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RESEARCH/REVIEW ARTICLE

Contrasting strategies of resistance vs. tolerance to desiccation in two polar dipterans

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Abstract

Low water availability is one of the principal stressors for terrestrial invertebrates in the polar regions, determining the survival of individuals, the success of species and the composition of communities. The Arctic and Antarctic dipterans *Heleomyza borealis* and *Eretmoptera murphyi* spend the majority of their biennial life cycles as larvae, and so are exposed to the full range of environmental conditions, including low water availability, over the annual cycle. In the current study, the desiccation resistance and desiccation tolerance of larvae were investigated, as well as their capacity for cross-tolerance to temperature stress. Larvae of *H. borealis* showed high levels of desiccation resistance, only losing 6.9% of their body water after 12 days at 98.2% relative humidity (RH). In contrast, larvae of *E. murphyi* lost 46.7% of their body water after 12 days at the same RH. Survival of *E. murphyi* larvae remained high in spite of this loss (>80% survival). Following exposure to 98.2% RH, larvae of *E. murphyi* showed enhanced survival at −18°C for 2 h. The supercooling point of larvae of both species was also lowered following prior treatment at 98.2% RH. Cross-tolerance to high temperatures (37 or 38.5°C) was not noted following desiccation in *E. murphyi*, and survival even fell at 37°C following a 12-day pre-treatment. The current study demonstrates two different strategies of responding to low water availability in the polar regions and indicates the potential for cross-tolerance, a capacity which is likely to be beneficial in the ever-changing polar climate.

Insects, which are largely of small size, have a high surface area to volume ratio and are vulnerable to water loss (Gibbs et al. 1997). Injuries resulting from the loss of water include protein denaturation and unwanted macromolecular interactions, crystalline to gel membrane phase transitions, oxidative damage and mechanical stress (Danks 2000). In order to protect against these injuries, invertebrates generally adopt one of two strategies, desiccation resistance or desiccation tolerance. The capacity to prevent water loss from the body (desiccation resistance) varies greatly among invertebrates and has led to three species classifications, namely hygric species, which have little or no control over their water loss, and transitional and mesic species, which are increasingly able to regulate the loss of body water (Danks 2000). The mesic status of invertebrates like the Antarctic mite *Alaskozetes antarcticus* is largely achieved through lowered cuticular permeability (e.g., Benoit, Yoder et al. 2007), though the regulation of water is also achieved in other invertebrates using methods of freezing (Convey 1992), membrane alteration and metabolic suppression (Michaud et al. 2008) and/or specialized respiration (Danks 2000). In hygric species, the loss of water is tolerated. *Dendrobaena octaedra* (earthworm) cocoons (Holmstrup & Zachariassen 1996) and larvae of the Antarctic dipteran *Belgica antarctica* (Hayward et al. 2007) are able to endure >75% loss of their water content, while some insects, such as the dipteran *Polypedilum vanderplaanki* and many nematodes and tardigrades, are able to survive the loss of

virtually all their osmotically active water, employing the tactic of anhydrobiosis (Crowe & Madin 1975; Wharton & Barclay 1993; Watanabe et al. 2002; Wharton 2003, 2011; Hengherr et al. 2010). Molecular mechanisms underpinning desiccation tolerance include the accumulation of polyhydric alcohols and sugars (Benoit, Lopez-Martinez et al. 2007; Hengherr et al. 2008), the utilization of HSP and LEA proteins (Bahrndorff et al. 2009; Lopez-Martinez et al. 2009; Popovic et al. 2011), shifts in metabolism (Danks 2000; Li et al. 2009), membrane remodelling (Lopez-Martinez et al. 2009), oxidative damage repair (Lopez-Martinez et al. 2008) and cytoskeletal reorganization (Li et al. 2009; Lopez-Martinez et al. 2009).

