

Responses of invertebrates to temperature and water stress

Everatt, Matthew J.; Convey, Pete; Bale, Jeffrey S.; Worland, M. Roger; Hayward, Scott A.I.

DOI:

[10.1016/j.jtherbio.2014.05.004](https://doi.org/10.1016/j.jtherbio.2014.05.004)

License:

Other (please specify with Rights Statement)

Document Version

Peer reviewed version

Citation for published version (Harvard):

Everatt, MJ, Convey, P, Bale, JS, Worland, MR & Hayward, SAL 2014, 'Responses of invertebrates to temperature and water stress: A polar perspective', *Journal of Thermal Biology*.
<https://doi.org/10.1016/j.jtherbio.2014.05.004>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:

NOTICE: this is the author's version of a work that was accepted for publication in Journal of Thermal Biology. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in Journal of Thermal Biology, DOI: 10.1016/j.jtherbio.2014.05.004.

Eligibility for repository checked March 2015.

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Author's Accepted Manuscript

Responses of invertebrates to temperature and water stress: A polar perspective

M.J. Everatt, P. Convey, J.S. Bale, M.R. Worland, S.A.L. Hayward



www.elsevier.com/locate/jtherbio

PII: S0306-4565(14)00071-0
DOI: <http://dx.doi.org/10.1016/j.jtherbio.2014.05.004>
Reference: TB1522

To appear in: *Journal of Thermal Biology*

Received date: 21 August 2013
Revised date: 22 January 2014
Accepted date: 22 January 2014

Cite this article as: M.J. Everatt, P. Convey, J.S. Bale, M.R. Worland, S.A.L. Hayward, Responses of invertebrates to temperature and water stress: A polar perspective, *Journal of Thermal Biology*, <http://dx.doi.org/10.1016/j.jtherbio.2014.05.004>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting galley proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Responses of invertebrates to temperature and water stress: A polar perspective

M. J. Everatt^a, P. Convey^{b, c, d}, J. S. Bale^a, M. R. Worland^b and S. A. L. Hayward^{a*}

^a*School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*

^b*British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge, CB3 0ET, UK*

^c*National Antarctic Research Center, IPS Building, University Malaya, 50603 Kuala Lumpur, Malaysia*

^d*Gateway Antarctica, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand*

*Corresponding author. Tel.: + 44 121 41 47147. Email address: s.a.hayward@bham.ac.uk (S. A. L. Hayward).

Abstract

As small bodied poikilothermic ectotherms, invertebrates, more so than any other animal group, are susceptible to extremes of temperature and low water availability. In few places is this more apparent than in the Arctic and Antarctic, where low temperatures predominate and water is unusable during winter and unavailable for parts of summer. Polar terrestrial invertebrates express a suite of physiological, biochemical and genomic features in response to these stressors. However, the situation is not as simple as responding to each stressor in isolation, as they are often faced in combination. We consider how polar terrestrial invertebrates manage this scenario in light of their physiology and ecology. Climate change is also leading to warmer summers in parts of the polar regions, concomitantly increasing the potential for drought. The interaction between high temperature and low water availability, and the invertebrates' response to them, are therefore also explored.

Keywords: Cross tolerance; rapid cold hardening; anhydrobiosis; cryoprotective dehydration; sub-lethal characteristics; climate warming

1. Introduction

1.1. The trials of being an invertebrate

Invertebrates, more so than any other animal group, are at the whim of their environment. Unlike birds and mammals, which are able to regulate their internal body temperature, invertebrates are poikilothermic ectotherms and their body temperature is highly influenced by, and varies markedly with, the environmental temperature (Speight *et al.* 2008). While cold-blooded vertebrates, such as fish, reptiles and amphibians, are also poikilothermic ectotherms, they are not generally as diminutive

39 as invertebrates. Even the smallest vertebrate recorded, the Papua New Guinea frog *Paedophryne*
40 *amanuensis* (7.7 mm in length), dwarfs the vast majority of invertebrates (Rittmeyer *et al.* 2012).
41 Cold-blooded vertebrates accordingly have a smaller surface area to volume ratio than invertebrates
42 and therefore have more time to respond to changes in temperature. This means that invertebrates are
43 more susceptible to injuries following either rapid cooling (Czajka 1990) or warming (Chidawanyika
44 and Terblanche 2011). A small body size also means invertebrates are generally more vulnerable to
45 desiccation than their larger-bodied vertebrate relatives.

46 1.2. Polar climate

47 In few places are invertebrates more directly impacted by their environment than in the Arctic tundra
48 (Strathdee and Bale 1998) or the fellfields of the Antarctic (Block *et al.* 2009; Hogg *et al.* 2006). Air
49 temperatures regularly fall below -10°C during the winter in the maritime Antarctic and, in regions
50 such as the continental Antarctic and High Arctic, frequently drop below -40°C (Block *et al.* 2009;
51 Convey 2013; Sformo *et al.* 2010; Strathdee and Bale 1998). Invertebrates buffer these temperatures
52 behaviourally to some extent (Hayward *et al.*, 2003) by moving beneath the snow, within the soil
53 profile, or into cryptogams like mosses, lichen and algae (Bengtson *et al.* 1974; Burn 1986; Convey
54 1996; Convey and Smith 1997; Spaul 1973). However, even within these microhabitats, they can still
55 be subjected to sub-zero temperatures on a daily basis throughout the winter (Davey *et al.* 1990;
56 Block *et al.* 2009; Strathdee and Bale 1998). Microhabitat temperatures during the summer are also
57 very low and rarely rise above 5°C in the maritime and continental Antarctic, and slightly higher in
58 the High Arctic (Block *et al.* 2009; Coulson *et al.* 1993; Strathdee and Bale 1998). The availability of
59 liquid water also presents an important challenge. During the winter, water is locked up as snow and
60 ice where it is inaccessible (Block *et al.* 2009) while, in summer, streams, lakes and rock pools, which
61 form from melted ice and snow in spring, evaporate, resulting in drought (Convey *et al.* 2003). Again,
62 behavioural responses can help reduce desiccation stress (Hayward *et al.*, 2000, 2001). However,
63 because access to moisture is so restricted in both space and time at polar latitudes, physiological
64 responses play a dominant role in determining species survival.

65 1.3. Overview

66 In response to low temperatures and water stress, polar terrestrial invertebrates express a suite of
67 responses and strategies. However, these two stressors are often faced concurrently and the level of
68 crossover between the strategies employed in response is considerable. A further interaction that may
69 be faced currently, and will likely occur more frequently in the future, is that between high
70 temperature and low water availability. Climate change is resulting in higher temperatures in summer
71 and throughout the year in some polar regions (Arctic Council, 2005; Convey *et al.* 2009; Turner *et al.*
72 *et al.* 2009), increasing the potential for summer drought. The manner in which the resident invertebrate
73 fauna, and potential colonisers, are able to tolerate and respond to this combination of stressors is
74 therefore also pertinent.

75 It is important to note that the adaptations shown by polar terrestrial invertebrates are not necessarily
76 uniquely different from non-polar species, simply that their adaptations are, in some cases, more
77 developed because of the more extreme conditions they experience (Convey 1996). Studies on non-
78 polar invertebrates are therefore also highly informative, and throughout this review these will be used
79 to complement and expand on the concepts introduced for their polar counterparts. Further, there are
80 certain stress tolerance strategies that are potentially relevant to polar systems that have only been
81 described in non-polar invertebrates to date.

82 2. Responses to low temperature

83 Invertebrates that live in the polar regions can be at constant risk of their body fluids freezing and any
84 associated injury (Mazur 1977). This risk is generally ameliorated by adoption one of two strategies -
85 freeze-tolerance (= tolerance of internal ice formation) or freeze-avoidance (= avoidance of internal
86 ice formation) (Bale 2002; Cannon and Block 1988; Convey 1996; Storey and Storey 1988;
87 Zachariassen 1985).

88 2.1. Freeze-tolerance

89 Various polar invertebrates have been shown to use this strategy, including Diptera (e.g. *Belgica*
90 *antarctica* [Benoit *et al.* 2009a], *Eretmoptera murphyi* [Worland 2010] and *Heleomyza borealis*
91 [Worland *et al.* 2000]), Lepidoptera (e.g. *Gynaephora groenlandica* [Strathdee and Bale 1998]),
92 Coleoptera (e.g. *Hydromedion sparsutum* and *Perimylops antarcticus* [Worland and Block 1999]) and
93 nematoda (e.g. *Eudorylaimus coniceps* [Convey and Worland 2000]). While the continental Antarctic
94 nematode, *Panagrolaimus davidi* (Wharton and Ferns 1995), has been shown to survive intracellular
95 ice formation, perhaps indicative of a more general ability within polar nematodes, this form of injury
96 is thought to be lethal to most other invertebrates (Block 1990). The vast majority of freeze-tolerant
97 invertebrates therefore restrict ice formation to extracellular compartments. Key to this process is the
98 accumulation of ice nucleating agents (INAs), such as specialised proteins (Block *et al.* 1990), food
99 particles, crystalloid compounds (Lee *et al.* 1996) and microorganisms (Klok and Chown 1997;
100 Worland and Block 1999), which act as heterogeneous surfaces for the promotion of water molecule
101 aggregation (Bale 2002). By accumulating these agents in the haemolymph and gut, as well as in
102 other tissues (Izumi *et al.* 2009), ice formation (which occurs at the supercooling point or SCP) is
103 encouraged to take place extracellularly at high sub-zero temperatures (-3 to -10°C) (Duman and
104 Horwath 1983; Worland *et al.* 1992, 1993; Worland and Block 1999). At these temperatures, ice
105 crystal growth is relatively slow, allowing water to move from the cytoplasm of cells and join the
106 newly formed ice crystals. The cytoplasm therefore becomes more concentrated and the cell less
107 susceptible to lysis via intracellular freezing (Worland and Block 1999). It should be noted that some
108 invertebrates require an external trigger to survive internal ice formation. In the case of the wood
109 centipede, *Lithobius forficatus*, inoculative freezing occurs at approximately -1°C and is essential for
110 subsequent survival in the freeze-tolerant state (Tursman *et al.* 1994). Other invertebrates that require
111 or may require inoculative freezing include nematodes and the midge, *B. antarctica* (Convey and
112 Worland 2000; Elnitsky *et al.* 2008; Wharton 2003a, 2003b, 2011b).

113 However, freeze-tolerant invertebrates are still at risk from any one ice crystal in the extracellular
114 space becoming too large and puncturing cells from the outside. They therefore also produce
115 antifreeze proteins (AFPs) and/or antifreeze glycolipids (AFGLs). AFPs and AFGLs arrest the
116 expansion of large crystals and instead promote the growth of many small crystals in a process called
117 ice recrystallisation inhibition (Duman *et al.* 2004). AFGLs may also stabilise membranes and prevent
118 the propagation of ice into the cytosol, and slow the growth of extracellular ice, reducing the rate of
119 water flux and solutes across the cellular membrane (Walters *et al.* 2011). Even with the help of AFPs
120 and AFGLs, ice formation is still able to distort proteins, membranes and other structures. Freeze-
121 tolerant invertebrates thus accumulate polyhydric alcohols and sugars, such as glycerol, sorbitol and
122 trehalose. Intracellularly, these cryoprotectants stabilise proteins and membranes, and prevent
123 freezing, while extracellularly their function is to limit the osmotic imbalance that occurs during
124 freezing, by maintaining water content above the “critical minimum cell volume” (Calderon *et al.*
125 2009; Holmstrup *et al.* 1999; Montiel 1998). Polyols and sugars also provide other benefits and aid
126 metabolism.

127 2.2. Freeze-avoidance

128 In contrast to freeze-tolerant species, invertebrates which are freeze-avoiding are unable to withstand
129 any internal ice formation (Bale 1996; Cannon and Block 1988; Storey and Storey 1988; Zachariassen
130 1985). While seemingly disadvantageous in an environment which experiences temperatures close to
131 an invertebrate's SCP, these invertebrates avoid the dangers of both extracellular ice formation and
132 subsequent cellular dehydration that occur in freeze-tolerant species. Freeze-avoiding invertebrates
133 range from Alaska (e.g. the red flat bark beetle, *Cucujus clavipes puniceus* [Sformo *et al.* 2010]) and
134 the High Arctic (e.g. the mite, *Diapterobates notatus* [Coulson *et al.* 1995]) to the Antarctic continent
135 (e.g. *Cryptopygus antarcticus* [Block and Worland 2001; Cannon and Block 1988]), and outnumber
136 freeze-tolerant species in almost all cases. Freeze avoiding invertebrates can be separated into several
137 different categories to better define them ecologically and physiologically. These include, for
138 instance, true freeze-avoiding (lower lethal temperature [LLT] = SCP), chill tolerant (show minimal
139 pre-freeze mortality), chill susceptible (die well above their SCPs) and opportunistic survival (unable
140 to survive below their developmental threshold) (see Bale 1993). The SCP can also vary greatly
141 between and within species, and such classifications can thus become misleading. For example, the
142 summer-acclimatised polar collembolan, *Megaphorura arctica*, is classified as true freeze-avoiding or
143 chill tolerant, while the temperate aphid *Myzus persicae* is classified as chill susceptible. The reader
144 may therefore infer that summer acclimatised *M. arctica* is more cold tolerant. However, *M. arctica* in
145 summer has a high SCP of only -6°C (Worland 1996), which is higher than the LLT of *M. persicae*
146 (Clough *et al.* 1990).

147 Mechanistically, freeze-avoidance revolves around a process termed supercooling - the prevention of
148 internal ice formation below the environmental freezing point. Enhanced supercooling is principally
149 achieved via three processes, and thus involves fewer core stress response mechanisms than freeze
150 tolerance (Bale 2002). The first is the removal of INAs and has been shown to lower the SCP by up to
151 20°C in some invertebrates (Zachariassen *et al.* 1980; Burns *et al.* 2010). INAs are often removed by
152 moulting or ecdysis, the removal of the outer layer of the body and gut contents, which is a necessary
153 stage in the somatic development of arthropods (Hawes *et al.* 2007). Recent studies have shown that
154 moulting is highly dependent on temperature. For example, both the proportion of *C. antarcticus*
155 moulting at any one time (Worland and Convey, 2008) and the expression of moult-associated genes
156 (Burns *et al.* 2010) increase as temperatures fall, suggesting that the timing of moulting is an
157 important adaptive developmental trait. Starvation (Cannon and Block 1988; Sømme and Block 1982)
158 and food selection (Bokhorst *et al.* 2007; Worland and Lukešová 2000) may also be adaptive
159 processes which aid INA removal. While these processes help to rid INAs from the gut, or on the
160 cuticle, those in the rest of the body remain largely unaffected. To arrest ice nucleation here, as well
161 as any remaining INAs in the gut, freeze-avoiding invertebrates initiate a second element of the
162 supercooling process - the accumulation of AFPs. Through a non-colligative mechanism (thermal
163 hysteresis) of adsorption onto, and consequently inhibition of, embryonic ice crystals or INAs (Clark
164 and Worland 2008; Davies and Sykes 1997), AFPs reduce an organism's SCP relative to its melting
165 point (MP) (Bale 2002). Thermal hysteresis has been recorded in a number of polar terrestrial
166 invertebrates, including Antarctic and Arctic mites (*A. antarcticus* [Block *et al.* 2009] and *Phauloppia*
167 sp. [Sjursen and Sømme 2000]), Antarctic Collembola (e.g. *C. antarcticus* [Block *et al.* 2009] and
168 *Gressittacantha terranova* [Hawes *et al.* 2011]), Alaskan beetles (e.g. *Cucujus clavipes* [Sformo *et al.*
169 2010]) and Alaskan lacewings (e.g. *Hemerobius simulans* [Duman *et al.* 2004]). AFPs provide further
170 protection by stabilising the supercooled state and preventing inoculative freezing (Bale 1993), and
171 preserving membranes during phase transitions (Duman *et al.* 2004). In a similar manner to freeze-
172 tolerant species, freeze-avoiding invertebrates also utilise polyols, sugars and amino acids for

173 cryoprotection and the relative enhancement of metabolism at lower temperatures (Block *et al.* 2009;
 174 Clark and Worland 2008; Muise and Storey 2001). Polyols and sugars also help to lower the SCP in a
 175 non-colligative manner like AFPs (Lee *et al.* 1996).

176 2.3. Commonalities between freeze-tolerance and freeze-avoidance

177 2.3.1. Responses to chilling injury

178 Freeze-tolerance and freeze-avoidance are mechanistically distinct from each other. However, there is
 179 also commonality between the two strategies, as organisms deploying them are both susceptible to,
 180 and therefore must also guard against, chilling injury. Chilling is defined as cooling sufficient to
 181 induce damaging effects or even death in the absence of freezing (Hayward *et al.* 2014). Extreme
 182 chilling injury can result from rapid cooling (cold shock or acute stress), as well as long-term
 183 exposure to low temperatures (chronic stress) and/or experience of temperature extremes (Czajka
 184 1990). In truth, chilling and cold stress are relative terms, and the temperatures at which they occur
 185 will depend on multiple factors, ranging from the species' evolutionary history and geographic origin,
 186 to an individual's physiological status and recent thermal history. Chilling-induced damage includes
 187 the loss of integrity, fluidity, and thus function, of the membrane (Izumi *et al.* 2009), the deterioration
 188 of intracellular organelles (Strange and Dark 1962), the disruption of enzymes and electrochemical
 189 ion potentials (Denlinger and Lee 2010), and the destruction of whole cells through apoptosis (Yi *et*
 190 *al.* 2007).

