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REVIEW

Surviving in a frozen desert: environmental stress physiology of terrestrial Antarctic arthropods

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ABSTRACT

Abiotic stress is one of the primary constraints limiting the range and success of arthropods, and nowhere is this more apparent than Antarctica. Antarctic arthropods have evolved a suite of adaptations to cope with extremes in temperature and water availability. Here, we review the current state of knowledge regarding the environmental physiology of terrestrial arthropods in Antarctica. To survive low temperatures, mites and Collembola are freeze-intolerant and rely on deep supercooling, in some cases supercooling below –30°C. Also, some of these microarthropods are capable of cryoprotective dehydration to extend their supercooling capacity and reduce the risk of freezing. In contrast, the two best-studied Antarctic insects, the midges *Belgica antarctica* and *Eretmoptera murphyi*, are freeze-tolerant year-round and rely on both seasonal and rapid cold-hardening to cope with decreases in temperature. A common theme among Antarctic arthropods is extreme tolerance of dehydration; some accomplish this by cuticular mechanisms to minimize water loss across their cuticle, while a majority have highly permeable cuticles but tolerate upwards of 50–70% loss of body water. Molecular studies of Antarctic arthropod stress physiology are still in their infancy, but several recent studies are beginning to shed light on the underlying mechanisms that govern extreme stress tolerance. Some common themes that are emerging include the importance of cuticular and cytoskeletal rearrangements, heat shock proteins, metabolic restructuring and cell recycling pathways as key mediators of cold and water stress in the Antarctic.

KEY WORDS: Antarctica, Cold tolerance, Dehydration, Environmental stress, Physiology

Introduction

Environmental stress, in the form of both biotic and abiotic stress, is one of the primary constraints governing the abundance and distribution of terrestrial arthropods. While insects on all continents encounter some form of environmental stress, perhaps nowhere are the environmental onslaughts more challenging than the continent of Antarctica. Even in maritime Antarctica, where proximity to the sea limits temperature extremes, winter lows exceed –40°C and subzero temperatures can be experienced any time of year (Baust and Lee, 1981). Furthermore, water is frozen and biologically unavailable for much of the year, thus water availability is perhaps the biggest challenge confronting terrestrial arthropods in Antarctica (Kennedy, 1993). Whereas arthropods are the predominant terrestrial life form on Earth, only a handful of species have successfully established in Antarctica, and most of these are restricted to maritime regions (Convey, 1996a; Convey, 2013).

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In this review, we summarize the limits and mechanisms of environmental stress tolerance in Antarctic arthropods. In particular, we focus on the biochemical and molecular underpinnings of the stress response in Antarctic arthropods. As non-model (not to mention difficult to access) species, molecular studies of Antarctic arthropods have been limited in scope. However, recent advances in molecular biology, particularly the 'omics' revolution, have launched Antarctic research into a new era. In the final section, we provide a few suggestions for future directions, in particular the need to continue searching for the unique molecular characteristics that distinguish Antarctic arthropods from their temperate counterparts.

Overview of Antarctic arthropods

The predominant arthropods in Antarctica are soil microarthropods, dominated by mites and collembolans (Convey, 1996a; Convey, 2013). Acari is the most diverse taxon on the continent, represented by over 100 species (Marshall and Pugh, 1996), of which roughly half are parasitic ticks and mites. Nearly all free-living species are endemic and seem to have established prior to the last glacial maximum (Mortimer et al., 2011). Maritime and continental Antarctica are home to 15 species of Collembola, again most of which are endemic to the continent (Greenslade, 1995). Perhaps the most glaring aspect of Antarctica's terrestrial fauna is its lack of true insects; true insects native to Antarctica consist of two chironomid species, the midges *Belgica antarctica* and *Parochlus steinenii* (Convey and Block, 1996). As *P. stenenii* is also found in southern South America, *B. antarctica* is considered the only insect endemic to Antarctica. A third dipteran species, *Eretmoptera murphyi* [now considered by some to be a member of the genus *Belgica* (see Allegrucci et al., 2012)], was accidentally introduced to Signy Island in maritime Antarctica from subantarctic South Georgia in the 1960s (Convey, 1992). Despite the lack of species diversity in Antarctica, local abundance can be very high. For example, a mass of over 2 million collembolan eggs (from *Cryptopygus antarcticus* and *Friesea grisea*) was found under a single rock on the Antarctic Peninsula in an area less than 0.1 m^2 (Schulte et al., 2008).

Arthropods are generally concentrated in maritime environments, but there are some taxa of microfauna that, despite low overall species diversity, seem to flourish in harsh inland environments. For example, tardigrades, members of a closely related phylum basal to arthropods (Telford et al., 2008), are found in high abundance in ice-free areas throughout Antarctica, even in continental regions inhospitable to arthropods (Convey and McInnes, 2005). Additionally, a number of nematode species are found in both maritime and continental Antarctica, all of which appear to be endemic to Antarctica (Convey et al., 2008); the true species diversity of this group may be substantially underestimated (Nielsen et al., 2011). However, the physiological adaptations of these non-arthropod microfauna are beyond the scope of this review and will only be discussed when there is clear overlap with arthropod adaptations.

The Journal of Experimental Biology

Basic ecophysiological characteristics of Antarctic arthropods

While life history adaptations of Antarctic invertebrates have been reviewed extensively (Convey, 1996a; Convey, 2010), they are worth mentioning here. Perhaps the most conspicuous life history adaptation of Antarctic arthropods is lifespan extension. The low temperatures of Antarctica lead to extremely short growing seasons, so in many cases Antarctic arthropods take multiple years to complete their life cycle (Convey, 1996a). For example, the Antarctic collembolan *C. antarcticus* takes an estimated 3–7 years to complete its life cycle (Burn, 1981), whereas temperate collembolans typically complete multiple generations per year. Similarly, the Antarctic midge *B. antarctica* has a 2-year life cycle, which is rare among the chironomids (Convey and Block, 1996). In conjunction with long life cycles, Antarctic arthropods typically lack diapause and instead rely on quiescence to endure unfavorable periods (Convey, 1996b). This allows Antarctic species to take advantage of the extremely short, unpredictable growing seasons. However, the capacity for diapause has only been examined in non-insect taxa, which typically do not exhibit diapause, even in temperate regions.

