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# The relationship between water content and cold tolerance in the arctic collembolan *Onychiurus arcticus* (Collembola: Onychiuridae)

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**Collembola, *Onychiurus arcticus*, unfrozen water, melt, osmolarity, cold tolerance, desiccation, water content, Differential Scanning Calorimetry**

**Abstract.** The Arctic collembolan *Onychiurus arcticus* is freezing intolerant and experiences temperatures below –25°C during winter periods of low air temperatures and only light snow cover. Summer-collected individuals have a mean (±SE) supercooling point of –6.1 ± 0.1°C. This study was designed to measure the desiccation resistance and subsequent recovery of *O. arcticus* from partial dehydration and relate these to its cold-hardiness in terms of changes in the supercooling point (SCP) and solute concentration. Drying curves measured with a recording microbalance showed two distinct phases characteristic of the loss of free and chemically bound (osmotically inactive) water. Rates of water loss at 0°C and low relative humidity (< 5%) were similar to those measured for Antarctic Collembola (5% $h^{-1}$ of the initial total water content). *O. arcticus* survived losses of 40% of its total body water content and recovered within 36 h but could not survive losses of 50% of its original water content. Differential scanning calorimetry was used to investigate the nature of the body water, i.e. the proportion of freezable to unfreezable water and the nucleation temperature. The melt onset temperature correlated positively with the body water content. But no clear relationship was seen between the water content and the SCP, either because the springtails had low levels of cryoprotectants or because the ice nucleation activity was unaffected. However, long periods (7 months) at –2.5°C reduced the water content from 74 ± 10.1 to 43 ± 7.2% of fresh weight and lowered the SCP from –6.1 ± 2.1 to –15.5 ± 2.3°C. When given access to water these individuals re-gained their body weight within 24 h. During periods of desiccation water losses were attributed to the loss of freezable water with the unfreezable portion remaining almost constant at 16.5 ± 2.0%. It appears that *O. arcticus* may experience a reduction of body water during winter periods of sub-zero temperatures, which may lower its SCP and enhance its cold tolerance but that it can rapidly return to summer levels given access to free water during the spring melt.

## INTRODUCTION

Although insects show many adaptations to survive potentially freezing temperatures they are commonly divided into two categories i.e. freezing tolerant and freeze avoiding or intolerant (Block, 1991). Freezing intolerant insects survive sub-zero temperatures by remaining in an unfrozen meta-stable state, commonly referred to as supercooled, which can be enhanced by the presence of low molecular weight substances such as sugars and sugar alcohols. This group of animals frequently shows a cold hardening response at the onset of winter, reducing their supercooling point (SCP) from around –6°C for an active feeding insect down to –20°C or below (for reviews see Lee, 1991; Leather et al., 1993; Cannon & Block, 1988). Salt (1956) first proposed that insects may increase their cold tolerance appreciably by reducing their water content and hence increasing the solute concentration.

Freezing may be initiated by ice nucleators which can occur in any part of the insect body. For freeze tolerant species there may be advantages in producing active ice nucleators which initiate controlled freezing at relatively high temperatures thereby avoiding damage to cells. However for freeze intolerant species it is imperative to eliminate or mask ice nucleators as part of the cold hardening process. The Antarctic collembolan *Cryptopygus antarcticus* has been shown to significantly reduce its SCP when starved for 7 days (Sømme & Block, 1982). This decrease was attributed to the elimination of ice nucleators from the alimentary canal. However examples of insects which reduce their SCP by increasing cryoprotectant concentration, usually low molecular weight sugars and sugar alcohols such as glycerol, are much more common (see Baust et al., 1982 for a bibliography), their production being triggered by environmental conditions.

In polar environments insects will spend long periods during which they will be unable to feed or actively take up water. They may also experience substantial water loss due to the vapour pressure difference between the supercooled body fluids and the ice in their microhabitats. Desiccation and the subsequent increase in solute concentration will have only a limited effect on the SCP if ice nucleators are present. However cryoprotectants have a much higher capacity to reduce the SCP of ice nucleator-free systems (Zachariassen, 1992).