191 The plasma membranes which surround cells of all life forms allow for the selective transfer of
 192 solutes across the cell, intra- and inter-cellular communication, the application of energy harnessed
 193 through transmembrane ion gradients, and function as a barrier to pathogens and toxins (Hazel 1995).
 194 It is therefore necessary for plants, microbes and animals, including invertebrates, to maintain
 195 membrane fluidity and function as temperature falls. Homeoviscous adaptation is a process which
 196 permits the maintenance of membrane fluidity, in particular through alterations in the composition of
 197 membrane phospholipid fatty acid chains (Hazel 1995). Desaturase enzymes are known to play a
 198 fundamental role in membrane responses to low temperature, by raising the number of unsaturated
 199 fatty acids (UFAs) relative to saturated fatty acids (SFAs) (Murray *et al.* 2007; Hayward *et al.* 2007).
 200 These UFAs introduce more double bonds (or kinks) into the phospholipid matrix and so reduce
 201 phospholipid aggregation. The fluidity of the membrane and the transition phase (T_m = fluid to gel)
 202 are also influenced by the position of double bonds and the length of fatty acid chains (Baenzinger *et*
 203 *al.* 1992), and some invertebrates, including *M. arctica*, respond by augmenting these attributes
 204 (Bahrndorff *et al.* 2007).

205 HSPs also play an important role in response to chilling injury, as they are involved in refolding and
 206 stabilising denatured proteins, recovering microfilament dynamics and regulating apoptosis at low
 207 temperatures (Benoit and Lopez-Martinez 2012; Clark and Worland 2008; Tammariello *et al.* 1999;
 208 Yi *et al.* 2007). HSPs have been shown to be constitutively expressed in larva of the Antarctic midge,
 209 *B. antarctica*, which experience chronic cold, while adults, found in more variable environments, only
 210 expressed HSPs as a direct response to stress (Rinehart *et al.* 2006). The latter scenario represents the
 211 'classic' HSP response, as seen in non-polar species like the potato beetle, *Leptinotarsa decemlineata*
 212 (Yocum 2001) and the onion fly, *Delia antiqua* (Chen *et al.* 2006), amongst others. Direct evidence of
 213 their contribution to stress tolerance has also been shown in the firebug, *Pyrrhocoris apterus* (Kostal
 214 and Tollarova-Borowanska 2009), and the flesh fly, *S. crassipalpis* (Rinehart *et al.* 2007). In both
 215 species, injection with *hsp70* double-stranded RNA (RNAi) was used to suppress *hsp70* mRNA and

216 protein levels, which resulted in reduced survival (Rinehart *et al.* 2007), or restricted ability to repair
217 chilling injury and mate successfully (Kostal and Tollarova-Borowanska 2009).

218 Further mechanisms suggested to play roles in chilling injury include mitochondrial degradation,
219 which reduces metabolism and energy use (Levin *et al.* 2003), the accumulation of polyols and
220 sugars, which act as cryoprotectants (Montiel *et al.* 1998), and the build-up of amino acids, which
221 may serve as a reserve for HSPs or stabilise supercooling (Kostal *et al.* 2011). A modification of the
222 schematic representation of freeze-avoidance and freeze-tolerance strategies of Bale (2002), which
223 now includes the molecular and physiological responses to chilling (common to both strategies), is
224 presented in Fig. 1.

225 2.3.2. Vitrification

226 Under crystalline conditions, cells eventually degrade and lose their viability when exposed to chronic
227 chilling or cold shock (Katkov 2006). Freezing can also irreversibly damage cells. Vitrification (or the
228 attainment of a glass-like state) in contrast does not lead to such injuries. Vitrified fluids behave more
229 like a solid and yet also show little change in their molecular state. Under these conditions, an
230 organism's fluids are stable and immune from molecular degradation. It is speculated that vitrification
231 could further aid freeze-avoidance and freeze-tolerance strategies of polar terrestrial invertebrates.
232 Clarke *et al.* (2013) concluded that vitrification occurs in a number of unicellular organisms,
233 including bacteria, and photosynthetic and heterotrophic eukaryotes, when cooled slowly in the
234 presence of extracellular ice. Cellular dehydration resulting from the movement of water out of the
235 cell to join newly formed ice crystals and the subsequent increase in fluid viscosity inside the cell,
236 under slow rates of cooling, allows these unicellular organisms to vitrify prior to intracellular
237 freezing.

238 Clarke *et al.* (2013) hypothesised that vitrification would also occur in the cells of freeze-tolerant
239 multicellular organisms, where ice formation in the extracellular space, rather than in the
240 environment, would encourage intracellular dehydration and the consequential formation of a glass
241 state. As an example, Clarke *et al.* (2013) postulated that the Alaskan tenebrionid beetle, *Upis*
242 *ceramboides*, would vitrify. *Upis ceramboides* freezes at high sub-zero temperatures and is able to
243 survive in this frozen state to -60°C (Miller 1978). It is likely that the dehydration induced by
244 extracellular ice formation would eventually also lead to glass formation, and would only be limited
245 by the rate at which the beetle is cooled (Miller 1978).

246 In the presence of ice in the environment, several polar freeze-avoiding organisms are known to
247 desiccate in a process called cryoprotective dehydration, e.g. the beetle *Cucujus clavipes* (Bennet *et*
248 *al.* 2005) and *M. arctica* (Worland *et al.* 1998). These organisms also accumulate a number of
249 polyhydric alcohols and sugars, such as trehalose, which increase the viscosity of cellular fluid. It has
250 thus previously been speculated that they may also be capable of vitrification. A recent study (Sformo
251 *et al.* 2010) indicates that this is the case, having shown the Arctic red flat bark beetle, *C. clavipes*
252 *puniceus*, to vitrify at a very low temperature (-58°C). As a consequence, 43% of the beetles were able
253 to survive between -70 and -73°C , and a further 7% were able to survive -100°C (Sformo *et al.* 2010).
254 Although the ecological relevance of vitrification in this beetle is questionable, with temperatures in
255 Alaska rarely falling to -58°C , confirmation of the presence of this trait is significant.

256 2.3.3. Acclimation and cooling rates

257 The variation in temperature between summer and winter in the Antarctic and Arctic is great, and
258 temperatures annually can vary by tens of degrees in buffered microhabitats, and by as much as 100°C

259 on exposed soil and rock surfaces (Convey 1996). There are some invertebrates, such as the nunatak
260 inhabiting springtail, *Cryptopygus sverdrupi* (Sømme 1986), which manage this scenario by
261 remaining in a cold hardy state all year round. Larvae of *B. antarctica* also constitutively express Hs
262 (Rinehart *et al.* 2006). However, the vast majority of polar invertebrates are not in a constant state of
263 readiness for the winter months and instead acclimatise (Deere *et al.* 2006), preparing themselves
264 physiologically and improving their low temperature tolerance in the lead-up to winter. Examples of
265 acclimation have been shown in the chironomid *E. murphyi* (Worland 2010), the beetle, *Hydromedion*
266 *sparsutum* (Bale *et al.* 2000), the aphid, *Myzus polaris* (Hazell *et al.* 2010), the mite *H. belgicae*
267 (Hawes *et al.* 2007), the collembolan, *C. antarcticus* (Worland *et al.* 2007), the nematode, *Plectus*
268 *murrayi* (Adhikari *et al.* 2010), and a number of other polar invertebrates (and also temperate
269 invertebrates, which similarly experience a distinct summer to winter transition). The change in
270 temperature from summer to winter is gradual, allowing evolution to optimise the rate at which
271 invertebrates acclimatise to this slow rate of cooling. It is well known that faster cooling rates reduce
272 the survival of freeze-tolerant species, raise the SCP of freeze-avoiding species, and reduce the
273 capacity of these animals to respond to chilling injury (Sinclair *et al.* 2003).

274 Acclimation to low temperatures can also occur on a shorter timescale of minutes to hours via rapid
275 cold hardening (RCH) (Lee *et al.* 2006a; Yi *et al.* 2007). RCH was first described in the flesh fly, *S.*
276 *crassipalpis* (Lee *et al.* 1987), and has since been observed in a wide range of other organisms,
277 including polar species such as *B. antarctica* (Lee *et al.* 2006a), *E. murphyi* (Everatt *et al.* 2012), *H.*
278 *belgicae* (Hawes *et al.* 2007; Worland and Convey 2001), *C. antarcticus* and the mite, *Alaskozetes*
279 *antarcticus* (Worland and Convey 2001). The response generally provides only moderate survivorship
280 benefits, however, with survival typically being extended for, at most, 10 hours at the discriminating
281 temperature (DT = temperature at which there is between 10 and 20% survival upon exposure for 2
282 hours), or lowering the DT by just 2-3°C (Bale 2002). However, there are exceptions. Following
283 RCH, the LLT of *E. murphyi* larvae decreased by up to 6.5°C, and survival of larvae of the same
284 species was maintained above 80% for at least 22 h at the DT (Everatt *et al.* 2012).

285 RCH also impacts on sub-lethal characteristics, including at temperatures above 0°C. In the fruit fly,
286 *Drosophila melanogaster*, courting and reproduction were 35 and 55% greater at 16°C, respectively,
287 following RCH (Shreve *et al.* 2004). Further sub-lethal improvements have included the maintenance
288 of the proboscis extension reflex and grooming behaviour in flesh flies (Kelty *et al.* 1996), the
289 preservation of learning and spatial conditioning (Kim *et al.* 2005), and the sustenance of flight
290 (Larsen and Lee 1994). Similar improvements are likely to be found in polar invertebrates though, as
291 yet, they have not been explored.

292 The survival and behavioural improvements of RCH are likely to be highly advantageous and may
293 allow invertebrates to adjust quickly to, and track, environmental temperatures on both a temporal
294 (daily) and spatial (microhabitat) scale (Kelty and Lee 1999; Powell and Bale 2004, 2005, 2006;
295 Shreve *et al.* 2004; Worland and Convey 2001). Analogous to acclimation over weeks and months, a
296 gradual rate of cooling that is more in line with nature tends to elicit greater protection (Chidawanyika
297 and Terblanche 2011; Kelty and Lee 1999, 2001; McDonald *et al.* 1997; Wang and Kang 2003). As
298 suggested by Wang and Kang (2003) and others, this enhanced protection is presumably because of
299 the greater time these individuals spend at protection-inducing temperatures.

300 Although the ecological role of RCH is well established, relatively little is known about the
301 physiological mechanisms underlying the response. Recent studies suggest that RCH is driven by a
302 calcium signalling cascade involving calmodulin, which allows cells to sense changes in temperature
303 and trigger downstream physiological responses (Teets *et al.* 2008). Protection against cold-induced

304 apoptosis is likely to be one such physiological response. RCH is able to impair apoptosis by down-
305 regulating promoters of the response and up-regulating apoptosis inhibitors. In *D. melanogaster* and
306 *S. crassipalpis*, apoptosis was reduced by >34% following RCH (Yi *et al.* 2007; Yi and Lee 2011).
307 RCH also involves a homeoviscous adaptation response. With the use of solid state NMR
308 spectroscopy, Lee *et al.* (2006b) were able to demonstrate enhanced membrane unsaturation and a
309 subsequent rise in membrane fluidity in *B. antarctica* following RCH. Metabolic adjustments,
310 including the accumulation of polyols and sugars during RCH and the minimisation of metabolic
311 perturbations during cold shock recovery, may likewise play a key role (Michaud and Denlinger 2007;
312 Overgaard *et al.* 2007; Teets *et al.* 2012). However, the universality of homeoviscous adaptation and
313 metabolic adjustment is in question, as some invertebrates show an RCH response in the absence of
314 either the elevation of polyols and sugars or alterations in membrane composition (MacMillan *et al.*
315 2009). Because apoptosis inhibition and homeoviscous adaptation, as well as metabolic adjustments
316 to a large degree, concern responses to chilling injury, this suggests that chilling injury, rather than
317 freezing damage, is the primary target of RCH in the chill-susceptible and freeze-tolerant
318 invertebrates studied. The same cannot be said for some freeze-avoiding invertebrates, however, such
319 as *C. antarcticus*, in which the SCP is lowered during RCH (Worland and Convey 2001). Worland
320 and Convey (2001) also confirmed that the body water content and solute concentration of freeze-
321 avoiding invertebrates were unaffected by RCH, and hypothesised that RCH in these organisms could
322 be understood by the inhibition of INAs, though this remains unconfirmed.

323 **3. Responses to low water availability**

324 Water is a requirement for all life on Earth (Hodkinson *et al.* 1999). Without it, living organisms are
325 exposed to desiccation and its associated injuries, which include protein denaturation and unwanted
326 macromolecular interactions (Sano *et al.* 1999; Tang and Pikal 2005), crystalline to gel membrane
327 phase transitions (Hazel 1995), oxidative damage (Lopez-Martinez *et al.* 2008), mechanical stress and
328 the rapid influx of water following rehydration (Bayley and Holmstrup 1999). The possibility of such
329 injuries is particularly high in the Antarctic and Arctic, where water is unavailable for extended
330 periods of the year (Block *et al.* 2009). Invertebrates protect against this threat physiologically
331 through the adoption of one of two strategies, desiccation resistance or desiccation tolerance (Fig. 2,
332 Danks 2000).

333 *3.1. Desiccation resistance*

334 Desiccation resistance is defined as the capacity to prevent water loss from the body. The extent to
335 which this occurs varies greatly amongst polar invertebrates (and invertebrates in general), leading to
336 the recognition of three groups - hygric, which have little or no control over their water loss,
337 transitional and mesic, which are increasingly able to regulate the loss of their body water (Eisenbeis
338 1983). The mesic status of some invertebrates is partly due to their lowered cuticular permeability.
339 Reduced permeability is largely achieved through the modulation of the wax layer, which coats the
340 cuticle and consists of bipolar molecules with hydrophobic and hydrophilic ends (Speight *et al.* 2008).
341 In the majority of invertebrates, the hydrophobic ends face outward and limit the rate of water loss.
342 However, mesic species go a little further and tend to either accumulate or increase the length of
343 hydrocarbons or hydrophobic molecules, resulting in tighter packing and a greater reduction of water
344 loss (Benoit *et al.* 2007a). For instance, the mesic mite, *A. antarcticus*, experienced a lower rate of
345 water loss than the mites, *Hydrogamasellus antarcticus* and *Rhagidia gerlachei*, which had two to
346 three times less hydrocarbons. *A. antarcticus* was also shown to have a high critical transition
347 temperature of 25°C, below which hydrocarbons remained stable and cells remained relatively
348 watertight (Benoit *et al.* 2007a).

349 The accumulation of polyols and sugars, and subsequent absorption of water, has also proven a
350 beneficial strategy in a number of Antarctic species, such as *C. antarcticus* (Elnitsky *et al.* 2008b) and
351 *B. antarctica* (Benoit *et al.* 2009a), which are able to depress the rate of water loss through the
352 accumulation of osmolytes. Perhaps the best example of this is given by the non-polar collembolan,
353 *Folsomia candida*. Having lost almost half of its osmotically active water under 98.2% RH, this
354 collembolan was able to recover nearly all of the loss within 5-7 d, via the accumulation and synthesis
355 of myo-inositol, glucose and trehalose (Bayley and Holmstrup 1999; Timmermans *et al.* 2009). There
356 are also some species, including astigmatid mites, that are able to maintain an equilibrium with the
357 environment at between 70 and 98% RH from the outset (Benoit *et al.* 2007a, 2009a). Further means
358 of resisting desiccation are freezing (Convey 1992), membrane alterations and metabolic suppression
359 (Michaud *et al.* 2008) as well as specialised respiration (Convey *et al.* 2003; Danks 2000; Slama
360 1988).

361 Further, recent studies on species of *Drosophila* have shown a correlation between body melanisation
362 and desiccation resistance, which corresponds with the aridity of the flies' local climate. Parkash *et al.*
363 (2008a, b) demonstrated greater desiccation resistance in darker morphs of *Drosophila melanogaster*
364 and *D. immigrans* that were predominantly found in drier, high altitude habitats, as compared with
365 their lighter counterparts found at lower altitudes. Likewise, lower desiccation resistance of *D.*
366 *melanogaster* and *D. ananassae* during the rainy season was correlated with lower melanisation
367 (Parkash *et al.* 2009, 2012). Greater desiccation resistance in strains of *D. melanogaster* selected for
368 higher levels of melanisation, and the reverse in those selected for lower levels, has now provided
369 direct evidence of the phenomenon (Ramniwas *et al.* 2013).

370 3.2. Desiccation tolerance

371 For polar invertebrates that are less desiccation resistant, an ability to tolerate water loss is crucial for
372 survival (Danks 2000). Some invertebrates are particularly tolerant - *Dendrobaena octaedra* cocoons
373 (Holmstrup and Zachariassen 1996) and larvae of *B. antarctica* (Hayward *et al.* 2007) are able to
374 endure >75% loss of their water content, and some nematodes and tardigrades are able to survive the
375 loss of virtually all of their osmotically active water and most of their osmotically inactive water in a
376 process called anhydrobiosis during which metabolism ceases (Crowe and Madin 1975; Hengherr
377 *et al.* 2010; Watanabe *et al.* 2002; Wharton 1993, 2003b, 2011; Wharton and Worland 2001). The
378 mechanisms which these organisms utilise to confer tolerance are many and include the accumulation
379 of polyols and sugars, the activation of HSPs and Late Embryogenesis Abundant (LEA) proteins,
380 membrane remodelling and oxidative damage repair (Gusev *et al.* 2010; Watanabe 2006).