Aside from prolonged, flexible lifestyles, there are a couple other noteworthy features of Antarctic arthropods. While most representative species are from apterous taxa (i.e. mites and collembolans), of the three chironomid species on the continent, two (*B. antarctica* and *E. murphyi*) are secondarily brachypterous (Convey and Block, 1996). Brachyptery is a common adaptation in isolated, windswept regions, and serves to prevent accidental dispersal into unfavorable environments and to conserve heat by reducing the surface area to volume ratio. Because environmental stressors are considered the primary selective pressure in Antarctica, there is thought to be little inter- and intra-specific competition for resources (Hogg et al., 2006). A majority of Antarctic arthropods are generalist herbivores and detritivores (Davis, 1981), thriving on sporadic, inconsistent nutrient sources. A final noteworthy attribute of Antarctic arthropod life histories is the large investment of energy towards environmental stress protection. Many stress mechanisms (see below for examples) are energetically costly, thus diverting energy from growth and reproduction. For example, populations of the Antarctic mite *Alaskozetes antarcticus* from mild subantarctic climates are able to divert significantly more energy to reproduction than populations in maritime Antarctica, presumably because of differential resource allocation towards environmental stress tolerance (Convey, 1998). However, for a majority of Antarctic species, the fitness costs of elevated stress tolerance have not been quantified.

Environmental stress tolerance of Antarctic arthropods

As mentioned above, mechanisms of stress tolerance are a major physiological cost for Antarctic arthropods. Also, Antarctic arthropods are excellent models for the evolutionary physiology of stress tolerance, because so few species have become successfully established on the continent. Thus, the basic mechanisms of stress tolerance have been extensively studied in Antarctic arthropods, and recent studies have begun to unravel the molecular mechanisms of extreme stress tolerance. In this section, we review the basic strategies and limits of stress tolerance in Antarctic arthropods. Rather than comprehensively reviewing every study, we will provide specific examples and general principles from each of the predominant arthropod taxa on the continent (i.e. Acari, Collembola and Diptera).

With low temperature being the most conspicuous feature of Antarctic environments, the cold tolerance of Antarctic arthropods has been extensively studied. The most primitive group, ticks and mites (Order Acari), are all freeze-avoiding (Sømme, 1981) and rely on supercooling to survive subzero temperatures. For example, the Antarctic mite *Alaskozetes arcticus* has a winter supercooling point around −30°C, which is accomplished in part by accumulation of glycerol and removal of ice-nucleating particles from the gut (Young and Block, 1980). Another mite, *Styerotydeus mollis*, has a supercooling point around −20°C in early summer that increases over the course of the summer, likely because of feeding (Sjursen and Sinclair, 2002). While overwintering supercooling points have not been measured, they are assumed to be much lower than −20°C, as winter microhabitat temperatures can reach −40°C (Sinclair and Sjursen, 2001). The seabird tick *Ixodes uriae* has a supercooling capacity similar to that of mites (approximately –30°C), but can also tolerate high temperatures (>25°C), perhaps to permit survival and activity both on- and off-host (Lee and Baust, 1987). In addition to seasonal regulation of supercooling points, summer-acclimated mites (*A. antarcticus* and *Halozetes belgicae*) are capable of rapid cold-hardening (RCH; Fig. 1) (Worland and Convey, 2001), an acclimation response in which brief (minutes to hours) exposure to chilling significantly enhances cold tolerance (Lee et al., 1987).

Like mites, all Antarctic collembolans studied thus far are freeze-avoiding. Generally, collembolans have extensive supercooling capacities, with supercooling points around −30°C in some species (Cannon and Block, 1988; Worland and Convey, 2001; Worland and Block, 2003). Extensive supercooling in this group is achieved by a combination of extremely high hemolymph osmolality (Sinclair and Sjursen, 2001) and a moderate degree of thermal hysteresis (Sinclair et al., 2006). Seasonal regulation of supercooling points in Antarctic collembolans appears to be primarily regulated by the molt cycle. Decreasing temperatures trigger progression into a non-feeding, pre-molt period that results in gut clearance and decreased supercooling points (Worland and Convey, 2008). Like mites, Antarctic collembolans are capable of rapidly increasing cold tolerance by depressing their supercooling point (Worland and Convey, 2001). Gut clearance also plays a role during RCH, as rapid decreases in supercooling point are achieved primarily by gut clearance (Worland et al., 2000). In the field, diurnal variations in supercooling points can be observed as a result of daily rhythms of feeding and gut clearance; for example, in two species of Antarctic Collembola, nighttime supercooling points are >10°C lower than those during the day (Sinclair et al., 2003). While this phenomenon has been referred to as ‘RCH’, it appears to be distinct from RCH *sensu stricto*, which involves cellular protection against the damaging effects of low temperature (Fig. 1) (see Lee and Denlinger, 2010).

Among the true insects on Antarctica, there are notably different mechanisms of overwintering cold tolerance. The midge *P. steinenii* is freeze-susceptible and has relatively modest supercooling capacity, with summer supercooling points around −7°C for larvae and −15°C for pupae and adults (Shimada et al., 1991). Also, lower lethal temperatures of *P. steinenii* are only −3°C for summer larvae (and −9°C for pupae), making it significantly less cold hardy than other Antarctic dipterans (see below). However, the winter cold hardiness of *P. steinenii* has not been examined. Nonetheless, as an aquatic midge that overwinters as larvae at the bottom of freshwater ponds and lakes, *P. steinenii* is likely buffered from extremes in temperature, perhaps explaining its modest level of cold tolerance. In contrast, the other two Antarctic insects, endemic *B. antarctica* and introduced *E. murphyi*, are both freeze-tolerant, and in fact are the only known freeze-tolerant arthropods on the continent. Larvae of both *B. antarctica* and *E. murphyi* are freeze-tolerant down to approximately −20°C (Baust and Lee, 1987; Worland, 2010), which

