Antarctic Science 13 (3): 271–279 (2001) © Antarctic Science Ltd Printed in the United Kingdom

Cold tolerance of the Antarctic springtail *Gomphiocephalus hodgsoni* (Collembola, Hypogastruridae)

BRENT J. SINCLAIR[1]* and HEIDI SJURSEN[2]

[1]*Department of Zoology, University of Otago, Dunedin, New Zealand*
[2]*Department of Terrestrial Ecology, National Environmental Research Institute, Silkeborg, Denmark*
**present address: Department of Zoology, University of Stellenbosch, Private Bag x1, Matieland 7602, South Africa*
celatoblatta@hotmail.com

Abstract: Cold tolerance of the springtail *Gomphiocephalus hodgsoni* Carpenter (Collembola: Hypogastruridae) was studied at Cape Bird, Ross Island, Antarctica (77°13'S, 166°26'E). Microclimate temperatures indicate a highly seasonal thermal environment, with winter minima < -39°C. Snow cover significantly buffers both minimum temperatures and cooling rates. *Gomphiocephalus hodgsoni* survives low temperatures by avoiding freezing. Mean low group supercooling points (SCPs) ranged from -35.4°C in October to -28.3°C in January. The lowest SCP measured was -38.0°C. The high SCP group was very small, making up only 18% of the population in January. In October, *G. hodgsoni* had a very high glycerol content (> 80 μg mg^{-1} dry weight), although this declined rapidly to low levels (*c.* 7–10 μg mg^{-1} dry weight) in January. Quantities of glucose and trehalose were low during October, but steadily increased throughout the summer. Haemolymph osmolality was exceptionally high (up to 1755 mOsm kg^{-1}) at the end of November, but this rapidly declined to *c.* 500 mOsm kg^{-1} by late December. The presence of thermal hysteresis proteins was indicated by both osmometry on haemolymph samples and recrystallization inhibition studies of springtail homogenates. There was a strong relationship between glycerol content and SCP, but the relationship between haemolymph osmolality, SCP and carbohydrates is uncertain.

Received 16 October 2000 , accepted 23 March 2001

Key words: cryoprotectants, glycerol, overwintering, recrystallization inhibition, supercooling point, thermal hysteresis

Introduction

The Antarctic continent includes some of the harshest terrestrial habitats on earth, and is noted for extremely short summers, cold dark winters, and a lack of liquid water. Mites (Acari) and springtails (Collembola) are the only terrestrial arthropods present in the scattered ice-free areas of the continental region, although some higher insects are present in the milder maritime Antarctic (Block 1994).

Generally, springtails survive low temperatures by utilizing a freeze-avoidance strategy, whereby lethal freezing is avoided by depression of the freezing point of the animal to below the temperature of the environment. The lower lethal temperature of springtails may therefore be estimated by measuring the supercooling point (or temperature of crystallization), the temperature at which an animal freezes. Supercooling points are usually lower in winter (or when cold tolerance is required) and higher in summer. A number of authors have also reported bimodal distributions of supercooling points in springtails collected in summer, the high group of which are interpreted as having ice nucleators in the gut (Cannon & Block 1988).

Cryoprotective compounds in freeze-avoiding insects may be categorised by their action as colligative or non-colligative antifreezes (Lee 1991). Low molecular weight carbohydrate cryoprotectants serve to stabilize membranes, and to reduce the freezing point of body fluids by colligative action. Sømme (1986) reports accumulation of glycerol by the continental Antarctic isotomid *Cryptopygus sverdrupi* Lawrence, while Sømme & Block (1982) report a multi-component cryoprotectant system comprising glycerol, mannitol and trehalose in *C. antarcticus* Willem from the maritime Antarctic. Non-colligative freezing point depression by thermal hysteresis proteins (THPs, analogous to the antifreeze proteins in Antarctic fish, Duman *et al.* 1991) has been reported in several species of Collembola (Zettel 1984). By acting directly on ice embryos, these proteins reduce the risk of exogenous nucleation of ice, and help to stabilise the body fluids of highly supercooled insects (Zachariassen & Husby 1982, Duman *et al.* 1991).

Cryptopygus antarcticus from the maritime Antarctic is the most intensively studied collembolan with regards to cold tolerance. During the summer, over 90% of individuals were in the 'high group' (supercooling point >-15°C, mean = -7.2) (Sømme & Block 1982), but acclimation to low temperatures resulted in a downward shift in supercooling points, with low group means in the region of -24.5°C (Sømme & Block 1982) to -26.5°C (Sømme 1978). Sømme (1986) investigated *Cryptopygus sverdrupi* from isolated nunataks in Dronning Maud Land, finding that the summer mean supercooling point

of -28.6°C reduced to -34.6°C following acclimation. The lowest supercooling point for this species was -38°C. *Cryptopygus sverdrupi* accumulated large quantities of glycerol (up to 80 μg mg^{-1} fresh weight) during acclimation experiments. Pryor (1962) reports that individuals of another Antarctic isotomid species, *Isotoma klovstadi* Carpenter, could survive periods at temperatures below -50°C, although there are no quantitative data to suggest a mechanism for this exceptional cold tolerance.

Gomphiocephalus hodgsoni (Hypogastruridae) is the dominant collembolan in the McMurdo Sound region (*c.* 77°S). Janetschek (1963) reported that -28°C was lethal to *G. hodgsoni*, and Fitzsimons (1971) found -23°C to be lethal after 24 h. Block (1985) reports supercooling points of *G. hodgsoni* from McMurdo Sound locations of around -30°C during late November–mid January.