Low water availability is seen as being one of two principal stressors to terrestrial invertebrates in the polar regions, with the other being low temperature (Cannon & Block 1988; Convey 1996; Strathdee & Bale 1998; Block et al. 2009). In winter, water is locked up as ice and is inaccessible to invertebrates (Block et al. 2009), while in summer, evaporation of meltwater can lead to drought (Kennedy 1993). In some areas, such as the McMurdo Dry Valleys, soil water content can be as little as 2% (Treonis & Wall 2005). The Antarctic dipteran *Eretmoptera murphyi* and the High-Arctic dipteran *Heleomyza borealis* also experience aridity in their respective habitats. The larval stages of these two species comprise the majority of the life cycle duration, and thus experience the full spectra of environmental conditions over the annual cycle (Convey & Block 1996; Worland et al. 2000). *Eretmoptera murphyi* is locally highly abundant in the sub-Antarctic island of South Georgia and, since its introduction onto Signy Island (maritime Antarctic) in the 1960s, it has spread to occupy an area >2000 m^2, with densities as high as 410 000 ind. m^{-2} (Hughes & Worland 2010). Worland (2010) has shown that *E. murphyi* larvae possess good desiccation tolerance, but low desiccation resistance. *Heleomyza borealis* is also abundant at certain High-Arctic sites when found in association with bird colonies, but its desiccation tolerance has not been assessed thoroughly.

Because injuries to invertebrates, such as protein impairment and loss of membrane fluidity, result from desiccation as well as from other stresses like low temperature, the physiological adaptations induced in response to these stresses are analogous, or at least complementary (Ring & Danks 1998; Bayley et al. 2001). For example, the desaturation of membranes, up-regulation of heat shock proteins and the accumulation of polyols and sugars occur after both desiccation and low temperature treatments (Bayley et al. 2001; Holmstrup et al. 2002; Bahrndorff et al. 2007; Benoit et al. 2009). It therefore follows that a sub-lethal desiccation exposure can potentially afford protection for an invertebrate subsequently exposed to low temperatures. This phenomenon is termed cross-tolerance and has been observed in a number of organisms, though primarily in Diptera and Collembola (e.g., Holmstrup et al. 2002; Elnitsky et al. 2008; Levis et al. 2012). In the collembolan *Megaphorura arctica* desiccation in the presence of ice lowers the supercooling point (SCP) to such an extent that the collembolan is able to survive the low temperatures of the Arctic winter (Worland et al. 1998). This strategy, termed cryoprotective dehydration, is now seen to be fairly common, having been described in an increasing number of invertebrates (e.g., Pedersen & Holmstrup 2003; Elnitsky et al. 2008; Smith et al. 2008; Sørensen & Holmstrup 2011). Cross-tolerance also works independently of the SCP. In the freeze-tolerant dipteran *B. antarctica* survival was improved by 90% at −10°C following 48 h at 98% relative humidity (RH) and by 60% at −15°C following the loss of 50% of its body water (Benoit et al. 2009). Invertebrates that experience complete desiccation or anhydrobiosis are also conferred improved low temperature tolerance, and the extent to which it is improved is usually greater than in partially desiccated animals like *B. antarctica* (e.g., Ramløv & Westh 1992; Sømme & Meier 1995; Shuker 2001).

Climate change is leading to warmer summers in the polar regions, with evidence of increasing exposure to drought (Convey et al. 2003; Convey et al. 2009; Turner et al. 2009). Exploration of cross-tolerance between desiccation and low and high temperature in two additional dipteran species, therefore, provides further insight into how polar terrestrial invertebrates tolerate extreme conditions currently and may indicate how they will cope with climate warming in future.

Materials and methods

Insect collection and storage conditions

Summer acclimatized individuals of *Heleomyza borealis* were collected from the moss-covered slopes at Krykkjefjellet and Blomstrandhalvøya, near Ny-Ålesund, Spitsbergen, Svalbard (78°55'N, 11°56'E) in August 2011. Summer acclimatized individuals of *Eretmoptera murphyi* were collected from soil and moss on Signy Island (60°S, 45°W) close to the British Antarctic Survey Signy Research Station between January and March 2012. They were transported to the University of Birmingham under refrigerated conditions and then held in plastic boxes containing substratum from the site of collection at 4°C (0:24 L:D). The duration of travel was approximately two days from the Arctic and two months from the Antarctic. Numbers of *H. borealis* were limited and hence it was not possible to assess for cross-tolerance to high and low temperatures, except with respect to their SCPs.