381 3.2.1. Polyols and sugars

382 Polyols and sugars are produced in response to desiccation in a number of polar invertebrate groups,
383 including nematodes (e.g. *Plectus murrayi* [Adhikari *et al.* 2010]), midges (e.g. *B. antarctica* [Benoit
384 *et al.* 2009a]), beetles (e.g. *H. sparsutum* [Bale *et al.* 2000]) and Collembola (e.g. *C. antarcticus*
385 [Elnitsky *et al.* 2008b]). Of these, *B. antarctica* has been especially well studied. As a result of water
386 loss and *de novo* synthesis, larvae of this species raise the level of glycerol and trehalose two to three
387 fold (Benoit *et al.* 2007b). Two hypotheses have been put forward for the function of polyols and
388 sugars during desiccation. The first is that polyols and sugars, particularly trehalose, are used for the
389 replacement of water. Subsequently, cellular damage and deleterious protein interactions, which
390 would otherwise occur in the absence of water, are reduced (Benoit *et al.* 2009a). The second
391 hypothesis is that the low molecular weight compounds aid the production of amorphous sugar
392 glasses (e.g. through the formation of hydrogen bonds with membrane phospholipids [Sakurai *et al.*

393 2008]). These glasses stabilise proteins and membranes by minimising physical and chemical
394 reactions involving molecular diffusion, such as solute crystal nucleation (Bahrndorff *et al.* 2009;
395 Danks 2000; Hengherr *et al.* 2009). It should also be noted that the mechanisms responsible for the
396 survival of desiccation tolerance are not necessarily universal. There are some anhydrobiotic
397 invertebrates, such as the tardigrade, *Milnesium tardigradum* (Hengherr *et al.* 2008) and rotifers
398 (Caprioli *et al.* 2004; Lapinski and Tunnacliffe 2003), which do not seem to accumulate trehalose or
399 any other carbohydrate molecules. It has been suggested, because of the presence of anhydrobiosis in
400 many taxonomic groups, that the ability has evolved several times and, during each evolutionary
401 event, different mechanisms have been exploited (Alpert 2006).

402 3.2.2. HSPs and LEA proteins

403 Protein denaturation is a common injury following desiccation. HSPs are induced in direct response to
404 protein damage, and are well recognised as being involved in the reformation or degradation of
405 affected proteins (Benoit and Lopez-Martinez 2012; Feder and Hofmann 1999). Thus, it is
406 unsurprising that HSPs are up-regulated in response to desiccation in several invertebrates, including
407 tardigrades (Hengherr *et al.* 2008), Collembola (Timmermans *et al.* 2009) and Antarctic midges
408 (Lopez-Martinez *et al.* 2009). The group of proteins known as LEA proteins has also been shown to
409 play a role in desiccation tolerance in a number of invertebrates, including polar species (Bahrndorff
410 *et al.* 2009; Browne *et al.* 2002, 2004; Gal *et al.* 2004; Goyal *et al.* 2005; Watanabe *et al.* 2003). LEA
411 proteins possess many of the same attributes as HSPs, being able to prevent protein aggregation and
412 preserve enzymatic activity. These proteins may also be able to suppress unwanted macromolecular
413 interactions and maintain membrane fluidity *in vitro* (Bahrndorff *et al.* 2009), reduce water loss,
414 prevent ice crystal formation (Bokor *et al.* 2005) and stabilise sugar glasses (Wolkers *et al.* 2001).
415 There is even evidence that LEA proteins are fragmented into smaller, but still functional,
416 components in response to increased desiccation and are thereby better able to counteract damage
417 (Kikiwada *et al.* 2006).

418 3.2.3. Membrane remodelling

419 As with low temperature, the loss of water from cells and membranes leads to the transition of the
420 plasma membrane from a crystalline to a gel phase (Hazel 1995). Some invertebrates ameliorate this
421 stress via homeoviscous adaptation. In *B. antarctica*, enzymes such as $\Delta 9$ FAD and fatty acyl CoA $\Delta 9$
422 desaturases are used to increase unsaturation, and thus also fluidity, of the membrane (Lopez-
423 Martinez *et al.* 2009). However, membrane remodelling in this midge may also involve the
424 replacement of unsaturated membrane fatty acids with saturated forms. Although seemingly
425 counterintuitive, this substitution minimises the impact of singlet oxygen, a product of desiccation,
426 which reacts directly with polyunsaturated fatty acid side chains and subsequently causes lipid
427 peroxidation (Lopez-Martinez *et al.* 2009).

428 3.2.4. Oxidative damage repair

429 Desiccation of invertebrates results in the production of reactive oxygen species (ROS), such as
430 hydrogen peroxide and superoxide radicals. ROS cause numerous injuries, including the disruption of
431 membrane fluidity, apoptosis of mitochondria, denaturation of proteins and fragmentation of DNA
432 (Lopez-Martinez *et al.* 2008). Antioxidants, primarily superoxide dismutase (SOD), catalase and
433 glutathione peroxidase, are accumulated in organisms in response to these injuries and inhibit ROS.
434 Such accumulation has been observed in a number of organisms, including plants (Jenks and Wood
435 2007), nematodes (Reardon *et al.* 2010) and the Antarctic midge, *B. antarctica* (Lopez-Martinez *et al.*

436 2008). However, antioxidants are unable to completely arrest the effects of oxidation in some species.
 437 There is therefore a need for other defences, such as apoptosis of damaged cells or a DNA repair
 438 system. The latter is used in the midge, *Polypedilum vanderplanki*, and is achieved through the up-
 439 regulation of Rad23 and Rad51, which are genes associated with the repair of DNA breaks (Gusev *et*
 440 *al.* 2010).

441 3.2.5. Additional mechanisms

442 There are several other processes known to be involved in desiccation tolerance which may be utilised
 443 by polar invertebrates. These include cytoskeletal reorganization, such as the synthesis of actin,
 444 tropomyosin and myosin for the maintenance of the cytoskeleton (Lopez-Martinez *et al.* 2009; Li *et*
 445 *al.* 2009), the accumulation of aquaporins for the efficient transport of water and solutes from and into
 446 the body (Li *et al.* 2009; Philip *et al.* 2008, 2010), the removal and redistribution of osmolytes during
 447 rehydration (Bayley and Holmstrup 1999; Hayward *et al.* 2007), the regulation of autophagy (Teets *et*
 448 *al.* 2012), the down-regulation of metabolism and ATP production (Teets *et al.* 2012), and the
 449 possession of a high initial water content (Hayward *et al.* 2007).

450 4. Links between low water availability and low temperature tolerance

451 The responses of invertebrates to low temperature and low water availability are not exclusive, and
 452 there is considerable linkage between the two. This linkage is especially apparent in environments
 453 which frequently experience both stresses sequentially or in tandem. The fellfields of the Antarctic
 454 and tundra of the Arctic are prime examples of such environments (Block *et al.* 2009; Strathdee and
 455 Bale 1998).

456 4.1. Cross-tolerance following stress acclimation

457 There is increasing awareness that a sub-lethal exposure of an invertebrate to low relative humidity
 458 (RH) not only improves tolerance to low RH, but also to low temperature. This phenomenon can now
 459 be understood through the interrelationship that exists between low temperature and low water
 460 availability. Both stressors result in similar injuries and physiological challenges, including reduction
 461 of the fluidity, and thus stability and function, of plasma membranes (Bayley *et al.* 2001), impairment
 462 of protein folding (Ring and Danks 1994) and, in the case of freezing, increase of pH and osmolality
 463 of cellular fluid (Ring and Danks 1994). It therefore follows that the physiological mechanisms
 464 induced by an invertebrate in response to these stresses are also similar, or at least complementary.
 465 Several studies have suggested that the mechanisms used in response to low temperature evolved
 466 from those used in response to low water availability either as aquatic organisms colonised the land,
 467 or as they moved from generally less stressful tropical and temperate latitudes towards the poles
 468 (Block 1996; Ring and Danks 1994, 1998).

469 4.1.1. Polar examples

470 Perhaps the best-described example of cross-tolerance in a polar terrestrial invertebrate is that in the
 471 flightless Antarctic midge, *B. antarctica*. Following desiccation at 0-98.2% RH, larvae of *B.*
 472 *antarctica* show significantly higher survival at -10 and -15°C, as compared with fully hydrated larvae
 473 (Benoit *et al.* 2009a; Hayward *et al.* 2007). There is now also evidence that the closely related
 474 Antarctic midge, *E. murphyi*, and the Antarctic nematode, *Plectus murrayi*, are capable of cross-
 475 tolerance, with significantly improved survival of *E. murphyi* larvae at -18°C, and enhanced survival
 476 of the nematode at -10°C, following desiccation (Adhikari *et al.* 2010; Everatt *et al.* in press). Similar
 477 examples of cross-tolerance are found at lower latitudes in the goldenrod gall fly, *Eurosta solidaginis*,

478 which exhibits reduced water loss after low temperature acclimation, and in the collembolan, *F.*
479 *candida*, which shows enhanced low temperature tolerance after acclimation at 98.2% RH (Holmstrup
480 *et al.* 2002; Levis *et al.* 2012; Williams and Lee 2008).

481 4.1.2. Mechanisms of cross-tolerance

482 In *B. antarctica*, several physiological mechanisms that are common in response to both low
483 temperature and desiccation have been proposed to underlie cross-tolerance. Following a preliminary
484 analysis of desiccation-responsive solutes using Fourier Transform Infrared (FT-IR) spectroscopy and
485 discrimination function analysis, Hayward *et al.* (2007) found the polysaccharide region of the spectra
486 to change considerably. They went on to suggest that polyols and sugars, which serve as cryo- and
487 osmo-protectants, play an important role in conferring cross-tolerance. Benoit *et al.* (2007b, 2009a)
488 further demonstrated the level of glycerol and trehalose to increase in larvae of *B. antarctica*
489 following exposure to 75 and 98% RH, and later showed, by injecting exogenous trehalose into
490 larvae, that an enhanced concentration of trehalose leads to greater low temperature tolerance. Shared
491 metabolites produced in response to desiccation and low temperature were also shown in larvae of *B.*
492 *antarctica* by Michaud *et al.* (2008). These studies show that the accumulation of polyols and sugars
493 at the organismal level are important in desiccation and cross-tolerance. However, tolerance in *B.*
494 *antarctica* may not be as straightforward as a simple accumulation. Williams and Lee (2011) have
495 demonstrated that glycerol and sorbitol are not only accumulated in *E. solidaginis*, but are also moved
496 from extra- to intra-cellular fluids during exposure to low temperature or desiccation. This movement
497 may be especially important in freeze-tolerant invertebrates, such as *B. antarctica*, in which cells lose
498 water to the extracellular space and become susceptible to injuries associated with desiccation.
499 Polyols and sugars may subsequently act to replace water or aid in the formation of amorphous glass
500 inside the cell (Calderon *et al.* 2009). The transfer of these solutes across the cellular membrane is
501 likely orchestrated by water- and small uncharged solute specific-channels known as aquaporins or
502 aquaglyceroporins. These were first identified in human kidney and red blood cells, but have since
503 been found in yeast, plants, arthropods, birds, anurans and non-human mammals (Beuron *et al.* 1995;
504 Muller *et al.* 2006; Nielsen *et al.* 1993; Preston *et al.* 1992). Using the aquaporin inhibitor, mercuric
505 chloride (HgCl₂), Izumi *et al.* (2006) and Philip *et al.* (2008) demonstrated reduced freezing survival
506 and therefore a direct role of aquaporins in the rice stem borer, *Chilo suppressalis*, and *E. solidaginis*,
507 respectively. Philip and Lee (2010) further confirmed the role of aquaporins by showing that AQP3-
508 like proteins and AQP4-like proteins increase from summer to winter in *E. solidaginis* following low
509 temperature initiation.

510 Further mechanisms which have been suggested as important in the cross-tolerance of *B. antarctica*
511 include heat shock proteins (HSPs) and antioxidants. Although HSPs (smHsp, Hsp70 and Hsp90) are
512 constitutively expressed in larvae of *B. antarctica*, both slow and rapid desiccation are able to further
513 up-regulate them (Lopez-Martinez *et al.* 2009; Teets *et al.* 2012). HSPs are used during stress to
514 reform or degrade damaged proteins and other macromolecular structures. It is plausible that up-
515 regulation of these proteins following desiccation could repair proteins at low temperature and
516 improve low temperature tolerance (or *vice versa*). A caveat to this hypothesis is that, in *S.*
517 *crassipalpis*, the up-regulation of HSP transcripts after desiccation did not provide any further
518 tolerance to low temperature (Tammariello *et al.* 1999). However, it should be recognised that
519 transcript up-regulation is not direct evidence of protein synthesis. Thus, it remains unclear if the
520 utilisation of HSPs is a cross-tolerance mechanism universally utilised by invertebrates. LEA proteins
521 are also up-regulated under desiccation in a number of invertebrates, such as nematodes, rotifers,
522 chironomids and Collembola (Bahrndorff *et al.* 2009; Browne *et al.* 2002; Kikiwada *et al.* 2006;

523 Tunnacliffe *et al.* 2005). It has therefore been suggested that they may likewise play a role in cross-
524 tolerance, as has already been established for a number of plant species (Kosova *et al.* 2007).

525 The loss of water under freezing and desiccation leads to the production of ROS, and Lopez-Martinez
526 *et al.* (2008, 2009) have shown that antioxidants and detoxification enzymes, including superoxide
527 dismutase, catalase, metallothionein and cytochrome P450 monooxygenase, are up-regulated in
528 response to desiccation in larvae of *B. antarctica*. These same antioxidants and detoxification
529 enzymes would likely provide similar protection against oxidative damage in larvae that are frozen.

530 Similar physiological symptoms are also seen with respect to the membrane. Under desiccation and
531 low temperature, phospholipid fatty acid chains of the membrane pack together, resulting in the loss
532 of membrane fluidity and function (Hazel 1995). Most invertebrates arrest these changes by raising
533 the level of unsaturation of phospholipid fatty acids and introducing kinks into the membrane (Hazel
534 1995). In *B. antarctica*, evidence of unsaturation is shown in relation to both desiccation (Lopez-
535 Martinez *et al.* 2009) and low temperature (Lee *et al.* 2006b). It is therefore likely that the
536 unsaturation induced in response to one stress may provide protection in response to the other stress.
537 Further support for this hypothesis is provided by the collembolan, *F. candida*. Unsaturation of
538 phospholipid fatty acids takes place under desiccation (98.2% RH) in this species but, importantly, the
539 time spent at 98.2% RH which leads to a significant change in unsaturation (196 h) is the same as that
540 required at 98.2% RH to give a significant change in low temperature tolerance (Bayley *et al.* 2001;
541 Holmstrup *et al.* 2002).

542 The sub- Antarctic flightless midge *E. murphyi* is freeze-tolerant and is hypothesised to use similar
543 cross-tolerance mechanisms to *B. antarctica*. This is plausible, not only based on their close
544 phylogenetic relationship (Allegrucci *et al.* 2006, 2012), but also because the physiological
545 comparisons to date have been analogous. In particular, their low temperature tolerance and rapid cold
546 hardening responses are very similar (Everatt *et al.* 2012; Lee *et al.* 2006).

547 4.2. Cross-tolerance following anhydrobiosis

548 Examples of improved low temperature tolerance have so far been restricted to invertebrates
549 experiencing only partial desiccation. However, there are a number of invertebrates which are capable
550 of surviving the loss of virtually all of their body water (Crowe and Madin 1975). These invertebrates
551 are said to be cryptobiotic, or more specifically, anhydrobiotic (Sømme 1996), and include polar
552 nematodes (Treonis *et al.* 2000; Wharton and Barclay 1993; Wharton 2003b, 2011a), and non-polar
553 crustaceans (Crowe and Clegg 1973), rotifers (Tunnacliffe and Lapinski 2003), tardigrades (Hengherr
554 *et al.* 2008), and midge larvae (Gusev *et al.* 2010; Sakurai *et al.* 2008). When in a state of
555 anhydrobiosis, such invertebrates show a remarkable ability to survive low temperature. The
556 tardigrade, *Adorybiotus coronifer*, for example, is able to survive cooling to -196°C (Ramløv and
557 Westh 1992), while others are able to survive in liquid helium (-271°C) (Shuker 2001). Invertebrates
558 like tardigrades can also survive extreme conditions even in their hydrated state. The Antarctic
559 tardigrades, *Echiniscus jenningsi*, *Macrobiotus furciger* and *Diphyscon chilense*, show survival
560 after 590 days at -22°C, good survival at -80°C and even some survival at -180°C. Surprisingly when
561 anhydrobiotic, tolerance of low temperatures is actually attenuated in these species (Sømme and
562 Meier 1995).

563 The mechanisms which anhydrobiotic invertebrates use to survive the loss of all their osmotically
564 active water and confer tolerance at low temperature may be similar to those previously described,
565 though it is postulated that, because the level of desiccation is greater, the strength of these

566 mechanisms may be greater also. The accumulation of polyols and sugars, particularly trehalose,
567 conform to this hypothesis in a number of anhydrobiotic invertebrates, including nematodes (Crowe
568 and Madin 1975; Madin and Crowe 1975), midge larvae (Watanabe *et al.* 2002) and tardigrades
569 (Ramløv and Westh 1992). In the tardigrade, *A. coronifer*, and members of the Macrobiotidae family,
570 the accumulation during entry into anhydrobiosis is particularly pronounced, increasing the level of
571 trehalose by up to 20-fold (Hengherr *et al.* 2008; Ramløv and Westh 1992). Increases in trehalose
572 found in *B. antarctica* are, in contrast, less obvious (Benoit *et al.* 2007b). The role of polyols and
573 sugars, as with partially-desiccated invertebrates, has been attributed to water replacement and/or
574 vitrification (Danks 2000). The latter is now seen as being essential in some anhydrobiotic
575 invertebrates. Using FTIR analysis and differential scanning calorimetry (DSC), Sakurai *et al.* (2008)
576 show larvae of the midge *P. vanderplaanki* to form a glass-like state during anhydrobiosis.
577 Significantly, larvae were unable to successfully rehydrate when artificially taken out of this state.