Although environmental conditions are extreme in the continental Antarctic region, providing an excellent opportunity to study adaptations to harsh environments, logistic constraints are also great. Few studies have been carried out outside of the midsummer period, and there is no information about winter conditions of either the animals, or their habitats, for the continental Antarctic region. This paper aims to address some of these inadequacies, within the existing logistic constraints, by presenting cold tolerance data from *G. hodgsoni* gathered from a period encompassing spring and early autumn; we also provide the first winter recordings from the microhabitat of continental Antarctic terrestrial arthropods.

Methods

Winter access to field sites is currently unavailable in McMurdo Sound but spring (mid–late October) sampling is possible. This is a period of rapid change in temperature and day length, although low temperatures (below -20°C) are usually continuous with the coldest part of the winter period, allowing some conclusions to be drawn about the physiological state of animals in winter.

Gomphiocephalus hodgsoni were collected from the undersides of stones in "Keble valley" and "Robber's Roost", in the vicinity of the research hut at Cape Bird, Ross Island (77°13'S, 166°26'E). They were collected into small plastic containers, and stored in the shade outside the hut for up to 6 h prior to use in experiments.

Microclimate temperature recordings

Soil surface temperatures were recorded from two sites *c.* 10 m apart, one of which was covered by snow for both winters, while the other remained free of snow cover. Springtails and mites were present in high numbers at each site. Shielded air temperature at a height of 1.2m was also recorded. Temperatures were recorded from T107 thermistors connected to a Campbell CR10x datalogger (Campbell Scientific, Inc, Logan, UT, USA). Recordings were made every hour for the first year (1998), and every two hours subsequently (1999–January 2000). Memory limitations of the datalogger meant that data were not recorded from February–June in 1998 and February–March in 1999. Occasional programming errors resulted in loss of data from a few short periods during each summer. The soil fauna are confined to the upper *c.* 2 cm of soil (and are often seen active on the surface, or at the margins of stones), so soil surface temperatures provide a reasonable estimate of microclimatic conditions.

Daily mean, maximum and minimum temperatures were summarized from the data. Cooling rates during each winter were calculated for the 3 h (1998) or 4 h (1999) immediately prior to the microclimate temperature dropping below -25 and -30°C (temperatures at which 5 and 10% of springtails were likely to be at risk of freezing at the beginning of November).

Cooling apparatus

In February 1998, springtails were returned to the Crary Science and Engineering Centre at McMurdo station, and a Haake refrigerated circulator was used to cool specimens. Over the summers of 1998–99 and 1999–2000, samples were placed in polypropylene microcentrifuge tubes in holes drilled in an aluminium block. The block was cooled by Peltier modules powered from manually adjusted DC power supply units. Heat was conducted from the hot surface of the Peltier modules by iced water pumped from a reservoir. This apparatus could cool samples to temperatures below -45°C, with an initial cooling rate of *c.* 4°C min^{-1} (0 to -10°C), and thereafter at approximately 1.8°C min^{-1}. Several runs were conducted at a slower rate of 0.6°C min^{-1}.

Supercooling point and cold hardiness determination

Cold hardiness studies were carried out during five summer visits to Antarctica between January 1998 and January 2000.

Springtail temperatures were measured with K-Type thermocouples linked via a signal amplifier and Maclab analogue-digital interface (AD Instruments Ltd, Dunedin, New Zealand) to a Macintosh computer. Springtails were affixed to the thermocouple using a thin film of grease, and placed in microcentrifuge tubes in the cooling apparatus. They were allowed to equilibrate at *c.* 2°C, before cooling commenced. The supercooling point was taken as the temperature immediately prior to the upward inflection caused by the exotherm as the latent heat of crystallization is released.

This methodology assumes that the supercooling point represents a lethal temperature. This assumption was validated by checking for pre-freeze mortality and freezing survival. Groups of four animals were cooled to a temperature where two had frozen. All four animals were then warmed, and survival determined by looking for movement of each with a hand lens.

To examine the relationship between individual size and supercooling point, individuals were separated into 2°C blocks

on the basis of their supercooling point and preserved in ethanol. Individuals were measured in New Zealand under a dissecting microscope with an eyepiece graticule. Data were plotted to check for non-linearity, and Spearman's rank correlation was used to examine the relationship between animal size and supercooling point in October and December 1998 and February 1999.

Osmometry

The osmolality of springtail body fluids was measured with a Clifton Nanolitre Osmometer (Clifton Technical Physics, NJ, USA). Samples of haemolymph were extracted from a specimen by amputating an antenna or leg of a specimen immersed in liquid paraffin. Haemolymph samples were often clouded by the strong pigments of *G. hodgsoni*, but individual crystals could still be discerned. Haemolymph samples were snap-frozen, and the melting point of the body fluids was determined from the temperature at which the final ice crystal melted. All samples were screened for thermal hysteresis activity, which could be observed as the difference between the melting point of the sample, and the temperature at which growth of a single crystal resumed upon slow cooling.