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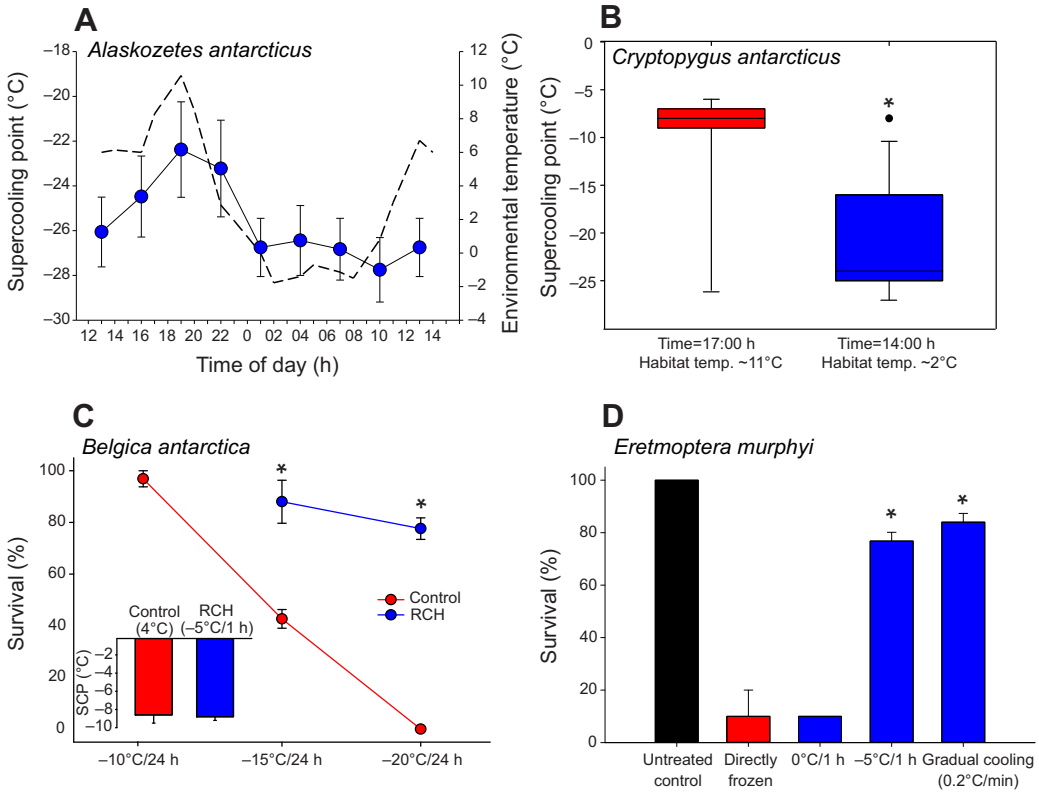

Fig. 1. Rapid cold-hardening (RCH) in Antarctic arthropods. RCH in (A) the Antarctic mite *Alaskozetes antarcticus* and (B) the Antarctic collembolan *Cryptpygus antarcticus* is manifested by diurnal shifts in supercooling points (SCPs), as individuals sampled during cooler parts of the day have a lower SCP. In contrast, RCH in the Antarctic midges (C) *Belgica antarctica* and (D) *Eretmoptera murphyi* involves physiological protection against the damaging effects of freezing with no effect on SCPs. Data are taken from Worland and Convey (Worland and Convey, 2001) for A and B, Lee et al. (Lee et al., 2006) for C, and Everatt et al. (Everatt et al., 2012) for D. In C, 1 h at −5°C was used to induce RCH prior to freezing at the indicated test temperature, while 'control' samples were directly exposed to the test temperature. In D, a discriminating temperature of −12.5°C for 8 h was used. Exposure to 0°C for 1 h failed to induce RCH, while −5°C for 1 h and gradual cooling at 2°C successfully enhanced freeze tolerance at −12.5°C. In B, an asterisk indicates a significant difference in SCP (rank sum test, $P<0.05$); in C and D, an asterisk indicates a significant improvement in survival in the RCH groups (ANOVA, Tukey, $P<0.05$). The inset in C illustrates that RCH has no effect on SCPs in *B. antarctica*.

is considerably lower than the minimum microhabitat temperatures experienced (Baust and Lee, 1981). In its native range, *E. murphyi* rarely experiences temperatures below −1.5°C, making this level of cold tolerance somewhat surprising, but this 'pre-adaptation' to colder climates is what allowed this species to successfully establish in maritime Antarctica (Worland, 2010; Everatt et al., 2012). Larvae of both species undergo seasonal cold acclimation, but it appears they remain freeze-tolerant year-round (Baust and Edwards, 1979).

Recently, RCH was described in *B. antarctica*, and this was the first report of RCH in a freeze-tolerant insect (Lee et al., 2006). Isolated tissues of *B. antarctica* are capable of RCH *ex vivo*, implying that RCH does not require input from the brain or hormones (Teets et al., 2008). Additionally, pharmacological evidence indicates a role of calcium signaling in mediating *ex vivo* RCH in this species (Teets et al., 2008). RCH has been subsequently characterized in *E. murphyi* (Everatt et al., 2012), suggesting that rapid enhancement of cold tolerance is a common adaptation in Antarctic arthropods. Interestingly, whereas RCH in *B. antarctica* can occur in a frozen state (Lee et al., 2006; Teets et al., 2008), RCH in *E. murphyi* is only effective when larvae remain supercooled during hardening (Everatt et al., 2012). As discussed above, RCH in Antarctic insects involves a different mechanism than that observed in mites and Collembola (Fig. 1). Antarctic mites and Collembola are chill-tolerant down to their supercooling points (Worland and Convey, 2001), so RCH is achieved by rapidly decreasing the supercooling point. However, *B. antarctica* and *E. murphyi* survive freezing well below the supercooling point, and in these species RCH improves the lower limit of freeze tolerance without affecting the supercooling point.

While low temperature is a significant stress limiting the range and fitness of Antarctic arthropods, water availability is perhaps the biggest challenge for Antarctic arthropods. Water is frozen and therefore unavailable for much of the year, and inland areas receive very little annual precipitation (Kennedy, 1993). Also, because of their small body size and high surface area to volume ratio, arthropods as a whole are susceptible to water loss (Gibbs et al., 2003). Thus, not surprisingly, Antarctic arthropods are typically extremely tolerant of desiccating conditions, with either mechanisms to reduce water loss or mechanisms to tolerate a dehydrated state.

To survive desiccating conditions, arthropods need to either reduce water loss or be able to tolerate cellular dehydration. Among Antarctic mites, both strategies seem to be in play. For example, *A. antarcticus* relies on water conservation, with a thick coating of waterproofing hydrocarbons that limit evaporative water loss to the environment (Benoit et al., 2008). In contrast, two predatory mites from the same habitat, *Hydrogamasellus antarcticus* and *Rhagidia gerlachei*, have high transpiration rates and thus seek moist microhabitats to maintain water balance. However, how these two

The Journal of Experimental Biology (2014) doi:10.1242/jeb.089490

mites tolerate winter desiccation, when liquid water is unavailable, has not been examined.