It has been suggested that loss of water and subsequent increase in solute concentration may contribute towards the survival of cold tolerant, freeze avoiding insects (Ring, 1982) but there have been few examples, particularly of soil-dwelling species, perhaps due to the difficulties encountered in accurately measuring the water content of insects encased in a frozen substrate. However Holmstrup (1992) found that cocoons of the lumbricid earthworm *Dendrobaena octaedra* showed an enhanced supercooling ability following dehydration at sub-zero temperatures. The physiology and ecology of the Arctic collembolan *Onychiurus arcticus* has been extensively studied (Hodkinson et al., 1994; Block et al., 1994). Although *O. arcticus* is known to survive winter soil temperatures of –20°C, the mean supercooling point in summer field-collected specimens is –6.1 ± 0.1°C (Block et al., 1994) and animals extracted from frozen blocks of substrate in the spring show little deviation from this figure. One possible explanation for this high supercooling point is that during the time taken for the substrate to thaw to extract the animals and to examine them for survival, they may have taken up water and even food, both of which may affect the whole body freezing point. *O. arcticus* acclimated for periods of up to 84 days at 0°C show no significant decrease in their SCP's (Block et al., 1994) unlike *C. antarcticus* from Antarctica, which reduces its SCP from –15 to –26°C after 14 days at 0°C (Sømme & Block, 1982). The aim of this study was to examine the relationship between body water content and cold tolerance to determine if *O. arcticus* could benefit in terms of cold hardiness from desiccation enhanced by sub-zero environmental conditions.

## METHODS

### Collection and culture of sample material

*Onychiurus arcticus* is a large (3.5 mm in length, 0.5 mg fresh weight), pale yellow collembolan which is abundant in high nutrient areas below bird cliffs in the Arctic especially in Svalbard, often forming large aggregations of hundreds of individuals. Samples were collected using an aspirator from beneath grass and moss turf growing around rocks at Krykkjefjellet and Stuphallet bird cliffs on the Brøggerhalvøya, near Ny-Ålesund, West Spitsbergen, Svalbard (78°56'N, 11°53'E). Cultures were established in the UK in Phytacon culture vessels with vented lids (Sigma-Aldrich). Culture pots contained a 1 cm thick base of moist plaster of Paris covered by a filter paper (Whatman number 1). A food supply of commercially available dried baker's yeast and algae (*Chlamydomonas* sp.) was provided as required. The cultures were maintained at 0°C unless otherwise stated.

Measurement of the rate of water loss

Changes in live weight of individual Collembola were measured using a recording microbalance (C. I. Electronics Ltd) with an accuracy of ± 1 μg. Individual collembolans were enclosed in aluminium pans, vented with small holes in the lids to allow free evaporation of water. The temperature of the sample was controlled by circulating water from a thermocirculator around an outer jacket surrounding the sample. For details see Worland & Block (1986). Desiccating conditions with low humidity levels (< 5% RH) were maintained by the addition of self-indicating silica gel in a container just below the sample pan and monitored using a Vaisala (HMP 13) humidity probe. The activity of the silica gel was maintained by heating at 140°C for 1 hour prior to each experiment. Sample weight, temperature and humidity close to the sample, were recorded at 20 sec intervals using sensors connected to a 12 bit Squirrel data logger (Grant Instruments). The duration of the experiments ranged from 8 to 60 h depending on the temperature regime and were continued until no further weight loss could be measured. The stored information was later transferred to a personal computer for analysis and plotted using Quattro Pro software (Borland). As the recording balance was designed to measure changes, rather than absolute weight, experimental animals were weighed using an electronic microbalance accurate to ± 1 μg (Sartorius MP3) prior to being placed in the balance, at the end of the desiccation period and finally after being dried in an oven at 60°C for 12 h.

Survival and recovery from partial desiccation

Survival of different levels of desiccation was assessed by placing weighed individual Collembola in aluminium pans in sealed glass jars (desiccators) containing freshly activated silica gel. The approximate time required to achieve particular levels of desiccation within a specific range (0 to 60% loss of fresh weight) was calculated from the rate of water loss measured previously. The desiccators were maintained at 0°C to reduce the rate of desiccation to manageable levels (4–5% $h^{-1}$ body weight loss). At the end of the treatment period samples were immediately examined for survival, weighed to determine the amount of desiccation and allowed to recover in a pot containing plaster of Paris, moistened so that a film of water covered its surface. The samples were further examined and weighed at intervals of 12, 24, 36 and 48 h. Survival was assessed on the basis of individuals having normal locomotory ability.

Similarly prepared samples of *O. arcticus* having known levels of desiccation were used to determine the effect of desiccation on their freezing and melting characteristics as described below.