Recrystallization inhibition

Recrystallization inhibiting activity was examined using the splat freezing technique (Knight *et al.* 1988). Ten *G. hodgsoni* collected on the 25 October 1998 (early spring) and 20 December 1998 (summer) were homogenized in 100 µl mg^{-1} fresh weight 5 mM Tris-Cl buffer (pH 7.8) containing 'Complete' protease inhibitor (Boehringer Mannheim, Germany). The homogenate was centrifuged at 10 000 g for 5 min, and the supernatant (plus a control containing only buffer) was used for splat freezing as described by Ramløv *et al.* (1996). Splats were transferred to a microscope cold stage at -20°C, photographed though crossed Polaroids, and annealed for 30 min at -8°C, before being photographed again. Median crystal diameter was assessed from randomized transects across photographs of the splat taken before and after annealment. The presence of discrete factors causing the observed recrystallization inhibition was tested using serial tenfold dilutions to dilute out the recrystallization inhibiting factors.

Gas-liquid chromatography

Springtails collected in the field were sorted within two hours into vials and immediately frozen in liquid nitrogen. Collections were made when liquid nitrogen was available, including periods in 1997 before supercooling point determinations were begun. No collections were made in October and early November 1999 because of the unavailability of liquid nitrogen in the field. Samples were transported to New Zealand on dry ice, and stored at -80°C until use.

Springtails were weighed, dried overnight at 55°C, and dry weight determined. They were then soaked for 1 h in 100 µl of 0.2 mg ml^{-1} Dulcitol (= 20 µg as an internal standard: Dulcitol is not present in *G. hodgsoni)* in 40% ethanol, before being homogenised. The homogenates were subjected to an ultrasound bath for 30 min and heated at 95°C for 5 min. Following centrifugation, the supernatant was kept, and the pellet resuspended three times in 40% or 20% ethanol and centrifuged. The pooled supernatants were then dried to a residue at 40°C under a constant stream of nitrogen (*c.* 0.5 l min^{-1}), and stored in a desiccator.

Sugar and polyol residues were converted to their trimethylsilyl derivatives with 20 µl Sil-prep (Alltech). A 5 µl subsample was injected into an Alltech Econocap EC-5 capillary column on a Hewlett Packard 5890 series II gas chromatograph. Recovery was estimated using the internal standard, and carbohydrates identified against peak times and sizes of known standards (Wharton *et al.* 2000). All polyol and sugar contents were standardised to the dry mass of the sample, and are presented as µg mg^{-1} dry weight. Samples containing an unidentified peak were sent to the Institute of Entomology, Czech Academy of Sciences for identification by GC-mass spectrometry.

Results

Microclimatic conditions

Air and microhabitat temperatures are presented in Fig. 1. Soil surface temperatures were frequently well above zero in the summer, with maxima often greater than 15°C. Air temperatures, particularly maxima and means, were considerably lower than soil surface temperatures during the summer, although this difference was smaller during the winter (Fig. 1, Table I). The relief of the site is low, and the small amount of precipitation meant that a snowpack of *c.* 20 cm depth had accumulated on the snow-covered site by the time it was visited in late October. Even this small amount of snow was enough to buffer the thermal microenvironment from the extreme minima and amplitude of variation seen in the snow-free site, although this buffering extended to periods of warm temperatures during the winter as well as delaying the beginning of the period of warm summer temperatures (Table I).

Table I. Extreme maximum and minimum temperatures, and the date of the start of the growing season at "Keble Valley", Cape Bird, Ross Island, in 1998 and 1999.

		Maximum (°C)	Minimum (°C)	Start of growing season (1st daily mean > 0°C)
1998	air	5.0	-35.2	24 December
	snow-free	22.2	-34.9	19 November
	snow-covered	18.6	-30.9	17 December
1999	air	5.7	-37.1	-
	snow-free	17.7	-39.6	25 November
	snow-covered	22.4	-30.1	1 December

https://doi.org/10.1017/S0954102001000384 Published online by Cambridge University Press

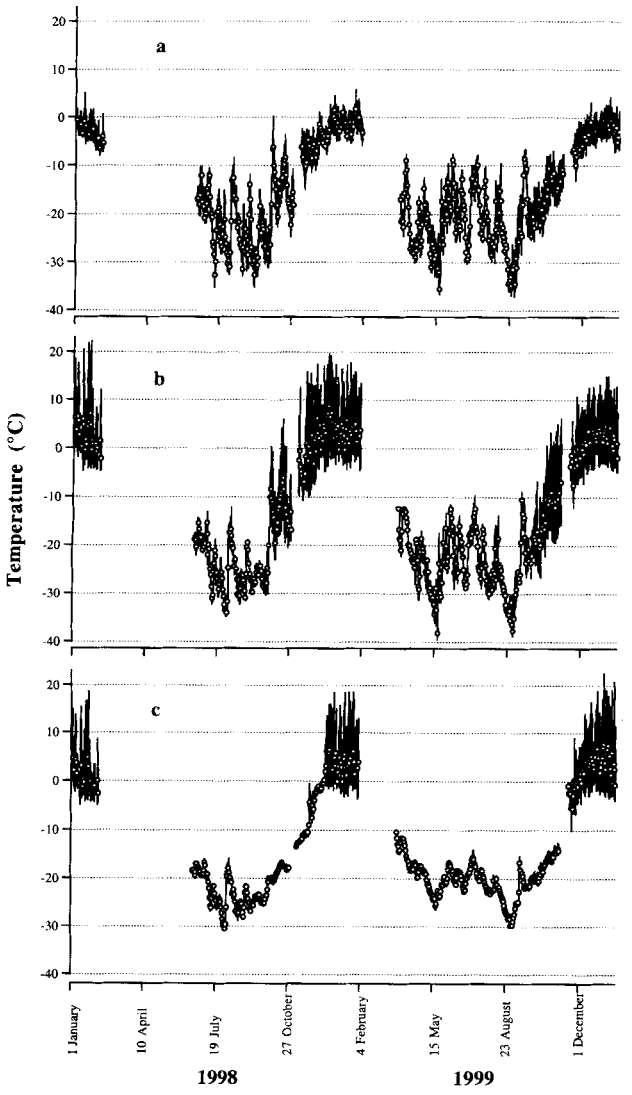

Fig. 1. Daily mean, maximum and minimum temperatures in "Keble Valley", Cape Bird, Ross Island, **a.** air, **b.** snow-free site, **c.** snow-covered site.