Water balance and desiccation tolerance

Bayley & Holmstrup (1999) highlighted the importance of performing desiccation experiments at more

ecologically relevant RH values, and in particular those close to the wilting point of plants (ca. 98.9% RH). The specific RH of 98.2% used here was produced using 150 ml of NaCl solution (31.60 g NaCl L^{-1}) in a plastic container. The RH was verified as being stable using a Hygrochron temperature and humidity logger iButton (Maxim, San Jose, CA, USA). Controls were maintained at 100% RH using purified water and were given access to water. Dipteran larvae were placed in small glass containers, covered with nylon gauze, which were then placed inside the plastic containers, and sealed with a tight fitting lid. Following Raoult's law, the air inside the closed system quickly equilibrated with the aqueous solution used.

Three replicates of 10 individuals of each species were removed from 98.2% RH at set intervals over a 12-day period (6 h, 2, 4, 8 and 12 days). Dipteran larvae were subsequently transferred into plastic universal tubes containing moist plaster of Paris and given substrate and water. Survival, defined as larvae which either moved spontaneously or in response to gentle contact stimulus, was assessed 72 h after treatment. The larvae were also weighed prior to desiccation, upon removal from each desiccation treatment, and following drying to constant mass at 60°C over 24 h. From these values, initial water content and percentage water loss or gain were calculated (see Hayward et al. 2007).

Desiccation induced low temperature tolerance

Effect of desiccation on the SCP

Individuals of *H. borealis* and *E. murphyi* were held at 98.2% RH for either 6 h, 2, 4, 8 or 12 days (only 12 days for *H. borealis*) prior to experimental treatment. Fifteen larvae were placed in contact with a thermocouple, within Beem capsules, in glass test tubes plugged with sponge, inside an alcohol bath (Haake Phoenix II C50P, Fisher Scientific UK Ltd, Loughborough, UK). Larvae were subsequently cooled from 4 to −30°C at 0.5 min^{-1}. SCPs, defined as the temperature at the onset of the freezing exotherm, were identified using an eight channel datalogger interfaced to a computer and recorded using Picolog Recorder software (Pico Technology Limited, St. Neots, UK; see Hawes et al. 2006).

Lower discriminating temperature

The temperature at which 10–20% survival occurred (Lee et al. 1987) was determined by cooling three replicates of 10 larvae at 0.2°C min^{-1} to progressively lower sub-zero temperatures (−15 to −19°C) for 2 h, before being re-warmed to the rearing temperature (4°C) at the same rate. Larvae were placed in Eppendorf tubes, inside glass test tubes plugged with sponge, in an alcohol bath prior to each experimental treatment. Control groups were handled, and exposed, in the same way at 4°C. The temperature experienced by the larvae was measured by placing a thermocouple within an identical Eppendorf tube into one of the glass test tubes. At the end of each experimental treatment, the larvae were rapidly transferred (over ice) from the Eppendorf tubes into plastic universal tubes containing moist plaster of Paris and substratum, and returned to the rearing conditions. Survival was assessed as described previously. The highest temperature at which survival was between 10 and 20% after 72 h recovery was defined as the discriminating temperature.

Effect of desiccation on low temperature tolerance

Larvae of *E. murphyi* only were held at 98.2% RH for 6 h, 2, 4, 8 or 12 days prior to experimental treatment. Three replicates of 10 larvae were subsequently cooled at 0.2°C min^{-1} to the discriminating temperature and held for 2 h before being re-warmed to the rearing temperature at the same rate. Larvae collection and handling, controls, thermocouple use, recovery and survival assessment were as described previously.

Desiccation-induced heat tolerance

Higher discriminating temperature

The temperature at which 10–20% survival occurred was determined by warming three replicates of 10 individuals at 0.2°C min^{-1} to progressively higher temperatures (30–40°C) for 2 h, before being re-cooled to the rearing temperature at the same rate. Larvae collection and handling, controls, thermocouple use, recovery and survival assessment were as described previously. The lowest temperature at which survival was between 10 and 20% after 72 h recovery was defined as the discriminating temperature. The lowest temperature at which survival was between 80 and 90% was also used to assess whether survival was lowered by a prior desiccation exposure.

Effect of desiccation on high temperature tolerance

Larvae of *E. murphyi* were held at 98.2% RH for 6 h, 2, 4, 8 and 12 days prior to experimental treatment. Three replicates of 10 larvae were subsequently warmed at 0.2°C min^{-1} to the discriminating temperature and

the 80–90% survival temperature. Larvae were held for 2 h and cooled to the rearing temperature at the same rate. Larvae collection and handling, controls, thermocouple use, recovery and survival assessment were as described previously.

