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Desiccation tolerance and drought acclimation in the Antarctic collembolan *Cryptopygus antarcticus*

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ABSTRACT

The availability of water is recognized as the most important determinant of the distribution and activity of terrestrial organisms within the maritime Antarctic. Within this environment, arthropods may be challenged by drought stress during both the austral summer, due to increased temperature, wind, insolation, and extended periods of reduced precipitation, and the winter, as a result of vapor pressure gradients between the surrounding icy environment and the body fluids. The purpose of the present study was to assess the desiccation tolerance of the Antarctic springtail, *Cryptopygus antarcticus*, under ecologically-relevant conditions characteristic of both summer and winter along the Antarctic Peninsula. In addition, this study examined the physiological changes and effects of mild drought acclimation on the subsequent desiccation tolerance of *C. antarcticus*. The collembolans possessed little resistance to water loss under dry air, as the rate of water loss was >20% h^{-1} at 0% relative humidity (RH) and 4 °C. Even under ecologically-relevant desiccating conditions, the springtails lost water at all relative humidities below saturation (100% RH). However, slow dehydration at high RH dramatically increased the desiccation tolerance of *C. antarcticus*, as the springtails tolerated a greater loss of body water. Relative to animals maintained at 100% RH, a mild drought acclimation at 98.2% RH significantly increased subsequent desiccation tolerance. Drought acclimation was accompanied by the synthesis and accumulation of several sugars and polyols that could function to stabilize membranes and proteins during dehydration. Drought acclimation may permit *C. antarcticus* to maintain activity and thereby allow sufficient time to utilize behavioral strategies to reduce water loss during periods of reduced moisture availability. The springtails were also susceptible to desiccation at subzero temperatures in equilibrium with the vapor pressure of ice; they lost ~40% of their total body water over 28 d when cooled to −3.0 °C. The concentration of solutes in the remaining body fluids as a result of dehydration, together with the synthesis of several osmolytes, dramatically increased the body fluid osmotic pressure. This increase corresponded to a depression of the melting point to approximately −2.2 °C, and may therefore allow *C. antarcticus* to survive much of the Antarctic winter in a cryoprotectively dehydrated state.

1. Introduction

The abundance and activity of many soil-dwelling organisms depends upon the moisture characteristics of their environment. This is especially true in the Antarctic where water availability, even more so than temperature, is recognized as the most important determinant of the distribution of Antarctic terrestrial organisms (Kennedy, 1993). During winter, habitat moisture is likely to be limited, as water is biologically unavailable in the form of ice. Similarly, during summer terrestrial microhabitats may dry depending on the vagaries of precipitation, wind, temperature and insolation in relation to soil and vegetation type (Kennedy, 1993). Therefore, desiccation resistance and/or tolerance of varying relative humidity (RH) conditions are likely to be as important as cold tolerance for the survival of terrestrial organisms in polar environments (Ring and Danks, 1994; Block, 1996).

Collembolans are among the most abundant and widespread terrestrial arthropods and have been classified into three groups based upon their response to desiccating conditions (Vannier, 1983).

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Because of their small size, and correspondingly high surface-to-{\GuliverItalicvee}olume ratio, and highly permeable cuticle, Type I species (hygrophilic) show little to no control over water loss. Type II (mesophilic) and III (xerophilic) species show increasing control, through morphological, behavioral, and/or physiological means, over water loss when exposed to a desiccating environment. However, most previous studies assessing rates of water loss and the survival of springtails under desiccating conditions were conducted at extremely low relative humidities, which often were not ecologically relevant (e.g., Block et al., 1990). Such experimental treatments may mask mechanisms involved in desiccation resistance/tolerance under a more ecologically-relevant setting.

Traditionally, studies on the desiccation physiology of springtails have focused on the role of the cuticle as a barrier for water loss (Verhoef and Witteveen, 1980; Block et al., 1990; Harrisson et al., 1991), while desiccation-induced osmolyte production has also received considerable attention (Verhoef, 1981; Verhoef and Prast, 1989; Bayley and Holmstrup, 1999; Holmstrup et al., 2001; Kaersgaard et al., 2004). Recently, a drought acclimation response that increases the tolerance of springtails to subsequent desiccation and cold shock was documented (Sjursen et al., 2001; Bayley et al., 2001; Holmstrup et al., 2002b). Drought acclimation is facilitated by the accumulation of sugars and polyols, which reduce the gradient for water loss and likely stabilize membranes and proteins during subsequent desiccation.

In polar and temperate regions, over-wintering arthropods within the soil column possessing a limited resistance to water loss, such as many species of Collembola, may also be challenged by dehydration. As temperatures decline and the soil water freezes, a vapor pressure gradient is established between the unfrozen body fluids of the arthropods and the surrounding frozen environment. Dehydration occurs because the vapor pressure of the environment is lower than that of the unfrozen body fluids at the same temperature. During such dehydration, the melting point of the body fluids may be depressed, as solutes become concentrated in the remaining body fluids and equilibrate with the surrounding environmental temperature, thereby eliminating any risk of freezing (Holmstrup et al., 2002a). Several arthropods are now known to have the capacity to use such cryoprotective dehydration as a viable strategy for winter survival (Holmstrup et al., 2002a; Holmstrup and Sømme, 1998; Worland and Block, 2003; Elnitsky et al., 2008).