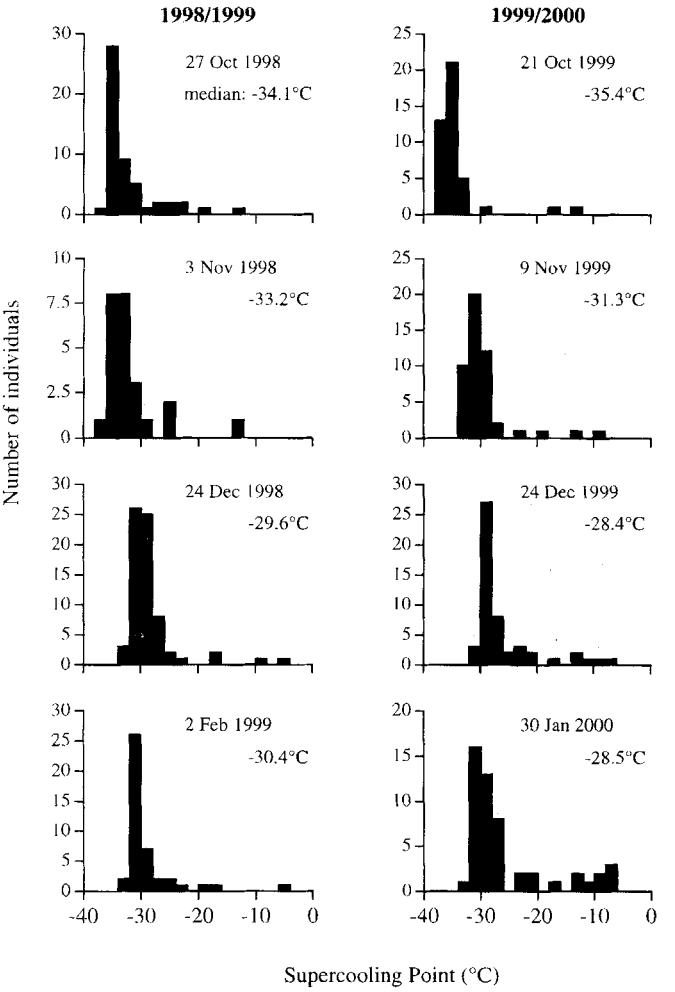

Fig. 2. Distributions of *Gomphiocephalus hodgsoni* supercooling points over two summers. The median supercooling point value is given on each graph.

There is a pronounced seasonality at the site as a result of insolation: thermal amplitude in summer is considerably higher at ground level than in winter, or air temperatures (Fig. 1). Cooling rates to potential freezing temperatures were relatively slow throughout the year. At the snow-free site, the fastest cooling rate was 0.021°C min^{-1}, at the snow-covered site, the fastest cooling rate was 0.003°C min^{-1}.

Cold tolerance

No pre-freeze mortality or survival of freezing events was detected. There was no significant difference between the low group (LG) supercooling point of *G. hodgsoni* cooled at 1.8 and 0.6°C min^{-1} (t_{55} = 0.977, P = 0.33). There was no correlation between the length of an animal and its supercooling point in October or December (October: r_s = 0.18, P = 0.27; December: r_s = -0.002, P = 0.98), but larger animals had

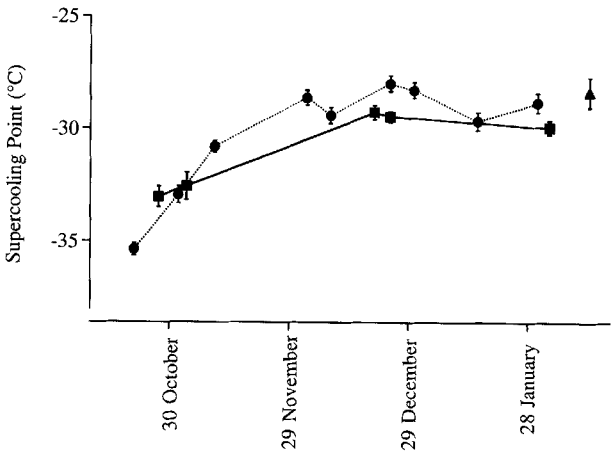

Fig. 3. Seasonal changes in mean (± s e) low group (< -20°C) supercooling points of *G. hodgsoni*. ▲ = 1997/98, —■— = 1998/99, ···●··· = 1999/2000.