Antarctic collembolans are typically found in moist environments and exhibit very little resistance to water loss. In a comparison of seven species of Antarctic mites and collembolans, Worland and Block (Worland and Block, 1986) found that collembolans had significantly higher rates of water loss than any of the mite species. At 5% relative humidity (RH) and 0°C, collembolans lost anywhere from 9 to 25% of their body water per hour, depending on species. Recently, the water balance of *C. antarcticus* was examined in more detail, revealing that this species is incapable of maintaining water balance at any relative humidity below saturation, and therefore relies on liquid water to maintain water balance (Elnitsky et al., 2008a). Because *C. antarcticus* is unable to prevent dehydration, it must be able to survive in a severely dehydrated condition. Indeed, more than 50% survived a 60% loss of body water at ecologically relevant conditions (Elnitsky et al., 2008a). Thus, in general, while Antarctic collembolans have high water loss rates, they are capable of tolerating extreme desiccation, surviving in a near-anhydrobiotic state during the Antarctic winter.

Like the collembolans, the Antarctic midges *E. murphyi* and *B. antarctica* lose water very rapidly but are extremely tolerant of dehydration. At ecologically relevant humidities, larvae of *E. murphyi* readily survive upwards of 50% water loss (Worland, 2010), while larvae of *B. antarctica* survive up to 70% loss of body water (Benoit et al., 2007b; Hayward et al., 2007). In response to desiccation, larvae of *B. antarctica* combat high water loss rates by behaviorally clustering and by increasing the waterproofing properties of their cuticle (Benoit et al., 2007b). Larvae are also highly tolerant of fluctuating moisture regimes. Survival following four cycles of 24 h dehydration (resulting in ~40% water loss) and 24 h rehydration is near 100%, although significant mortality occurs after five such cycles (Teets et al., 2012a). Also, fluctuating moisture regimes are energetically costly, as larvae exposed to five dehydration/rehydration cycles burn 67% of their carbohydrate energy stores. In *B. antarctica*, dehydration tolerance is restricted to the larval stage; adults, which are only active for a brief period during the summer, require moist habitats and succumb to a 30% loss of body water (Benoit et al., 2007a). In addition to dehydration, Antarctic arthropods experience other forms of osmotic stress. For example, because of their proximity to the sea, some larvae of *B. antarctica* are also at risk of immersion in seawater, but they are fully capable of surviving up to 10 days in 1000 mOsm seawater (Elnitsky et al., 2009).

Recently, it has been discovered that many Antarctic arthropods are capable of a distinct form of dehydration termed cryoprotective dehydration (Fig. 2). When arthropods with water-permeable cuticles are surrounded by environmental ice, a vapor pressure gradient draws water out of the body into the surrounding ice, thereby allowing the body fluid melting point to track environmental temperatures (Holmstrup et al., 2002). The prerequisites for cryoprotective dehydration are a permeable cuticle, extreme dehydration tolerance and the ability to resist inoculative freezing. Among Antarctic species, cryoprotective dehydration has been demonstrated in the collembolan *C. antarcticus* (Worland and Block, 2003; Elnitsky et al., 2008a) and the midge *B. antarctica* (Elnitsky et al., 2008b). Some non-arthropod soil-dwelling organisms in Antarctica, including nematodes, are also capable of cryoprotective dehydration (Wharton et al., 2005). Cryoprotective dehydration is now considered by some to be a third overwintering strategy (along with freeze tolerance and freeze avoidance), as during cryoprotective dehydration arthropods are neither frozen nor supercooled, as their melting point matches the environmental temperature.

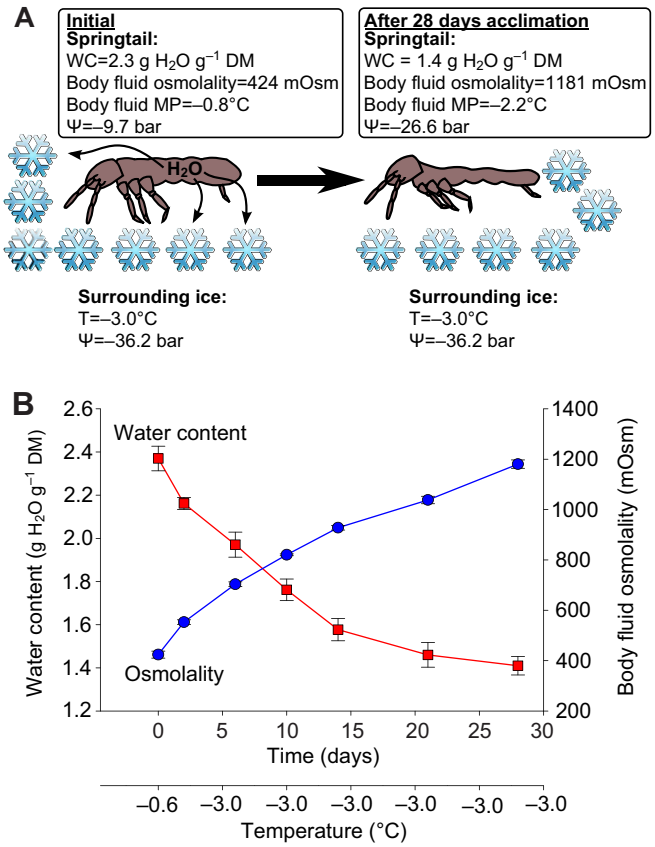

Fig. 2. Schematic illustration of cryoprotective dehydration in Antarctic arthropods. This example demonstrates cryoprotective dehydration in the Antarctic collembolan *Cryptopygus antarcticus*, using data from Elnitsky et al. (Elnitsky et al., 2008a). (A) Illustration of the underlying physics of cryoprotective dehydration. (B) Water content and osmolality data from *C. antarcticus* over the course of a 28-day exposure to gradually decreasing temperatures in the presence of ice. In A, when a fully hydrated springtail is placed in the presence of ice at −3°C, the water potential (Ψ; 1 bar=100 kPa) inside the body is considerably higher than that of the surrounding ice, which drives water out of the insect into the surrounding ice. After 28 days, the water content decreases while osmolality increases, such that the body fluid melting temperature and water potential approach that of the surrounding ice, which reduces the risk of internal freezing. WC, water content; MP, melting point; DM, dry mass.

