# Life without water

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From space our planet is blue, and life here has evolved in the presence of abundant water. However, on land, water remains one of life's major challenges. Fully two-fifths of the land surface is classified as arid: the hot and cold deserts, where water is largely unavailable. Even in biomes where water availability is generally good, seasonal, daily and sporadic conditions can mean that life has to be able to survive its absence. Surprisingly, some organisms are able to survive complete loss of all their body water, to undergo anhydrobiosis. This surprising ability has evolved many times, and is particularly prevalent in very small animals. The biochemistry of anhydrobiosis challenges ideas of what 'being alive' really means and promises exciting biotechnological applications.

Terrestrial life has evolved several general mechanisms for dealing with water loss. Larger organisms, such as mammals, avoid loss by reducing their surface area to volume ratio, having waterproof coverings and reducing water losses involved in essential processes such as respiration or excretion. Another method to avoid water loss in a variable environment is to migrate to better environments when times get hard, for example to burrow deeper into the mud to avoid the drying up of a river bed, or following the seasons across a savannah landscape. A third method is to go into stasis when times are hard, and to remain in stasis until times get better.

Many organisms follow an extreme version of this third option, and are able to survive complete loss of all their body water. When free water becomes available again, they rehydrate, and resume their lives. This process is called anhydrobiosis – life without water. Anhydrobiosis is found in bacteria, many 'protozoa', in fungi and in plants. Mosses and lichens on a desert rock, or on a British garden wall on a sunny day, can dry to a crisp and still come back to life when the rains come (rarely in the desert, frequently in the British summer).

## Dried-up animals?

Among animals, anhydrobiosis is quite common. Many members of the meiofauna – animals with body lengths less than a millimetre that live in the water films surrounding soil grains and on plants and lichens – are frequently challenged by drying of their local environment. Because they are very small, and lack the ability to escape, being able to survive a temporary loss of water is an effective strategy. Unlike larger animals, which can retain water within shells, cuticles or other coverings, meiofauna cannot both respire and prevent total water loss. Thus a zoo of animals can be revived from bone-dry moss by the simple addition of rainwater (See Box). The animals that make-up terrestrial meiofauna are not often seen unless one goes hunting for them. They include nematodes (roundworms), rotifers (wheel animals) and tardigrades (moss piglets or water bears). In each group, many species are able to undergo anhydrobiosis. Our research has focused on the tardigrades.

Not all tardigrades can undergo anhydrobiosis. Marine species, and species living in freshwater, are unable to perform this trick, and it is likely that many soil tardigrades are also not anhydrobiotic. However, many species are. When challenged by drying conditions (low relative humidity) they stop feeding and moving, and slowly contract as all their body water evaporates. At the completion of drying out, essentially all their body water has gone, and they form 'tuns'– contracted barrel-shaped specks. These tuns can be stored dry for years. The current record is held by some freeze-dried Antarctic tardigrades revived 30 years after they were collected and preserved as part of a herbarium moss specimen. Some species – slow anhydrobionts – need hours of warning that dry times are coming (a period of reduced humidity), while others – rapid anhydrobionts – can be dried in 30 minutes, without problems. Rehydration in both groups is rapid. A tun transforms into a walking tardigrade in half an hour. We have mainly studied two tardigrade species, *Ramazzottius varieornatus* and *Hypsibius dujardini*. These are relatively closely related (they are both members of the Hypsibiidae) but *R. varieornatus* is a rapid anhydrobiont, while *H. dujardini* needs a physiological warning. We have generated genome sequence data for both, and explored the dynamics of transcription during anhydrobiosis, identifying the likely players in the process.

## How do they do it?

So how do tardigrades (and other anhydrobionts) achieve this remarkable feat? If we take mammalian cells, or other small animals and dry them up, they do not survive. Water loss leads to three major, catastrophic effects in normal living cells. Membranes rely on the interaction between amphipathic lipids and water for their stability: in the absence of water, membranes collapse and fuse. Proteins rely on water for stability and function, and many water molecules are coordinated with each protein, keeping

## Tardigrade hunting

To catch your own tardigrades and other anhydrobiotic animals you need a clump or tuft of moss (one centimetre in diameter) from a richly covered wall or roof, some still spring water, two dessert spoons, a small Petri dish or watch glass and a microscope. A good binocular 'dissection' microscope is best, but a simple compound scope works fine – it is just harder to find the animals in the small field of view.

Take the button of moss and place it in one of the spoons. Pour on a full spoonful of still water. Leave for 30 minutes or more if the moss was dry when you got it. Force the water out of the moss and into the Petri dish by squeezing the two spoons together. Let the sediment settle in the Petri dish before looking at the dish under the microscope. Illuminate from below, if possible slightly obliquely.

Under medium power (so the field of view is about a centimetre) slowly scan across the dish, focusing on the sediment, sand grains and moss leaves on the bottom. You are looking for movement – signs of animal life. When you see something moving, stop. Focus up and down, and change the lighting. If you can, zoom in.

After a while, once you have got your eye in, you will identify a menagerie of life in your miniscule world. Nematodes are long, see-through worms, thrashing about, often with their tails attached to the surface of the dish or a sand grain. Rotifers move by looping like caterpillars, and feed by attaching their tails and unfurling their 'wheel organs', coronae of cilia that waft food into their mouths. Single-celled ciliates, some as big as the rotifers, swim around bumping into things and occasionally settling to feed.

Then you will spot a tardigrade, clinging onto a leaf or clawing vainly to get traction on the bottom of the dish. They are between ~1 mm and 0.2 mm when adults, have eight stumpy legs, and often paired eyespots on their heads. Each leg ends in claws, which allow the animals to grip and traverse their miniature world.