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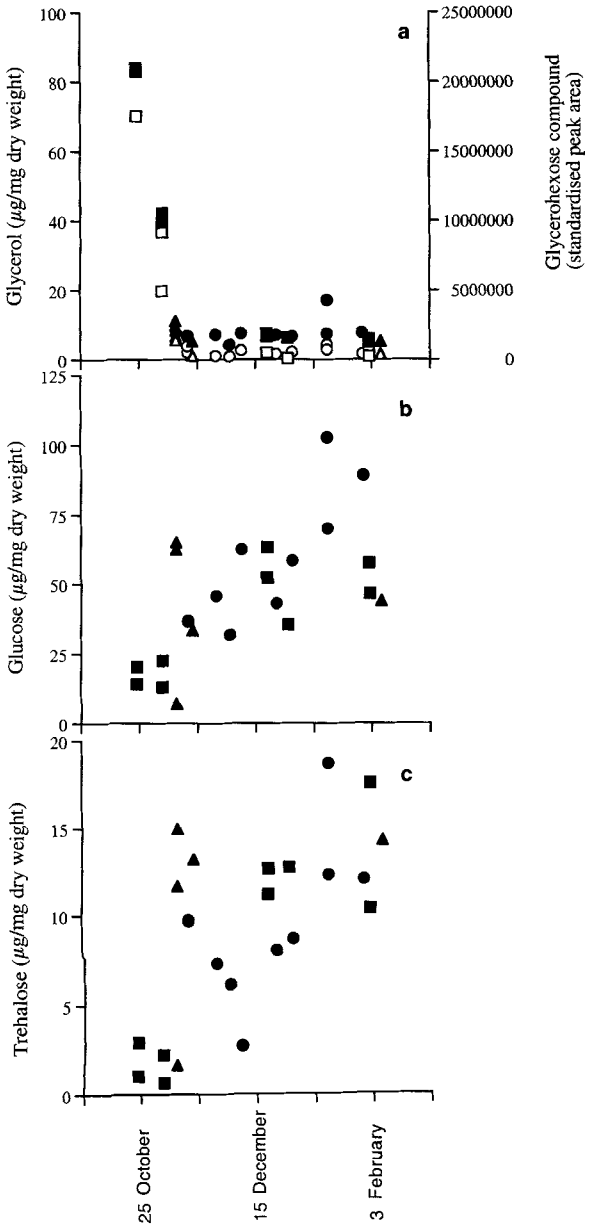

Fig. 4. Seasonal changes in sugar and polyol contents of *G. hodgsoni* collected from Cape Bird. ▲ = 1997/98, ■ = 1998/99, ● = 1999/2000. Unfilled symbols are the glycerohexose compound.

significantly higher supercooling points in February ($r_s = 0.39$, $P = 0.02$).

The minimum supercooling point recorded was -38°C on 21 October 1999 at which time the highest supercooling point was -13.2°C. During the height of summer (December and January), the minimum supercooling point was -32.9°C (24 December 1998) while the maximum was -4.3°C. Distributions of supercooling points are displayed in Fig. 2, while Fig. 3 shows the changes in mean low group supercooling points over the progression of the summer. Supercooling points were lowest (< -32°C) in October and early November, before

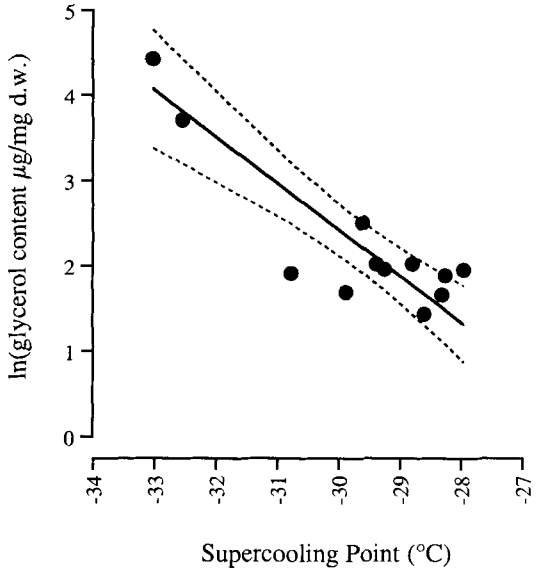

Fig. 5. Relationship between glycerol content and supercooling point of *Gomphiocephalus hodgsoni*. The line is a reduced major axis regression line with 95% confidence intervals (Sokal & Rohlf 1981).

undergoing a significant upwards shift in mid-November, and remaining more or less stable around -30°C for the remainder of the summer. This pattern of supercooling point change was consistent between summers (Figs 2 & 3).

Cryoprotectants

The main carbohydrates detected by gas chromatography were glycerol, glucose and trehalose. The concentrations of all of these varied across the season. Glycerol was present in extremely high quantities (> 80 μg mg^{-1} dry weight, corresponding to 35.5 μg mg^{-1} fresh weight) in October and early November, but rapidly declined to trace quantities (*c.* 7–10 μg g^{-1} dry weight) by mid November (Fig. 4a). Glucose and trehalose were at low levels in October, but both showed patterns of steady increase as the summer progressed (Fig. 4b & c). An unidentified carbohydrate, possibly a glycerohexose compound (P. Simek, personal communication 2000) was present in October, but not in midsummer samples (Fig. 4a). The relative quantity of this compound was very strongly correlated to quantities of glycerol ($r = 0.978$, $P << 0.01$). Glycerol content was strongly negatively related to supercooling point (Fig. 5). Assuming the relationship remains linear, extrapolation of the regression line predicts a glycerol content of 213.9 μg mg^{-1} dry weight (95% confidence interval: 70.1–652.8 μg mg^{-1} dry weight) for the mean LG supercooling point of -35.3 measured on 21 October 1999.

