Blake B. J.** (1996) *Microclimate and prediction of photosynthesis at Marion Island*. M.Sc. Thesis. University of the Orange Free State
- Block W.** (1984) Terrestrial microbiology, invertebrates and ecosystems. *Antarctic ecology*. Ed. Laws R. M., Academic Press, London. **1**: pp 163-236
- Boelhouwers J., Holness S. & Sumner P.** (2003) The maritime Subantarctic: a distinct periglacial environment. *Geomorphology* **52**: 39-55
- Cannon R. J. C., Block W. & Collett G. D.** (1985) Loss of supercooling ability in *Cryptopygus antarcticus* (Collembola: Isotomidae) associated with water uptake. *Cryo-Letters* **6**: 73-80
- Chown S. L. & Crafford J. E.** (1992) Microhabitat temperatures at Marion Island. *South African Journal of Antarctic Research* **22**: 51-58

- Chown S. L., Klok C. J. & McGeoch M. A.** (2004) Weather to go out: activity of *Bothriometopus brevis* at Heard Island. *Polar Biology* **27**: 217-221
- Chown S. L., McGeoch M. A. & Marshall D. J.** (2002) Diversity and conservation of invertebrates on the sub-Antarctic Prince Edward Islands. *African Entomology* **10**: 67-82
- Chown S. L. & Nicolson S. W.** (2004) *Insect physiological ecology. Mechanisms and patterns*. Oxford University Press, Oxford
- Convey P.** (1996) Overwintering strategies of terrestrial invertebrates in Antarctica - the significance of flexibility in extremely seasonal environments. *European Journal of Entomology* **93**: 489-505
- Coulson S. J., Leinaas H. P., Ims R. A. & Søvik G.** (2000) Experimental manipulation of the winter surface ice layer: the effects on a High Arctic soil microarthropod community. *Ecography* **23**: 299-306
- Crafford J. E. & Chown S. L.** (1990) The introduction and establishment of the diamondback moth (*Plutella xylostella* L., Plutellidae) on Marion Island. *Antarctic ecosystems. Ecological change and conservation*. Ed. Kerry K. R. & Hempel G. pp 354-358
- Crafford J. E., Scholtz C. H. & Chown S. L.** (1986) The insects of sub-Antarctic Marion and Prince Edward Islands; with a bibliography of entomology of the Kerguelen Biogeographical Province. *South African Journal of Antarctic Research* **16**: 42-84
- Davenport J. & MacAlister H.** (1996) Environmental conditions and physiological tolerances of intertidal fauna in relation to shore zonation at Husvik, South Georgia. *Journal of Marine Biological Association of the United Kingdom* **76**: 985-1002
- Gabriel A. G. A., Chown S. L., Barendse J., Marshall D. J., Mercer R. D., Pugh P. J. A. & Smith V. R.** (2001) Biological invasions of southern ocean islands: the Collembola of Marion Island as a test of generalities. *Ecography* **24**: 421-430
- Gaines S. D. & Denny M. W.** (1993) The largest, smallest, highest, lowest, longest, and shortest: extremes in ecology. *Ecology* **74**: 1677-1692
- Gaston K. J.** (2003) *The structure and dynamics of geographic ranges*. Oxford University Press, Oxford
- Hänel C. & Chown S. L.** (1999) *An introductory guide to the Marion and Prince Edward Island special nature reserves. Fifty years after annexation*. Department of Environmental Affairs & Tourism, Pretoria

