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

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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.


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

### 1.2. Polar climate

In few places are invertebrates more directly impacted by their environment than in the Arctic tundra (Strathdee and Bale, 1998) or the fellfields of the Antarctic (Block et al., 2009; Hogg et al., 2006). Air temperatures regularly fall below −10 °C during the winter in the maritime Antarctic and, in regions such as the continental Antarctic and High Arctic, frequently drop below −40 °C (Block et al., 2009; Convey, 2013; Sformo et al., 2010; Strathdee and Bale, 1998). Invertebrates buffer these temperatures behaviourally to some extent (Hayward et al., 2003) by moving beneath the snow, within the soil profile, or into cryptogams like mosses, lichen and algae (Bengtson et al., 1974; Burn, 1986; Convey, 1996a, 1996b; Convey and Smith, 1997; Spaull, 1973). However, even within these microhabitats, they can still be subjected to sub-zero temperatures on a daily basis throughout the winter (Davey et al., 1992; Block et al., 2009; Strathdee and Bale, 1998). Microhabitat temperatures during the summer are also very low and rarely rise above 5 °C in the maritime and continental Antarctic, and slightly higher in the High Arctic (Block et al., 2009; Coulson et al., 1993; Strathdee and Bale, 1998).

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The availability of liquid water also presents an important challenge. During the winter, water is locked up as snow and ice where it is inaccessible (Block et al., 2009) while, in summer, streams, lakes and rock pools, which form from melted ice and snow in spring, evaporate, resulting in drought (Convey et al., 2003). Again, behavioural responses can help reduce desiccation stress (Hayward et al., 2000, 2001). However, because access to moisture is so restricted in both space and time at polar latitudes, physiological responses play a dominant role in determining species survival.

### 1.3. Overview

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

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

## 2. Responses to low temperature

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

### 2.1. Freeze-tolerance

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

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

### 2.2. Freeze-avoidance

In contrast to freeze-tolerant species, invertebrates which are freeze-avoiding are unable to withstand any internal ice formation (Bale, 1996; Cannon and Block, 1988; Storey and Storey, 1988; Zachariassen, 1985). While seemingly disadvantageous in an environment which experiences temperatures close to an invertebrate's SCP, these invertebrates avoid the dangers of both extracellular ice formation and subsequent cellular dehydration that occur in freeze-tolerant species. Freeze-avoiding invertebrates range from Alaska (e.g., the red flat bark beetle, *Cucujus clavipes puniceus* (Sformo et al., 2010)) and the High Arctic (e.g., the mite, *Diapterobates notatus* (Coulson et al., 1995)) to the Antarctic continent (e.g., *Cryptopygus antarcticus* (Block and Worland, 2001; Cannon and Block, 1988)), and outnumber freeze-tolerant species in almost all cases. Freeze avoiding invertebrates can be separated into several different categories to better define them ecologically and physiologically. These include, for instance, true freeze-avoiding (lower lethal temperature [LLT]=SCP), chill tolerant (show minimal pre-freeze mortality), chill susceptible (die well above their SCPs) and opportunistic survival (unable to survive below their developmental threshold) (see Bale, 1993).