Haemolymph osmolality was very high in late November (1502–1755 mOsm kg^{-1}), but rapidly declined over the following month to 485–530 mOsm kg^{-1} (Fig. 6a). Thermal hysteresis activity of *c.* 1.1°C was present in late November, but this rapidly declined, and disappeared by late December

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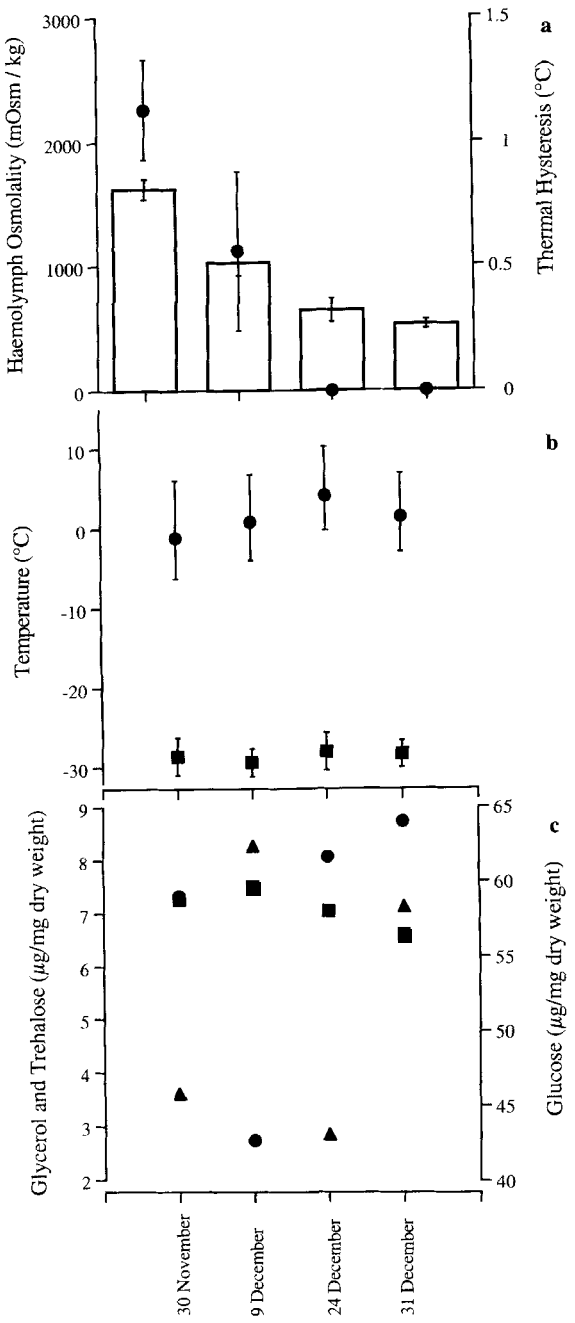

Fig. 6. Changes in **a.** haemolymph osmolality (bars = mean ± s e), thermal hysteresis (● = mean ± s e), **b.** supercooling point (■ = mean ± s e, with ● = daily mean, maximum and minimum air temperatures), and **c.** carbohydrate contents (■ = glycerol, ▲ = glucose, ● = trehalose) of *Gomphiocephalus hodgsoni* at four points between the end of November and end of December 1999.

(Fig. 6a). Over this period of rapid haemolymph change, there were no consistent changes in ambient temperatures or supercooling points (Fig. 6b), nor in carbohydrates, which had already declined from spring levels (Figs 4 & 6c). Further, there was no significant correlation between supercooling point, air temperatures or carbohydrates and haemolymph

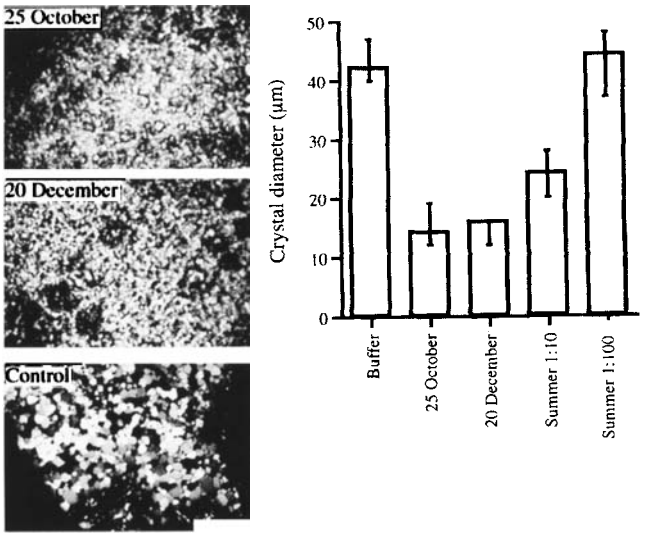

Fig. 7. Recrystallization inhibiting factors in *Gomphiocephalus hodgsoni* homogenates. Photographs provide examples of crystal size in ice splats after 30 min annealment at -8°C (scale bar = 250 µm). Graph shows median ± upper and lower quartiles of control, spring, summer and diluted summer samples.

osmolality (Spearman's rank correlation, $P > 0.2$ in all cases).

Recrystallization inhibiting factors were present in springtail homogenate, and could be diluted out (Fig. 7). Recrystallization inhibiting factors were present in both October and late December samples, even though osmometry indicates the disappearance of thermal hysteresis activity by that time.