Measurement of the temperature and enthalpy of freezing and melting

Single, weighed Collembola were sealed in aluminium pans (40 μl) and cooled in a differential scanning calorimeter (Mettler Toledo DSC820). A standard temperature programme cooling from 5 to –20°C at 1°C $min^{-1}$ and returning to the original temperature at the same rate was used for all the determinations. The DSC was calibrated using indium as an upper temperature and enthalpy standard (melting point 156.6°C, enthalpy 28.45 $Jg^{-1}$) and do-decane as a lower point temperature calibrant (melting point –9.65°C, enthalpy 216.73 $Jg^{-1}$). The supercooling point (temperature of crystallisation), melt onset and enthalpy of both freezing and melting events were calculated using the automatic evaluation programmes provided with the instrument using an enthalpy value of 334.5 $Jg^{-1}$ for the crystallisation of water. The melt onset was taken as the point where the base line is crossed by a tangent drawn to the steepest point of the endotherm peak. For concentrated aqueous solutions this measurement may not represent the true melting point i.e. the point at which the last crystal in the solution melts, but is used to show relative changes in melting point. The enthalpy value of the freeze was corrected according to the amount of undercooling.

Effect of starvation on freezing

To allow springtails access to free water but not food, they were placed in a small tray with a base of porous polythene sheet. The tray was placed at an angle in a sealed chamber containing distilled water so that the lower edge of the tray dipped below the level of the water. The inert plastic base ensured that the Collembola had access to free water and constant high humidity but did not have a source of food. The culture was held at a temperature of 10°C for 3 days to encourage the Collembola to use food reserves in the gut, then returned to 0°C for 3 weeks. The individuals were examined for survival and only active

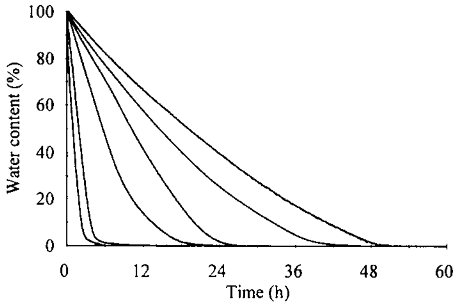

Fig. 1. Drying curves obtained from a recording microbalance for individual *O. arcticus* at different constant temperatures and R.H. < 5%. Curves from right to left: –10, –5, 0, 5, 10, 15 and 20°C.

individuals were used for freezing studies using DSC.

Long term survival of sub-zero temperatures

Culture pots containing about 50 Collembola were placed in a cabinet and the temperature reduced from 0 to –2.5°C in steps of 0.5 degrees day$^{-1}$. After 7 months the springtails were immobile on the ice covered substratum of the culture pot. Individuals were removed from the culture pot using a fine paint brush and observed under a low power microscope to check for survival. Only individuals showing some signs of movement were used for measurements of freezing and melting point studies using differential scanning calorimetry. Water contents were determined by DSC and direct weighing.

## RESULTS

### Rates of water loss

Drying curves plotted from data obtained from the recording microbalance for individual Collembola at temperatures ranging from –10 to 20°C are shown in Fig 1. The drying curves, particularly those for the higher temperatures (20 and 15°C), show two distinct phases of water loss similar to those described by Scheuplein & Morgan (1967) distinguishing the transition between the loss of free and bound water. Working with tardigrades, Wright (1989) introduced the concept of "permeability slump" to describe a similar phenomena. Bound water is better described as osmotically inactive or unfreezable water (Franks, 1986). This recording microbalance technique has been used to measure "bound" water in the soluble subcellular components in *Eurosta solidaginis* (Storey et al., 1981) but has not been used for whole animals. On the basis of these results it was decided to use a temperature of 0°C for the desiccation experiments giving a reduction in the water content of samples in desiccators of about 5% h$^{-1}$.

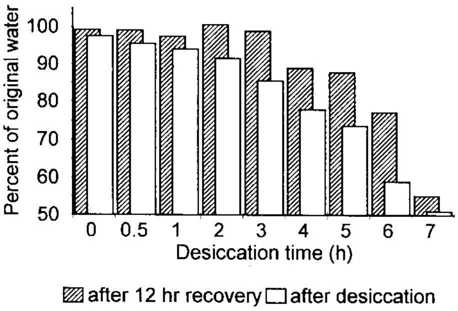

Fig. 2. Water contents of *O. arcticus* after rapid desiccation at 0°C and < 5% R.H. to a range of moisture contents and subsequent recovery after a period of 12 h with access to water.