- Hill J. K. & Fox R.** (2003) Climate change and British butterfly distributions. *Biologist* **50**: 106-110
- Hugo A. E., McGeoch M. A., Marshall D. J. & Chown S. L.** (2004) Fine scale variation in microarthropod communities inhabiting the keystone species *Azorella selago* on Marion Island. *Polar Biology* **27**: 466-473
- Huntley B.** (1971) Vegetation. *Marion and Prince Edward Islands: Report on the South African Biological and Geological Expedition / 1965-1966*. Ed. Van Zinderen Bakker Sr E. M., Winterbottom J. M. & Dyer R. A., Balkema, Cape Town. pp 98-160
- IPCC** (2001) *Climate change 2001: synthesis report. A contribution of working groups I, II, and III to the third assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge
- Irwin J. T. & Lee R. E.** (2000) Mild winter temperatures reduce survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis* (Diptera: Tephritidae). *Journal of Insect Physiology* **46**: 655-661
- Irwin J. T. & Lee R. E.** (2002) Energy and water conservation in frozen vs. supercooled larvae of the goldenrod gall fly, *Eurosta solidaginis* (Fitch) (Diptera: Tephritidae). *Journal of Experimental Zoology* **292**: 345-350
- Irwin J. T. & Lee R. E.** (2003) Cold winter microenvironments conserve energy and improve overwintering survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis*. *Oikos* **100**: 71-78
- Kennedy A. D.** (1995) Antarctic terrestrial ecosystem response to global environmental change. *Annual Review of Ecology and Systematics* **26**: 683-704
- Kingsolver J. G.** (1989) Weather and the population dynamics of insects: integrating physiological and population ecology. *Physiological Zoology* **62**: 314-334
- Klok C. J. & Chown S. L.** (1997) Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic caterpillar, *Pringleophaga marioni* Viette (Lepidoptera: Tineidae). *Journal of Insect Physiology* **43**: 685-694
- Klok C. J. & Chown S. L.** (1998) Interactions between desiccation resistance, host-plant contact and the thermal biology of a leaf-dwelling sub-Antarctic caterpillar, *Embryonopsis halticella* (Lepidoptera: Yponomeutidae). *Journal of Insect Physiology* **44**: 615-628
- Klok C. J. & Chown S. L.** (2000) Lack of cold tolerance in a small, brachypterous sub-Antarctic fly, *Apetaemus litoralis* Eaton (Diptera: Tethinidae) from Marion Island. *African Entomology* **8**: 305-308

- Klok C. J. & Chown S. L.** (2001) Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic kelp fly, *Paractora dreuxi* (Diptera: Helcomyzidae). *Journal of Insect Physiology* **47**: 95-109
- Klok C. J. & Chown S. L.** (2005) Inertia in physiological traits: *Embryonopsis halticella* caterpillars (Yponomeutidae) across the Antarctic Polar Frontal Zone. *Journal of Insect Physiology* **51**: 87-97
- McDougall I., Verwoerd W. & Chevallier L.** (2001) K-Ar geochronology of Marion Island, Southern Ocean. *Geological Magazine* **138**: 1-17
- Merriam G., Wegner J. & Caldwell D.** (1983) Invertebrate activity under snow in deciduous woods. *Holarctic Ecology* **6**: 89-94
- Miller L. K.** (1978) Freezing tolerance in relation to cooling rate in an adult insect. *Cryobiology* **15**: 345-349
- NOAA-CIRES** (2005) Climate Diagnostics Center, Boulder, Colorado, USA. <http://www.cdc.noaa.gov>.
- Parmesan C., Root T. L. & Willig M. R.** (2000) Impacts of extreme weather and climate on terrestrial biota. *Bulletin of the American Meteorological Society* **81**: 443-450
- Parmesan C., Ryrholm N., Stefanescu C., Hill J. K., Thomas C. D., Descimon H., Huntley B., Kaila L., Kullberg J., Tammaru T., Tennent W. J., Thomas J. A. & Warren M.** (1999) Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* **399**: 579-583
- Pimentel D., McNair W., Jancecka J., Wightman J., Simmonds C., O'Connell E., Wong E., Russel L., Zern J., Aquino T. & Tsomondo T.** (2002) Economic and environmental threats of alien plant, animal and microbe invasions. *Biological invasions. Economic and environmental costs of alien plant, animal and microbe species*. Ed. Pimentel D., CRC Press LCC, Boca Raton. pp 307-329
- Pugh P. J. A. & MacAlister H. E.** (1994) Acari of the supralittoral zone on sub-Antarctic South Georgia. *Pedobiologia* **38**: 552-565
- Rosenberg N. J., Blad B. L. & Verma S. B.** (1983) *Microclimate. The biological environment*. John Wiley & Sons, New York
- Roy D. B., Rothery P., Moss D., Pollard E. & Thomas J. A.** (2001) Butterfly numbers and weather: predicting historical trends in abundance and the future effects of climate change. *Journal of Animal Ecology* **70**: 201-217
- Salt R. W.** (1966) Effect of cooling rate on the freezing temperatures of supercooled insects. *Canadian Journal of Zoology* **44**: 655-659