The springtail *Cryptopygus antarcticus* is the most widespread and abundant terrestrial arthropod in the maritime Antarctic (Block, 1984). Large aggregations are found on the underside of rocks, beneath mats of terrestrial algae (i.e., *Prasiola crispa*), and in association with mosses (Worland and Block, 1986). Previous studies have focused on the desiccation resistance/tolerance of *C. antarcticus*, suggesting that this species has a limited resistance to desiccation and no physiological control over water loss (Block et al., 1990; Harrisson et al., 1991; Block and Harrison, 1995). These studies exposed *C. antarcticus* to extremely low, constant RH environments (i.e., 0–35% RH). However, Worland and Block (1986) reported diurnal ranges of 37–100% RH under stones in springtail microhabitats. As yet, no study has assessed the response of *C. antarcticus* under these more ecologically-relevant conditions of dehydration characteristic of the austral summer. Similarly, Worland and Block (2003) demonstrated that *C. antarcticus* is susceptible to desiccation at subzero temperatures corresponding to conditions during winter on the Antarctic Peninsula, but did not assess the physiological response to such dehydration.

Therefore, the purpose of the present study was to assess the desiccation tolerance and physiological response to dehydration of *C. antarcticus* under ecologically-relevant conditions characteristic of both the austral summer and winter. In addition, this study examined the physiological changes and effects of drought acclimation on the subsequent desiccation tolerance of *C. antarcticus*.

2. Materials and methods

2.1. Source of animals

C. antarcticus (Willem) (Collembola, Isotomidae) were collected from sites on Humble and Torgersen Islands, near Palmer Station on the Antarctic Peninsula (64°46′S, 64°04′W). Collembola were subsequently stored at 100% RH and 4 °C (0L: 24D) for a minimum of 2 d to ensure animals were fully hydrated prior to experimental use. Only adult *C. antarcticus* (>1.0 mm long and typically >50 μg; Worland and Block, 2003) were used in all experiments.

2.2. Water balance and desiccation tolerance

The desiccation tolerance and changes in water content (WC) of *C. antarcticus* were assessed at 4 °C at several constant relative humidities. Specific relative humidities were produced in 3.8-L glass desiccators containing 500 mL NaCl solutions. The air inside the closed system quickly equilibrated with the salt solution (following Raoult's law) to create a 98.2 (31.60 g NaCl L^{-1}), 96.0 (71.20 g NaCl L^{-1}), 93.0 (126.57 g NaCl L^{-1}), or 75.0% (supersaturated NaCl solution) RH environment. Control animals were maintained at 100% RH (double-distilled water). For tests of desiccation tolerance, groups of 10 *C. antarcticus* were placed within mesh-covered cages (20 μm mesh size), which allowed the free movement of water vapor, and transferred to the experimental RH. Thirty individuals for measurement of total body WC and five groups of 10 *C. antarcticus* for assessment of survival were removed daily from each RH treatment. The WC of individual springtails was assessed gravimetrically from measurements (to the nearest 1 μg; Cahn C-31 electrobalance, Ventron Co., Cerritos, CA, USA) of fresh weight at the time of sampling and dry mass (DM) after drying to constant mass at 65 °C. Prior to assessing survival, *C. antarcticus* were rehydrated for 24 h at 100% RH. Animals were considered to have survived if they displayed coordinated walking behavior following rehydration.

For comparison to previous studies of water balance in arthropods, the rate of water loss at 0% RH (dry calcium sulfate) and 4 °C was also determined by fitting measurements taken at 0.5 h intervals to Wharton's (1985) model for exponential water loss

$$m_t = m_0 e^{-kt} \quad \text{or} \quad \ln\left(\frac{m_t}{m_0}\right) = -k_t$$

where m_0 is the initial water mass, m_t is the water mass at time t, and k_t is the amount of water lost between the measurements m_0 and m_t. The slope of $\ln(m_t/m_0)$ plotted against time is the water loss rate expressed as percent of the total WC per hour. The water mass of individually maintained springtails was assessed gravimetrically as the difference between the mass at time t and DM values.

2.3. Drought acclimation

To determine the effect of a mild drought stress on the subsequent tolerance of desiccation, *C. antarcticus* were drought-acclimated at 98.2 or 75.0% RH prior to tests of desiccation tolerance. Collembola were drought-acclimated at 4 °C and 98.2% RH for 24, 48, or 96 h, or at 75.0% RH for 24 h, in glass desiccators as described above. A control group of *C. antarcticus* was maintained at 100% RH prior to tests of desiccation tolerance. Springtails were

subsequently transferred to 96.0 or 93.0% RH for 5 d prior to assessment of survival as described above.

During drought acclimation, samples were also removed to assess the role of sugar and polyol accumulation. Glycerol, trehalose, and glucose were measured in *C. antarcticus* drought-acclimated at 98.2% RH for 24, 48, and 96 h, or 75.0% RH for 24 h, as described above. A control group was maintained at 100% RH prior to assessment of sugar and polyol content. Groups of ~500 springtails were weighed and immediately frozen at −80 °C until whole body concentrations of sugars and polyols were determined. Animals were subsequently homogenized in 1N perchloric acid and neutralized with an equal volume of 1N potassium hydroxide prior to determining sugar and polyol content. Glycerol concentration was determined enzymatically as described by Holmstrup et al. (1999). Trehalose content was determined following digestion with trehalase as described by Chen et al. (2002). Glucose concentration was determined using a glucose oxidase procedure (no. 510; Sigma, St. Louis, MO, USA).