578 4.3. Cross-tolerance following selection

579 To examine whether cross-tolerance to one stress could be enhanced by selecting for improved basal
580 tolerance to another stress (i.e. without prior acclimation), Bublly and Loeschcke (2005) artificially
581 selected for either cold or desiccation tolerance in the fruit fly, *D. melanogaster*, and subsequently
582 exposed selected individuals to the opposing stress. They found that individuals selected for
583 desiccation resistance had greater low temperature tolerance at 0.5°C. Sinclair *et al.* (2007) also found
584 that selection for desiccation resistance in *D. melanogaster* resulted in a decreased chill coma
585 recovery time, approximately 2 min less (15% reduction) than control flies. However, Sinclair *et al.*
586 (2007) did not find a discernible improvement in the low temperature survival of *D. melanogaster*
587 following desiccation resistance selection. Likewise, selection for low temperature tolerance did not
588 result in an improvement in the flies' desiccation resistance/tolerance (MacMillan *et al.* 2009). The
589 lack of cross-tolerance was suggested by MacMillan *et al.* (2009) to indicate that changes in basal
590 tolerance are not responsible for cross-tolerance. Yet, because there are some studies, including those
591 mentioned (e.g. Bublly and Loeschcke 2005), which do show cross-tolerance through selection of
592 basal tolerance, it was also suggested that other selective pressures as part of the experimental design
593 may act to break up linkage gene groups associated with multiple stress tolerance (MacMillan *et al.*
594 2009). As outlined in this review, multiple physiological processes underpin both cold and desiccation
595 tolerance, and artificial selection experiments are unlikely to target all the underlying genetic
596 mechanisms.

597 4.4. Cryoprotective dehydration

598 Cryoprotective dehydration was first discovered by Holmstrup (1992) in cocoons of the earthworm,
599 *Dendrobaena octaedra*, and has since been described in nematodes (e.g. *Panagrolaimus davidi*
600 [Smith *et al.* 2008]), enchytraeid worms (e.g. *Fridericia ratzeli* [Pedersen and Holmstrup 2003]),
601 Collembola (Elnitsky *et al.* 2008b; Sorensen and Holmstrup 2011; Worland *et al.* 1998) and even
602 mammalian cells (Pegg 2001). This process occurs in an environment in equilibrium with the vapour
603 pressure of ice. Under these conditions, invertebrates continue to lose water along a diffusion gradient
604 between their supercooled body fluids and the surrounding ice until the vapour pressure of their body
605 fluids is equal to that of the environment (Wharton *et al.* 2003a). The subsequent concentration and *de*
606 *novo* synthesis of solutes (Elnitsky *et al.* 2008a) causes the SCPs of invertebrates to be reduced and
607 their MPs to become equilibrated with the ambient temperature (Elnitsky *et al.* 2008a; Holmstrup *et*
608 *al.* 2002; Pedersen and Holmstrup 2003). In this state, the risk of freezing is eliminated (Elnitsky *et al.*
609 2008).

610 Cryoprotective dehydration is perhaps best exemplified in the Arctic collembolan, *M. arctica*. The
 611 response was first described in this collembolan by Worland *et al.* (1998) and Holmstrup and Sømme
 612 (1998), who showed the SCP of *M. arctica* to fall as low as -30°C when the temperature was reduced
 613 to -12.4°C. The melting point (MP) was also shown to decrease with temperature (Holmstrup and
 614 Sømme 1998), and was later shown by Holmstrup *et al.* (2002) to decline in parallel with the
 615 environmental temperature, before equilibrating with this temperature after a 1-6 day lag period.
 616 Cryoprotective dehydration is not restricted to freeze-avoiding invertebrates such as *M. arctica*, but
 617 also extends to freeze-tolerant species. For instance, larvae of *B. antarctica* have been demonstrated to
 618 lose water in the presence of ice when cooled to -3°C, and have subsequently shown to have a three-
 619 fold depression of their MP (Elnitsky *et al.* 2008a).

620 The solutes accumulated during cryoprotective dehydration are similar in *B. antarctica* and *M. arctica*
 621 and include glucose and trehalose (Elnitsky *et al.* 2008a; Holmstrup *et al.* 2002). Glucose is likewise
 622 accumulated during cryoprotective dehydration in the earthworm, *F. ratzelei* (Pedersen and Holmstrup
 623 2003). Other polyols, sugars and amino acids may also be involved in the process (Elnitsky *et al.*
 624 2008a). The accumulation of these solutes has already been shown to lower the SCP and MP, and they
 625 may also lead to a vitrified state, as has been shown in at least one invertebrate, the red flat bark
 626 beetle, *C. c. puniceus* (Sformo *et al.* 2010).

627 For cryoprotective dehydration to be a viable strategy, invertebrates must possess low desiccation
 628 resistance (cuticular permeability) and high desiccation tolerance (Bahrdorff *et al.* 2007). The former
 629 is required for the transport of water from the supercooled body fluids to the external environment at a
 630 rate equivalent to that of the lowering of ice vapour pressure with temperature (Holmstrup *et al.*
 631 2002), while desiccation tolerance is imperative if the organism is to survive considerable water loss.

632 **5. Links between low water availability and high temperature tolerance, and tolerance** 633 **to other stresses**

634 Cross-tolerance between desiccation and high temperature has been identified in a number of
 635 organisms, including plants, cysts of crustaceans, rotifers (Mertens *et al.* 2008), tardigrades (Hengherr
 636 *et al.* 2009; Hinton 1951, 1960), and midges, including *B. antarctica* (Benoit *et al.* 2009a; Sakurai
 637 *et al.* 2008). However, examples such as these are infrequent, especially for polar organisms. This is
 638 partly explained by the lack of studies that have investigated the effect of desiccation on heat
 639 tolerance, but also by the fact that some organisms seem not to show cross-tolerance. For example,
 640 desiccation failed to improve heat tolerance in the bed bug, *Cimex lectularius* (Benoit *et al.* 2009b). In
 641 addition, larvae of the Antarctic midge, *E. murphyi*, failed to show greater survival at 37 and 38.5°C
 642 following 2, 4 and 8 days at 98.2% RH, and even exhibited lowered survival at 37°C following 12
 643 days of desiccation (Everatt *et al.* in press).

644 Unlike the response to desiccation and low temperature stresses, some of the physiological changes
 645 that occur as a result of desiccation are different, and opposing, to those incurred as a result of high
 646 temperature. For example, the phospholipid fatty acids of the cell membrane under low temperature
 647 pack together, and transition from crystalline to gel phase, resulting in lowered membrane fluidity.
 648 Under high temperature, the phospholipid fatty acids spread apart, leading to the opposite outcome
 649 (Hazel 1995). Membrane remodelling in response to desiccation and high temperature is subsequently
 650 also antagonistic, and a prior desiccation exposure will therefore result in lowered protection. The
 651 lowered protection with regard to membrane fluidity and other processes may counteract the
 652 protection gained through physiological mechanisms that are similar between desiccation and high

653 temperature, such as the activation of HSPs and accumulation of polyols and sugars (Benoit *et al.*
654 2009a).

655 If this is generally the case, then cross-tolerance between desiccation and high temperature would not
656 be expected in any organism. Yet, it does occur. It may be significant that a large proportion of the
657 organisms that to date have shown cross-tolerance between these two stresses are anhydrobiotic
658 (Hengherr *et al.* 2009; Hinton 1951, 1960; Mertens *et al.* 2008; Sakurai *et al.* 2008). In general,
659 metazoan animals are only able to survive above 47°C temporarily (Addo-Bediako *et al.* 2000;
660 Nicholas 1984; Schmidt-Nielsen 1997), and any further improvement in heat tolerance requires an
661 animal to enter an anhydrobiotic state. This has been shown in *Polypedilum vanderplanki*, which
662 when in anhydrobiosis was able to survive temperatures 59°C higher than when it was in its hydrated
663 state (Hinton 1951, 1960; Sakurai *et al.* 2008). It is known that anhydrobiotic organisms have a
664 tendency to vitrify, and also that any damage incurred as a result of denaturation, aggregation and
665 disintegration by immobilisation ceases or slows when in this glass-like state (Crowe *et al.* 1998,
666 2002). These organisms may therefore not suffer the injurious changes to the membrane that would
667 otherwise occur for non-anhydrobiotic species, and would instead only receive greater tolerance at
668 high temperatures from being in a vitrified state. While this explanation is appropriate for
669 anhydrobiotic organisms, the same is not true of partially desiccated organisms like *B. antarctica*,
670 which are not vitrified when cross-tolerance is conferred to high temperatures. It is possible that the
671 relative contribution of membrane remodelling and other opposing processes to cross tolerance is
672 lower in these organisms. Instead, other physiological responses, including the accumulation of
673 trehalose and the activation of HSPs (Benoit *et al.* 2009a), which are similar between desiccation and
674 high temperature tolerance, may compensate.

675 In addition to cross-tolerance between desiccation and low and high temperature, cross-tolerance has
676 also been observed between desiccation and other stresses. In particular, desiccation has been
677 observed to improve tolerance of low-Linear Energy Transfer (LET) and high-LET radiation
678 (Gladyshev and Meselson 2008; Gusev *et al.* 2010; Jonsson *et al.* 2008; Watanabe *et al.* 2007), CO₂
679 and N₂ exposure (Ricci *et al.* 2005), and high hydrostatic pressure (Seki and Toyoshima 1988). While
680 for low temperature exposures, cross-tolerance has been reported to salinity (Elnitsky *et al.* 2009),
681 anoxia (Yoder *et al.* 2006) and high temperature (Yoder *et al.* 2006).

682 **6. Ecology of cross-tolerance**

683 *6.1. Ecological conditions*

684 Although cross-tolerance has been shown to be of benefit to several polar invertebrates under
685 laboratory conditions, there remain a number of ecological conditions that must be met for it to be
686 successful in the field. One of these concerns the rate at which an organism is desiccated prior to or
687 during low temperature exposure. Benoit *et al.* (2009a) discovered that faster rates of desiccation (2-
688 13%/h) resulted in significantly less protection at -15°C in *B. antarctica*, than rates of 0.4-0.5%/h,
689 even though percentage water loss was equivalent between treatments during low temperature
690 exposure. It was subsequently shown that the level of trehalose was considerably lower in larvae
691 desiccated more quickly (Benoit *et al.* 2009a). Other processes, such as the accumulation of
692 alternative polyols and sugars, are also speculated to be affected in a similar way in *B. antarctica*.
693 Completely desiccated or anhydrobiotic organisms are likewise affected by the rate of desiccation.
694 Tardigrades, before entering anhydrobiosis, must first restructure their bodies into a tun-like form
695 (Baumann 1992; Crowe 1972; Wright 1989). The formation of this tun shape is essential for the

696 protection of internal organs and for the reduction of water loss during anhydrobiosis (Sømme 1996).
697 Tun formation can only take place under slow desiccation (Wright 1989).

698 The rate at which an organism is cooled is also important for cross-tolerance, particularly for freeze-
699 avoiding invertebrates which utilise cryoprotective dehydration. If the SCP of these invertebrates is to
700 remain below the ambient temperature, desiccation must proceed at a pace equivalent to that of the
701 rate of cooling. This cannot occur if the rate of cooling is too rapid (Elnitsky *et al.* 2008b; Wharton *et al.*
702 2003a). The importance of a slow cooling rate is also observed during low temperature
703 acclimation, over the long-term (Sinclair *et al.* 2003), as well as over the short-term in the form of
704 RCH (Chidawanyika and Terblanche 2011; Kelty and Lee 1999, 2001; McDonald *et al.* 1997; Wang
705 and Kang 2003). Rates of cooling in the Arctic and Antarctic generally tend to be slow and are
706 sufficient to permit cryoprotective dehydration. For example, in the McMurdo Dry Valleys of
707 continental Antarctica, natural cooling rates can average $0.021^{\circ}\text{C min}^{-1}$ (Sinclair and Sjørnsen 2001).

708 A further ecological condition that must be met by partially and severely desiccated, or
709 cryoprotectively dehydrated, organisms, is the avoidance of inoculative freezing (= induction of
710 freezing whilst in direct contact with ice). This is because inoculative freezing can initiate ice
711 nucleation above an organism's SCP. For freeze-avoiding organisms this poses an inherent survival
712 risk, while for freeze-tolerant organisms, inoculative freezing terminates desiccation and subsequently
713 reduces the potential for cross-tolerance. Inoculative freezing is most likely to occur under moist
714 conditions, in which ice crystals regularly pass through the cuticle and into orifices (Olsen *et al.* 1998;
715 Salt 1963; Zachariassen and Kristiansen 2000). This increase in freezing under moist conditions has
716 been shown in both the enchytraeid worm, *Fridericia ratzeli* (Pedersen and Holmstrup 2003), and the
717 midge *B. antarctica* (Elnitsky *et al.* 2008). These authors therefore speculate that the microhabitat
718 preference of some invertebrates may be influenced by the risk of inoculative freezing.

719 6.2. Sub-lethal characteristics

720 While survival is relevant to the "success" or fitness of a species, there are a number of other
721 attributes which may be impacted upon by the sequential or simultaneous occurrence of stresses.
722 These attributes are referred to as sub-lethal characteristics and include courtship, reproduction,
723 foraging, predator avoidance and activity (e.g. Kelty and Lee 1999; Korenko *et al.* 2010). Because
724 sub-lethal characteristics are affected by stresses less extreme and therefore more regularly
725 encountered in nature than those which cause mortality, the importance of sequential or
726 simultaneously occurring stresses may be greater than that of simple survival of extremes. However,
727 there are only a handful of studies which have addressed this subject. Sinclair *et al.* (2007)
728 demonstrated that *D. melanogaster* which had previously been selected for greater desiccation
729 resistance/tolerance had a shorter, and thus improved, chill coma recovery time as compared with
730 controls. Everatt *et al.* (2013) showed that the critical thermal minimum (CT_{min}, the low temperature
731 at which an organism no longer shows coordinated movement) and chill coma (low temperature at
732 which electrophysiological activity ceases completely) of *C. antarcticus* were negatively affected by
733 prior desiccation on concentrated sea water. However, due to the limited number of such studies, and
734 that they have focussed only on the thermal thresholds of activity to date, it is not yet possible to draw
735 conclusions on the effect of sequential and simultaneous stresses on sub-lethal characteristics.

736 6.3. Climate warming

737 Over the last two to three decades, climate warming has received considerable public attention and
738 has become the focus of the largest scientific collaboration in human history. There is now an almost

739 universal consensus that atmospheric CO₂ levels are rising as a result of human activity and are
740 leading to warming on a global scale. Temperatures have so far risen, on average, by 0.85°C across
741 the Earth's surface over the last century (IPCC 2013). The rate of increase in temperature has been
742 particularly high in certain polar regions ('polar amplification'), averaging 2°C over the past 50 years
743 (Arctic Council 2005; Convey *et al.* 2009; Turner *et al.* 2009). Furthermore, general circulation
744 models suggest these temperature trends will continue (Convey *et al.* 2009; Turner *et al.* 2009). Water
745 availability is also likely to change as a result of climate warming. Precipitation is predicted to
746 increase by 0.5-1% per decade at higher latitudes (Walther *et al.* 2002; Turner *et al.* 2009). However,
747 as temperatures rise, snow cover is expected to decrease and melt is expected to occur earlier in the
748 season. In turn, the thawing of glaciers and evaporation of meltwater are also expected to take place
749 earlier in the summer season (Avila-Jimenez *et al.* 2010; Walther *et al.* 2002).

750 Polar terrestrial invertebrates will therefore increasingly experience warmer and drier summers.
751 Understanding the capability of these invertebrates to tolerate high temperature and low water
752 availability either sequentially or simultaneously thus becomes increasingly significant. As discussed
753 above, the level of cross-tolerance between high temperature and low water availability is variable.
754 While invertebrates like the midge, *B. antarctica*, are afforded greater heat tolerance following prior
755 desiccation, others including the closely related midge, *E. murphyi*, and the collembolan, *C.*
756 *antarcticus*, are incapacitated once desiccated (Benoit *et al.* 2009a; Everatt *et al.* 2013, in press). The
757 success of polar invertebrates in a warming climate will therefore be species specific. However, recent
758 studies have demonstrated that some of these invertebrates possess considerable basal heat tolerance
759 over both short and long timescales, and the heat tolerance shown far exceeds that required during
760 current and foreseeable Antarctic and Arctic summers (Deere *et al.* 2006; Everatt *et al.* 2013, in press;
761 Sinclair *et al.* 2006; Slabber *et al.* 2007). The Collembola, *C. antarcticus* and *M. arctica*, for example,
762 are able to survive above 30°C for a number of hours and at 10°C for over 40 days (Block *et al.*, 1994;
763 Hodkinson *et al.*, 1996; Everatt *et al.* 2013, in press). The level of tolerance these invertebrates show
764 is in line with the thermal sensitivity hypothesis, that the sensitivity of terrestrial invertebrates to
765 higher temperature exposure decreases with increasing latitude (Addo-Bediako *et al.* 2000; Deutsch *et*
766 *al.* 2008).