Statistical analysis

The Kolmogorov-Smirnov test was used to check for normality in the survival, SCP and percentage water loss data. Normally distributed data were analysed using analysis of variance (ANOVA) and Tukey's multiple range test; data that were not normally distributed were analysed using the Kruskal-Wallis test.

Results

Water balance and desiccation tolerance

Larvae of *Heleomyza borealis* were significantly more desiccation resistant than those of *Eretmoptera murphyi* (Fig. 1). After 12 days, larvae of *H. borealis* had lost only 6.9% of their water content, as compared with 46.7% in larvae of *E. murphyi*. Water loss rate was not constant in larvae of *E. murphyi*; between 2 and 4 days, they regained 13.6% of their initial water content, before losing water rapidly again thereafter. Survival following 6 h, 2 days, 4 days, 8 days and 12 days at 98.2% RH was high in larvae of both *H. borealis* and *E. murphyi*, and was not significantly different from survival in the control (Fig. 2).
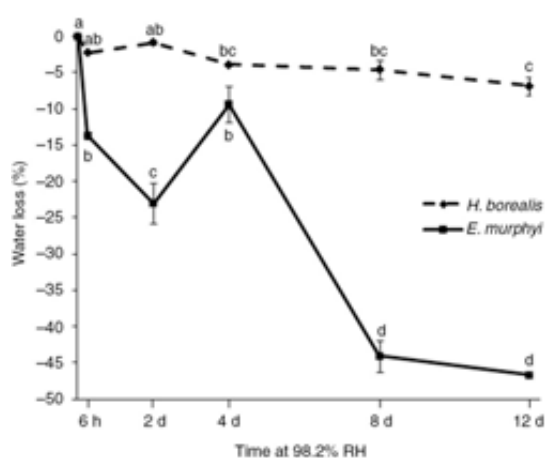
Fig. 1 Percentage water loss or gain of larvae of *H. borealis* and *E. murphyi* following exposure to 98.2% relative humidity (RH) for 6 h, 2 days, 4 days, 8 days and 12 days. Means±standard error of the mean are presented for three replicates of 10 individuals. Means with the same letter are not significantly different within each species group at $p<0.05$ (Tukey's multiple range test).
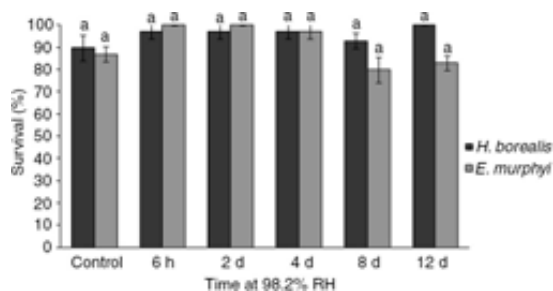
Fig. 2 Survival (%) of larvae of *H. borealis* and *E. murphyi* following exposure to 98.2% relative humidity (RH) for 6 h, 2 days, 4 days, 8 days and 12 days. Means±standard error of the mean are presented for three replicates of 10 individuals. Survival was assessed 72 h after treatment. Means with the same letter are not significantly different from the control within each species group at $p<0.05$ (Tukey's multiple range test).

Desiccation-induced cold tolerance

Effect of desiccation on the SCP

Prior exposure to 98.2% RH significantly lowered the SCP in larvae of both species (Table 1). In larvae of *H. borealis* the SCP fell by 1.6°C after 12 days at 98.2% RH, while in larvae of *E. murphyi* the SCP fell by up to 2.5°C.

Table 1 *H. borealis* and *E. murphyi* larval supercooling points (SCPs) following exposure to 98.2% relative

humidity (RH) for 6 h, 2 days, 4 days, 8 days and 12 days (only 12 days for *H. borealis*). Means±standard error of the mean are presented for 15 replicates. Asterisks indicate a treatment significantly different from the control (0 h) at $p<0.05$ (Tukey's multiple range test).

Species	SCP (°C)					
	0 h	6 h	2 days	4 days	8 days	12 days
H. borealis	−7.70±0.28	–	–	–	–	−9.29±0.38*
E. murphyi	−5.05±0.29	−6.16±0.11	−6.21±0.29	−5.36±0.40	−7.52±0.22*	−6.74±0.46*

Lower discriminating temperature and the effect of desiccation on low temperature tolerance

Survival of *E. murphyi* larvae declined gradually following exposure to progressively lower temperatures (Fig. 3a). The discriminating temperature (20% survival) was determined to be −18°C. At this temperature, survival of *E. murphyi* larvae was raised following all acclimation treatments (6 h, 2 days, 4 days, 8 days and 12 days) at 98.2% RH (Fig. 3b). The increase in survival was significant following 4 days.

