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Review

The ins and outs of water dynamics in cold tolerant soil invertebrates

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ABSTRACT

Many soil invertebrates have physiological characteristics in common with freshwater animals and represent an evolutionary transition from aquatic to terrestrial life forms. Their high cuticular permeability and ability to tolerate large modifications of internal osmolality are of particular importance for their cold tolerance. A number of cold region species that spend some or most of their life-time in soil are in more or less intimate contact with soil ice during overwintering. Unless such species have effective barriers against cuticular water-transport, they have only two options for survival: tolerate internal freezing or dehydrate. The risk of internal ice formation may be substantial due to inoculative freezing and many species rely on freeze-tolerance for overwintering. If freezing does not occur, the desiccating power of external ice will cause the animal to dehydrate until vapor pressure equilibrium between body fluids and external ice has been reached. This cold tolerance mechanism is termed *cryoprotective dehydration* (CPD) and requires that the animal must be able to tolerate substantial dehydration. Even though CPD is essentially a freeze-avoidance strategy the associated physiological traits are more or less the same as those found in freeze tolerant species. The most well-known are accumulation of compatible osmolytes and molecular chaperones reducing or protecting against the stress caused by cellular dehydration. Environmental moisture levels of the habitat are important for which type of cold tolerance is employed, not only in an evolutionary context, but also within a single population. Some species use CPD under relatively dry conditions, but freeze tolerance when soil moisture is high.

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1. Introduction

Animals of temperate regions living permanently in the soil or overwintering there may never or rarely experience temperatures low enough to produce a risk of freezing of their body fluids because thermal conditions in the soil are often much less extreme than air temperatures. However, in cold regions of the world, soil will freeze every winter, and if insulating snow cover is low, or if permafrost prevails, soil animals cannot evade the low temperatures and must rely on physiological adaptations for survival. Cold

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hardy ectothermic animals have evolved two major strategies for survival of sub-zero temperatures. The freeze avoiding species, for which freezing of body fluids is lethal, depend on extensive supercooling of their body fluids, whereas a second strategy is deployed by the freeze tolerant species that are able to tolerate freezing of their extracellular body fluids. Intracellular freezing is generally considered to be lethal (Asahina, 1969; Zachariassen, 1985) although a few examples of tolerance of intracellular freezing have been shown in insects and nematodes (Lee et al., 1993; Wharton and Ferns, 1995). A comprehensive outline of general cold hardiness strategies of ectothermic animals will not be given here since several excellent reviews are available (Block, 1990; Denlinger and Lee, 2010; Ramløv, 2000; Storey and Storey, 1996; Zachariassen, 1985).

This paper will focus on a third cold hardiness strategy termed *cryoprotective dehydration* (CPD), which is relevant for those smaller soil invertebrates that have only little cuticular resistance to desiccating conditions (Holmstrup et al., 2002). Since the first mechanistic description of CPD (Holmstrup and Westh, 1994), this cold tolerance strategy has been found in a growing number of species, and several previous observations support the (likely) widespread occurrence of CPD in soil invertebrates. The paper will discuss the physical conditions prevailing in soil, and the distinct relationships these have with water balance of soil invertebrates. Further, I will discuss which physiological traits are of importance for CPD, and show the similarities to adaptations making animals freeze tolerant. Lastly, I will present an inventory of species presently known to use CPD and from that attempt to predict characteristics of typical species employing this cold tolerance mechanism.

2. The soil environment and organisms

Soil invertebrates are important components of soil ecosystems worldwide, with the exception of deserts and land permanently covered by ice. This diverse group of animals covers a range of taxa, the most important being protozoans, tardigrades, nematodes, oligochaete worms (earthworms and enchytraeids), mites, springtails (Collembola), millipedes, centipedes, and a range of insects (mostly belonging to Diptera and Coleoptera) whose larval stages complete their development in the soil. Many of these organisms such as tardigrades, nematodes, oligochaetes and soil-dwelling springtails have several characteristics that distinguish them from surface living forms, in particular with respect to water balance. These characteristics include small size, epidermal respiration, and high integumental permeability to water resembling aquatic animals more than truly terrestrial. These characters seem to match the soil environment, where the pore humidity is normally very close to 100% relative humidity (RH), and it could be argued that the soil has provided a habitat for an evolutionary transition from aquatic to terrestrial life forms (Ghilarov, 1958). Extreme winter (and summer) temperatures are also buffered due to insulation from snow, vegetation and dead plant litter, and from deeper soil layers that provide either a sink or a source of heat during warming and cooling from the air, respectively (Berry, 1981; Isard et al., 2007). This results in cooling rates that are mostly much slower than found aboveground, except in permafrost soils with little insulation by snow. In that case cooling rates in soil may be similar to those in air once all water in the soil has frozen (Coulson et al., 1995).

