Tardigrades and anhydrobiosis

Water bears and

Ian M. Kinchin (King's College London)

The Phylum Tardigrada consists of about 900 known species of microscopic animals that are often referred to by their endearing popular names, water bears or moss piglets (translated from the German expressions *Wasser Bär* and *Mooschweinchen*). The group is considered a sister group to the arthropods, with animals typically less than 0.5 mm in length[1]. As tardigrades are of no direct medical or agricultural importance, their study is confined to a small, but highly productive, international community of researchers.

Tardigrades are often a favourite of the amateur microscopist as they are so easy to find and rewarding to observe. Their slow lumbering gait (*tardi*=slow, *grado*=walker) is not only amusing to watch, but also makes their movements easy to track under the microscope. Many of the species are more or less transparent, so the inner workings of the intricate buccal apparatus and the transition of the food through the gut can be observed without requiring any special preparation. Other species are bright orange (Figure 1), carrying an intricate arrangement of dorsal armour plating (Figure 2).

Figure 1. Fresh specimen of *Echiniscus granulatus* (Tardigrada) showing the natural orange colouration of this species. The animal's body length is 350 µm. (Photo by author.)

The paradox of their biology is that, although all tardigrades are strictly aquatic (needing to be surrounded by water to support their activities), those occupying terrestrial habitats account for the bulk of species within the group. Tardigrades feature among the fauna found in a variety of habitats where water is often scarce[2]. Suitable habitats need to hold temporary water films. It is the thickness of this water film that appears to be important in aiding tardigrade locomotion over the substratum[3]. A thin water film presses the tardigrade's body close to the substratum and allows the claws to gain purchase and move the animal along. When the microhabitat is saturated, the animals can lose this purchase and drift uncontrollably in the water. Suitable microhabitats include lichens, leaf axils of some plants, soils and sediments, and most famously moss cushions[4]. Whether the mosses are growing in urban environments (roofs and walls) or natural environments (rock surfaces or tree bark), they are typically subjected to repeated episodes of desiccation and rehydration. The tardigrades cope with this by entering a state of suspended animation during dry periods. This is a characteristic that has a significant impact on the ecology of tardigrades[1,5] and is shared with other animals inhabiting these microenvironments, including nematodes and rotifers.

Latent or dormant states are quite common in the Animal and Plant Kingdoms. Typically they occur at set stages in the life cycle of the organisms, particularly the seeds of various flowering plants and the resistant eggs of animal species such as tapeworms and brine shrimps. However, in tardigrades, the latent state can occur at any stage of the life cycle (egg or adult) and on repeated occasions. Their ability to employ latent states (in which metabolism is virtually undetectable) has been a noted feature of tardigrade biology since the animals were first observed by the pioneering microscopists of the 18th Century[6]. This has been one of the enduring attractions of tardigrades as an object of study, leading early observers to describe tardigrades as exhibiting 'resurrection'[1]. More recently, it has been asked, if specimens failed to emerge from dormancy, was it because *they died while they were dead*?[7] Excitement over the remarkable physiology of these animals has, however, resulted in some claims that may have been a little exaggerated and given rise to sensational journalism[8]. Such overenthusiastic claims include suggestions that tardigrades could survive space travel; be transported around the globe in high-level wind currents or survive desiccation for periods in excess of a century. However, it appears that hard

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water loss

evidence to support such claims is conspicuously absent. Even so, when tardigrades are in dormant states, they exhibit increased resistance to environmental extremes (such as cold, heat, drought and ionizing radiation), allowing them to colonize habitats that would be too hostile and unpredictable for most aquatic animals.

Although tardigrades can enter various latent states (cryptobiosis) that are triggered by adverse changes in various environmental factors, including temperature (cryobiosis), salinity (osmobiosis) and oxygen (anoxybiosis), it is the response to the removal of water (anhydrobiosis) that has more often been the focus of study. Various factors influence the effectiveness of anhydrobiosis by promoting a low rate of drying that is crucial for the animals to survive the experience. Lowering the rate of desiccation facilitates a metabolic preparatory stage during which the animals undergo anatomical and metabolic adjustments that are required for them to successfully see through periods of drought when activity is not possible.