### Survival and recovery from partial desiccation

The results shown in Fig. 2 express the water content as a percentage of the original water to allow comparison of samples which had different initial water contents. During the desiccation phase of this experiment *O. arcticus* lost water at approximately the rate predicted from the microbalance determinations (5% h$^{-1}$ at 0°C and relative humidity < 5%). The recovery rate was much slower than the rate of water loss but animals desiccated for periods of up to 180 min recovered after 24 h given access to free water. Individuals losing up to 40% of their original

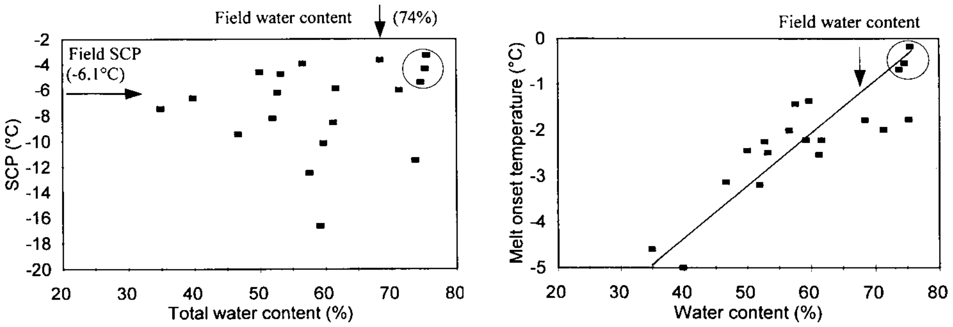

Fig. 3. The effect of rapid desiccation on individual *O. arcticus* to a range of moisture contents: (a) the effect of water content on SCP and (b) the effect of water content on the melt onset of the same individuals measured using DSC. The three data points enclosed by a circle are for non-desiccated controls. Arrows indicate mean field levels of water content and SCP. The fitted regression line for water content on melt onset has been included in (b).

water took over 36 h to recover but mortality was high (50%), while samples desiccated for 7 h lost almost 50% of their original water content (n = 10) and although these samples gained weight due to passive absorption of water, no specimens survived.

## Effect of desiccation on cold tolerance

The DSC determinations of the SCP and melting point of individual collembolan desiccated to different levels are shown in Fig. 3a, b with mean field levels of water content and SCP indicated by arrows. The regression of the water content against the melt onset has been calculated and indicated by a line in Fig. 3b. A distinct relationship can be seen due to the concentration of solutes as water is lost from the insect with an intercept of –7.42 ± 0.6°C a slope of 0.09 ± 0.01 ($r^2$ = 0.76) between the melt onset and the water content. However, there is no such relationship between SCP and the water content (Fig. 3a) although the SCPs are scattered. The SCPs ranged from –2.5 to –16.0°C with water contents covering the range from field levels (74%) down to 34%.

## Effect of starvation on the SCP

Starvation for 3 weeks reduced the mean (± SD) SCP slightly from -6.09 ± 2.1°C to -6.70 ± 3.66°C. Although these animals were not treated to induce desiccation % water contents ranged from 75.82 to 61.17 which is similar to field distributions (74 ± 10%).

## Effect of acclimation at –2.5°C for 7 months

The mean (± SE) SCP for this group of springtails was reduced from -6.1 ± 2.1°C to -15.5 ± 2.3°C and the melt onset from -0.5 ± 0.3°C to -7.9 ± 1.9°C (Fig. 4a, b). Water contents ranged from 55 to as low as 25% with a mean (± SD) of 43 ± 7.2% of fresh weight. All individuals were alive prior to DSC determinations but their bodies appeared to be concave in places and much darker in colour than *O. arcticus* cultured at 5°C and a higher humidity.

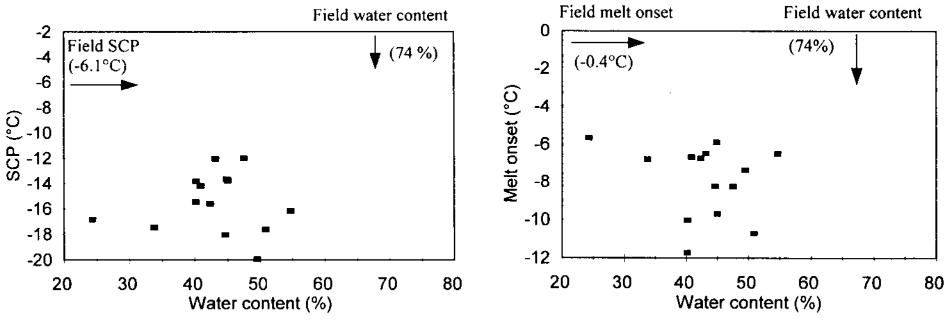

Fig. 4. The desiccating effect of acclimation at –2.5°C for 7 months in the presence of ice on *O. arcticus*. Water content, SCP (a) and the melt onset (b) are reduced from field levels indicated by arrows.