767 **7. Conclusions and future directions**

768 Many polar terrestrial invertebrates are ancient and have likely spent the last few million years honing
769 their physiology to the extreme environments in which they live (Convey and Stevens 2007; Marshall
770 1996; McGaughan *et al.* 2010). The tolerance these animals have to sub-zero temperatures and
771 desiccation stress has been known for many decades, yet it is only relatively recently that
772 investigations into the molecular mechanisms underpinning these physiological adaptations have
773 begun. Molecular responses to sub-lethal chilling also remain poorly characterised, and disentangling
774 the processes underpinning chill vs. extreme cold or freezing damage is an important future challenge.
775 A possible route to addressing this would be to undertake more detailed time series investigations of
776 the stress response cascade to declining temperature. This includes key functional, i.e. not just
777 tolerance, phenotypes such as activity thresholds and metabolic adaptation as set out in Fig. 1.
778 Crucially, future studies need to more directly address mechanism, rather than simple correlations
779 between molecular and phenotypic changes that currently dominate the literature. This extends to
780 understanding responses to multiple stressors in tandem, which is another current knowledge gap. The
781 protection afforded against one stress following exposure to another was no doubt pivotal in the
782 persistence of many species during historic climate transitions, as well as important currently during
783 winter in the polar regions. This is perhaps most evident in animals which cryoprotectively dehydrate

784 like *M. arctica*, whose sole strategy of surviving winter relies on cross-tolerance to cold and
785 desiccation.

786 A benefit of cross-tolerance between high temperature and desiccation has received even less
787 attention, but is a combination of stressors which may occur more frequently in polar environments
788 under continued climate warming. While some species show cross-tolerance, others do not, and
789 further work is required to establish a baseline for different species groups, as well as for polar
790 terrestrial invertebrates generally. However, it should be noted that many of these invertebrates show
791 a level of heat tolerance that is far greater than is required for even medium term predictions of
792 climate warming. Thus, physiological approaches must be guided by more detailed studies of current
793 microclimate conditions, and models forecasting rates of environmental change, to better predict
794 winners and losers under different climate scenarios. A greater threat to survival may in fact be
795 competition from newly colonising species – and investigating the physiological ‘suitability’ of
796 species whose distribution boundaries place them on the doorstep of polar environments is another
797 important research objective (Everatt *et al.* 2012; Frenot *et al.* 2005).

798 To end, this review has shown the almost boundless flexibility and adaptability enlaced into the
799 physiology of invertebrates, when faced with a single stressor or multiple stressors. Such is their
800 adaptability that they have been able to thrive in many habitats, even those as extreme and
801 inhospitable as the Arctic and Antarctic. The review has also highlighted the importance of looking at
802 all factors together and not simply investigating single factors in isolation. As the field of invertebrate
803 stress ecophysiology grows, so will the need for a cross-disciplinary approach.

804 **Acknowledgements**

805 MJE is funded through NERC PhD studentship (RRBN15266), which is a CASE partnership between
806 the University of Birmingham and British Antarctic Survey. PC is a member of the BAS
807 ‘Ecosystems’ programme, which receives core funding through NERC to BAS. This paper also
808 contributes to the SCAR ‘Antarctic Thresholds – Ecosystem Resilience and Adaptation’ programme.
809 Two anonymous reviewers are thanked for their constructive suggestions.

810 **References**

- 811 Addo-Bediako, A., Chown, S. L. and Gaston, K. J. (2000) Thermal tolerance, climatic variability and
812 latitude. *Proceedings of the Royal Society of London B*. 267, 739-745.
- 813 Adhikari, B. N., Wall, D. H. and Adams, B. J. (2010) Effect of slow desiccation and freezing on gene
814 transcription and stress survival of an Antarctic nematode. *The Journal of Experimental*
815 *Biology*. 213, 1803-1812.
- 816 Allegrucci, G., Carchini, G., Todisco, V., Convey, P. and Sbordoni, V. (2006) A molecular phylogeny
817 of antarctic chironomidae and its implications for biogeographical history. *Polar Biology*. 29,
818 320-326.
- 819 Allegrucci, G., Carchini, G., Convey, P. and Sbordoni, V. (2012) Evolutionary geographic
820 relationships among orthocladine chironomid midges from maritime Antarctic and sub-
821 Antarctic islands. *Biological Journal of the Linnean Society*. 106, 258-274.
- 822 Alpert, P. (2006) Constraints of tolerance: Why are desiccation-tolerant organisms so small or rare?.
823 *Journal of Experimental Biology*. 209, 1575–1584.
- 824 Arctic Council (2005) Arctic climate impact assessment - scientific report. Cambridge: Cambridge
825 University Press, 1046 pp (available at <http://www.acia.uaf.edu/pages/scientific.html>).

- 826 Ávila-Jiménez, M. L., Coulson, S. J., Solhøy, T. and Sjöblom, A. (2010) Overwintering of terrestrial
 827 Arctic arthropods: the fauna of Svalbard now and in the future. *Polar Research*, 29, 127-137.
- 828 Baenziger, J. E., Jarrell, H. C. and Smith, I. C. (1992) Molecular motions and dynamics of a
 829 diunsaturated acyl chain in a lipid bilayer: implications for the role of polyunsaturation in
 830 biological membranes. *Biochemistry*. 31, 3377-3385.
- 831 Bahrndorff, S., Petersen, S. O., Loeschcke, V., Overgaard, J. and Holmstrup, M. (2007) Differences in
 832 cold and drought tolerance of high arctic and sub-arctic populations of *Megaphorura arctica*
 833 Tullberg 1876 (Onychiuridae: Collembola). *Cryobiology*. 55, 315-23.
- 834 Bahrndorff, S., Tunnacliffe, A., Wise, M. J., McGee, B., Holmstrup, M. and Loeschcke, V. (2009)
 835 Bioinformatics and protein expression analyses implicate LEA proteins in the drought
 836 response of Collembola. *Journal of Insect Physiology*. 55, 210-217.
- 837 Bale, J. S. (1993) Insects in the cold. *Endeavour*. 17, 132-137.
- 838 Bale, J. S. (1996) Insect cold hardiness: A matter of life and death. *European Journal of Entomology*.
 839 93, 369-382.
- 840 Bale, J. S. (2002) Insects and low temperatures: from molecular biology to distributions and
 841 abundance. *Philosophical Transactions of the Royal Society of London. Series B, Biological*
 842 *Sciences*. 357, 849-62.
- 843 Bale, J. S., Block, W. and Worland, M. R. (2000) Thermal tolerance and acclimation response of
 844 larvae of the sub-Antarctic beetle *Hydromedion sparsutum* (Coleoptera: Perimylopidae).
 845 *Polar Biology*. 23, 77-84.
- 846 Bauer, R., Worland, M. R. and Block, W. (2001) Experimental studies on cold survival of enchytraeid
 847 cocoons. *Pedobiologia*. 45, 561-571.
- 848 Baumann, H. (1922) Die anabiose der Tardigraden. *Zool. Jahresber*. 45, 501-566.
- 849 Bayley, M. and Holmstrup, M. (1999) Water Vapor Absorption in Arthropods by Accumulation of
 850 Myoinositol and Glucose. *Science*. 285, 1909-1911.
- 851 Bayley, M., Petersen, S. O., Knigge, T., Köhler, H-R. and Holmstrup, M. (2001) Drought acclimation
 852 confers cold tolerance in the soil collembolan *Folsomia candida*. *Journal of Insect*
 853 *Physiology*. 47, 1197-1204.
- 854 Bengtson, S. A., Fjellberg, A., and Solhøy, T. (1974) Abundance of tundra arthropods in Spitsbergen.
 855 *Scandinavian Entomology*. 5, 137-142.
- 856 Bennett, V. A., Sformo, T., Walters, K. Toien, O., Kennan, J., Hochstrasser, R., Pan, Q, Serianni, A.,
 857 Barnes, B. M., Duman, J. G. (2005) Comparative overwintering physiology of Alaska and
 858 Indiana populations of the beetle *Cucujus clavipes* (Fabricius): roles of antifreeze proteins,
 859 polyols, dehydration and diapause. *The Journal of Experimental Biology*. 208, 4467-4477.
- 860 Benoit, J. B. and Lopez-Martinez, G. (2012) Role of conventional and unconventional stress proteins
 861 during the response of insects to traumatic environmental conditions. In: *Heamolymph*
 862 *Proteins and Functional Peptides: Recent Advances in Insects and other Arthropods* (Ed. by
 863 M. Tufail and M. Takeda), pp. 128-160. Bentham Science.
- 864 Benoit, J. B., Lopez-Martinez, G., Elnitsky, M. A., Lee, R. E. and Denlinger, D. L. (2009a)
 865 Dehydration-induced cross tolerance of *Belgica antarctica* larvae to cold and heat is
 866 facilitated by trehalose accumulation. *Comparative biochemistry and physiology. Part A,*
 867 *Molecular & integrative physiology*. 152, 518-523.
- 868 Benoit, J. B., Lopez-Martinez, G., Michaud, M. R., Elnitsky, M.A., Lee, R. E. and Denlinger, D. L.
 869 (2007b) Mechanisms to reduce dehydration stress in larvae of the Antarctic midge, *Belgica*
 870 *antarctica*. *Journal of Insect Physiology*. 53, 656-67.
- 871 Benoit, J. B., Lopez-Martinez, G., Teets, N. M., Phillips, S. A. and Denlinger, D. L. (2009b)
 872 Responses of the bed bug, *Cimex lectularius*, to temperature extremes and dehydration: levels

- 873 of tolerance, rapid cold hardening and expression of heat shock proteins. *Medical and*
874 *Veterinary Entomology.* 23, 418-425.
- 875 Benoit, J. B., Yoder, J. A., Lopez-Martinez, G., Elnitsky, M. A., Lee, R. E. and Denlinger, D. L.
876 (2007a) Adaptations for the maintenance of water balance by three species of Antarctic mites.
877 31, 539-547.
- 878 Beuron, F., Le Caherec, F., Guillam, M., Cavilier, A., Garret, A., Tassan, J., Delmarche, C., Schultz,
879 P., Mallouh, V., Roland, J.-P., Hubert, J-F., Gouranton, J. and Thomas, D. (1995). Structural
880 analysis of a MIP family protein from the digestive tract of *Cicadella viridis*. *The Journal of*
881 *Biological Chemistry.* 270, 17414-17422.
- 882 Block, W. (1982) Cold hardiness in invertebrate poikilotherms. *Comparative Biochemistry and*
883 *Physiology A.* 73, 581-593.
- 884 Block, W. (1990) Cold tolerance of insects and other arthropods. *Philosophical transactions of the*
885 *Royal Society of London. Series B, Biological Sciences.* 326, 613-633.
- 886 Block, W. (1996) Cold or drought – the lesser of two evils for terrestrial arthropods. *European Journal*
887 *of Entomology.* 93, 325-339.
- 888 Block, W. and Worland, M. R. (2001) Experimental studies of ice nucleation in an Antarctic
889 springtail (Collembola, Isotomidae). *Cryobiology.* 42, 170-181.
- 890 Block, W., Harrison, P. M. and Vannier, G. (1990) A comparative study of patterns of water loss
891 from two Antarctic springtails (Insecta, Collembola). *Journal of Insect Physiology.* 36, 181–
892 187.
- 893 Block, W., Smith, R. I. L. and Kennedy, A. D. (2009) Strategies of survival and resource exploitation
894 in the Antarctic fellfield ecosystem. *Biological Reviews of the Cambridge Philosophical*
895 *Society.* 84, 449-84.
- 896 Block, W., Webb, N. R., Coulson, S, Hodkinson, I. D. and Worland, M. R. (1994) Thermal adaptation
897 in the Arctic collembolan *Onychiurus arcticus* (Tullberg). *Journal of Insect Physiology.* 40,
898 715-722.
- 899 Bokhorst, S., Huiskes, A., Convey, P. and Aerts, R. (2007) External nutrient inputs into terrestrial
900 ecosystems of the Falkland Islands and the Maritime Antarctic region. *Polar Biology.* 13,
901 2642-2653.
- 902 Bokor, M., Csizmok, V., Kovacs, D., Banki, P., Friedrich, P., Tompa, P. and Tompa, K. (2005) NMR
903 relaxation studies on the hydrate layer of intrinsically unstructured proteins. *Biophysical*
904 *Journal.* 88, 2030-2037.
- 905 Browne, J. A., Dolan, K. M., Tyson, T., Goyal, K., Tunnacliffe, A. and Burnell, A. M. (2004)
906 Dehydration-specific induction of hydrophilic protein genes in the anhydrobiotic nematode
907 *Aphelenchus avenae*. *Eukaryotic Cell.* 3, 966-975.
- 908 Browne, J., Tunnacliffe, A. and Burnell, A. (2002) Plant desiccation gene found in a nematode.
909 *Nature.* 416, 38.
- 910 Bublly, O. A. and Loeschcke, V. (2005) Correlated responses to selection for stress resistance and
911 longevity in a laboratory population of *Drosophila melanogaster*. *Journal of Evolutionary*
912 *Biology.* 18, 789–803.
- 913 Burn, A. (1986) Feeding rates of the cryptostigmatid mite *Alaskozetes antarcticus* (Michael). *British*
914 *Antarctic Survey Bulletin.* 71, 11-17.
- 915 Burns, G., Thorne, M. A. S., Hillyard, G., Clark, M. S., Convey, P. and Worland, M. R. (2010) Gene
916 expression associated with changes in cold tolerance levels of the Antarctic springtail,
917 *Cryptopygus antarcticus*. *Insect Molecular Biology.* 19, 113-120.
- 918 Calderon, S., Holmstrup, M., Westh, P. and Overgaard, J. (2009) Dual roles of glucose in the freeze-
919 tolerant earthworm *Dendrobaena octaedra*: cryoprotection and fuel for metabolism. *The*
920 *Journal of Experimental Biology.* 212, 859-866.

- 921 Callaghan, T. V., Sonesson, M., Sømme, L., Walton, D. W. H., Christensen, T. and Block, W. (1992)
 922 Responses of Terrestrial Plants and Invertebrates to Environmental Change at High Latitudes
 923 [and Discussion]. *Philosophical Transactions of the Royal Society of London B.* 338, 279-
 924 288.
- 925 Cannon, R. J. C. and Block, W. (1988) Cold tolerance of microarthropods. *Biological Reviews.* 63,
 926 23-77.
- 927 Caprioli, M., Kathiölm, A. K., Melno, G., Ramlöv, H., Ricci, C. and Santo, N. (2004) Trehalose in
 928 desiccated rotifers: a comparison between a bdelloid and a monogonont species. *Comparative*
 929 *Biochemistry and Physiology Part A.* 139, 527-532.
- 930 Chen, B., Kayukawa, T., Monteiro, A. and Ishikawa, Y. (2006) Cloning and characterization of the
 931 HSP70 gene, and its expression in response to diapauses and thermal stress in the onion
 932 maggot, *Delia antiqua*. *Journal of Biochemistry and Molecular Biology.* 39, 749-758.
- 933 Chidawanyika, F. and Terblanche, J. S. (2011) Rapid thermal responses and thermal tolerance in adult
 934 codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). *Journal of Insect Physiology.* 57,
 935 108-117.
- 936 Clarke, A., Morris, G. J., Fonseca, F., Murray, B. J., Acton, E. and Price, H. C. (2013) A low
 937 temperature limit for life on Earth. *PLOS One.* 8, e66207.
- 938 Clark, M. S. and Worland, M. R. (2008) How insects survive the cold: molecular mechanisms-a
 939 review. *Journal of comparative physiology. B, Biochemical, Systemic, and Environmental*
 940 *Physiology.* 178, 917-33.
- 941 Clark, M. S., Thorne, M. A. S., Purać, J., Burns, G., Hillyard, G., Popović, Z. D., Grubor-Lajsić, G.
 942 and Worland, M. R. (2009) Surviving the cold: molecular analyses of insect cryoprotective
 943 dehydration in the Arctic springtail *Megaphorura arctica* (Tullberg). *BMC genomics.* 10,
 944 328.
- 945 Clough, M. S., Bale, J. S. and Harrington, R. (1990) Differential cold hardiness in adults and nymphs
 946 of the peach-potato aphid *Myzus persicae*. *Annals of Applied Biology.* 116, 1-9.
- 947 Convey, P. (1992) Aspects of the biology of the midge, *Eretmoptera murphyi* Schaeffer (Diptera:
 948 Chironomidae), introduced to Signy Island, maritime Antarctic. *Polar Biology.* 12, 653-657.
- 949 Convey, P. (1996a) Overwintering strategies of terrestrial invertebrates in Antarctica - the
 950 significance of flexibility in extremely seasonal environments. *European Journal of*
 951 *Entomology.* 93, 489-505.
- 952 Convey, P. (1996b) The influence of environmental characteristics on life history attributes of
 953 Antarctic terrestrial biota. *Biological Reviews.* 71, 191-225.
- 954 Convey, P. (1997) How are the life history strategies of Antarctic terrestrial invertebrates influenced
 955 by extreme environmental conditions?. *Journal of Thermal Biology.* 22, 429-440.
- 956 Convey, P. (2013) Antarctic Ecosystems. *Encyclopedia of Biodiversity.* Vol. 1, 2nd edition, ed. S.A.
 957 Levin. Elsevier, San Diego, pp. 179-188.
- 958 Convey, P. and Smith, R. I. L. (1997) The terrestrial arthropod fauna and its habitats in northern
 959 Maguerite Bay and Alexander Island, maritime Antarctic. *Antarctic Science.* 9, 12-26.
- 960 Convey, P. and Stevens, M. I. (2007) Antarctic biodiversity. *Science.* 317, 1877-1878.
- 961 Convey, P. and Worland, M. R. (2000) Survival of freezing by free-living Antarctic soil nematodes.
 962 *Cryo-Letters.* 21, 327-332.
- 963 Convey, P., Bindschadler, R., di Prisco, G., Fahrbach, E., Gutt, J., Hodgson, D. A., Mayewski, P. A.,
 964 Summerhayes, C.P. and Turner, J. (2009) Antarctic climate change and the environment.
 965 *Antarctic Science.* 21, 541-563.
- 966 Convey, P., Block, W. and Peat, H. J. (2003) Soil arthropods as indicators of water stress in Antarctic
 967 terrestrial habitats?. *Global Change Biology.* 9, 718-730.