2.4. Subzero temperature-induced desiccation

C. antarcticus were also desiccated by exposure to an environment at equilibrium with the vapor pressure of ice while monitoring changes in WC and the osmotic pressure of the body fluids. Groups of ~30 springtails were placed within 0.6-mL microcentrifuge tubes and confined by means of fine plastic mesh (20 μm mesh size) which allowed the free movement of water vapor. Microcentrifuge tubes were in turn placed within 10-mL glass vials containing ~4 g of crushed ice and closed with tightly fitting lids. Vials containing *C. antarcticus* were allowed to equilibrate in refrigerated baths at −0.6 °C for 24 h. The temperature of the bath was subsequently lowered incrementally (~0.5 °C d^{-1}) to −3.0 °C and remained at this temperature until termination of the experiment (day 28). A control group of *C. antarcticus* was held at −0.6 °C throughout the experiment. Samples were removed at 2–7-d intervals for assessment of WC, as described above, and osmotic pressure of the body fluids. Determinations of the body fluid osmotic pressure were made using a vapor pressure depression technique (Holmstrup and Sømme, 1998). Groups of ~30 *C. antarcticus* were placed in a sample holder and quickly crushed with a Teflon rod to expose the body fluids. Samples were then allowed to equilibrate for 30 min following placement within a C-52 sample chamber (Wescor Inc., Logan, UT, USA). The osmotic pressure of the body fluids was measured using a Wescor HR-33T Dew Point Microvoltmeter (Wescor Inc., Logan, UT, USA) operated in the dew point mode.

Additionally, sugar and polyol accumulation during subzero temperature-induced desiccation was examined. On days 0, 6, 15, and 28 during exposure to −3.0 °C in an environment at equilibrium with the vapor pressure of ice, groups of *C. antarcticus* were removed and stored for subsequent analysis of glycerol, trehalose, and glucose as described above. Control springtails were maintained at −0.6 °C until termination of the experiment.

2.5. Statistical analysis

Variations in survival, WC, and DM during dehydration under constant RH environments were analyzed with two-way (treatment × time) analysis of variance (ANOVA) following tests of parametric assumptions. When there were significant treatment effects, Bonferroni–Dunn multiple comparison tests were used to test for significant differences over time. Differences in survival of desiccation between control and drought-acclimated Collembola were compared with Student's *t*-tests. Mean sugar and polyol concentrations and changes in WC and osmotic pressure during subzero temperature-induced desiccation were analyzed with one-way ANOVA and Bonferroni–Dunn tests. Percentage data were arcsin-square root transformed prior to analysis. Data not meeting parametric assumptions were log transformed to correct for non-normality or heteroscedasticity. All data are presented as mean ± S.E.M. with statistical significance set at $P < 0.05$.

3. Results

3.1. Water content and desiccation tolerance

When tested at 4 °C, *C. antarcticus* lost water at all relative humidities except at water saturation (100% RH; Fig. 1A). Within a RH treatment, the DM of *C. antarcticus* did not differ significantly during the course of the experiment, suggesting that differences in fresh mass were due solely to water loss. The WC of live Collembola declined significantly (treatment × time interaction, $F_{55, 1016} = 6.72$; $P < 0.0001$) at all relative humidities below water saturation through day 5, prior to leveling throughout the remainder of the experiment. Rates of water loss from *C. antarcticus*, at least over the first few experimental days, were inversely related to the desiccation treatment (i.e., higher rates of water loss occurred at lower relative humidities). By day 10 of desiccation, only the 98.2% RH treatment had a sufficient number of surviving collembola to allow accurate measurement of WC. The WC of *C. antarcticus* maintained at

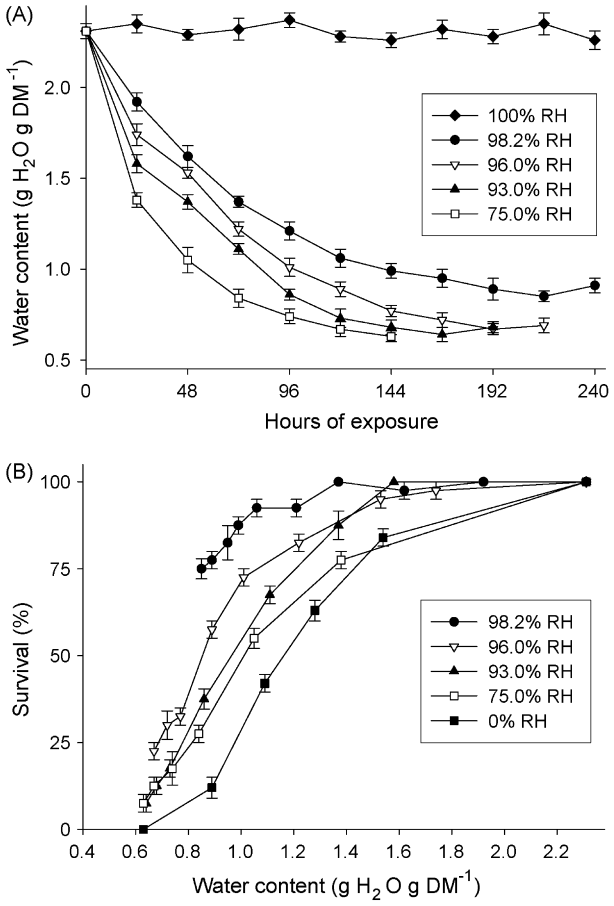

Fig. 1. (A) Changes in total body water content of *Cryptopygus antarcticus* during desiccation exposure within various relative humidity (RH) environments at 4 °C. Values are mean ± S.E.M. of 25–30 individuals. (B) Percent survival as a function of total body water content of *C. antarcticus* during desiccation in various constant relative humidity environments. Values are mean ± S.E.M. of five groups of 10 individuals.