In the case of *B. antarctica*, the discovery of cryoprotective dehydration was somewhat surprising, because this species is freeze-tolerant. Also, *B. antarctica* is the first true insect in which cryoprotective dehydration has been demonstrated. Cryoprotective dehydration was originally considered an adaptation for freeze-intolerant species, as it allows arthropods to remain unfrozen at subzero temperatures. Cryoprotective dehydration was described in a freeze-tolerant nematode (Wharton et al., 2003) and an enchytraeid worm (Pedersen and Holmstrup, 2003) but was considered a laboratory artifact, because neither of these species is likely to be able to avoid inoculative freezing in the field. Antarctic tardigrades also appear to be capable of surviving low temperature in both frozen and dehydrated states (Sømme, 1996), although the ability of tardigrades to undergo cryoprotective dehydration *sensu stricto* has not been examined. While cryoprotective dehydration in *B. antarctica* has yet to be examined in a field setting, larvae are capable of avoiding inoculative freezing at ecologically relevant soil moisture levels (Elnitsky et

al., 2008b). Thus, *B. antarctica* represents a unique case among arthropods where both freeze tolerance and cryoprotective dehydration may be used within a single species. Furthermore, the supercooling point of *B. antarctica* larvae (−7°C) is lower than typical microhabitat temperatures, meaning that during brief subzero temperature exposures larvae are capable of remaining supercooled, provided larvae avoid inoculative freezing. Recent evidence indicates that it is preferable for larvae to remain supercooled at subzero temperatures. Following a 60 h exposure to −5°C, larvae that are inoculatively frozen experience approximately sevenfold higher mortality and have ~20% less energy reserves than their supercooled counterparts (Teets et al., 2011), and similar results are observed when larvae are exposed to repeated diurnal cold cycles. Thus, the cellular dehydration associated with freezing imposes additional cellular stress and energetic demands beyond that of low temperature alone.

Biochemical and molecular mechanisms of stress tolerance in Antarctic arthropods

In comparison with temperate insects, the molecular mechanisms of stress tolerance in Antarctic arthropods have received little attention. Nonetheless, early studies in the 1980s characterized biochemical markers of environmental stress, and recent studies have capitalized on advances in molecular biology. Here, we will review the physiological mechanisms of stress tolerance in Antarctic terrestrial arthropods and highlight some avenues for future research.

Like their temperate counterparts, seasonal and stress-induced accumulation of low-molecular-weight osmoprotectants is a hallmark of Antarctic arthropods. Every species profiled thus far accumulates some sort of osmoprotective compound, although the type and amount vary from species to species. For example, the mite *A. antarcticus* primarily uses glycerol as an osmoprotectant, accumulating levels upwards of 0.5 mol l^{-1} (Block and Convey, 1995). Likewise, the collembolan *C. antarcticus* uses glycerol as its chief cryoprotectant and also accumulates glucose and trehalose in response to desiccation (Elnitsky et al., 2008a). In contrast, the midge *B. antarctica* accumulates very low levels of glycerol and instead relies primarily on glucose, erythritol and trehalose as osmoprotectants (Lee and Baust, 1981; Baust and Lee, 1983). One theme that is emerging from studies of both polar and tropical desiccation-tolerant organisms is the importance of trehalose as an osmoprotectant during dehydration. A range of organisms, including certain bacteria, fungi, plants and metazoans, have been demonstrated to accumulate trehalose in response to desiccation (Elbein et al., 2003). In arthropods, trehalose typically functions as the blood sugar and is the chief osmoprotectant in arthropods capable of anhydrobiosis (Clegg, 2001). Recent studies in *C. antarcticus* (Elnitsky et al., 2008a) and *B. antarctica* (Benoit et al., 2007b; Elnitsky et al., 2008b) have implicated its importance during extreme dehydration in Antarctic arthropods as well.

In recent years, physiological studies of Antarctic arthropods have benefited from advances in molecular biology and 'omics' technology, although molecular experiments have only been conducted in two species, the collembolan *C. antarcticus* and the midge *B. antarctica*. Regardless, these studies are beginning to provide clues about the mechanisms of stress tolerance in some of the world's most extreme arthropods. A summary of the stress-responsive genes identified in Antarctic arthropods is provided in Table 1.

Molecular experiments in *C. antarcticus* are restricted to two microarray studies of cold tolerance. In the first, animals with 'low' supercooling points (<–15°C) were compared with those with 'high' supercooling points (>–15°C), to determine which genes are responsible for lowering supercooling points (Purać et al., 2008). This microarray contained a subset of 672 expressed sequence tags (ESTs), and thus was not comprehensive. Nonetheless, expression patterns indicate upregulation of a number of cuticular proteins and other structural constituents in the 'low' group, confirming the importance of the cuticle and molt cycle in regulating supercooling capacity in Antarctic collembolans (Worland and Convey, 2008). Other genes upregulated in the 'low' group relative to the 'high' group include several mitochondrial genes involved in ATP synthesis, suggesting that boosting energy production may be a component of low temperature survival. Specific upregulated genes confirmed by qPCR include a cuticular protein and CHK1, a checkpoint homolog involved in cell cycle regulation. A similar experiment was conducted later with a larger microarray (containing 5400 ESTs), and once again, a number of cuticular genes and genes involved in the molt cycle were upregulated in cold-acclimated collembolans (Burns et al., 2010). In this study, three genes (endocuticle structural glycoprotein SgAbd-4, cuticular protein 49Ah and chitin-binding peritrophin A) were confirmed to be upregulated by qPCR, while an mRNA encoding the extracellular matrix protein tenebrin was verified to be downregulated.

Relative to other Antarctic arthropods, the Antarctic midge *B. antarctica* has been subjected to the largest number of molecular studies. As in temperate insects, the heat shock proteins are important mediators of stress tolerance in *B. antarctica*. However, whereas most insects express genes encoding heat shock proteins at very low levels until the proteins are needed, larvae of *B. antarctica* constitutively express genes encoding heat shock proteins at high levels all the time (Rinehart et al., 2006). While adults of *B. antarctica* have a typical heat shock protein response, neither heat nor cold increased expression of three different heat shock proteins (small hsp, hsp70 and hsp90) in larvae. This constant presence of heat shock proteins likely provides year-round protection against environmental stress, which can be frequent and unpredictable in maritime Antarctica. Whereas high expression of heat shock proteins typically hinders growth and development (Krebs and Feder, 1997), larvae of *B. antarctica* are able to circumvent this and produce heat shock proteins at high levels even while they are feeding and growing.