- 968 Convey, P., Pugh, P. J. A., Jackson, C., Murray, A. W., Ruhland, C. T., Xiong, F. S. and Day, A.
 969 (2002) Response of Antarctic Terrestrial Microarthropods to Long-Term Climate
 970 Manipulations. *Ecology*. 83, 3130-3140.
- 971 Coulson, S. J., Hodkinson, I. D., Block, W., Webb, N. R. and Worland, M. R. (1995) Low summer
 972 temperatures: a potential mortality factor for high arctic soil microarthropods? *Journal of*
 973 *Insect Physiology*. 41, 783-792.
- 974 Coulson, S. J., Hodkinson, I. D., Strathdee, A., Bale, J. S., Block, W., Worland, M. R. and Webb, N.
 975 R. (1993) Simulated climate change: the interaction between vegetation type and microhabitat
 976 temperatures at Ny Ålesund, Svalbard. *Polar Biology*. 13, 67-70.
- 977 Crowe, J. H. (1972) Evaporative water loss by tardigrades under controlled relative humidities.
 978 *Biological Bulletin, Woods Hole*. 142, 407-416.
- 979 Crowe, J. H., Clegg, J. S. (Eds.), 1973. *Anhydrobiosis*. Dowden, Hutchinson and Ross, Stroudsburg,
 980 PA.
- 981 Crowe, J. H. and Madin, K. A. C. (1975) Anhydrobiosis in nematodes: Evaporative water loss and
 982 survival. *Journal of Experimental Zoology*. 193, 323-334.
- 983 Crowe, J. H., Carpenter, J. F. and Crowe, L. M. (1998) The role of vitrification in anhydrobiosis.
 984 *Annual Review of Physiology*. 60, 73-103.
- 985 Crowe, J.H., Oliver, A.E. and Tablin, F. (2002) Is there a single biochemical adaptation to
 986 anhydrobiosis? *Integrative and Comparative Biology*. 42, 497-503.
- 987 Czajka, M. C. and Lee, R. E. (1990) A rapid cold-hardening response protecting against cold shock
 988 injury in *Drosophila melanogaster*. *Journal of Experimental Biology*. 148, 245-254.
- 989 Danks, H. V. (2000) Dehydration in dormant insects. *Journal of Insect Physiology*. 46, 837-852.
- 990 Davies, P. and Sykes B. D. (1997) Antifreeze proteins. *Current Opinion in Structural Biology*. 7, 828-
 991 834.
- 992 Day, T. A., Ruhland, C. T., Strauss, S. L., Park, J., Krieg, M. L., Krna, M. A. and Bryant, D. M.
 993 (2009) Response of plants and the dominant microarthropod, *Cryptopygus antarcticus*, to
 994 warming and contrasting precipitation regimes in Antarctic tundra. *Global Change Biology*.
 995 15, 1640-1651.
- 996 Deere, J. A., Sinclair, B. J., Marshall, D. J. and Chown, S. L. (2006) Phenotypic plasticity of thermal
 997 tolerances in five oribatid mite species from sub-Antarctic Marion Island. *Journal of Insect*
 998 *Physiology*. 52, 693-700.
- 999 Denlinger, D. L. and Lee, R. E. (2010) Rapid cold-hardening: Ecological significance and
 1000 underpinning mechanisms. In: *Low Temperature Biology of Insects* (Ed. by D. L. Denlinger
 1001 and R. E. Lee), pp 35-58. Cambridge University Press.
- 1002 Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, David C. and
 1003 Martin, P. R. (2008) Impacts of climate warming on terrestrial ectotherms across latitude
 1004 Thermal Safety margin. *Proceedings of the National Academy of Sciences*. 105, 6668-6672.
- 1005 Duman, J. G. and Horwath, K. L. (1983) The role of haemolymph proteins in the cold tolerance of
 1006 insects. *Annual Review of Physiology*. 45, 261-270.
- 1007 Duman, J. G. Bennett, V., Sformo, T., Hochstrasser, R. and Barnes, B. M. (2004) Antifreeze proteins
 1008 in Alaskan insects and spiders. *Journal of Insect Physiology*. 50, 259-66.
- 1009 Eisenbeis, G. (1983) Kinetics of transpiration in soil arthropods. *New Trends in Soil Biology* (ed. by
 1010 P. Lebrun, H. M. andré, A. De Medts, C. Gregoire-Wibo and G. Wauthy), pp. 626-627. Dieu-
 1011 Brichart, Lovain-la-Neuve.
- 1012 Elnitsky, M. A., Benoit, J. B., Denlinger, D. L. and Lee, R. E. (2008b) Desiccation tolerance and
 1013 drought acclimation in the Antarctic collembolan *Cryptopygus antarcticus*. *Journal of Insect*
 1014 *Physiology*. 54, 1432-1439.

- 1015 Elnitsky, M. A., Benoit, J. B. Lopez-Martinez, G., Denlinger, D. L. and Lee, R. E. (2009)
 1016 Osmoregulation and salinity tolerance in the Antarctic midge, *Belgica antarctica*: seawater
 1017 exposure confers enhanced tolerance to freezing and dehydration. *Journal of Experimental*
 1018 *Biology*. 212, 2864-2871.
- 1019 Elnitsky, M. A., Hayward, S. A. L., Rinehart, J. P., Denlinger, D. L. and Lee, R. E. (2008a)
 1020 Cryoprotective dehydration and the resistance to inoculative freezing in the Antarctic midge,
 1021 *Belgica antarctica*. *Journal of Experimental Biology*. 211, 524-30.
- 1022 Everatt, M. J., Convey, P., Worland, M. R., Bale, J. S. and Hayward, S. A. L. (2013) Heat tolerance
 1023 and physiological plasticity in the Antarctic collembolan, *Cryptopygus antarcticus*, and the
 1024 mite, *Alaskozetes antarcticus*. *Journal of Thermal Biology*. 38, 264-271.
- 1025 Everatt, M. J., Worland, M. R., Bale, J. S., Convey, P. and Hayward, S. A. L. (2012) Pre-adapted to
 1026 the maritime Antarctic? – Rapid cold hardening of the midge, *Eretmoptera murphyi*. *Journal*
 1027 *of Insect Physiology*. 58, 1104-1111.
- 1028 Everatt, M. J., Worland, M. R., Convey, P., Bale, J. S. and Hayward, S. A. L. (2013) The impact of
 1029 salinity on survival and temperature tolerance of the Antarctic collembolan, *Cryptopygus*
 1030 *antarcticus*. *Physiological Entomology*. 38, 202-210.
- 1031 Feder, M. E., and Hofmann, G. E. (1999) Heat-shock proteins, molecular chaperones, and the stress
 1032 response: evolutionary and ecological physiology. *Annual Review of Physiology*. 61, 243-
 1033 282.
- 1034 Frenot, Y., Chown, S. L., Whinam, J., Selkirk, P. M., Convey, P., Skotnicki, M. and Bergstrom, D. M.
 1035 (2005) Biological invasions in the Antarctic: extent, impacts and implications. *Biological*
 1036 *reviews of the Cambridge Philosophical Society*. 80, 45-72.
- 1037 Gal, T. Z., Glazer, I. and Koltai, H. (2004) An LEA group 3 family member is involved in survival of
 1038 *C. elegans* during exposure to stress. *FEBS Letters*. 577, 21-26.
- 1039 Gladyshev, E. and Meselson, M. (2008) Extreme resistance of bdelloid rotifers to ionizing radiation.
 1040 *Proceedings of the National Academy of Sciences USA*. 105, 5139–5144.
- 1041 Goyal, K., Walton, L. J., Browne, J. A., Burnell, A. M. and Tunnacliffe, A. (2005) Molecular
 1042 anhydrobiosis: identifying molecules implicated in invertebrate anhydrobiosis. *Integrative and*
 1043 *Comparative Biology*. 45, 702-709.
- 1044 Gusev, O., Nakahara, Y., Vanyagina, V., Malutina, L., Cornette, R., Sakashita, T., Hamada, N.,
 1045 Kikawada, T., Kobayashi, Y. and Okuda, T. (2010) Anhydrobiosis-associated nuclear DNA
 1046 damage and repair in the sleeping chironomid: linkage with radioresistance. *PLoS One*. 5,
 1047 14008.
- 1048 Hawes, T. C., Bale, J. S., Worland, M. R. and Convey, P. (2007) Plasticity and superplasticity in the
 1049 acclimation potential of the Antarctic mite *Halozetes belgicae* (Michael). *The Journal of*
 1050 *Experimental Biology*. 210, 593.
- 1051 Hawes, T. C., Marshall, C. J. and Wharton, D. A. (2011) Antifreeze proteins in the Antarctic
 1052 springtail, *Gressittacantha terranova*. *Journal of Comparative Physiology. B, Biochemical,*
 1053 *Systemic, and Environmental Physiology*. 181, 713-719.
- 1054 Hayward, S. A. L., Worland, M.R., Bale, J. S. and Convey, P. (2000) Temperature and the
 1055 hygropreference of the Arctic Collembolan *Onychiurus arcticus* and mite *Lauroppia*
 1056 *translamellata*. *Physiological Entomology*. 25, 266-272.
- 1057 Hayward, S. A. L., Bale, J. S., Worland, M.R. and Convey, P. (2001) Influence of temperature on the
 1058 hygropreference of the Collembolan, *Cryptopygus antarcticus*, and the mite, *Alaskozetes*
 1059 *antarcticus* from the maritime Antarctic. *Journal of Insect Physiology*. 47. 11-18.
- 1060 Hayward, S. A. L., Worland, M.R., Convey, P. and Bale, J. S. (2003) Temperature preferences of the
 1061 mite *Alaskozetes antarcticus*, and the collembolan, *Cryptopygus antarcticus* from the
 1062 maritime Antarctic. *Physiological Entomology*. 28, 114-121.

- 1063 Hayward, S. A. L., Murray, P. A., Gracey, A. Y. and Cossins, A. R. (2007) Beyond the lipid
 1064 hypothesis: mechanisms underlying phenotypic plasticity in inducible cold tolerance.
 1065 Advances in Experimental Medicine and Biology. 594, 132-142
- 1066 Hayward, S. A. L., Rinehart, J. P., Sandro, L. H., Lee, R. E. and Denlinger, D. L. (2007) Slow
 1067 dehydration promotes desiccation and freeze tolerance in the Antarctic midge *Belgica*
 1068 *antarctica*. The Journal of Experimental Biology. 210, 836-844.
- 1069 Hayward, S. A. L., Manso, B. and Cossins, A. R. (2014) Molecular basis of chill resistance
 1070 adaptations in poikilothermic animals. Journal of Experimental Biology. 217, 6-15.
- 1071 Hazel, J. R. (1995) Thermal adaptation in biological membranes: is homeoviscous adaptation the
 1072 explanation?. Annual Review of Physiology. 57, 19-42.
- 1073 Hazell, S. P., Groutides, C., Neve, B. P., Blackburn, T. M. and Bale, J. S. (2010) A comparison of low
 1074 temperature tolerance traits between closely related aphids from the tropics, temperate zone,
 1075 and Arctic. Journal of Insect Physiology. 56, 115-122.
- 1076 Hengherr, S., Heyer, A. G., Köhler, H-R. and Schill, R. O. (2008) Trehalose and anhydrobiosis in
 1077 tardigrades--evidence for divergence in responses to dehydration. The FEBS Journal. 275,
 1078 281-88.
- 1079 Hengherr, S., Reuner, A., Brümmer, F. and Schill, R. O. (2010) Ice crystallization and freeze
 1080 tolerance in embryonic stages of the tardigrade *Milnesium tardigradum*. Comparative
 1081 Biochemistry and Physiology. Part A, Molecular & Integrative Physiology. 156, 151-155.
- 1082 Hengherr, S., Worland, M. R., Reuner, A., Brümmer, F. and Schill, R. O. (2009) High-temperature
 1083 tolerance in anhydrobiotic tardigrades is limited by glass transition. Physiological and
 1084 Biochemical Zoology. 82, 749-755.
- 1085 Hinton H. E. (1951) A new chironomid from Africa, the larvae of which can be dehydrated without
 1086 injury. Proceedings of the Zoological Society (Calcutta). 121, 371-380.
- 1087 Hinton, H. E. (1960) Cryptobiosis in the larva of *Polypedilum vanderplanki* Hint (Chironomidae).
 1088 Journal of Insect Physiology. 5, 286-300.
- 1089 Hodkinson, I. D., Coulson, S., Webb, N. R. and Block, W. (1996) Can High Arctic soil
 1090 microarthropods survive elevated summer temperatures?. Functional Ecology. 10, 314-321.
- 1091 Hodkinson, I. D., Webb, N. R., Bale, J. S. and Block, W. (1999) Hydrology, water availability and
 1092 tundra ecosystem function in a changing climate: the need for a closer integration of ideas?.
 1093 Global Change Biology. 5, 359-369.
- 1094 Hogg, I., Craigcary, S., Convey, P., Newsham, K., Odonnell, A., Adams, B., Aislabie, J., Frati, F.,
 1095 Stevens, M. and Wall, D. (2006) Biotic interactions in Antarctic terrestrial ecosystems: Are
 1096 they a factor?. Soil Biology and Biochemistry. 38, 3035-40.
- 1097 Holmstrup, M. (1992) Cold hardiness strategy in cocoons of the lumbricid earthworm *Dendrobaena*
 1098 *octaedra* (Savigny). Comparative Biochemistry and Physiology Part A: Physiology. 102A,
 1099 49-54.
- 1100 Holmstrup, M. and Sømme, L. (1998) Dehydration and cold hardiness in the Arctic Collembolan
 1101 *Onychiurus arcticus* Tullberg 1876. Journal of Comparative Physiology B: Biochemical,
 1102 Systemic, and Environmental Physiology. 168, 197-203.
- 1103 Holmstrup, M. and Zachariassen, K. E. (1996) Physiology of cold hardiness in earthworms.
 1104 Comparative Biochemistry and Physiology Part A: Physiology. 115, 91-101.
- 1105 Holmstrup, M., Costanzo, J. P. and Lee, R. E. (1999) Cryoprotective and osmotic responses to cold
 1106 acclimation and freezing in freeze-tolerant and freeze-intolerant earthworms. Journal of
 1107 Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology. 169,
 1108 207-214.

- 1109 Holmstrup, M., Hedlund, K. and Boriss, H. (2002) Drought acclimation and lipid composition in
 1110 *Folsomia candida*: implications for cold shock, heat shock and acute desiccation stress.
 1111 *Journal of Insect Physiology*. 48, 961-970.
- 1112 IPCC (2013) Summary for Policymakers. In: *Climate Change 2013: The Physical Science Basis*.
 1113 Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental
 1114 Panel on Climate Change [Stocker, T.F., D. Qin, G-K. Plattner, M. Tignor, S. K. Allen, J.
 1115 Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press,
 1116 Cambridge, United Kingdom and New York, NY, USA.
- 1117 Izumi, Y., Katagiri, C., Sonoda, S. and Tsumuki, H. (2009) Seasonal changes of phospholipids in last
 1118 instar larvae of rice stem borer *Chilo suppressalis* Walker (Lepidoptera: Pyralidae).
 1119 *Entomological Science*. 12, 376-381.
- 1120 Izumi, Y., Sonoda, S., Yoshida, H., Danks, H.V. and Tsumuki, H. (2006) Role of membrane transport
 1121 of water and glycerol in the freeze tolerance of the rice stem borer, *Chilo suppressalis* Walker
 1122 (Lepidoptera: Pyralidae). *Journal of Insect Physiology*. 52, 215–220.
- 1123 Jenks M. A. and Wood A. J. (2007) *Plant desiccation tolerance*. Ames, Iowa: Blackwell Publishing.
- 1124 Jonsson, K. I., Rabbow, E., Schill, R. O., Harms-Ringdahl, M. and Rettberg, P. (2008) Tardigrades
 1125 survive exposure to space in low Earth orbit. *Current Biology*. 18, 729–731.
- 1126 Kelty, J. D. and Lee, R. E. (1999) Induction of rapid cold hardening by cooling at ecologically
 1127 relevant rates in *Drosophila melanogaster*. *Journal of Insect Physiology*. 45, 719-26.
- 1128 Kelty, J. D. and Lee, R. E. (2001) Rapid cold-hardening of *Drosophila melanogaster* (Diptera:
 1129 Drosophilidae) during ecologically based thermoperiodic cycles. *The Journal of Experimental*
 1130 *Biology*, 204, 1659-1666.
- 1131 Kelty, J. D., Killian, K. A. and Lee, R. E. (1996) Cold shock and rapid cold- hardening of pharate
 1132 adult flesh flies (*Sarcophaga grassipalpis*) effects on behaviour and neuromuscular function
 1133 following eclosion. *Physiological Entomology*. 21, 283–288.
- 1134 Kikawada, T., Nakahara, Y., Kanamori, Y., Iwata, K-I, Watanabe, M., McGee, B., Tunnacliffe, A.
 1135 and Okuda, T. (2006) Dehydration-induced expression of LEA proteins in an anhydrobiotic
 1136 chironomid. *Biochemical and Biophysical Research Communications*. 348, 56-61.
- 1137 Kim, Y-S, Denlinger, D. L. and Smith, B. (2005) Spatial conditioning in the flesh fly, *Sarcophaga*
 1138 *crassipalpis*: disruption of learning by cold shock and protection by rapid cold hardening.
 1139 *Journal of Asia-Pacific Entomology*. 8, 345-351.
- 1140 Klok, C. J. and Chown, S. L. (1997) Critical thermal limits, temperature tolerance and water balance
 1141 of a sub-Antarctic caterpillar, *Pringleophaga marioni* (Lepidoptera: Tineidae). *Journal of*
 1142 *Insect Physiology*. 43, 685-694.
- 1143 Korenko, S., Pekar, S. and Honek, A. (2010) Predation activity of two winter-active spiders (Araneae:
 1144 Anyphaenidae, Philodromidae). *Journal of Thermal Biology*. 35, 112-116.
- 1145 Kostál, V. and Tollarová-Borovanská, M. (2009) The 70 kDa heat shock protein assists during the
 1146 repair of chilling injury in the insect, *Pyrrhocoris apterus*. *PLoS One*. 4, e4546.
- 1147 Kostál, V., Renault, D. and Rozsypal, J. (2011) Seasonal changes of free amino acids and thermal
 1148 hysteresis in overwintering heteropteran insect, *Pyrrhocoris apterus*. *Comparative*
 1149 *biochemistry and physiology. Part A, Molecular & integrative physiology*. 160, 245-251.
- 1150 Larsen, K. J. and Lee, R. E. (1994) Cold tolerance including rapid cold-hardening and inoculative
 1151 freezing in migrant monarch butterflies in Ohio. *Journal of Insect Physiology*. 40, 859-864.
- 1152 Lee, R. E., Chen, C. P. and Denlinger, D. L. (1987) A rapid cold-hardening process in insects. *Science*
 1153 (New York, N.Y.). 238, 1415-17.
- 1154 Lee, R. E., Costanzo, J. and Mugnano, J. (1996) Regulation of supercooling and ice nucleation in
 1155 insects. *European Journal of Entomology*. 93, 405-418.