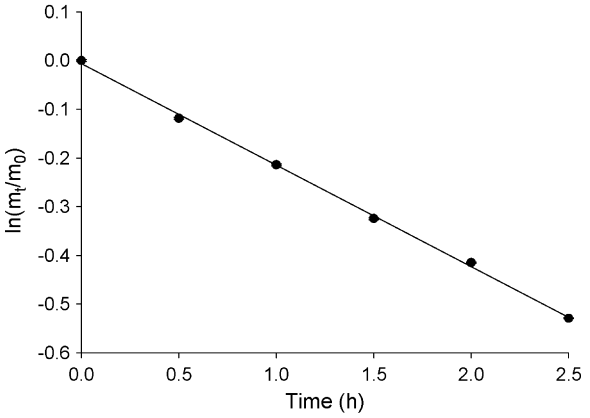

Fig. 2. Water loss rate of *Cryptopygus antarcticus* at 4 °C and 0% RH. Wharton's (1995) model for exponential water loss was fitted to the points [$y = -0.208(\pm 0.003)x - 0.00650(\pm 0.005)$, $R^2 = 0.998$; $P < 0.0001$], where m_0 is the initial water mass, m_t is the water mass at time t, and the slope of the regression represents the water loss rate in percent of total body water per hour. Values are mean ± S.E.M. of 25–30 individuals.

100% RH did not change ($F_{10, 252} = 0.93$; $P = 0.452$) during the course of the experiment.

The survival of *C. antarcticus* during desiccation declined as a non-linear function of WC (Fig. 1B). At 96.0, 93.0, and 75.0% RH, survival dropped rapidly as WC dropped below 1.1 g H_2O g DM^{-1}, and only at 96.0% RH did >50% of the adults survive at a WC below 0.95 g H_2O g DM^{-1}. This contrasts with *C. antarcticus* exposed to 98.2% RH, of which >75% of individuals survived at a WC below 0.90 g H_2O g DM^{-1}. These results suggest that the desiccation tolerance of *C. antarcticus* is highly dependent upon the severity of the desiccation stress and rate of dehydration; desiccation tolerance was increased at lower rates of water loss. At the termination of the experiment (day 10), the osmotic pressure of body fluids in springtails exposed to 98.2% RH had equilibrated to that of the environment (approximately −25 bar; $n = 5$) and, therefore, they would not have been expected to lose more water.

Springtails displayed little resistance to water loss in dry air (0% RH). Applying Wharton's (1985) model, the hourly rate of water loss at 0% RH and 4 °C of *C. antarcticus* was ~21% h^{-1} (Fig. 2). Under such extreme conditions of desiccation, survival dropped rapidly, as fewer than 50% of the individuals survived body WCs below ~1.1 g H_2O g DM^{-1} (Fig. 1B), corresponding to only 1.5 h of desiccation at 0% RH.

3.2. Drought acclimation

Mild drought stress at 98.2% RH significantly increased the subsequent tolerance of desiccation of *C. antarcticus* to both 96.0 and 93.0% RH (Fig. 3A and B). The survival of springtails acclimated at 100% RH (controls) was ~55 and <20%, respectively, when exposed to 96.0 or 93.0% RH for 5 d. Drought acclimation at 98.2% RH reduced the body WC of the springtails (Table 1); WC was reduced by nearly 45% in the 96 h acclimation. Relative to the controls, acclimation at 98.2% RH for 48 or 96 h significantly increased subsequent survival of exposure to either 96.0 (48 h, $t_4 = 3.92$; $P = 0.012$; 96 h, $t_4 = 4.23$; $P = 0.007$) or 93.0% RH (48 h, $t_4 = 4.04$; $P = 0.008$; 96 h, $t_4 = 12.78$; $P < 0.0001$) for 5 d. Acclimation at 98.2% RH for 24 h had no significant effect (at 96% RH,