- Sax D. F.** (2001) Latitudinal gradients and geographic ranges of exotic species: implications for biogeography. *Journal of Biogeography* **28**: 139-150
- Schulze B. R.** (1971) The climate of Marion Island. Marion and Prince Edward Islands: Report on the South African Biological and Geological Expedition / 1965-1966. Ed. Dyer R. A., Balkema, Cape Town. pp 16-31
- Sinclair B. J.** (1997) Seasonal variation in freezing tolerance of the New Zealand alpine cockroach *Celatoblatta quinque maculata*. *Ecological Entomology* **22**: 462-467
- Sinclair B. J.** (1999) Insect cold tolerance: How many kinds of frozen? *European Journal of Entomology* **96**: 157-164
- Sinclair B. J.** (2001b) Biologically relevant environmental data: Macros to make the most of microclimate recordings. *Cryo-Letters* **22**: 125-134
- Sinclair B. J.** (2001a) Field ecology of freeze tolerance: interannual variation in cooling rates, freeze-thaw and thermal stress in the microhabitat of the alpine cockroach *Celatoblatta quinque maculata*. *Oikos* **93**: 286-293
- Sinclair B. J., Addo-Bediako A. & Chown S. L.** (2003b) Climatic variability and the evolution of insect freeze tolerance. *Biological Reviews* **78**: 181-195
- Sinclair B. J. & Chown S. L.** (2003) Rapid responses to high temperature and desiccation but not to low temperature in the freeze tolerant sub-Antarctic caterpillar *Pringleophaga marioni* (Lepidoptera, Tineidae). *Journal of Insect Physiology* **49**: 45-52
- Sinclair B. J., Klok C. J., Scott M. B., Terblanche J. S. & Chown S. L.** (2003a) Diurnal variation in supercooling points of three species of Collembola from Cape Hallett, Antarctica. *Journal of Insect Physiology* **49**: 1049-1061
- Slabber S. & Chown S. L.** (2002) The first record of a terrestrial crustacean, *Porcellio scaber* (Isopoda, Porcellionidae), from sub-Antarctic Marion Island. *Polar Biology* **25**: 855-858
- Slabber S. & Chown S. L.** (2004) Thermal tolerance and cold hardiness strategy of the sub-Antarctic psocid *Antarctopsocus jeanneli* Badonnel. *Polar Biology* **28**: 56-61
- Slabber S. & Chown S. L.** (2005) Thermal tolerance responses to acclimation in the sub-Antarctic rove beetle *Halmaeus atriceps* (C.O. Waterhouse). *Physiological Entomology*: **30**: 195-204
- Smith V. R.** (1978) Animal-plant-soil nutrient relationships on Marion Island (Subantarctic). *Oecologia* **32**: 239-253

- Smith V. R.** (1987) The environment and biota of Marion Island. *South African Journal of Science* **83**: 211-220
- Smith V. R.** (1992) Surface air temperatures at Marion Island, sub-Antarctic. *South African Journal of Science* **88**: 575-578
- Smith V. R.** (2002) Climate change in the sub-Antarctic: An illustration from Marion Island. *Climatic Change* **52**: 345-357
- Smith V. R. & French D. D.** (1988) Patterns of variation in the climates, soils and vegetation of some subantarctic and antarctic islands. *South African Journal of Botany* **54**: 35-46
- Smith V. R. & Steenkamp M.** (1990) Climatic change and its ecological implications at a sub-Antarctic island. *Oecologia* **85**: 14-24
- Stevenson R. D.** (1985) The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. *American Naturalist* **126**: 362-386
- Thomas C. D., Cameron A., Green R., Bakkenes M., Beaumont L. J., Collingham Y., Erasmus B. F. N., de Siqueira M. F., Grainger, A. Hannah, L., Hughes L., Huntley B., van Jaarsveld A. S., Midgley G. F., Miles L., Ortega-Huerta M. A., Peterson A. T., Phillips O. L. & Williams S. E.** (2004) Extinction risk from climate change. *Nature* **427**: 145-148
- Todd C. M. & Block W.** (1997) Responses to desiccation in four coleopterans from Sub-Antarctic South Georgia. *Journal of Insect Physiology* **43**: 905-913
- Tréhen P., Bouché M., Vernon P. & Frenot Y.** (1985) Organization and dynamics of Oligochaeta and Diptera on Possession Island. *Antarctic nutrient cycles and food webs*. Ed. Siegfried W. R., Condy P. R. & Laws R. M., Springer, Berlin. pp 606-613
- Tweedie C. E.** (2000) *Climate change and the autecology of six plant species along an altitudinal gradient on subantarctic Macquarie Island*. PhD Thesis. University of Queensland
- Tweedie C. E. & Bergstrom D. M.** (2000) A climate change scenario for surface air temperature at subantarctic Macquarie Island. *Antarctic ecosystems: Models for wider ecological understanding*. Ed. Davison W., Howard-Williams C. & Broady P. A., New Zealand Natural Sciences, Christchurch. pp 272-281
- Unwin D. M. & Corbet S. A.** (1991) *Insects, plants and microclimate*. The Richmond Publishing Co. Ltd., Great Britain