- 1156 Lee, R. E., Damodaran, K., Yi, S-X. and Lorigan, G. A. (2006b) Rapid cold-hardening increases
1157 membrane fluidity and cold tolerance of insect cells. *Cryobiology*. 52, 459-463.
- 1158 Lee, R. E., Elnitsky, M. A., Rinehart, J. P. Hayward, S. A. L., Sandro, L. H. and Denlinger, D. L.
1159 (2006a) Rapid cold-hardening increases the freezing tolerance of the Antarctic midge *Belgica*
1160 *antarctica*. *The Journal of Experimental Biology*. 209, 399-406.
- 1161 Levin, D. B., Danks, H. V. and Barber, S. A. (2003) Variations in mitochondrial DNA and gene
1162 transcription in freezing-tolerant larvae of *Eurosta solidaginis* (Diptera: Tephritidae) and
1163 *Gynaephora groenlandica* (Lepidoptera: Lymantriidae). *Insect Molecular Biology*. 12, 281-
1164 289.
- 1165 Levis, N. A., Yi, S. and Lee, R. E. (2012) Mild desiccation rapidly increases freeze tolerance of the
1166 goldenrod gall fly, *Eurosta solidaginis*: evidence for drought-induced rapid cold-hardening.
1167 *The Journal of Experimental Biology*. 215, 3768-3773.
- 1168 Li, A., Benoit, J. B., Lopez-Martinez, G., Elnitsky, M. A., Lee, R. E. and Denlinger, D. L. (2009)
1169 Distinct contractile and cytoskeletal protein patterns in the Antarctic midge are elicited by
1170 desiccation and rehydration. *Proteomics*. 9, 2788-2798.
- 1171 Lopez-Martinez, G., Benoit, J. B., Rinehart, J. P., Elnitsky, M. A., Lee, R. E. and Denlinger, D. L.
1172 (2009) Dehydration, rehydration, and overhydration alter patterns of gene expression in the
1173 Antarctic midge, *Belgica antarctica*. *Journal of Comparative Physiology. B, Biochemical,*
1174 *Systemic, and Environmental Physiology*. 179, 481-491.
- 1175 Lopez-Martinez, G., Elnitsky, M. A., Benoit, J. B., Lee, R. E. and Denlinger, D. L. (2008) High
1176 resistance to oxidative damage in the Antarctic midge *Belgica antarctica*, and
1177 developmentally linked expression of genes encoding superoxide dismutase, catalase and heat
1178 shock proteins. *Insect Biochemistry and Molecular Biology*. 38, 796-804.
- 1179 MacMillan, H. A., Walsh, J. P. and Sinclair, J. B. (2009) The effects of selection for cold tolerance on
1180 cross-tolerance to other environmental stressors in *Drosophila melanogaster*. *Insect Science*.
1181 16, 263-276.
- 1182 Madin, K. A. C. and Crowe, J. H. (1975) Anhydrobiosis in nematode: Carbohydrate and lipid
1183 metabolism during dehydration. *Journal of Experimental Zoology*. 193, 335-342.
- 1184 Marshall, W. A. (1996) Biological particles over Antarctica. *Nature* 383, 680.
- 1185 Mazur, P. (1977) The role of intracellular freezing in the death of cells cooled at supraoptimal rates.
1186 *Cryobiology*. 14, 251-272.
- 1187 McDonald, J. R., Bale, J. S. and Walters, K. F. A. (1997) Rapid cold hardening in the western flower
1188 thrips *Frankliniella occidentalis*. *Journal of Insect Physiology*, 43, 759-766.
- 1189 McGaughan, A., Stevens, M. I. and Holland, B. R. (2010) Biogeography of circum-Antarctic
1190 springtails. *Molecular Phylogenetics and Evolution*. 57, 48-58.
- 1191 Mertens J., Beladjal, L., Alcantara, A., Fougnes, L., Van Der Straeten, D. and Clegg, J. S. (2008)
1192 Survival of dried eukaryotes (anhydrobiotes) after exposure to very high temperatures.
1193 *Biological Journal of the Linnean Society*. 93, 15-22.
- 1194 Michaud, M. R., Benoit, J. B., Lopez-Martinez, G., Elnitsky, M. A., Lee, R. E. and Denlinger, D. L.
1195 (2008). Metabolomics reveals unique and shared metabolic changes in response to heat shock,
1196 freezing, and desiccation in the Antarctic midge, *Belgica antarctica*. *Journal of Insect*
1197 *Physiology*. 54, 645-655.
- 1198 Miller, L. K. (1978) Freezing tolerance in relation to cooling rate in an adult insect. *Cryobiology*. 15,
1199 345-349.
- 1200 Montiel, P. O. (1998) Profiles of soluble carbohydrates and their adaptive role in maritime Antarctic
1201 terrestrial arthropods. *Polar Biology*. 19, 250-256.
- 1202 Muise, A. and Storey, K. (2001) Regulation of hexokinase in a freeze avoiding insect: role in the
1203 winter production of glycerol. *Archives of Insect Biochemistry and Physiology*. 47, 29-34.

- 1204 Müller, C., Sendler, M. and Hildebrandt, J. (2006). Downregulation of aquaporins 1 and 5 in nasal
 1205 gland by osmotic stress in ducklings, *Anas platyrhynchos*: Implications for the production of
 1206 hypertonic fluid. *Journal of Experimental Biology*. 209, 4067-4076.
- 1207 Murray, P. A., Hayward, S. A. L., Govan, G. G., Gracey, A. Y. and Cossins, A. R. (2007) Acquired
 1208 cold tolerance in *Caenorhabditis elegans*: and explicit test of the phospholipid saturation
 1209 hypothesis. *Proceedings of the National Academy of Sciences USA*. 104, 5489-5494
- 1210 Nicholas, W. L. (1984) *The Biology of Free Living Nematodes*. Clarendon Press, Oxford.
- 1211 Nielsen, S., Smith, B. L., Christensen, E. I., Knepper, M. A. and Agre, P. (1993). CHIP28 water
 1212 channels are localized in constitutively water-permeable segments of the nephron. *Journal of*
 1213 *Cell Biology*. 120, 317-383.
- 1214 Olsen, T. M., Sass, S. J., Li, N. and Duman, J. G. (1998) Factors contributing to seasonal increases in
 1215 inoculative freezing resistance in overwintering fire-coloured beetle larvae *Dendroides*
 1216 *cnadensis* (Pyrochroidae). *Journal of Experimental Biology*. 201, 1585-1594.
- 1217 Parkash, R., Rajpurohit, S. and Ramniwas, S. (2008a) Changes in body melanisation and desiccation
 1218 resistance in highland vs. lowland populations of *D. melanogaster*. *Journal of Insect*
 1219 *Physiology*. 54, 1050-1056.
- 1220 Parkash, R., Ramniwas, S., Rajpurohit, S. and Sharma, V. (2008b) Variations in body melanisation
 1221 impact desiccation resistance in *Drosophila immigrans* in Western Himalayas. *Journal of*
 1222 *Zoology*. 276, 219-227.
- 1223 Parkash, R., Sharma, V. and Kalra, B. (2009) Impact of body melanisation on desiccation resistance
 1224 in montane populations of *D. melanogaster*: Analysis of seasonal variation. *Journal of Insect*
 1225 *Physiology*. 55, 898-908.
- 1226 Parkash, R., Chahal, J., Sharma, V. and Dev, K. (2012) Adaptive associations between total body
 1227 color dimorphism and climatic stress related traits in a stenothermal circumtropical
 1228 *Drosophila* species. *Insect Science*. 19, 247-262.
- 1229 Pedersen, P. G. and Holmstrup, M. (2003) Freeze or dehydrate: only two options for the survival of
 1230 subzero temperatures in the arctic enchytraeid *Fridericia ratzeli*. *Journal of Comparative*
 1231 *Physiology. B, Biochemical, Systemic, and Environmental Physiology*. 173, 601-609.
- 1232 Pegg, D. E. (2001). The current status of tissue cryopreservation. *Cryo-letters*. 22, 105-114.
- 1233 Philip, B. N. and Lee, R. E. (2010) Changes in abundance of aquaporin-like proteins occurs
 1234 concomitantly with seasonal acquisition of freeze tolerance in the goldenrod gall fly, *Eurosta*
 1235 *solidaginis*. *Journal of Insect physiology*. 56, 679-685.
- 1236 Philip, B. N., Yi, S-X., Elnitsky, M. A. and Lee, R. E. (2008) Aquaporins play a role in desiccation
 1237 and freeze tolerance in larvae of the goldenrod gall fly, *Eurosta solidaginis*. *The Journal of*
 1238 *Experimental Biology*. 211, 1114-1119.
- 1239 Powell, S. J. and Bale, J. S. (2004) Cold shock injury and ecological costs of rapid cold hardening in
 1240 the grain aphid *Sitobion avenae* (Hemiptera: Aphididae). *Journal of Insect Physiology*. 50,
 1241 277-84.
- 1242 Powell, S. J. and Bale, J. S. (2005) Low temperature acclimated populations of the grain aphid
 1243 *Sitobion avenae* retain ability to rapidly cold harden with enhanced fitness. *The Journal of*
 1244 *Experimental Biology*. 208, 2615-20.
- 1245 Powell, S. J. and Bale, J. S. (2006) Effect of long-term and rapid cold hardening on the cold torpor
 1246 temperature of an aphid. *Physiological Entomology*. 31, 348-352.
- 1247 Preston, G. M., Carroll, T. P., Guggino, W. B. and Agre, P. (1992). Appearance of water channels in
 1248 *Xenopus* oocytes expressing red cell CHIP28 protein. *Science*. 256, 385-387.
- 1249 Ramløv, H. and Westh, P. (1992) Survival of the cryobiotic eutardigrade *Adorybiotus coronifer*
 1250 during cooling to -196°C: effect of cooling rate, trehalose level and short term acclimation.
 1251 *Cryobiology*. 19, 125-130.

- 1252 Ramniwas, S., Kajla, B., Dev, K. and Parkash, R. (2013) Direct and correlated responses to laboratory
 1253 selection for body melanisation in *Drosophila melanogaster*: support for the melanisation-
 1254 desiccation resistance hypothesis. *The Journal of Experimental Biology*. 216, 1244-1254.
- 1255 Reardon, W., Chakrabortee, S., Pereira, T. C., Tyson, T., Banton, M. C., Dolan, K. M., Culleton, B.
 1256 A., Wise, M. J., Burnell, A. M. and Tunnacliffe, A. (2010) Expression profiling and cross-
 1257 species RNA interference (RNAi) of desiccation- induced transcripts in the anhydrobiotic
 1258 nematode *Aphelenchus avenae*. *BMC Molecular Biology*. 11.
- 1259 Ricci, C., Caprioli, M., Boschetti, C. and Santo, N. *Macrotrachela quadricornifera* featured in a space
 1260 experiment. *Hydrobiologia*. 534, 239-244.
- 1261 Rinehart, J. P., Hayward, S. A. L., Elnitsky, M. A., Sandro, L. H., Lee, R. E. and Denlinger, D. L.
 1262 (2006) Continuous up-regulation of heat shock proteins in larvae, but not adults, of a polar
 1263 insect. *Proceedings of the National Academy of Sciences of the United States of America*.
 1264 103, 14223-14227.
- 1265 Rinehart, J. P., Li, A., Yocum, G. D., Robich, R. M., Hayward, S. A. L. and Denlinger, D. L. (2007).
 1266 Up-regulation of heat shock proteins is essential for cold survival during insect diapause.
 1267 *Proceedings of the National Academy of Sciences of the United States of America*. 104,
 1268 11130-11137.
- 1269 Ring, R. and Danks, H. (1994) Desiccation and cryoprotection: Overlapping adaptations. *Cryo-*
 1270 *Letters*. 15, 181-190.
- 1271 Ring, R. and Danks, H. (1998) The role of trehalose in cold-hardiness and desiccation. *Cryo-letters*.
 1272 19, 275-282.
- 1273 Rittmeyer, E. N., Allison, A., Gründler, M. C., Thompson, D. K. and Austin, C. C. (2012) Ecological
 1274 guild evolution and the discovery of the world's smallest vertebrate. *PloS ONE*. 7, e29797.
- 1275 Sakurai, M., Furuki, T., Akao, K., Tanaka, D., Nakahara, Y., Kikawada, T., Watanabe, M. and Okuda,
 1276 T. (2008) Vitrification is essential for anhydrobiosis in an African chironomid, *Polypedilum*
 1277 *vanderplanki*. *Proceedings of the National Academy of Sciences of the United States of*
 1278 *America*. 105, 5093-5098.
- 1279 Salt, R. W. (1963) Delayed inoculative freezing of insects. *Canadian Entomologist*. 95, 1190–1202.
- 1280 Sano, F., Asakawa, N., Inoue, Y. and Sakurai, M. (1999) A dual role for intracellular trehalose in the
 1281 resistance of yeast cells to water stress. *Cryobiology*. 39, 80–87.
- 1282 Schmidt-Nielsen, K. (1997) *Animal Physiology. Adaptation and Environment*. fifth ed. Cambridge
 1283 University Press, Cambridge 607 pp.
- 1284 Seki, K. and Toyoshima, M. (1988) Preserving tardigrades under pressure. *Nature*. 395, 853-854.
- 1285 Sformo, T., Walters, K., Jeannet, K., Wowk, B., Fahy, G. M., Barnes, B. M. and Duman, J. G. (2010)
 1286 Deep supercooling, vitrification and limited survival to -100{degrees}C in the Alaskan beetle
 1287 *Cucujus clavipes puniceus* (Coleoptera: Cucujidae) larvae. *The Journal of Experimental*
 1288 *Biology*. 213, 502-9.
- 1289 Shreve, S. M. (2004) Preservation of reproductive behaviors during modest cooling: rapid cold-
 1290 hardening fine-tunes organismal response. *Journal of Experimental Biology*. 207, 1797-1802.
- 1291 Shuker, K. P. N. (2001) *The Hidden Powers of Animals: Uncovering the Secrets of Nature*. London:
 1292 Marshall Editions Ltd. 240 p.
- 1293 Sinclair, B. J. and Sjørnsen, H. (2001). Cold tolerance of the Antarctic springtail *Gomphiocephalus*
 1294 *hodgsoni* (Collembola, Hypogastruridae). *Antarctic Science*. 13, 271-279.
- 1295 Sinclair, B. J., Nelson, S., Nilson, T. L., Roberts, S. P. and Gibbs, A. G. (2007) The effect of selection
 1296 for desiccation resistance on cold tolerance of *Drosophila melanogaster*. *Physiological*
 1297 *Entomology*. 32, 322-327.