First, the nature of the surrounding microhabitat can help to control the rate of water loss. Moss cushions have their own adaptations to water loss (including the curling of leaves as they dry), providing ideal microenvironments for the resident fauna. Other microhabitats such as soils tend to dry unevenly, resulting in the encapsulation of animals in small pockets of water. It has also been reported that tardigrades exhibit clumping behaviour to retard water loss[1], although it is not clear whether the patterns observed are merely the results of tardigrades being trapped together in receding water films.

The most obvious anatomical feature of desiccated tardigrades is the way they fold up with the result of reducing the exposed surface area. This is described as tun formation. The animals undergo considerable longitudinal contraction that is facilitated by regions of thinner cuticle across the dorsum, allowing folds in the skin. The legs are also withdrawn into the body.

The adult animals are not the only focus of interest in tardigrade biology. The complexity of tardigrade egg shells is presumed to be a factor in their survival (Figure 3). The function of the elaborate ornamentation that is a feature of the eggs of so many species is not really understood, but a number of hypotheses have been suggested[1]. The projections on the surface may help maintain the egg's position within an unstable substratum or they may

Figure 2. *Echiniscus madonnae* scanning electron photomicrograph ©Lukasz Michalczyk and Lukasz Kaczmarek (www.tardigrada.net).

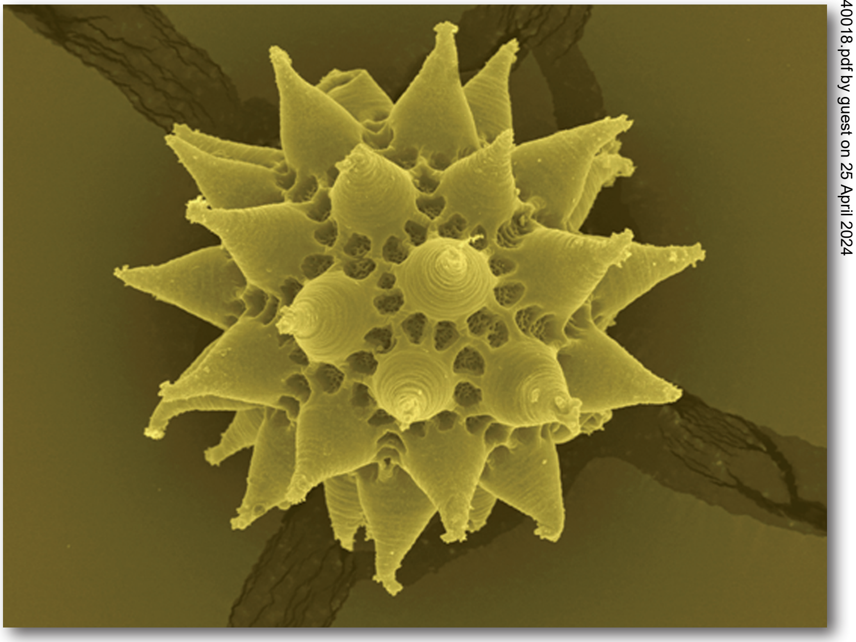

Figure 3. Egg of *Macrobiotus magdalenae* scanning electron photomicrograph ©Lukasz Michalczyk and Lukasz Kaczmarek (www.tardigrada.net).