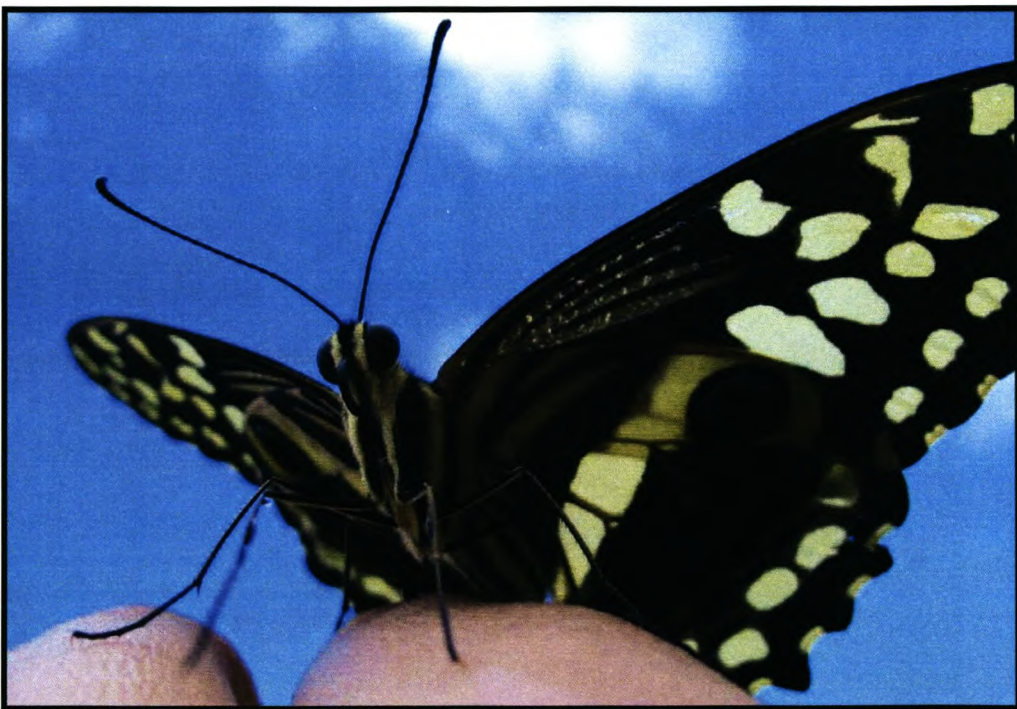
- Van der Merwe M., Chown S. L. & Smith V. R.** (1997) Thermal tolerance limits in six weevil species (Coleoptera, Curculionidae) from sub-Antarctic Marion Island. *Polar Biology* **18**: 331-336
- Van der Woude H. A.** (1987) Seasonal changes in cold hardiness of temperate Collembola. *Oikos* **50**: 231-238
- Van Wilgen B. W., Richardson D. M., Le Maitre D. C., Marais C. & Magadlela D.** (2002) The economic consequences of alien plant invasions: examples of impacts and approaches to sustainable management in South Africa. *Biological Invasions. Economic and environmental costs of alien plant, animal and microbe species*. Ed. Pimentel D., CRC Press LCC, Boca Raton. pp 243-265
- Vernon P. & Vannier G.** (1996) Developmental patterns of supercooling capacity in a subantarctic wingless fly. *Experientia* **52**: 155-158
- Walther G.-R., Post E., Convey P., Menzel A., Parmesan C., Beebee T. J. C., Fromentin J. M., Hoegh-Guldberg O. & Bairlein F.** (2002) Ecological responses to recent climate change. *Nature* **416**: 389-395
- Walton D. W. H.** (1984) The terrestrial environment. *Antarctic ecology*. Ed. Laws R. M., Academic Press, London. pp 1-60
- Willmer P. G. & Unwin D. M.** (1981) Field analyses of insect heat budgets: reflectance, size and heating rates. *Oecologia* **50**: 250-255