- 1298 Sinclair, B. J., Terblanche, J. S. and Scott, M. B. (2006) Environmental physiology of three species of
 1299 springtail at Cape Hallett, North Victoria Land, Antarctica. *Journal of Insect Physiology*. 52,
 1300 29-50.
- 1301 Sinclair, B. J., Vernon, P., Klok, C. J. and Chown, S. L. (2003) Insects at low temperatures: an
 1302 ecological perspective. *Trends in Ecology and Evolution*. 18, 257-262.
- 1303 Sjørnsen, H. and Sømme, L. (2000) Seasonal changes in tolerance to cold and desiccation in
 1304 *Phauloppia* sp. (Acari, Oribatida) from Finse, Norway. *Journal of Insect Physiology*. 46,
 1305 1387-1396.
- 1306 Slabber, S., Worland, M. R., Leinaas, H. P. and Chown, S. L. (2007) Acclimation effects on thermal
 1307 tolerances of springtails from sub-Antarctic Marion Island: indigenous and invasive species.
 1308 *Journal of Insect Physiology*. 53, 113-125.
- 1309 Slama, K. (1988) A new look at insect respiration. *Biological Bulletin*. 175, 289-300.
- 1310 Smith, T., Wharton, D. A. and Marshal, C. J. (2008) Cold tolerance of an Antarctic nematode that
 1311 survives intracellular freezing: comparisons with other nematode species. *Journal of*
 1312 *comparative physiology. B, Biochemical, systemic, and environmental physiology*. 178, 93-
 1313 100.
- 1314 Sømme, L. (1982) Supercooling and winter survival in terrestrial arthropods. *Comparative*
 1315 *Biochemistry and Physiology A*. 73, 519-543.
- 1316 Sømme, L. (1986) Ecology of *Cryptopygus sverdrupi* (Insecta: Collembola) from Dronning Maud
 1317 Land, Antarctica. *Polar Biology*. 6, 179-184.
- 1318 Sømme, L. (1996) Anhydrobiosis and cold tolerance in tardigrades. *European Journal of Entomology*.
 1319 93, 349-357.
- 1320 Sømme, L. and Block, W. (1982) Cold hardiness of Collembola at Signy Island, maritime Antarctic.
 1321 *Oikos*. 38, 168-176.
- 1322 Sømme, L. and Meier, T. (1995) Cold hardiness of Tardigrada from Dronning Maud Land,
 1323 Antarctica. *Polar Biology*. 15, 221-224.
- 1324 Sørensen, J. G. and Holmstrup, M. (2011) Cryoprotective dehydration is widespread in Arctic
 1325 springtails. *Journal of Insect Physiology*. 57, 1147-1153.
- 1326 Sørensen, J. G., Heckmann, L.-H. and Holmstrup, M. (2010) Temporal gene expression profiles in a
 1327 palaeartic springtail as induced by desiccation, cold exposure and during recovery.
 1328 *Functional Ecology*. 24, 838-846.
- 1329 Spaul, V. W. (1973) Qualitative and quantitative distribution of soil nematodes of Signy Island,
 1330 South Orkney Islands. *British Antarctic Survey Bulletin*. 34, 177-184.
- 1331 Speight, M. R., Hunter, M. D. and Watt, A. D. (2008) Insects and climate. In: *Ecology of Insects*
 1332 *Concepts and Applications* (ed. by M. R. Speight, M. D. Hunter and A. D. Watt), pp 33-60.
 1333 Wiley-Blackwell.
- 1334 Storey, K. B. and Storey, J. M. (1988) Freeze tolerance in animals. *Physiological Reviews*. 68, 27-84.
- 1335 Strange, R. E. and Dark, F. A. (1962) Effect of chilling on *Aerobacter aerogenes* in aqueous
 1336 suspension. *Journal of General Microbiology*. 29, 719-730.
- 1337 Strathdee, A. T. and Bale, J. S. (1998) Life on the edge: insect ecology in arctic environments. *Annual*
 1338 *Review of Entomology*. 43, 85-106.
- 1339 Tammariello, S. P., Rinehart, J. P. and Denlinger, D. L. (1999) Desiccation elicits heat shock protein
 1340 transcription in the flesh fly, *Sarcophaga crassipalpis*, but does not enhance tolerance to high
 1341 or low temperatures. *Journal of Insect Physiology*. 45, 933-938.
- 1342 Tang, X. and Pikal, M. J. (2005) The effects of stabilizers and denaturants on the cold denaturation
 1343 temperatures of proteins and implications for freeze-drying. *Pharmacological Research*. 22,
 1344 1167-1175.

- 1345 Teets, N. M., Elnitsky, M. A., Benoit, J. B., Lopez-Martinez, G., Denlinger, D. L. and Lee, R. E.
 1346 (2008) Rapid cold-hardening in larvae of the Antarctic midge *Belgica antarctica*: cellular
 1347 cold-sensing and a role for calcium. *American Journal of Physiology. Regulatory, Integrative*
 1348 *and Comparative Physiology*. 294, 1938-1946.
- 1349 Teets, N. M., Peyton, J. T., Colinet, H., Renault, D., Kelley, J. L., Kawarasaki, Y., Lee, R. E. and
 1350 Denlinger, D. L. (2012) Gene expression changes governing extreme dehydration tolerance in
 1351 an Antarctic insect. *Proceedings of the National Academy of Sciences*. 109, 20744-20709.
- 1352 Timmermans, M. J. T. N., Roelofs, D., Nota, B., Ylstra, B. and Holmstrup, M. (2009) Sugar sweet
 1353 springtails: on the transcriptional response of *Folsomia candida* (Collembola) to desiccation
 1354 stress. *Insect Molecular Biology*. 18, 737-746.
- 1355 Treonis, A. M., Wall, D. H., Virginia, R. A. (2000) The use of anhydrobiosis by soil nematodes in the
 1356 Antarctic Dry Valley. *Functional Ecology*. 14, 460-467.
- 1357 Tunnacliffe, A. and Lapinski, J. (2003). Resurrecting Van Leeuwenhoek's rotifers: a reappraisal of
 1358 the role of disaccharides in anhydrobiosis. *Philosophical Transactions of the Royal Society*
 1359 *London Series B*. 358, 1755-1771.
- 1360 Tunnacliffe, A., Lapinski, J. and McGee, B. (2005) A putative LEA protein, but no trehalose, is
 1361 present in anhydrobiotic rotifers. *Hydrobiologia*. 546, 315-321.
- 1362 Turner, J., Bindschadler, R., Convey, P., Di Prisco, G., Fahrbach, E., Gutt, J., Hodgson, D. A.,
 1363 Mayewski, P. A. and Summerhayes, C. P. eds. (2009). *Antarctic climate change and the*
 1364 *environment*. Cambridge: Scientific Committee for Antarctic Research, 554 pp.
- 1365 Waller, C. L., Worland, M. R., Convey, P. and Barnes, D. K. A. (2006) Ecophysiological strategies of
 1366 Antarctic intertidal invertebrates faced with freezing stress. *Polar Biology*. 29, 1077-1083.
- 1367 Walters, K. R., Serianni, A. S., Voituron, Y., Sformo, T., Barnes, B. M. and Duman, J. G. (2011) A
 1368 thermal hysteresis-producing xylomannan glycolipid antifreeze associated with cold tolerance
 1369 is found in diverse taxa. *Journal of Comparative Physiology. B, Biochemical, Systemic, and*
 1370 *Environmental Physiology*. 181, 631-640.
- 1371 Walther, G., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., Fromentin, J. I., Ove,
 1372 H. and Bairlein, F. (2002) Ecological responses to recent climate change. *Nature*. 416, 389-
 1373 395.
- 1374 Wang, X-H. and Kang, L. (2003) Rapid cold hardening in young hoppers of the migratory locust
 1375 *Locusta migratoria* L. (Orthoptera: Acridiidae). *Cryo-letters*. 24, 331-40.
- 1376 Watanabe, M. (2006) Anhydrobiosis in invertebrates. *Applied Entomology and Zoology*. 41, 15-31.
- 1377 Watanabe, M., Kikawada, T., Minagawa, N., Yukuhiro, F. and Okuda, T. (2002) Mechanism allowing
 1378 an insect to survive complete dehydration and extreme temperatures. *Journal of Experimental*
 1379 *Biology*. 205, 2799-2802.
- 1380 Watanabe, M., Kikawada, T. and Okuda, T. (2003) Increase of internal ion concentration triggers
 1381 trehalose synthesis associated with cryptobiosis in larvae of *Polypedilum vanderplanki*.
 1382 *Journal of Experimental Biology*. 206, 2281-2286.
- 1383 Watanabe, M., Nakahara, Y., Sakashita, T., Kikawada, T., Fujita, A., Hamada, N., Horikawa, D. D.,
 1384 Wada, S., Kobayashi, Y. And Okuda, T. (2007) Physiological changes leading to
 1385 anhydrobiosis improve radiation tolerance in *Polypedilum vanderplanki* larvae. *Journal of*
 1386 *Insect Physiology*. 53, 573-579.
- 1387 Wharton, D. A. (2003b) The environmental physiology of Antarctic terrestrial nematodes: a review.
 1388 173, 621-628.
- 1389 Wharton, D. A. (2011a) Anhydrobiosis: The model worm as the model? *Current Biology*. 21, R578-
 1390 R579.
- 1391 Wharton, D.A. (2011b) Cold tolerance, In: *Molecular and Physiological Basis of Nematode Survival*
 1392 (Ed. by R.N. Perry and D.A. Wharton), pp. 182-204. CABI Publishing, Wallingford.

- 1393 Wharton, D. A. and Ferns, D. J. (1995) Survival of intracellular freezing by the Antarctic nematode
1394 *Panagrolaimus davidi*. Journal of Experimental Biology. 198, 1381-1387.
- 1395 Wharton, D. A. and Barclay, S. (1993) Anhydrobiosis in the free-living Antarctic nematode
1396 *Panagrolaimus davidi*. Fundamental and Applied Nematology. 16, 17–22.
- 1397 Wharton, D. A. and Worland, M. R. (2001) Water relations during desiccation of cysts of the potato-
1398 cyst nematode *Globodera rostochiensis*. Journal of Comparative Physiology - B,
1399 Biochemical, Systemic, & Environmental Physiology. 171, 121-126.
- 1400 Wharton, D. A., Goodall, G. and Marshall, C. J. (2003a) Freezing survival and cryoprotective
1401 dehydration as cold tolerance mechanisms in the Antarctic nematode *Panagrolaimus davidi*.
1402 Journal of Experimental Biology. 206, 215-221.
- 1403 Williams, J. B. and Lee, R. E. (2008) Differences in cold tolerance, desiccation resistance, and
1404 cryoprotectant production between three populations of *Eurosta solidaginis* collected from
1405 different latitudes. Journal of comparative physiology. B, Biochemical, systemic, and
1406 environmental physiology. 178, 365-375.
- 1407 Williams, J. B. and Lee, R. E. (2011) Effect of freezing and dehydration on ion and cryoprotectant
1408 distribution and hemolymph volume in the goldenrod gall fly, *Eurosta solidaginis*. Journal of
1409 Insect Physiology. 57, 1163-1169.
- 1410 Wolkers, W. F., Walker, N. J., Tablin, F. and Crowe, J. H. (2001) Human platelets loaded with
1411 trehalose survive freeze-drying. Cryobiology. 4279-4287.
- 1412 Worland, M. R. (1996) The relationship between water content and cold tolerance in the arctic
1413 collembolan *Onychiurus arcticus* (Collembola: Onychiuridae). European Journal of
1414 Entomology. 93, 341-348.
- 1415 Worland, M. R. (2010) *Eretmoptera murphyi*: pre-adapted to survive a colder climate. Physiological
1416 Entomology. 29, 127-137.
- 1417 Worland, M. R. and Block, W. (1999) Ice-nucleating bacteria from the guts of two sub-antarctic
1418 beetles, *Hydromedion sparsutum* and *Perimylops antarcticus* (Perimylopidae). Cryobiology.
1419 38, 60-67.
- 1420 Worland, R. and Block, W. (2003) Desiccation stress at sub-zero temperatures in polar terrestrial
1421 arthropods. Journal of Insect Physiology. 49, 193-203.
- 1422 Worland, M. R. and Convey, P. (2001) Rapid cold hardening in Antarctic microarthropods.
1423 Functional Ecology. 15, 515-24.
- 1424 Worland, M. R. and Convey, P. (2008) The significance of the moult cycle to cold tolerance in the
1425 Antarctic collembolan *Cryptopygus antarcticus*. Journal of Insect Physiology. 54, 1281-85.
- 1426 Worland, M. R. and Lukešová, A. (2000) The effect of feeding on specific soil algae on the cold-
1427 hardiness of two Antarctic micro-arthropods (*Alaskozetes antarcticus* and *Cryptopygus*
1428 *antarcticus*). Polar Biology. 23, 766-74.
- 1429 Worland, M., Block, W. and Grubor-Lajsic, G. O. (2000) Survival of *Heleomyza borealis* (Diptera,
1430 Heleomyzidae) larvae down to -60°C. Physiological Entomology. 25, 1-5.
- 1431 Worland, M. R., Block, W., and Rothery, P. (1992) Survival of sub-zero temperatures by South
1432 Georgian beetles (Coleoptera, Perimylopidae). Polar Biology. 11, 607–613.
- 1433 Worland, M. R., Block, W., and Rothery, P. (1993) Ice nucleation studies of two beetles from sub-
1434 Antarctic South Georgia. Polar Biology. 13, 105–112.
- 1435 Worland, M. R., Grubor-Lajsic, G. and Montiel, P. (1998). Partial desiccation induced by sub-zero
1436 temperatures as a component of the survival strategy of the Arctic collembolan *Onychiurus*
1437 *arcticus* (Tullberg). Journal of Insect Physiology. 44, 211-219.
- 1438 Worland, M. R., Hawes, T. C. and Bale, J. S. (2007) Temporal resolution of cold acclimation and de-
1439 acclimation in the Antarctic collembolan, *Cryptopygus antarcticus*. Physiological
1440 Entomology. 32, 233-239.

- 1441 Wright, J. C. (1989) Desiccation tolerance and water-retentive mechanisms in tardigrades. *Journal of*
 1442 *Experimental Biology*. 142, 267-292.
- 1443 Yi, S-X and Lee, R. E. (2011) Rapid cold-hardening blocks cold-induced apoptosis by inhibiting the
 1444 activation of pro-caspases in the flesh fly *Sarcophaga crassipalpis*. *Apoptosis*. 16, 249-255.
- 1445 Yi, S-X., Moore, C. W. and Lee, R. E. (2007) Rapid cold-hardening protects *Drosophila*
 1446 *melanogaster* from cold-induced apoptosis. *Apoptosis: an International Journal on*
 1447 *Programmed Cell Death*. 12, 1183-93.
- 1448 Yocum, G. D. (2001) Differential expression of two HSP70 transcripts in response to cold shock,
 1449 thermoperiod, and adult diapause in the Colorado potato beetle. *Journal of Insect Physiology*.
 1450 47, 1139–1145
- 1451 Yoder, J. A. Benoit, J. B., Denlinger, D. L. and Rivers, D. B. (2006) Stress-induced accumulation of
 1452 glycerol in the flesh fly, *Sarcophaga bullata*: evidence indicating anti-desiccant and
 1453 cryoprotectant functions of this polyol and a role for the brain in coordinating the response.
 1454 *Journal of Insect Physiology*. 52, 202-214.
- 1455 Zachariassen, K. E. (1980) The role of polyols and nucleating agents in cold-hardy beetles. *Journal of*
 1456 *Comparative Physiology*. 140, 227–234.
- 1457 Zachariassen, K. E. (1985) Physiology of cold tolerance in insects. *Physiological Reviews*. 65, 799-
 1458 832.
- 1459 Zachariassen, K. E. and Kristiansen, E. (2000). Ice nucleation and antinucleation in nature.
 1460 *Cryobiology*. 41, 257-279.

1461

1462 **Highlights**

- 1463 - Invertebrates are vulnerable to extremes of temperature and low water availability.
- 1464 - They express a suite of features in response to each stressor individually.
- 1465 - However, stresses usually occur simultaneously in nature.
- 1466 - Cross-tolerance is observed between temperature and water stress.
- 1467 - This is discussed in light of climate warming and the invertebrates' ecology.

1468

1469 **Figure Captions**

1470

1471 **Figure 1** Schematic representation of the molecular and physiological processes underpinning
 1472 chilling, Freeze Tolerance (FT) and Freeze Avoidance (FA) strategies in terrestrial invertebrates.
 1473 Chilling/cold stress responses can be induced in parallel or more sequentially - the order in which
 1474 temperature induces each mechanism will be species specific and potentially vary depending on the
 1475 rate of temperature change. Common chilling responses (light grey) are shared by both FT and FA
 1476 strategies to limit chilling injury. Fundamental differences between FT (white) and FA (dark grey)
 1477 include the synthesis of ice nucleating agents (INAs) in FT (white) vs. INA removal in FA. FT
 1478 insects also uniquely produce osmoprotectants to control cellular dehydration during extracellular
 1479 freezing. Both strategies employ cryoprotectants (e.g. glycerol and trehalose) and antifreeze proteins
 1480 (AFPs); and can potentially undergo vitrification. For FT species these strategies facilitate controlled
 1481 freezing and limiting freezing damage, while in FA species these adaptations enhance the supercooled
 1482 state/reduce the risk of ice-crystal formation. See main text for details of relevant studies. Adapted
 1483 from Bale (2002).

1484

1485 **Figure 2** Schematic representation of the molecular and physiological processes underpinning
1486 Desiccation Tolerance (DT) and Desiccation Resistance (DR). The three categories of desiccation
1487 resistance are Mesic (highly resistant to water loss), Transitional (intermediate resistance) and Hygric
1488 (limited desiccation resistance). At polar latitudes in particular, limited desiccation resistance
1489 necessitates increased desiccation tolerance. The purpose of DR mechanisms (white) is to reduce
1490 water loss. The purpose of DT mechanisms (dark grey) is to limit desiccation-induced damage.
1491 Some strategies are used by both DR and DT (light grey). See main text for further details.
1492

Accepted manuscript