Constitutive defenses in *B. antarctica* are not restricted to heat shock proteins. Likewise, larvae express genes encoding the antioxidant enzyme superoxide dismutase at high levels even in the absence of overt oxidative stress (Lopez-Martinez et al., 2008). Superoxide dismutase mRNA levels, as well as the mRNAs encoding catalase and three heat shock proteins, modestly increase after exposure to sunlight. Indeed, expression of these genes confers extremely high resistance to oxidative damage in *B. antarctica*, as the antioxidant capacity of *B. antarctica* larvae is five times greater than that of a temperate freeze-tolerant insect, *E. solidaginis* (Lopez-Martinez et al., 2008). Adults of *B. antarctica* have even higher levels of antioxidant capacity, probably because of their near-constant exposure to sunlight as they walk on the surface in search of mates. Resistance to oxidative damage is crucial for Antarctic arthropods, as Antarctic sunlight contains very high levels of UV radiation (Liao and Frederick, 2005), which is intensifying as a result of ozone damage (Weatherhead and Andersen, 2006). Furthermore, repeated bouts of freeze–thaw exposure, which are common in Antarctica, are known to cause oxidative damage in insects (Lalouette et al., 2011).

Recently, aquaporins have been implicated as key regulators of water movement during stressful conditions such as dehydration

The Journal of Experimental Biology

Table 1. List of stress-upregulated genes identified in Antarctic arthropods

Functional category	Species	Gene	Type of stress	Reference(s)
Structural protein	Ca	cuticular protein	C	Purac et al., 2008
	Ca	endocuticle structural glycoprotein SgAbd-4	C	Burns et al., 2010
	Ca	cuticular protein 49Ah	C	"
	Ca	chitin-binding peritrophin A	C	"
	Ba	actin	D	Lopez-Martinez et al., 2009
	Ba	muscle-specific actin	D	"
	Ba	myosin light-chain kinase	D	"
Heat shock proteins	*Ba*	small heat shock protein	H,A,S,D	Rinehart et al., 2006; Lopez-Martinez et al., 2008; Lopez-Martinez et al., 2009; Teets et al., 2012b
	Ba	70 kDa heat shock protein	H,A,S,D,C	Rinehart et al., 2006; Lopez-Martinez et al., 2008; Lopez-Martinez et al., 2009; Teets et al., 2011; Teets et al., 2012b
	Ba	90 kDa heat shock protein	H,S,D	Rinehart et al., 2006; Lopez-Martinez et al., 2008; Lopez-Martinez et al., 2009
	Ba	40 kDa heat shock protein	D	Teets et al., 2012b
Antioxidant enzymes	*Ba*	catalase	A,S,D	Lopez-Martinez et al., 2008; Lopez-Martinez et al., 2009
	Ba	superoxide dismutase	S,D	"
Detoxifcation	*Ba*	cytochrome P450 28a5	D	Lopez-Martinez et al., 2009
	Ba	metallothionein 2	D	"
	Ba	UDP-glycosyltransferase	D	Teets et al., 2012b
	Ba	cytochrome P450 6a23	D	"
Lipid modification	*Ba*	fatty acid delta Δ9 desaturase	D	Lopez-Martinez et al., 2009
	Ba	phospholipase A2 activating protein	D	"
	Ba	fatty acyl CoA Δ9 desaturase	D	"
Cryoprotectant mobilization	*Ba*	glycogen phosphorylase	H,C,D	Teets et al., 2013
	Ba	phosphoenolpyruvate carboxykinase	H,C,D	"
	Ba	trehalase	C,D	"
	Ba	glucose-6-phosphatase	D	"
	Ba	trehalose-6-phosphate synthase	D	"
	Ba	trehalose-6-phosphate phosphatase	D	"
	Ba	trehalose transporter 1	D	"
	Ba	aldehyde/ketone reductase	D	"
	Ba	glycerol-3-phosphate dehydrogenase	D	"
	Ba	pyrroline-5-carboxylate reductase	D	"
Cell death/longevity	*Ba*	sestrin	D	Teets et al., 2012b
	Ba	relish	D	"
	Ba	thread	D	"
	Ba	spermidine synthase	D	"
Other	*Ca*	CHK1 checkpoint homologue	C	Purac et al., 2008
	Ba	zinc finger protein	D	Lopez-Martinez et al., 2009
	Ba	pacifastin-related serine protease inhibitor	D	"
	Ba	vacuolar (H^+) ATPase	D	"

Only genes confirmed with a targeted approach (i.e. northern blot or qPCR) are included in the table.
Species: *Ca*, *Cryptopygus antarcticus*; *Ba*, *Belgica antarctica*. Type of stress: C, cold; D, dehydration; H, heat; A, anoxia; S, direct sunlight.

(Liu et al., 2011) and freezing (Philip et al., 2008; Philip and Lee, 2010). Aquaporins are pore-forming proteins that carry water, and sometimes other solutes, across the cell membrane (Borgnia et al., 1999). Because Antarctic arthropods are challenged by numerous forms of osmotic stress, including freezing, dehydration and immersion in seawater, aquaporins likely play an important role in mediating stress tolerance. Goto et al. (Goto et al., 2011) cloned and characterized the first aquaporin from an Antarctic arthropod, an aquaporin-1 like gene from *B. antarctica*. When expressed in *Xenopus* oocytes, this protein is capable of transmitting water, but not urea or glycerol, across the cell membrane. This specific aquaporin gene is expressed in several different tissues, indicating that it may play a general role in water movement across cells. However, mRNA expression did not change in response to dehydration, so it is unclear what, if any, role this gene plays in mediating stress tolerance. A second study of *B. antarctica* aquaporins found immunoreactivity to four different aquaporin antibodies from different species, and some of these were stress inducible (Yi et al., 2011). However, the sequence identity of these aquaporin genes has not been established. In the same study, blocking aquaporins pharmacologically with mercuric chloride reduced the *ex vivo* freezing tolerance of fat body, midgut and Malpighian tubule tissue, indicating that aquaporins are crucial for water redistribution during freezing. Additionally, mercuric chloride reduced the water loss of midgut tissue, suggesting that aquaporins also play a crucial role in mediating dehydration stress.