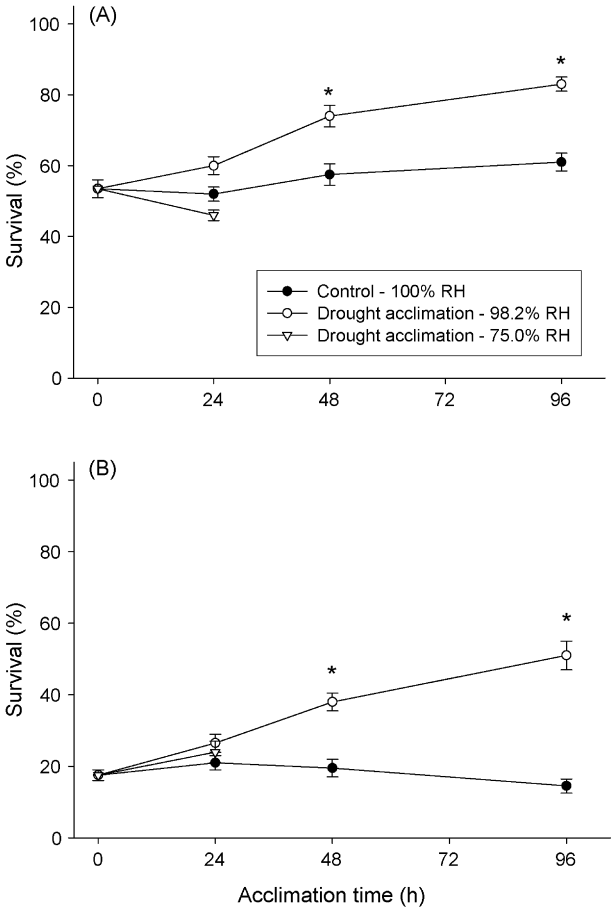

Fig. 3. Survival of *Cryptopygus antarcticus* desiccated for 5 d at either 96.0% RH (A) or 93.0% RH (B) at 4 °C. Collembola were previously acclimated at 100% RH (control) or drought-acclimated at 98.2 or 75.0% RH for 0, 24, 48, or 96 h prior to assessment of desiccation tolerance. Values are mean ± S.E.M. of five groups of 10 individuals. Asterisks denote a significant difference relative to the control treatment (Student's *t*-test).

Table 1
Mean (±S.E.M.) total body water content ($n = 25$–30 per treatment per time point) and osmolyte concentrations ($n = 6$ per treatment per time point) of *Cryptopygus antarcticus* during drought acclimation at 4 °C and 98.2 or 75.0% RH

	Hours of exposure				
	98.2% RH				75.0% RH
	0	24	48	96	24
Water content (g H_2O g DM^{-1})	2.27 ± 0.03[a]	1.96 ± 0.06[b]	1.67 ± 0.04[c]	1.26 ± 0.05[d]	1.42 ± 0.06[e]
Osmolyte concentration (μg mg DM^{-1})					
Glycerol	9.2 ± 1.2[a]	19.7 ± 2.6[b]	31.3 ± 3.1[c]	47.4 ± 5.2[d]	13.8 ± 0.9[a]
Glucose	5.6 ± 0.9[a]	6.2 ± 1.2[a]	12.3 ± 1.1[b]	19.5 ± 2.2[c]	7.8 ± 1.4[a]
Trehalose	4.1 ± 0.7[a]	6.4 ± 1.1[a]	10.6 ± 1.4[b]	18.6 ± 1.9[c]	6.8 ± 1.5[a]

Different letters denote significant differences between treatment groups (ANOVA; Bonferroni–Dunn test).

$t_4 = 1.06$; $P = 0.193$; at 93% RH, $t_4 = 0.31$; $P = 0.386$) on subsequent desiccation tolerance. Drought acclimation at 75.0% RH for 24 h similarly reduced WC by ~35% (Table 1); however, it had no significant effect on the subsequent survival of *C. antarcticus* at 96.0 ($t_4 = -0.92$; $P = 0.214$) or 93.0% RH ($t_4 = 0.23$; $P = 0.445$; Fig. 3A and B). Insufficient numbers of springtails survived longer drought acclimations at 75.0% RH; therefore, their subsequent tolerance of dehydration was not tested.

Drought acclimation was accompanied by a significant *de novo* synthesis and accumulation of several osmolytes within the body fluids (Table 1). Glycerol concentrations ($F_{4,\ 25} = 82.46$; $P < 0.0001$) were increased more than 5-fold to >45 μg mg DM^{-1} after a 96 h acclimation at 98.2% RH. Glucose ($F_{4,\ 25} = 29.17$; $P = 0.0021$) and trehalose concentrations ($F_{4,\ 25} = 37.34$; $P = 0.0017$) were more modestly elevated by ~2 and 3-fold, respectively, following acclimation for 48 or 96 h. Acclimation at 75.0% RH resulted in a small, but significant, increase in glycerol concentration, but failed to increase the synthesis of glucose or trehalose.

3.3. Subzero temperature-induced desiccation

Slow cooling of *C. antarcticus* to −3.0 °C in an environment at equilibrium with the vapor pressure of ice resulted in a significant ($F_{6,\ 116} = 87.64$; $P < 0.0001$) reduction of WC (Fig. 4A). The WC decreased from day 0 to 14, prior to leveling off over the remainder of the experiment. By day 28, the WC had been reduced by ~40% to 1.41 ± 0.03 g H_2O g DM^{-1}. The DM of *C. antarcticus* did not change significantly over the course of the experiment, suggesting all mass changes were due solely to water loss. At the termination of the experiment collembolan survival was nearly 75% ($n = 150$). Control *C. antarcticus* equilibrated to −0.6 °C had a body WC of 2.36 ± 0.05 g H_2O g DM^{-1}, with no significant change in WC ($F_{6,\ 114} = 0.86$; $P = 0.427$) or DM ($F_{6,\ 114} = 1.18$; $P = 0.369$) during the experiment. Similarly, at day 28 survival of control animals was >81% ($n = 150$).