Fig. 3 Survival (%) of larvae of *E. murphyi* (a) after exposure to progressively lower sub-zero temperatures (−15 to −19°C) for 2 h, and (b) after exposure to −18°C, following prior exposure to 98.2% relative humidity (RH) for 6 h, 2 days, 4 days, 8 days and 12 days. Means±standard error of the mean are presented for three replicates of 10 individuals. Survival was assessed 72 h after treatment. Means with the same letter are not significantly different at $p<0.05$ (Tukey's multiple range test).

Desiccation-induced heat tolerance

Higher discriminating temperature and the effect of desiccation on high temperature tolerance

Survival of *E. murphyi* larvae remained at 100% up to 35°C, but declined rapidly at temperatures near to the upper lethal temperature (ULT), falling by 80% between 37 and 40°C (Fig. 4a). The discriminating temperatures were determined to be 38.5 and 37°C, giving 20 and 80% survival, respectively. At 37°C, survival of *E. murphyi* larvae was unchanged at around 80% following 2, 4 and 8 days at 98.2% RH, but declined to 55% after 12 days (Fig. 4b). There was also no significant difference between non-acclimated and acclimated larvae of *E. murphyi* at 38.5°C.
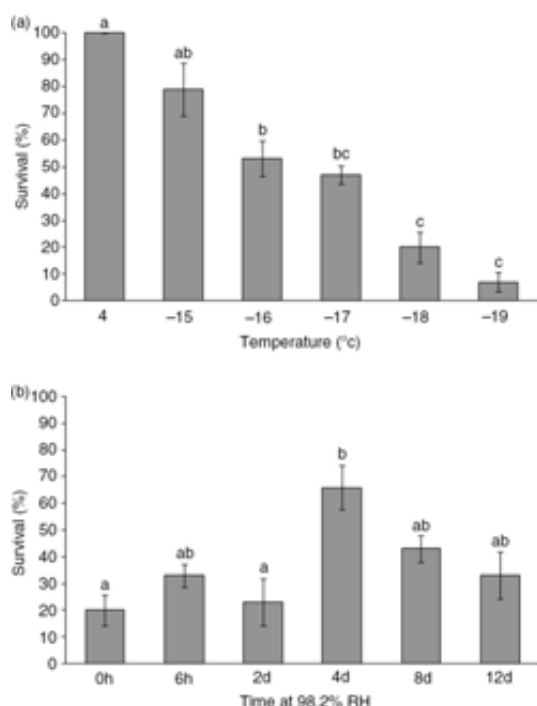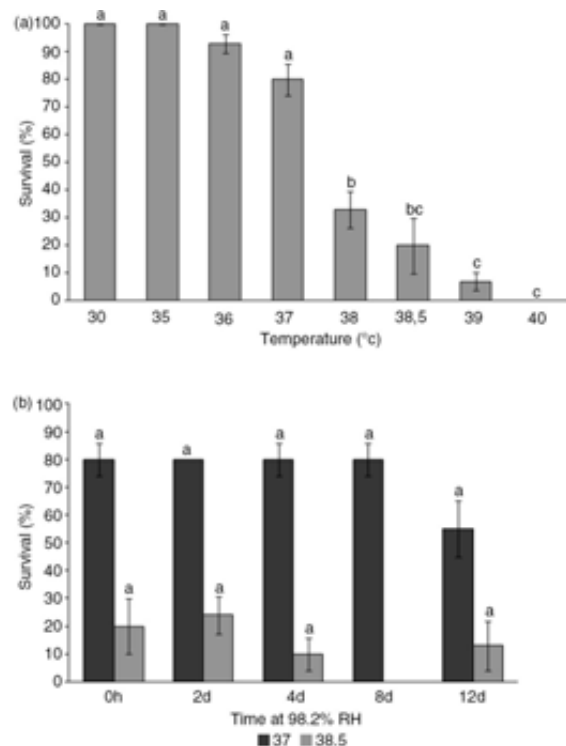
Fig. 4 Survival (%) of larvae of *E. murphyi* (a) after exposure to progressively higher temperatures (30–40°C) for 2 h and (b) after exposure to 37 or 38.5°C, following prior exposure to 98.2% relative humidity (RH) for 6 h, 2 days, 4 days, 8 days and 12 days. Means±standard error of the mean are presented for three replicates of 10 individuals. Survival was assessed 72 h after treatment. Means with the same letter are not significantly different across temperature treatments (a) and between temperature treatments (b) at *p*<0.05 (Tukey's multiple range test).