## Chapter 7

### Conclusions

*If we had a keen vision of all that is ordinary in human life, it would be like hearing the grass grow or the squirrel's heart beat, and we should die of that roar which is the other side of silence*

- Marion Evans Cross -





In this study it was found that despite the recommendations and strict enforcement of quarantine procedures stipulated by the Prince Edward Islands Management Plan (Anonymous 1996), the number of successful alien invertebrate species introductions are increasing at the Prince Edward Islands (PEIs). The recent discovery of the alien isopod *Porcellio scaber*, which represents the first record of a terrestrial crustacean from Marion Island (Slabber & Chown 2002), and an unknown wasp species along the east coast of Marion Island indicate that these procedures and regulations will have to be strictly adhered to in order to further prevent the accidental introduction of species to the PEIs, especially so during the construction of the new Scientific Research Station. It is of major concern that several studies have recorded a warming and drying trend at the Southern Ocean Islands (Smith & Steenkamp 1990; Frenot *et al.* 1997; Tweedie 2000; Smith 2002), which is set to continue (IPCC 2001) and will lead to further successful introductions, establishment and spread of alien species (Gaston *et al.* 2003; Frenot *et al.* 2005). This study was thus aimed at investigating the thermal physiological tolerances displayed by indigenous and alien invertebrates from Marion Island and the extent to which phenotypic flexibility (Via *et al.* 1995) could explain thermal responses to acclimation, which are important in light of rapid recent climate change (Smith & Steenkamp 1990; Smith 2002).

Several approaches were used in this study to elucidate the effects of acclimation on invertebrate upper and lower thermal tolerances, and have indicated that indigenous and alien species on Marion Island do differ in their abilities to withstand physiologically stressful environmental conditions, as had been predicted previously (Barendse & Chown

2000; Gabriel *et al.* 2001). This study also examined the thermal tolerances, and responses to acclimation, of two of the smaller invertebrate species from Marion Island that have thus far escaped investigation. It was found that the small freeze-intolerant psocid *Antarctopsocus jeanneli* was able to mount a strong response to acclimation despite showing only moderate chill tolerance (see Bale 1993) and that the upper thermal temperatures of *A. jeanneli* are similar to that of a range of other invertebrates on Marion Island (e.g. Klok & Chown 1997; 2001). Thermal tolerances in this species closely mirrored microclimates experienced by it on Marion Island.

*Halmaeus atriceps* adults and larvae showed similar responses to acclimation. A large increase in the level of cold hardiness with decreasing acclimation temperatures was found, with relatively small changes in upper thermal limits in response to acclimation, which is in keeping with what is generally accepted to be the norm in insects (a larger response to acclimation in lower than in upper thermal limits (Chown 2001)). There was a clear seasonal difference in the cold hardiness strategy in both adults and larvae of this species, as supercooling points (SCPs) drastically increased to above lower lethal temperatures in winter, and moderate freeze tolerance (Bale 1993) thus allowed survival, compared to their chill susceptibility in summer, where substantial prefreeze mortality occurred in this species. Lower lethal temperatures were thus more labile in this species than upper ones, and thermal tolerance ranges in both adults and larvae fell within the range of microhabitat temperatures experienced by them.