The osmotic pressure of the body fluids of *C. antarcticus* equilibrated to −0.6 °C was −9.4 ± 0.4 bar ($n = 6$) and did not change significantly ($F_{6,\ 35} = 1.13$; $P = 0.213$) in control animals during the course of the experiment. However, cooling at equilibrium with the vapor pressure of ice resulted in a significant increase ($F_{6,\ 35} = 1152.71$; $P < 0.0001$) of the hemolymph osmotic pressure (Fig. 4B). Relative to controls, the osmotic pressure of *C. antarcticus* exposed to the vapor pressure of ice increased nearly 3-fold, to −26.4 ± 0.3 bar ($n = 6$) by day 28. However, at termination of the experiment there remained a vapor pressure deficit of approximately −10 bar between the collembolans and the surrounding environment. Therefore, the springtails would have likely continued to lose water to their environment.

Water loss alone could not account for the observed increase in the osmotic pressure of the body fluids during cooling at equilibrium with the vapor pressure of ice. Desiccation at subzero temperatures also induced the *de novo* synthesis of several osmolytes (Table 2). During desiccation, the concentrations of

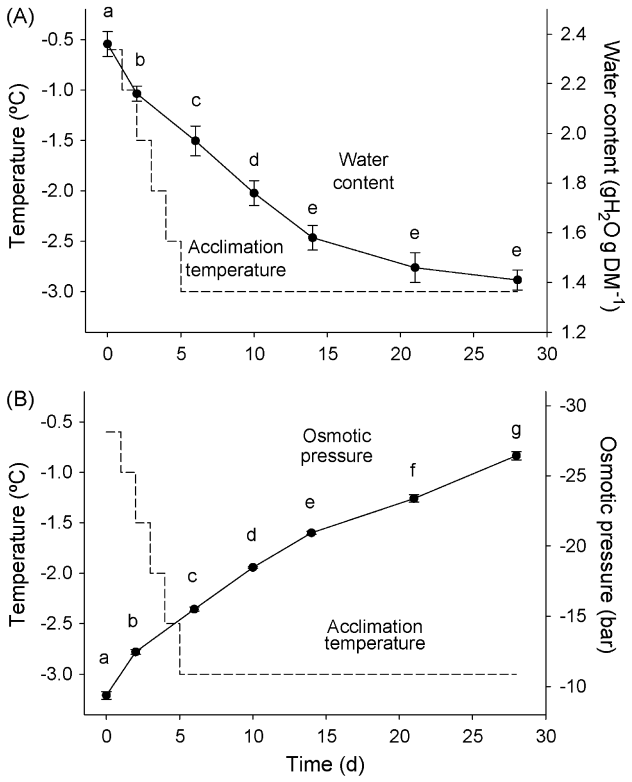

Fig. 4. Changes in (A) body water content ($n = 15$–20 individuals) and (B) osmotic pressure of the body fluids ($n = 6$) of *Cryptopygus antarcticus* during slow cooling to −3.0 °C in an environment at equilibrium with the vapor pressure of ice. Values are mean ± S.E.M. Different letters denote significant differences between days of exposure (ANOVA; Bonferroni–Dunn test).

trehalose ($F_{4,\ 25} = 26.82$; $P = 0.0023$) and glucose ($F_{4,\ 25} = 43.58$; $P = 0.0006$) increased significantly by ~4 and 5-fold, respectively, by day 28 of exposure to −3.0 °C. At termination of the experiment, glycerol ($F_{4,\ 25} = 123.83$; $P < 0.0001$) concentration had increased ~8-fold to nearly 70 μg mg DM^{-1}. Such osmolyte synthesis likely contributed substantially toward the observed increase in the osmotic pressure of the body fluids, especially at low body WCs. Glucose and trehalose concentrations were unchanged in control individuals maintained at −0.6 °C throughout the experiment, whereas glycerol concentration increased ~2-fold.

4. Discussion

Long-term field monitoring of *C. antarcticus* populations on maritime Antarctic Signy Island suggests that springtails are faced with drought stress during both the austral summer and winter (Block and Convey, 2001; Convey et al., 2003). Monthly sampling over an 11-year period from 1984–1995 revealed a distinct

Table 2
Mean (±S.E.M.) osmolyte concentrations ($n = 6$ per treatment per time point) of *Cryptopygus antarcticus* during exposure at −3.0 °C in an environment at equilibrium with the vapor pressure of ice

	Days of exposure				
	Desiccation at −3.0 °C				Control (−0.6 °C)
	0	6	15	28	28
Osmolyte concentration (μg mg DM^{-1})					
Glycerol	8.7 ± 1.3^a	15.3 ± 2.2^b	27.6 ± 2.7^c	68.4 ± 5.6^d	16.5 ± 2.0^b
Glucose	5.4 ± 0.8^a	8.3 ± 1.1^a	16.7 ± 2.1^b	26.8 ± 3.3^c	7.8 ± 1.2^a
Trehalose	3.8 ± 0.7^a	7.1 ± 0.9^a	10.1 ± 1.6^b	16.2 ± 1.4^c	6.1 ± 1.1^a

Different letters denote significant differences between days of exposure (ANOVA; Bonferroni–Dunn test).

seasonal pattern of body WC in *C. antarcticus*; WC was lowest during mid-summer (December–January) and mid-winter (June–August). During summer, increased solar radiation, temperature, wind, and periods of reduced precipitation may contribute to the drying of microhabitat sites. In winter, when ice is present within the soil matrix, springtails may lose water to the surrounding environment due to the lower vapor pressure of ice compared to the unfrozen body fluids (Holmstrup et al., 2002a). Such conditions necessitate an inherent resistance to and/or tolerance of desiccation. The present study sought to characterize the desiccation tolerance of *C. antarcticus* under ecologically-relevant conditions characteristic of both summer and winter along the Antarctic Peninsula, and also to identify physiological mechanisms that may reduce water loss during drought stress and/or increase the inherent tolerance for dehydration. These findings are discussed in the context of our current knowledge regarding the physiology and ecology of this species.