### Recovery from desiccation after low temperature acclimation

*Onychiurus arcticus* recovered rapidly from the desiccated state which had been caused by long periods at sub-zero temperatures. Mean water contents had almost returned to field levels within 24 h after being given access to water (Fig. 5), showing a very rapid recovery within the first few hours and complete recovery within 48 h.

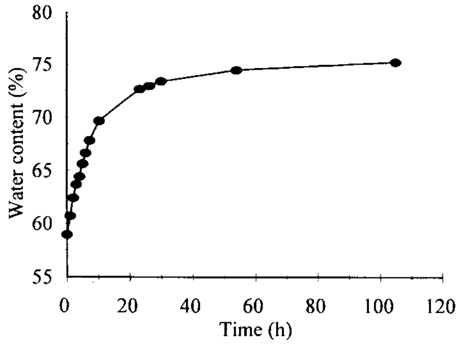

Fig. 5. Moisture uptake (recovery) by *O. arcticus* after loss of body water during acclimation at –2.5°C for 7 months and given access to free water.

### DSC determination of unfreezable water

The amount of unfreezable water calculated as the difference between gravimetric measurements of total body water content and DSC determination of the quantity of water freezing showed no significant change with desiccation. As the total water content reduced due to the treatment, either desiccation or long periods at sub-zero temperatures, the amount of unfreezable water remained constant at 16.5 ± 2.0%.

## DISCUSSION

Rates of water loss under desiccating conditions for *O. arcticus* are similar to those obtained for soil-dwelling Antarctic Collembola examined by Worland & Block (1986) and are typical of transitional species as classified by Eisenbeis (1983). *O. arcticus* was found to survive and recover from water losses of 40% of initial water content (44% of fresh weight), but did not survive levels of 50% initial water loss (37% of fresh weight). These values agree with those of Hodkinson et al. (1994) who suggested that *O. arcticus* is limited to moist mesic microhabitats. Due to its large size it may be unable to move down into the soil to avoid desiccation during dry periods when temperatures at the soil surface may rise to 30°C (Coulson et al., in press). However *O. arcticus* survived levels of desiccation down to 25% (of water content on a fresh weight basis ) when held for 7 months at

–2.5°C (Fig. 4a, b). Desiccation under these conditions was not caused by low humidity but was due to the vapour pressure difference between the supercooled liquid in the collembolan and the ice in the substrate of the hibernaculum. In this case desiccation rates will be slow and more similar to those occurring in the natural microhabitat.

The lack of correlation between the water content and the SCP of the rapidly desiccated samples, may be due to the continued presence of ice nucleators within the body. Sømme & Block (1982) showed that starvation of *Cryptopygus antarcticus* for periods of 6 days caused a significant decrease in the SCP, but it is unlikely that an insect will be able to remove or mask ice nucleators in a few hours. Responses of this type may also rely on a decrease in ambient temperature to act as a trigger although it is possible that a reduction in water content may also have an effect. Starvation did not have a significant effect on the SCP over a 3 week period but it was not possible to combine the effects of desiccation and starvation in this study.

Long periods at sub-zero temperatures have been shown to significantly reduce the SCP of *O. arcticus* by increasing the solute concentration as indicated by the decrease in the melt onset temperature, but it is likely that a reduction in ice nucleators also occurred. Levels of unfreezable water remained constant at about 16.5%, therefore the bulk of the water lost was from body fluids while the osmotically inactive water remained constant. Similar levels of osmotically inactive water have been measured in other insects (Zachariassen, 1979 [beetles]; Williams, 1970 [mussels]) where it has been attributed to the presence of large macromolecules. The cocoons of the lumbricid earthworm, *Dendrobaena octaedra* have been shown to utilise a protective dehydration mechanism (Holmstrup & Westh, 1994), losing > 97% of osmotically active water but with only a slight decrease in osmotically inactive water. Holmstrup and Westh proposed that this mechanism may be important for winter survival in other semi-terrestrial invertebrates, particularly those with a high permeability to water.

*O. arcticus* is a freeze intolerant collembolan but when active and feeding in summer it can only survive a few degrees below its chill-coma temperature of –4°C (Block et al., 1994). Hodkinson et al. (1994) suggested that *O. arcticus* will experience drying conditions towards the end of summer which may lead to a gradual loss of water, the process being reversed with the spring melt. This study provides evidence for this hypothesis although the rate of water loss and increase in solute concentration in the initial period, and hence the time taken for the cryoprotective system in the body to come to equilibrium with external frozen water, has yet to be determined.

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