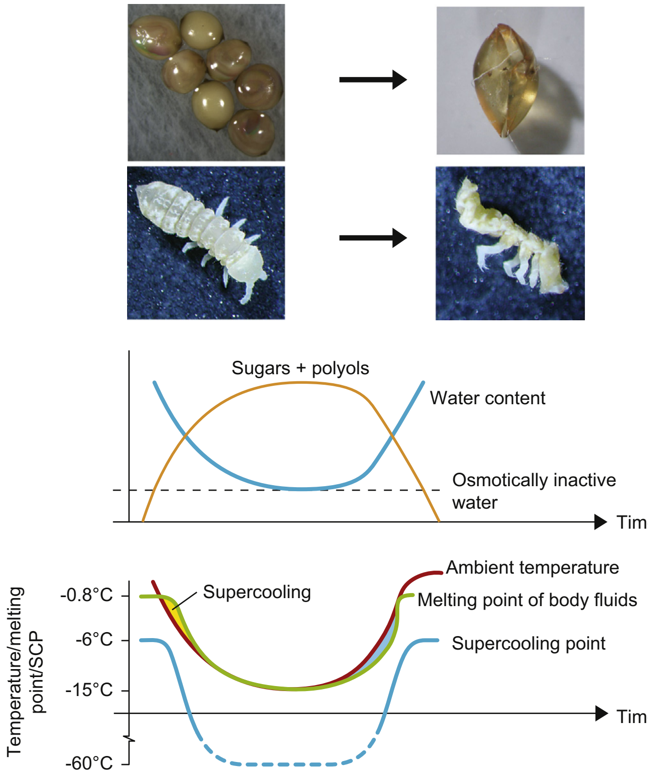

Fig. 1. A general model of cryoprotective dehydration. Decreasing soil temperature causes dehydration of the organism, which in turn induces accumulation of sugars and polyols (SP: upper panel). At low temperature (−15 to −20 °C) practically all osmotically active water is lost. Dehydration and SP accumulation bring about a lowering of melting point (MP), largely at the same rate as soil temperature decreases. Supercooling is therefore restricted to only a few degrees and only during a short initial period at relatively high subzero temperatures (lower panel). A temperature rise causes the animal to take up water, and increases the body fluid MP. Fully hydrated animals have supercooling points at about −6 °C. Dehydration result in a depression of the supercooling point. When dehydration approaches the level of osmotically inactive water, freezing cannot occur at environmental temperatures. The photos show fully hydrated cocoons of *Dendrobaena octaedra* and collembolan *Megaphorura arctica* (left), and animals dehydrated at −6 °C for 7 days (right).

3. Protective dehydration strategy – a different kind of cold tolerance mechanism

Permeable invertebrates that are trapped in frozen soil or sediment may become dehydrated. Scholander et al. (1953) and Danks (1971) observed that chironomid larvae found in frozen sediment were wrinkled and appeared to have become dehydrated, and later Gehrken and Sømme (1987) reported that overwintering eggs of stoneflies became severely desiccated when kept in frozen creek water. Other organisms like Collembola and earthworm egg capsules (“cocoons”) also became severely desiccated if subjected to sub-zero temperatures in small vials where the air humidity was defined by ice, i.e. simulating the conditions in frozen soil (Holmstrup, 1992; Worland, 1996). In these early reports it was suggested that dehydration of the organism enhanced the supercooling capacity as a primary cold tolerance strategy. Later, using earthworm cocoons as models, Holmstrup and Westh (1994) showed that supercooling was of minor importance whereas the dehydration-induced lowering of body fluid melting point played a central role in the cold hardiness of permeable soil invertebrates. The difference in vapor pressure between ice and supercooled body fluids drives an outflux of water vapor. The force of this vapor pressure difference is so large (see following section) that even a few degrees of supercooling