Discussion

Overwinter survival of terrestrial invertebrates is of particular interest to polar biologists, yet there is little data on the winter conditions they must endure, especially in the less accessible continental Antarctic region. On Ross Island, soil surface microhabitat temperatures are quite dissimilar to the air temperatures recorded for meteorological purposes. Snow is particularly important as a buffer of extreme conditions in the winter, but the presence of springtails in areas that do not accumulate a snowpack suggests that snow is not essential for winter survival, at least of *G. hodgsoni*. By contrast, snow cover is considered to be extremely important for winter survival of invertebrates in Arctic (Coulson *et al.* 1995), sub-Antarctic (Bale *et al.* 2000) and alpine (Sinclair, unpublished data) habitats. During the summer, insolation onto the dark substrate elevates surface temperatures considerably above air temperatures, providing microhabitat temperatures much higher than meteorological records would suggest. During the spring, snow cover has the effect of delaying the onset of the summer warm period, effectively reducing the length of the growing season (this is also the case in the Arctic, Coulson *et al.* 1995).

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Winter surface temperatures were very low, especially in the snow-free sites, but even beneath snowpack, the temperature dropped below -30°C several times over the two year recording period. At the snow-free site, the minimum temperature recorded (-39.6°C) was below the lowest recorded supercooling point for *G. hodgsoni*, suggesting potential mortality. However, live springtails were abundant in the snow-free sites in October after both winters (Sinclair, personal observation), so it is reasonable to assume that they were able to survive these temperatures. This is in contrast to sub-Antarctic (Bale *et al.* 2000) and alpine (Sinclair *et al.* 2001) habitats, where there is strong evidence of microhabitat selection, possibly to avoid temperature extremes.

Gomphiocephalus hodgsoni is the most southern species of insect that has been subject to physiological investigation, so it is unsurprising that it has the lowest recorded field supercooling points for any springtail (other studies, e.g. Sømme 1986, have utilized acclimation experiments). The persistently low microhabitat temperatures recorded suggest that *G. hodgsoni* must spend the bulk of the year in a highly supercooled state. Nevertheless, the supercooling points measured in mid October are not lower than the lowest recorded microhabitat temperatures. Clearly, a greater level of cold hardiness is required to survive the temperatures recorded.

The late October–early November period is one of major changes. Daylength changes rapidly (to 24 h daylight), and with it come rising air temperatures and increasing insolation, which warms dark surfaces rapidly and encourages snowmelt. It is possible that even in mid-October, warm acclimation responses are beginning to affect the physiology of these animals, resulting in higher supercooling points and decreased polyol levels. Certainly, there is a distinct shift in supercooling points between mid-late October and early November (Fig. 3), even though air and microhabitat temperatures remain below 0°C for this period. It is also possible that there is some other interplay between the environment and internal physiological conditions that affect cold hardiness at these very low temperatures. For example, *Onychiurus arcticus* Tullberg has a relatively high supercooling point, but when it is in its natural (moist moss) habitat, cryoprotective desiccation prevents freezing (Worland *et al.* 1998, Holmstrup & Sømme 1998). Whilst there is no current evidence that *G. hodgsoni* may employ this strategy, there is very little information about the biological significanceof moisture in Antarctic mineral soils, especially during the winter.

The apparently very similar lower lethal temperatures (albeit measured in spring and not midwinter) and minimum microhabitat temperatures suggests that minimum winter temperature could be an important factor limiting localized distribution. The known distribution of *Gomphiocephalus hodgsoni* extends south of the Ross Ice Shelf to Minna Bluff (78°28'S), as well as to >1000 m on Mount England and in the McMurdo Dry Valleys (Wise 1967). Whilst there is no information on winter conditions or microhabitat at either of these locations, it is reasonable to assume that minimum temperatures there are considerably lower than at Cape Bird. *Gomphiocephalus hodgsoni* distribution may be limited by mortality from extreme winter conditions, and we predict that its presence in southern or high altitude locations may be limited to microhabitats that accumulate some snow pack which buffers the winter temperatures.

In contrast to studies on Collembola in the maritime Antarctic (Cannon & Block 1988), but consistent with Block's (1985) study of this species, *G. hodgsoni* presents a relatively small 'high group' of supercooling points, even in the summer. In *Cryptopygus antarcticus*, the high group comprises > 90% of individuals in midsummer (Sømme & Block 1982), yet in the present study, the maximum proportion of *G. hodgsoni* in the high group was 18%. Since the existence of a high group is postulated to be due to the presence of ice nucleators from the diet (Cannon & Block 1988), it is possible that this discrepancy is simply because *G. hodgsoni* has a diet relatively free of nucleating agents. However, all of the individuals for this and Block's (1985) study were collected from aggregations on the undersurface of stones. As Davidson & Broady (1996) point out, the significance of these aggregations is unclear. It is not known whether aggregated animals are feeding, and if aggregation is principally for moulting or mating purposes, it is possible that the animals are in fact 'starved'. Further investigation of the nature of these aggregations, is necessary to understand the factors affecting measured cold tolerance of *G. hodgsoni* during the summer.