4.1. Desiccation tolerance

C. antarcticus showed little or no resistance to desiccation under dry-air conditions, thus confirming the results from previous investigations (Worland and Block, 1986; Block et al., 1990; Harrisson et al., 1991). At 4 °C and 0% RH, the collembolans lost ~20% of their total body water per hour and succumbed to dehydration stress within 2 h. This high rate of water loss is comparable to that of other hygrophilic collembolans desiccated under conditions of dry air (Vannier, 1983). However, while useful for comparative purposes in assessing the absolute rates of water loss, such extreme conditions of desiccation are likely of little ecological relevance. Further, these tests of desiccation resistance may mask processes that operate to increase the tolerance and/or resistance to dehydration under more ecologically-relevant conditions. Within their microhabitat, *C. antarcticus* rarely, if ever, experiences conditions below ~35% RH (Worland and Block, 1986) and when presented with drought stress within their natural environment, they may employ behavioral [e.g., the tendency to aggregate in large numbers or actively seek moist refuges (Hayward et al., 2001, 2004)], and/or physiological mechanisms [e.g., through the accumulation of osmolytes (present study)] to limit water loss.

Due to their high surface area-to-{\GuliverItalicvee}olume ratio and cuticular permeability (Harrisson et al., 1991) to water, *C. antarcticus* are especially susceptible to dehydration. This was evident even at high relative humidities, as the springtails lost water under all conditions below saturation, with the rate of water loss inversely proportional to the desiccation treatment. No evidence of water vapor absorption from subsaturated air was observed, as has been reported for some other species of Collembola (Bayley and Holmstrup, 1999). However, springtails maintained at 98.2% RH for 10 d had equilibrated the osmotic pressure of their body fluids to that of the environment (approximately −25 bar). These individuals would, therefore, not be expected to undergo further reductions of body water. At this time survival was >75% (compared to ~90% for control springtails maintained at 100% RH), suggesting *C. antarcticus* can tolerate extended periods in the absence of free water at high relative humidities.

Under mild desiccation stress at high relative humidities, corresponding to lower rates of water loss, *C. antarcticus* tolerated a greater loss of body water. When desiccated at 0% RH, survival declined to below 50% at ~1.15 g H_2O g DM^{-1}, corresponding to a loss of only ~50% of the total body water. However, at 93.0% RH, springtails survived the loss of ~58% of their body water and >60% at the highest RH tested (98.2%). Such increases in desiccation tolerance at lower rates of water loss are well known amongst arthropods (Hadley, 1994). Similarly in the maritime Antarctic, Benoit and colleagues (2007) recently reported that the Antarctic midge, *Belgica antarctica*, tolerates a significantly greater loss of body water when dehydrated at higher relative humidities. However, the mechanisms accounting for this increased tolerance of dehydration are unknown. It has been suggested that desiccation at lower rates of water loss permits adjustments of the water stores, in an attempt to maintain sufficient tissue hydration and preserve cellular metabolic activity, by shifting water from the extracellular compartment to the cells (Hadley, 1994). Slow dehydration of both *C. antarcticus* (present study) and *B. antarctica* (Benoit et al., 2007) also stimulated the synthesis and accumulation of higher concentrations of several sugars and polyols. In addition to further reducing the gradient for water loss, even modest concentrations of these osmolytes are well known to stabilize and protect membranes and proteins thereby preventing/reducing dehydration-induced cellular damage (Crowe et al., 1992; Yancey, 2005).

4.2. Drought acclimation increases desiccation tolerance

Mild drought acclimation further increased the desiccation tolerance of *C. antarcticus*. Acclimation for 48 or 96 h at 98.2% RH increased the subsequent survival of springtails at either 96.0 or 93.0% RH for 5 d. Whereas analogous thermal acclimations are well known to confer increased tolerance to high or low temperature (e.g., Worland and Convey, 2001), drought acclimation is less well studied. However, increases of dehydration tolerance following acclimation are known from nematodes (Crowe and Madin, 1975), earthworm cocoons (Petersen et al., 2008), reptiles (Lillywhite, 2004), insects (Hoffmann, 1990; Benoit et al., 2007), and other collembolan species (Sjursen et al., 2001; Chown et al., 2007). For example, the survival of drought stress, as low as 94% RH, of the soil-dwelling springtail *Folsomia candida* was increased following acclimation at 98.2% RH for 6 d (Sjursen et al., 2001). Among other Antarctic organisms, workers recently reported a similar drought acclimation response in the midge *B. antarctica* (Benoit et al., 2007).