Discussion

Desiccation resistance

Water availability is limited for much of the year in the Arctic and Antarctic (Strathdee & Bale 1998; Block et al. 2009), and polar terrestrial invertebrates respond with one of two strategies, desiccation resistance or desiccation tolerance. Our data suggest that the Arctic dipteran *Heleomyza borealis* responds through desiccation resistance, while the Antarctic species *Eretmoptera murphyi* tolerates substantial desiccation (Figs. 1, 2). The rate of water loss in *E. murphyi* was seven times more rapid than in *H. borealis* and was similar to that reported by Worland (2010) at 88% RH. The closely related dipteran *Belgica antarctica* which also inhabits the maritime Antarctic, likewise shows a high rate of water loss. When exposed to 98% RH, larvae of this dipteran took around 5 days to lose 50%, and 8–10 days to lose over 60%, of their body water (Benoit, Lopez-Martinez et al. 2007).

There is therefore a clear difference in the level of desiccation resistance between *H. borealis* and the Antarctic Diptera *E. murphyi* and *B. antarctica*. The physiology of the cuticular layer between these species provides a possible, albeit unexplored, explanation for the difference, and lowered cuticular permeability is a widespread adaptation that invertebrates use to raise their desiccation resistance (Danks 2000). Invertebrates achieve this by increasing the number and/or length of hydrocarbons or hydrophobic molecules in the wax layer surrounding their cuticle, resulting in tighter packing and a greater reduction of water loss rate (Gibbs et al. 1997; Benoit, Yoder et al. 2007; Speight et al. 2008). Benoit, Lopez-Martinez et al. (2007) showed that the length of hydrocarbons increased in desiccated larvae of *B. antarctica*, but this change was only slight and there was no change in the number of hydrocarbons. *Eretmoptera murphyi* is closely related to *B. antarctica* (Allegrucci et al. 2006; Allegrucci et al. 2012) and may therefore possess similar physiological adaptations. We speculate that the initial composition and change in the cuticle layer is more biased towards a greater number and length of hydrocarbons in *H. borealis* than either Antarctic dipteran. Differences in melanization between the two Diptera may also offer an explanation for the differing levels of resistance in the current study, as has been shown between *Drosophila* (Parkash, Rajpurohit et al. 2008; Parkash, Ramniwas et al. 2008; Parkash et al. 2009; Parkash et al. 2012; Ramniwas et al. 2013).

It should also be noted that 98.2% RH may be a sufficiently high humidity that larvae of *H. borealis* are able to absorb water from the atmosphere. This may also underlie our observation that, between 2 and 4 days' exposure at 98.2% RH, larvae of *E. murphyi* exhibited reduced water loss and even rehydration. Rehydration has also been observed in other species. In the collembolan *Folsomia candida* nearly all of the water lost initially at 98.2% RH was recovered within 5–7 days, despite being continually held at 98.2% RH (Bayley &

Holmstrup <u>1999</u>). As confirmed by microarray, this recovery was supplemented by accumulating and synthesising myo-inositol, glucose and trehalose, which allowed the collembolan to become hyperosmotic to the environment and absorb moisture (Timmermans et al. <u>2009</u>). An analogous response may be present in *E. murphyi*. However, such a response has not been observed in the closely related *B. antarctica*, which was unable to absorb water from the atmosphere at any RH, except complete saturation, i.e., 100% RH (Benoit, Lopez-Martinez et al. <u>2007</u>; Hayward et al. <u>2007</u>).