A large number and variety of insects produce low molecular weight cryoprotectants, particularly polyols like glycerol, or sugars such as trehalose, in response to cold or desiccation (Lee 1991). Like *Cryptopygus sverdrupi* (which produces glycerol in response to acclimation, Sømme 1986), glycerol appears to be the main carbohydrate cryoprotectant in *G. hodgsoni*. A second carbohydrate, tentatively identified as a novel glycerohexose compound is also present in relatively high quantities in October, when conditions are cold, yet disappears in the summer. *Cryptopygus antarcticus* also has a multi-component cryoprotectant system, including glycerol, mannitol and trehalose (Cannon & Block 1988). In *G. hodgsoni*, the supercooling point is strongly related to glycerol levels, although the linearity of this relationship outside the bounds investigated is unknown. Nevertheless, it appears that winter levels of glycerol may be considerably higher than those observed in late October. Trehalose and glucose, standard sugars in insect intermediary metabolism (Storey & Storey 1991), are at low levels at the start of summer, and build up gradually, suggesting that the winter glycerol may not be converted directly back into glucose and trehalose. It seems likely that low winter levels of trehalose and glucose are due to wholesale conversion of these precursors into glycerol (and the unknown carbohydrate) at the onset of winter.

The very high haemolymph osmolality (up to 1755 mOsm kg^{-1}) observed at the end of November is

comparable among Collembola only to salt marsh springtails that have been acclimated to unnaturally high environmental salinities (Witteveen *et al.* 1987). Block & Harrisson (1995) report haemolymph osmolalities for *C. antarcticus* of *c.* 284 mOsm kg^{-1}, and predict that winter levels may be as high as 669 mOsm kg^{-1}. The apparent lack of relationship between measured haemolymph osmolality and carbohydrate contents (or of wider environmental conditions) in *G. hodgsoni* is somewhat puzzling. A reduction in water content can increase osmolality (e.g. Worland *et al.* 1998, Holmstrup & Sømme 1998), but limited measurements suggest that water content of *G. hodgsoni* is higher at the beginning of December (when osmolality is high) than at the end of December (Sinclair & Sjursen, unpublished data). The relationship between haemolymph osmolality, environmental conditions and carbohydrates clearly remains to be elucidated for this species.

Thermal hysteresis proteins have been reported in several species of Collembola from the Swiss Alps (Zettel 1984), as well as a number of other insect species (Duman *et al.* 1991). Although they do have an effect as depressors of freezing point, in this context, THPs (whose presence in *G. hodgsoni* is indicated by both osmometry and recrystallization inhibition) are probably most important in stabilizing insects when they are in a highly supercooled state (Zachariassen & Husby 1982). Microclimate recordings indicate that a significant proportion of the year is spent below -20°C, and risk of freezing increases with duration at low temperature (Sømme 1996). There is a rapid decline in thermal hysteresis activity over the month of December, suggesting that this may be the final process of summer acclimation. Recrystallization inhibition suggested that the recrystallization inhibiting factor was still present during the summer. Recrystallization inhibition is considerably more sensitive to thermal hysteresis activity than osmometry, so it may represent a much lower concentration of THPs (or an unrelated factor that does not cause thermal hysteresis). Alternatively, thermal hysteresis agents may be sequestered in tissues during the summer, where they will be detectable after homogenization for recrystallization inhibition, but not in the insect's haemolymph, which is used for osmometry.

Summary and future directions

Gomphiocephalus hodgsoni is a freeze avoiding insect, utilizing thermal hysteresis activity and massive quantities of glycerol to depress its supercooling point. The environment in which it lives is cold enough to provide potential thermal stress, which may be partly mitigated by the buffering effects of snow cover (although this may result in a delayed onset of spring). Nevertheless, *G. hodgsoni* must undergo some further cold hardening beyond the level observed in late October in order to survive the very low temperatures in exposed habitats. There are major changes in carbohydrate contents and supercooling points over spring, which are probably cued by the rapid shifts in both temperatures and daylength over this period. The physiology of this species during the winter and during the summer–winter transition still remains to be investigated. Cold hardiness of this species may be best understood by investigating the factors affecting regulation of carbohydrate and THP production, and the relationship between these, supercooling points and haemolymph osmolality during seasonal transitions.

Acknowledgements

Thanks to David Wharton, Steve Trewick, Jennifer Rock, Brendan Haigh, Caren Shrubshall, Kristi Skebo and Leigh Marshall for assistance in the field. We are grateful to Murray McKenzie, Ian Dickson, Mathew Dooher and Janine Grant for building, obtaining and repairing equipment, and to David Wharton, Karen Judge, Carleen Mitchell, Jane Kennedy and Roger Worland for advice and/or assistance in many aspects of the laboratory work. Professor Carolyn Burns kindly allowed us to use the gas chromatograph (which was purchased through a grant to her from the NZ Lotteries Science Board), and David Wharton generously made his protocols and standards available for Gas Chromatography. Olda Nedved, Vladimir Kostal and Petr Simek of the Institute of Entomology, Czech Academy of Sciences are thanked for their assistance and advice in attempts to identify the unknown compound in winter samples. The nanolitre osmometer was on loan from the Department of Biology, University of Oslo. Thanks to the staff at Scott Base and the Crary Science and Engineering Centre, McMurdo station for help, and to Marj Wright for valuable administrative advice and assistance. Travel to New Zealand by HS was generously funded by the Nordic Arctic Research Program; BJS was supported in part by the Sir Robin Irvine Antarctic Scholarship. This project was part of Antarctica New Zealand Event K067, and was supported by the University of Otago and National Geographic Society Committee for Research and Exploration Grant 6106-98.

We thank the referees (Dr R.J.C. Cannon and one unnamed) for their helpful comments.

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