Genes involved in mobilization of energy reserves and synthesis of osmoprotectants also appear to be essential components of the stress response in *B. antarctica*. Using qPCR, Teets et al. (Teets et al., 2013) profiled the expression of 11 genes involved in glycogen breakdown, gluconeogenesis, polyol and trehalose metabolism, and proline synthesis. High and low temperature induces rapid upregulation of genes involved in glucose mobilization, including transcripts encoding glycogen phosphorylase and

The Journal of Experimental Biology (2014) doi:10.1242/jeb.089490

phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting enzyme of gluconeogenesis. These results are consistent with previous observations of cold-induced glucose mobilization in larvae of *B. antarctica* (Teets et al., 2011). In contrast, acute high and low temperature result in a general downregulation of genes involved in trehalose and proline synthesis. In response to dehydration, gene expression patterns are highly dependent on the type of dehydration experienced. Rapid dehydration at 75% RH has a similar transcriptional signature as that observed in response to heat and cold, namely upregulation of genes encoding glucose mobilizing enzymes (i.e. PEPCK and glycogen phosphorylase) with concurrent downregulation of genes involved in trehalose and proline synthesis. In contrast, while slow dehydration at 98% RH and cryoprotective dehydration also induce expression of *pepck*, these treatments upregulate genes involved in trehalose and proline synthesis, consistent with accumulation of trehalose (Benoit et al., 2007b; Elnitsky et al., 2008b) and proline (Teets et al., 2012b) during prolonged dehydration.

Non-targeted, 'omics' approaches have also benefited our understanding of stress tolerance in *B. antarctica*. Using suppressive subtractive hybridization, Lopez-Martinez et al. (Lopez-Martinez et al., 2009) obtained a number of dehydration-responsive clones, and northern blots confirmed that 23 of these were indeed differentially expressed either during dehydration or rehydration. Upregulated genes include those encoding three heat shock proteins (hsp26, hsp70 and hsp90) and genes encoding two antioxidant enzymes (superoxide dismutase and catalase), indicating that protein denaturation and oxidative damage are symptoms of dehydration stress. Other genes upregulated during dehydration include genes coding for cytoskeletal proteins and membrane restructuring, consistent with previous observations that dehydration causes cytoskeletal reorganization (Chen et al., 2005) and membrane lipid remodeling (Bayley et al., 2001). In addition, several genes are downregulated in response to dehydration, including two electron transport chain genes, suggesting a shutdown of metabolism during dehydration.

To date, a single genome-wide expression study has been conducted in Antarctic arthropods. Using Illumina-based RNA-seq, Teets et al. (Teets et al., 2012b) profiled the expression of ~13,500 transcripts in response to both desiccation at a constant temperature and cryoprotective dehydration. Both treatments result in sweeping changes in gene expression, as desiccation and cryoprotective dehydration resulted in ~24 and ~18%, respectively, of all genes being differentially expressed. These results confirmed the crucial role of heat shock proteins during environmental stress in *B. antarctica*, as 15 different heat shock protein transcripts were upregulated by one or both dehydration treatments. Concurrently, desiccation caused upregulation of genes involved in the recycling/degradation of proteins and cellular macromolecules, including significant enrichment of proteasomal and autophagy genes. Taken together, these results suggest that coordinated upregulation of heat shock proteins, ubiquitin-mediated proteasome and autophagy function to recycle and remove damaged cellular components during dehydration, thereby conserving energy and promoting cell survival (Fig. 3). We hypothesize that autophagy is particularly important for surviving the Antarctic winter, as this pathway inhibits apoptosis and other forms of cell death during prolonged periods of cellular stress (Teets and Denlinger, 2013). Desiccation and cryoprotective dehydration also cause a general downregulation of central metabolic genes, including genes involved in glycolysis, the tricarboxylic acid cycle and lipid metabolism, suggesting that dehydration causes a molecular shift to hypometabolism that conserves energy and prevents the toxic build-up of metabolic end products (Teets et al., 2012b). Indeed, changes in the metabolome correlate well with gene expression changes, indicating that coordinated changes in gene expression and metabolism govern responses to extreme environmental conditions.

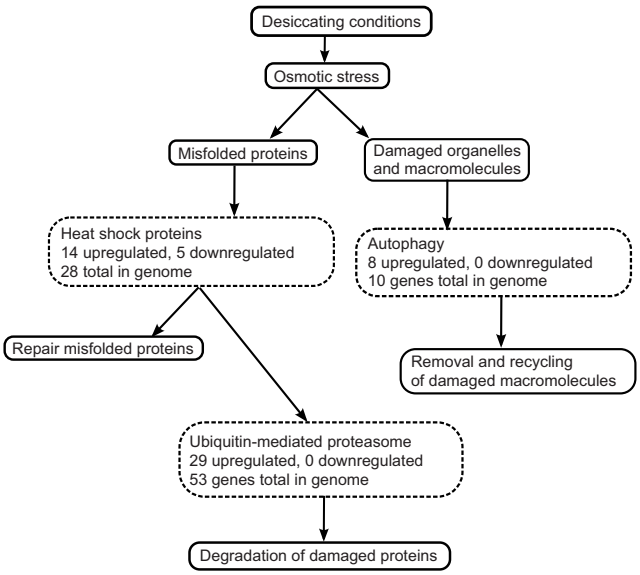

Fig. 3. Flow chart illustrating the importance of cell recycling pathways during extreme dehydration in the Antarctic midge *Belgica antarctica*. Results are taken from an RNA-seq study of dehydration-induced gene expression in larvae of *B. antarctica* (Teets et al., 2012b). In this diagram, the damaging effects of dehydration cause upregulation of key cell recycling genes, outlined in dashed lines. In the dashed boxes, the number of genes upregulated and downregulated in response to dehydration is included. These genes, in turn, carry out essential cell recycling functions to remove damaged proteins and organelles, thereby promoting cell survival during prolonged periods of environmental stress.