This study also sought to compare the baseline physiological tolerances of two indigenous and three alien springtails, and to explicitly investigate the effects of acclimation on thermal tolerances (high and low temperature), and desiccation resistance. In addition, a test of the beneficial acclimation hypothesis (Leroi *et al.* 1994) and its alternatives (Huey *et al.* 1999) was conducted using these parameters. It was found that alien springtail species were less sensitive to high temperatures and actually displayed increased physiological tolerance following high temperature acclimation, contrasting with indigenous springtail species, which displayed increased tolerance following low temperature acclimation, and large tolerances to acute low temperature exposure. This finding supports the prediction by Gabriel *et al.* (2001) that invasive springtails should perform better at high than low temperatures, and that indigenous species prefer low temperatures based on their distribution in cool, dry fellfield areas on Marion Island. The beneficial acclimation hypothesis did not receive much support from the acclimation responses of the indigenous and invasive springtail species. Rather, indigenous springtails tended to perform better at low rather than high temperatures (colder is better hypothesis (Huey *et al.* 1999)), and alien species performed better at high temperatures and following high temperature acclimation (warmer is better hypothesis (Huey *et al.* 1999)).

In an investigation of microhabitat temperatures along an altitudinal gradient (0 – 800 m above sea level) along the east coast of Marion Island, it was found that interannual variability in temperatures may lead to increased mortality among invertebrates in some years compared to milder years (also see Sinclair 2001), and that climate warming may lead to an amelioration in temperatures, which will favour alien invertebrates. However,

an amelioration of temperature may well also increase the occurrence of extreme high and low absolute temperatures at higher altitudes, which might prevent alien species from increasing their ranges to high altitude habitats. High altitude sites had consistently lower mean microhabitat temperatures than low altitude sites, further indicating that warmer microclimate conditions at low altitude sites would favour the establishment of alien species. High altitude sites had a higher frequency of freeze-thaw events, and more extreme absolute minima and maxima, thus indicating that indigenous invertebrates are able to survive these extremes to a greater extent than alien species, which prefer warmer conditions. It also seems likely that increased establishment of alien species, a climate warming trend at the Southern Ocean Islands, and the fact that alien species perform better at higher temperatures are interrelated. It is thus imperative that more stringent control measures to prevent alien introductions to Southern Ocean Islands are put in place.

From the research undertaken in this study, the following issues arose which require research attention or thought.

1. Physiological tolerances displayed by invertebrates in this study provide a basis from which certain conclusions could be drawn in light of rapid recent climate change (e.g. that low temperatures limit the altitudinal distribution of alien species). Should invasive species on Marion Island have shorter generation times than indigenous species, they may be at a further advantage during warm years compared to indigenous species, some of which are known to have life cycles

exceeding two years (e.g. *Pringleophaga marioni* (Crafford *et al.* 1986)). Knowledge of the rate of population increase of indigenous and invasive species, and especially alien springtails will prove invaluable and could also possibly explain patterns in distribution and abundance of invertebrates on Marion Island.

2. Lower lethal temperatures in this study were variable amongst springtail species and acclimation treatments. From the rapid cooling (plunge) method employed in Chapter 5 to test for prefreeze mortality in springtails, it became clear that lower lethal temperatures were sensitive to the protocol adopted (see Chown & Nicolson 2004). In other words, springtails died before freezing in plunge experiments (which is not considered to be the norm for springtails - Sømme 1982), whereas mortality only occurred upon freezing using a slow-cooling approach. The plunge method is thus not an appropriate method to investigate lower lethal limits in springtails.
  
3. That there was an effect of temperature acclimation on the desiccation tolerance in most of the springtail species studied in Chapter 5 is noteworthy. Future research should consider springtail cuticular attributes in conjunction with desiccation resistance, as well as investigating the accumulation of solutes (such as glycerol) in response to desiccation. Investigations should aim to elucidate the mechanisms underlying water loss rate changes following acclimation to low or high temperature. It would also be worthwhile to investigate the responses of

springtails species to desiccation following acclimation at different relative humidity treatments.