Desiccation tolerance

Heleomyza borealis showed >90% survival following 12 days of desiccation (<u>Fig. 2</u>). However, because water loss was so slight, even after 12 days at 98.2% RH, larvae cannot be said to have tolerated desiccation. Instead, it suggests that larvae of *H. borealis* are able to survive well under ecologically relevant relative humidities using a desiccation resistance strategy. Conversely, larvae of *E. murphyi* tolerated desiccation, having shown considerable water loss, but also survival, following 12 days at 98.2% RH (<u>Fig. 2</u>). *Belgica antarctica* also principally uses a desiccation tolerance strategy and has been shown to survive well following a 75% loss of initial water content (Benoit, Lopez-Martinez et al. <u>2007</u>; Hayward et al. <u>2007</u>).

One means of tolerating desiccation is through possessing high initial water content, as an organism must subsequently lose more water before reaching a point at which damage occurs or energy intensive mechanisms are induced (Hayward et al. <u>2007</u>). This argument is reinforced by the increased water content observed in selected desiccation tolerant lines of *Drosophila melanogaster* (Gibbs 1997). In *E. murphyi* larvae, the initial water content was high, averaging 74.3% (73.28–75.40%) of body mass (Benoit et al. <u>2009</u>). We did not assess osmotically active water in this study, though *B. antarctica* is known to have very high osmotically active water content relative to temperate species (Hayward et al. <u>2007</u>). Once considerable water loss does occur, as was the case in the current study, the potential for injury is great and an organism must adapt accordingly. Injuries that result from desiccation include protein denaturation and unwanted macromolecular interactions (Benoit et al. <u>2009</u>), crystalline to gel phase transitions (Hazel <u>1995</u>), oxidative damage (Lopez-Martinez et al. <u>2008</u>) and mechanical stress (Li et al. <u>2009</u>). The responses of *B. antarctica* in this regard have been particularly well studied. Larvae accumulate glycerol and trehalose, which are suggested as being replacements for lost water and/or an aid to the production of amorphous sugar glasses (Danks <u>2000</u>; Benoit, Lopez-Martinez et al. <u>2007</u>; Michaud et al. <u>2008</u>; Bahrndorff et al. <u>2009</u>; Benoit et al. <u>2009</u>; Hengherr et al. <u>2009</u>; Clarke et al. <u>2013</u>). Protein denaturation is also ameliorated via the up-regulation of HSPs in response to desiccation (Lopez-Martinez et al. <u>2009</u>; Teets et al. <u>2012</u>), and the fluidity of the membrane maintained using enzymes such as Δ9 FAD desaturase (Lopez-Martinez et al. <u>2009</u>). Further physiological mechanisms induced in response to desiccation include oxidative damage repair through the accumulation of antioxidants (Lopez-Martinez et al. <u>2008</u>), the minimization of mechanical stress via the restructuring of the cytoskeleton (Li et al. <u>2009</u>), the inhibition of apoptosis through the regulation of autophagy and the suppression of metabolism (Teets et al. <u>2012</u>). Larvae of *B. antarctica* therefore possess a suite of physiological responses against injuries resulting from desiccation. It is possible that *E. murphyi* possesses similar physiological adaptations that underlie its high level of desiccation tolerance. Indeed, the capacity to which they respond to temperature is very similar (Lee et al. <u>2006</u>; Everatt et al. <u>2012</u>).

Desiccation-induced cross-tolerance

Low temperatures

Survival of *E. murphyi* at −18°C was significantly raised following desiccation at 98.2% RH (<u>Fig. 3</u>b). Greater survivorship at low temperatures, following pre-exposure to unsaturated conditions, has also been observed for a number of other invertebrates, including *B. antarctica* (Benoit et al. <u>2009</u>) and the springtails *Cryptopygus antarcticus* (Elnitsky et al. <u>2008</u>; Everatt, Worland et al. <u>2013</u>) and *Folsomia candida* (Holmstrup et al. <u>2002</u>). Cross-tolerance is thought to occur between desiccation and low temperature because injuries that result from the two stresses are similar. Consequently, the physiological mechanisms induced in response to desiccation and low temperatures are often analogous (e.g., Bayley et al. <u>2001</u>), and act in concert to give greater protection. Even a mild desiccation treatment resulting in 6–10% water loss has been shown to confer significant gains in cold tolerance in the goldenrod gall fly, *Eurosta solidaginis* (Levis et al. <u>2012</u>). Interestingly, survival of −18°C was highest after 4 days at 98.2% RH and not after longer durations of 8 and 12 days (<u>Fig. 3</u>b). This corresponds with the time period at which larvae of *E. murphyi* exhibited rehydration (<u>Fig. 1</u>), suggesting physiological processes associated with rehydration provide cold tolerance, and that these are additive to the protection provided by those solely concerned with desiccation tolerance.