At the protein level, a proteomics study of dehydration and rehydration in *B. antarctica* supported the idea that cytoskeletal and cell structural rearrangements are essential during dehydration (Li et al., 2009). Of the 18 proteins more abundant during dehydration, 13 are cell structural proteins, including several isoforms of actin and myosin. Interestingly, several contractile proteins are also less abundant during desiccation, indicating that certain contractile proteins are synthesized while others are degraded. Finally, our understanding of the stress physiology of *B. antarctica* has benefited from recent advances in metabolomics. Using non-targeted GC-MS metabolomics, Michaud et al. (Michaud et al., 2008) profiled metabolic adaptations to heat, freezing and dehydration. Several metabolites show similar responses to cold and desiccation, including the osmoprotectants glycerol and erythritol, which may in part explain the cross-tolerance between these two stresses in *B. antarctica* (Hayward et al., 2007). A second metabolomics study of dehydration in this species observed accumulation of three amino acids (proline, glutamine and lysine), a single sugar (fructose) and three polyols (erythritol, sorbitol and mannitol) in response to both desiccation at a constant temperature and cryoprotective dehydration (Teets et al., 2012b).

Conclusions and future directions

In contrast to the abundance of arthropods on other continents, the Antarctic arthropod community is depauperate and consists of only a handful of species. In the past 30 years, researchers have been

intently studying the physiological ecology of these arthropods, and recent advances in molecular biology have fostered significant advances in our knowledge of the world's most extreme arthropods. However, what is still lacking is an understanding of the unique adaptations that distinguish Antarctic arthropods from their tropical and temperate counterparts. Most adaptations described in Antarctic arthropods, such as accumulation of osmoprotectants (e.g. Baust and Lee, 1983) and the role of aquaporins during freeze tolerance (Yi et al., 2011), have been previously described in temperate species. Thus, despite recent advances in the molecular physiology of Antarctic arthropods, unique Antarctic adaptations, if they exist, have been elusive to physiologists. Discoveries such as the constitutively high expression of heat shock proteins in *B. antarctica* (Rinehart et al., 2006) and the prevalence of cryoprotective dehydration in polar arthropods (Worland and Block, 2003) are promising starts, but there is still much to be learned.

One way to potentially uncover unique Antarctic adaptations would be an increased reliance on comparative physiology. Basic ecophysiological studies, such as the cold tolerance of Antarctic collembolans (Sinclair et al., 2003; Sinclair et al., 2006) and the water balance of Antarctic mites (Benoit et al., 2008), have successfully used comparative approaches to elucidate similarities and differences among Antarctic species. However, to date there have not been any molecular studies in Antarctic arthropods that take advantage of a comparative design. Comparative physiological genomics of stress responses across multiple Antarctic species would reveal whether there are crucial, conserved molecular adaptations to environmental stress, or whether each species relies on a unique suite of molecular mechanisms. For example, Teets et al. (Teets et al., 2012b) compared dehydration-induced gene expression changes in *B. antarctica* with those of an Arctic collembolan, *Megaphorura arctica* (Clark et al., 2009), and found that these two arthropods rely on distinct molecular adaptations to combat dehydration stress, despite occupying similar microhabitats. Such comparisons could also be conducted between Antarctic arthropods and closely related temperate species, to identify which molecular adaptations to stress are 'Antarctic specific'. There are clear physiological and life history differences between Antarctic and temperate species (see Convey, 2010), and tools are now available to reveal the molecular underpinnings of these differences.

Understanding the environmental physiology of Antarctic arthropods is particularly important in the face of a changing climate. The Antarctic Peninsula, which is home to much of Antarctica's arthropod diversity, is experiencing one of the most rapid warming rates on the planet over the last 50 years (Turner et al., 2009). For example, at Palmer Station, over a short period of 20 years from 1990 to 2010, there was a steady, consistent rise in average daily temperature of ~0.1°C every 2 years (Fig. 4). Warming is largely manifested in the winter; the mean August temperature (the coldest month of the year) at Palmer Station is a full degree higher in the decade 2000–2009 than it was from 1990 to 1999. However, what this rapid warming trend means for Antarctic arthropods remains to be seen. While milder winters would suggest reduced overwintering mortality, higher temperatures would also increase metabolic rate, depleting energy reserves normally reserved for reproduction (Bale and Hayward, 2010). Climate models also predict an increased frequency of extreme temperature and precipitation events, which can further influence the range and distribution of terrestrial arthropods (Bale and Hayward, 2010). In addition to direct effects of temperature and precipitation, climate warming would increase snowmelt, which would reduce microhabitat thermal buffering, thereby increasing the number of freeze–thaw cycles experienced. Also, increased soil moisture due to snowmelt could increase the risk of inoculative freezing (Teets et al., 2011). Thus, understanding the plasticity of Antarctic arthropods to perturbations in the environment is essential for forecasting future ranges and population dynamics of Antarctic arthropods.

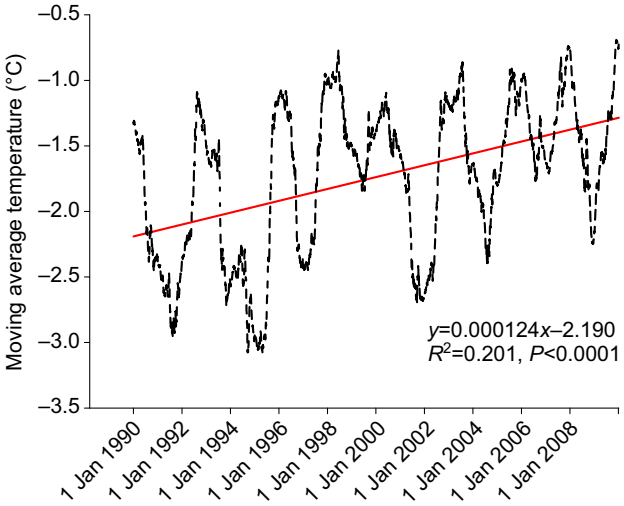

Fig. 4. Recent surface air temperature data from Palmer Station on the Antarctic Peninsula illustrating warming trends. Daily temperature data from 1 January 1990 to 1 January 2010 were obtained from the US Long Term Ecological Research Network data repository (https://metacat.lternet.edu/das/lter/index.jsp). Data were transformed with a moving average to reduce noise and highlight general trends in temperature. A yearly moving average for each day was calculated by taking the mean temperature for that day plus the next 364 days after.

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Competing interests
The authors declare no competing financial interests.

Author contributions
N.M.T. and D.L.D. conceived the manuscript and wrote the paper. N.M.T. prepared the figures.

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