## References

- Anonymous** (1996) *Prince Edward Islands management plan*. Department of Environmental Affairs and Tourism, Pretoria
- Bale J. S.** (1993) Classes of insect cold hardiness. *Functional Ecology* **7**: 751-753
- Barendse J. & Chown S. L.** (2000) The biology of *Bothrometopus elongatus* (Coleoptera, Curculionidae) in a mid-altitude fellfield on sub-Antarctic Marion Island. *Polar Biology* **23**: 346-351
- Chown S. L.** (2001) Physiological variation in insects: hierarchical levels and implications. *Journal of Insect Physiology* **47**: 649-660
- Chown S. L. & Nicolson S. W.** (2004) *Insect physiological ecology. Mechanisms and patterns*. Oxford University Press, Oxford
- Crafford J. E., Scholtz C. H. & Chown S. L.** (1986) The insects of sub-Antarctic Marion and Prince Edward Islands; with a bibliography of entomology of the Kerguelen Biogeographical Province. *South African Journal of Antarctic Research* **16**: 42-84
- Frenot Y., Chown S. L., Whinam J., Selkirk P. M., Convey P., Skotnicki M. & Bergstrom D. M.** (2005) Biological invasions in the Antarctic: extent, impacts and implications. *Biological Reviews* **80**: 45-72
- Frenot Y., Gloaguen J.-C. & Trehen P.** (1997) Climate change in Kerguelen Islands and colonization of recently deglaciated areas by *Poa kerguelensis* and *P. annua*. *Antarctic communities: species, structure and survival*. Ed. Battaglia B., Valencia J. & Walton D. W. H., Cambridge University Press, Cambridge. pp 358-366
- Gabriel A. G. A., Chown S. L., Barendse J., Marshall D. J., Mercer R. D., Pugh P. J. A. & Smith V. R.** (2001) Biological invasions of southern ocean islands: the Collembola of Marion Island as a test of generalities. *Ecography* **24**: 421-430
- Gaston K. J., Jones A. G., Hänel C. & Chown S. L.** (2003) Rates of species introductions to a remote island. *Proceedings of the Royal Society of London B*: 1091-1098
- Huey R. B., Berrigan D., Gilchrist G. W. & Herron J. C.** (1999) Testing the adaptive significance of acclimation: A strong inference approach. *American Zoologist* **39**: 323-336
- IPCC** (2001) *Climate change 2001: synthesis report. A contribution of working groups I, II, and III to the third assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge

- Klok C. J. & Chown S. L.** (1997) Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic caterpillar, *Pringleophaga marioni* Viette (Lepidoptera: Tineidae). *Journal of Insect Physiology* **43**: 685-694
- Klok C. J. & Chown S. L.** (2001) Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic kelp fly, *Paractora dreuxi* (Diptera: Helcomyzidae). *Journal of Insect Physiology* **47**: 95-109
- Leroi A. M., Bennett A. F. & Lenski R. E.** (1994) Temperature acclimation and competitive fitness: An experimental test of the beneficial acclimation assumption. *Proceedings of the National Academy of Sciences of the USA* **91**: 1917-1921
- Sinclair B. J.** (2001) Field ecology of freeze tolerance: interannual variation in cooling rates, freeze-thaw and thermal stress in the microhabitat of the alpine cockroach *Celatoblatta quinquemaculata*. *Oikos* **93**: 286-293
- Slabber S. & Chown S. L.** (2002) The first record of a terrestrial crustacean, *Porcellio scaber* (Isopoda, Porcellionidae), from sub-Antarctic Marion Island. *Polar Biology* **25**: 855-858
- Smith V. R.** (2002) Climate change in the sub-Antarctic: An illustration from Marion Island. *Climatic Change* **52**: 345-357
- Smith V. R. & Steenkamp M.** (1990) Climatic change and its ecological implications at a sub-Antarctic island. *Oecologia* **85**: 14-24
- Sømme L.** (1982) Supercooling and winter survival in terrestrial arthropods. *Comparative Biochemistry and Physiology A* **73**: 519-543
- Tweedie C. E.** (2000) *Climate change and the autecology of six plant species along an altitudinal gradient on subantarctic Macquarie Island*. PhD Thesis. University of Queensland
- Via S., Gomulkiewicz R., Scheiner S. M., Schlichting C. D. & Van Tienderen P. H.** (1995) Adaptive phenotypic plasticity: consensus and controversy. *Trends in Ecology and Evolution* **10**: 212-217