An effect on the SCP was also observed in the current study for both *H. borealis* and *E. murphyi*. Following a pre-exposure to 98.2% RH, the SCP was significantly reduced (<u>Table 1</u>). Both dipteran species are freeze-tolerant and it is therefore preferable for extracellular ice formation to take place at higher sub-zero temperatures, as it occurs more slowly and decreases the chance of tissue damage (Worland & Block <u>1999</u>). The lowering of the SCP in *H. borealis* and *E. murphyi* was therefore more likely a by-product of, rather than an adaptation to, desiccation. Water loss passively increases the concentration of solutes already present and

results in the colligative lowering of the SCP (e.g., Holmstrup & Zachariassen 1996). The dipteran larvae were also starved during the desiccation treatments and, during periods of starvation, ice-nucleating gut contents may be removed, reducing the likelihood of ice formation (Sømme & Block 1982; Cannon & Block 1988). Worland et al. (2006) also reported that time spent without access to food on water surfaces lowered the SCP of the collembolan *Ceratophysella denticulata*.

High temperatures

Prior exposure to desiccation at 98.2% RH had either no effect or a negative effect on the heat tolerance of *E. murphyi* larvae (Fig. 4b). Unlike low temperatures, injuries incurred as a result of high temperatures are dissimilar to those of desiccation, and physiological defences mounted in response to desiccation are therefore also different, and could even be conflicting. Consequently, little protection is afforded by prior acclimation to desiccation. A similar response has been observed in nematodes (Holmstrup & Zachariassen 1996) and Collembola (Everatt, Worland et al. 2013). However, improved heat tolerance has been noted in other invertebrates, particularly those which are anhydrobiotic (e.g., Hinton 1951, 1960; Sakurai et al. 2008). It is speculated that anhydrobiotic organisms, because of their tendency to vitrify, are less susceptible to injuries in general and that conflicting injuries are therefore less important. While this explanation is appropriate for anhydrobiotic organisms, the same is not true of partially desiccated organisms, which tend not to vitrify. At 30°C, heat tolerance was improved in partially desiccated larvae of *B. antarctica* following pre-exposure to 0, 75 and 98% RH (Benoit et al. 2009). In this instance, the up-regulation of heat shock proteins and the accumulation of trehalose were suggested as being possible explanations for the enhanced heat tolerance by overcompensating for any opposing injuries.

The heat tolerance of *E. murphyi* has been little explored, except in a study by Everatt et al. (2014), which showed larval survival up to 39°C for 1 h. In the current study, larvae of *E. murphyi* showed 100% survival up to 35°C and also survived temperatures as high as 39°C for the longer period of 2 h (Fig. 4). Larvae of *B. antarctica* are likewise able to survive temperatures above 30°C (Benoit et al. 2009). Although these organisms rarely, if ever, experience temperatures nearing 30 or even 25°C, their heat tolerance is not surprising. A number of other studies, including those by Deere et al. (2006), Everatt, Convey et al. (2013), Sinclair et al. (2006) and Slabber et al. (2007) have similarly shown appreciable heat tolerance in polar invertebrates. Such findings are consistent with the "thermal sensitivity hypothesis," which states that the thermal sensitivity of invertebrates to a temperature rise declines with increasing latitude (Addo-Bediako et al. 2000; Deutsch et al. 2008).

Conclusion

The Arctic and Antarctic are similar in that they include both cold and arid landscapes. However, the Diptera of these regions, based on the evidence presented here, have not adapted similarly. Two strategies of living in a dry environment have been identified. The Arctic dipteran *Heleomyza borealis* utilizes desiccation resistance, while the Antarctic dipteran *Eretmoptera murphyi* principally uses desiccation tolerance. Divergence between Antarctic and Arctic invertebrates has also been shown between *Belgica antarctica* and *Megaphorura arctica*, which utilize distinct molecular mechanisms in response to desiccation (Teets et al. 2012). Desiccation was found to induce cross-tolerance to low temperatures, but not high temperatures, in *E. murphyi*. An ability to acclimate in this way would likely be beneficial in the variable climates typical of polar terrestrial habitats, where low temperature and low water availability are commonly encountered simultaneously.

Acknowledgements

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