The reduction of the total body WC during drought acclimation of *C. antarcticus* was accompanied by the synthesis and accumulation of several osmolytes. This contrasts with previous investigations that suggested this species does not accumulate osmolytes during dehydration (Worland and Block, 1986; Block and Harrison, 1995). The reason for these differing results is unknown. However, in previous studies dehydration likely occurred rapidly, as springtails were exposed to extremely low RH environments. Our results suggest that *C. antarcticus* can accumulate significant concentrations of osmolytes if desiccation occurs at low rates of water loss and high RH. This point is exemplified by the finding that springtails accumulated significantly higher concentrations of osmolytes when desiccated to a similar extent at 98.2% RH (for 96 h) compared to those desiccated at 75.0% RH (for 24 h). A similar pattern is observed in *B. antarctica*, in which midge larvae accumulate greater osmolyte concentrations when desiccated at high relative humidities (Benoit et al., 2007).

The accumulation of osmolytes appears to be a common component to the drought acclimation response and likely contributes mechanistically, by protecting membranes and proteins, to the increased tolerance of dehydration (Sjursen et al., 2001; Benoit et al., 2007; present study). In *F. candida*, drought acclimation also results in a higher degree of unsaturation of membrane phospholipid fatty acids (Bayley et al., 2001; Holmstrup et al., 2002b), a change that resembles membrane alterations seen in ectothermic animals acclimated to low temperature (Hazel, 1995; Los and Murata, 2004). Holmstrup et al. (2002b) suggest

such membrane desaturation may counter the increased packing of membrane lipids that occurs as water is removed from the cell during dehydration, thereby maintaining membrane fluidity and metabolic function. Whether similar changes in the cellular membranes occur during and contribute to the drought acclimation observed in *C. antarcticus* deserves investigation.

In the maritime Antarctic, the availability of water is widely recognized as the most important determinant of the distribution and activity of terrestrial organisms (Kennedy, 1993; Sinclair and Stevens, 2006). The ecological significance of the enhanced desiccation tolerance of *C. antarcticus* may allow springtails to make use of microhabitats prone to dehydration. Further, Sjursen et al. (2001) suggested that drought acclimation likely permits springtails to maintain activity in the face of increasing environmental drought stress. This may be especially important as microhabitats continue to dry, allowing *C. antarcticus* to use behavioral mechanisms, such as aggregation or dispersal to moist refuges, to reduce further water loss.

4.3. Subzero temperature-induced desiccation

C. antarcticus is believed to over-winter within air spaces in the upper soil layers under vegetation, rocks and stones (Convey et al., 2003). The springtails are, therefore, vulnerable to desiccation at subzero temperatures during winter as the vapor pressure of the surrounding environment is lower than that of the unfrozen body fluids at the same temperature. Our results confirm previous reports (Worland and Block, 2003) and demonstrate that *C. antarcticus* may lose a considerable portion of their total body water when exposed to the vapor pressure of ice at subzero temperatures. In the present study, springtails lost ~40% of their total body water over the course of 28 d at −3.0 °C. Similar to their response to drought acclimation, desiccation at subzero temperatures was accompanied by the synthesis and accumulation of several osmolytes. Long-term monitoring of field populations suggests *C. antarcticus* is regularly challenged by drought stress during winter as WC may be reduced by 25% or more to as low as 1.2 g H_2O g DM^{-1} (Block and Harrison, 1995; Convey et al., 2003). Additionally, over-wintering field populations are known to accumulate significant concentrations of various osmolytes, including glycerol, glucose, and trehalose (Montiel, 1998). Taken together, it appears that partial dehydration and the accumulation of several sugars and polyols form an important component to the over-wintering strategy of *C. antarcticus*.

Desiccation at subzero temperatures and the resulting concentration of solutes in the remaining body fluids, together with the *de novo* synthesis of osmolytes, dramatically increased the osmotic pressure of the body fluids of *C. antarcticus* to approximately −25 bar by day 28 at −3.0 °C. Such an increase in the osmotic pressure corresponds to a depression of the melting point to approximately −2.2 °C. However, at termination of the experiment there remained a vapor pressure deficient (~10 bar) between the body fluids of the animals and the environment. Therefore, the springtails would likely have continued to lose water further depressing the body fluid melting point toward equilibrium with the surrounding environment. These results suggest that at least near Palmer Station, Antarctica where springtail microhabitat temperatures remain between −1 and −3 °C throughout much of the austral winter (Elnitsky et al., 2008), due to oceanic buffering, *C. antarcticus* may be able to remain in a cryoprotectively-dehydrated state. Even if temperatures subsequently decline forcing reliance upon supercooling for survival, such dehydration would likely only increase the supercooling capacity of the springtails and reduce the risk of lethal freezing (Sømme and Block, 1982; Worland and Block, 2003).

4.4. Conclusions

On the Antarctic Peninsula, *C. antarcticus* may be challenged by drought stress throughout the year. In addition to behavioral strategies, our results suggest that the springtails may also rely upon a number of physiological mechanisms to limit water loss and/or increase desiccation tolerance. When presented with water stress, the selection of moist microhabitat sites would not only reduce the rate of water loss, but may enhance desiccation tolerance through the synthesis and accumulation of various organic osmolytes. Additionally, the drought acclimation response may permit *C. antarcticus* to maintain metabolic and cellular activity, thereby extending survival during subsequent desiccation stress. During winter, the collembolans likely rely upon the partial dehydration and accumulation of sugars and polyols, similar to the Arctic collembolan *Onychiurus arcticus* (Worland et al., 1998), as important components of their over-wintering strategy. These physiological responses and their effects on activity and survival likely contribute to the success of *C. antarcticus* in this harsh environment.

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