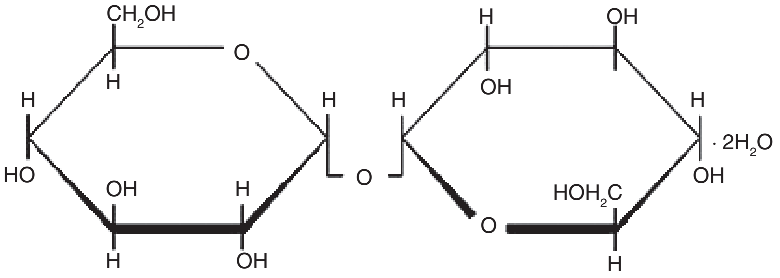

Figure 4. The structural formula of the non-reducing disaccharide, trehalose. One of a suite of compounds described as compatible solutes or osmolytes that confer tolerance to dehydration of living tissues.

help to avoid predation by preventing penetration by the stylets of predatory nematodes. Alternatively, the ornamentation may have a role in the control of water loss by trapping pockets of water between the projections.

Tardigrades achieve a degree of dehydration that not only involves the loss of free water forming the aqueous solutions of the body, but also the loss of bound water, which is required to maintain the structure of vital hydrated macromolecules such as proteins, membrane phospholipids and nucleic acids. The problem for anhydrobiotic tardigrades is to maintain structural integrity while simultaneously removing the water. Destruction of these hydrated macromolecules would cause irreversible damage and result in death. The bound water is replaced by compounds to maintain structural integrity during dehydration and which are easily removed during rehydration. Non-reducing sugars, such as trehalose, have been identified in high concentrations in anhydrobiotic animals, performing a protective role in tardigrades[9,10].

Various hypotheses for the mechanism of membrane protection mediated by sugars such as trehalose have been developed[9]. In the water-replacement model, the central argument is that the hydroxy groups within the trehalose molecule (Figure 4) form hydrogen bonds with polar surfaces on cellular membranes to replace the lost water. This then inhibits the structural deformations that would normally accompany dehydration. Other hypotheses consider the entrapment of water by sugar molecules aggregating at water surfaces and the vitrification of tissues (trehalose can form an amorphous glass upon dehydration) in which structural deformations would be minimized. There is probably a combination of these effects at work to protect the tissues from damage and degradation in the anhydrobiotic organism[9].

Observations on tardigrades and nematodes (in which the process of anhydrobiosis appears to have developed in a similar manner, as an example of convergent evolution) suggest that anhydrobiotic success is related to the nutritional health of the animals, particularly the possession of adequate lipid reserves that, in tardigrades, are stored in free-floating body cavity cells. The anatomic and metabolic preparations for anhydrobiosis consume a considerable amount of energy and so, although tardigrades can successfully undergo repeated episodes of dehydration, they need sufficient time in between to feed and build up their food reserves. The lipids in the body cavity cells serve a number of functions, including the maintenance of spatial distribution of the tissues in the shrunken animals to prevent reactions between molecules that are usually separated in the active animals as well as providing a reserve from which trehalose (and other membrane-protectant chemicals) are synthesized. Those animals which apparently fail to remove all of their water appear to succumb to a variety of fungal infections upon rehydration of the surrounding environment[1].

Although there has been considerable research undertaken to reveal the secrets of anhydrobiosis in tardigrades over the last 40 years, there are still gaps in our understanding of the process. Understanding the details of molecular interactions that facilitate the tolerance to the stresses exerted by extreme water loss is of interest not only to those studying the ecology and physiology of tardigrades, but also for the fundamental understanding of the importance of water to biology in general, and the field of biostabilization[11] in particular. The promise of this field in the future, and the real possibility of employing trehalose for the stabilization of macromolecules, cells and tissues for medical benefits seems a million miles away from Antonie van Leeuwenhoek and his peers marvelling at the *little animalcules* they could observe in water droplets, showing that you can never tell where an interest in natural history may lead. ■

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Ian Kinchin gained his first degree in biological sciences and followed this with a Masters thesis on the ultrastructure and taxonomy of tardigrades. He taught biology at various levels for nearly 20 years before moving into teacher education. He gained a PhD in science education and currently works as a senior lecturer in higher education within King's Institute of Learning and Teaching at King's College London, where he supports the development of teaching skills among university lecturers. email: ian.kinchin@kcl.ac.uk

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