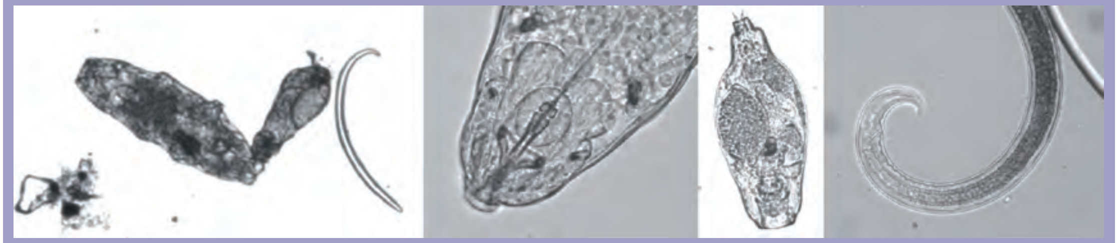

Image: A tardigrade, a rotifer and a nematode from a moss clump on a drystone wall in the eastern Cairngorms of Scotland. Close-ups of the tardigrade head, the rotifer containing a large egg and the nematode's tail. The tardigrade is 0.3 mm long.

it in solution; in the absence of water, proteins denature and precipitate. Lastly, water is the solvent for most small molecules and salts in the cell: loss of water results in extreme and damaging effective concentrations of ions and other metabolites. To survive drying out, tardigrades must avoid these three lethal effects.

Our, and others', analyses of slow anhydrobiont tardigrades entering anhydrobiosis indicates that this triggers expression of a set of genes with revealing features. Some of these genes are members of known heat-shock protein (HSP) families. Many HSPs in other organisms are induced under stressful conditions, but some are constitutively expressed. In general, HSPs serve as chaperones, assisting in the correct folding of newly expressed proteins or in the refolding of damaged proteins. They prevent misfolded or unfolded proteins from interacting with other, functioning parts of the cell machinery and permit recovery from damage. It is likely that the tardigrade HSPs are performing the same function. In a similar vein, a second set of stress-induced protein genes is also found to be up-regulated in drying tardigrades. These antioxidant and detoxification enzymes have roles in protecting proteins from attack by oxyradicals and other moieties generated as a result of, for example, breakdown in the compartmentalization of metabolism in the cell. Surprisingly, both our species have lost some peroxisomal and hypoxia pathways found near-universally in other animals, a loss we attribute to the need to avoid catastrophic recruitment of apoptotic damage processes during anhydrobiosis.

The third group of genes, up-regulated upon entering anhydrobiosis, express small proteins that have no clear domain similarities to other proteins, but share a particular structural property: they are predicted to be natively disordered. Natively disordered proteins are ones that do not appear to have any favoured, stable, three-dimensional structure. They are poorly crystallizable, if at all, and under nuclear magnetic resonance spectroscopy display few stable intramolecular interactions. Natively disordered proteins have also been described in other anhydrobiotic organisms, such as plant seeds (where anhydrins are abundant), in nematodes and in rotifers. While these natively disordered

*Hypsibius dujardini* adult. Photomicrograph by Aziz Aboobaker, Blaxter lab.

*Ramazzottius varieornatus* adult. SEM image by Kazuharu Arakawa.

proteins from different phyla and kingdoms are not orthologous (i.e. they do not derive from a single common ancestor) they share certain properties, such as a sub-repetitive sequence with abundant representation of alanine and valine amino acids , and adoption of an amphiphilic alpha-helical structure upon water loss. These proteins are extremely abundant in dried up tardigrades.

What are these disordered proteins doing? It is likely that they are replacing the water molecules lost to evaporation. Proteins are not volatile, and polar side groups in the anhydrins and the tardigrade anhydrobiosis proteins are well placed to replace the structural water molecules that coat cell membranes and perform essential structural roles in maintaining enzyme and other protein integrity. Excitingly, the rapid anhydrobiosis species constitutively express high levels of just these proteins, explaining their lack of requirement for preconditioning.

## A dry tardigrade is a supertardigrade

Water is a dangerous molecule on which to base life. It is liquid over a small temperature range, and most biochemistry is possible only in a small fraction of this range. Freezing kills cells and organisms as ice crystals puncture membranes and force proteins and metabolites into concentrated salt-rich phases. Heat kills organisms as proteins denature and membranes solubilize. However, once water is excluded, and the cells' machinery is stabilized by non-volatile proteins, these temperature barriers are opened.

Anhydrobiont tardigrades have superpowers. They are also cryobionts – they can be frozen with no adverse effect on recovery as there is no water to form ice crystals. They can be heated to above the boiling point of water, as there is no water to lose. They are resistant to pressure changes, as one of the major effects of low atmospheric pressure is loss of water vapour. Dried tuns can be blown like specks of dust into the upper atmosphere and distributed globally, immune to the lack of air pressure and the low temperatures. Biological systems are damaged by radiation, often through the interaction between high energy particles and cellular water, creating oxyradicals that attack DNA and proteins. In the absence of water, anhydrobiotic tardigrades are also resistant to radiation at levels that kill hydrated ones in minutes. *R. varieornatus* improves its radiation resistance by expressing a protein that traffics to the nucleus and protects the chromosomal DNA. When expressed in human cells this protein reduced the DNA damage caused by X-rays by 50%.

Immune to heat and cold, surviving in the vacuum of near space and resistant to radiation, it has been suggested that the tardigrade tun could be the spore of life's diaspora to other planets. Could they even have been transported here from another planet? Sadly for this attractive science fiction meme, the DNA of tardigrades clearly and unequivocally identifies them as earth animals, related to arthropods (insects and relatives) and nematodes. As not all tardigrades have anhydrobiotic abilities, and the ones that do are nested within non-anhydrobionts, including many marine species, it seems most parsimonious that tardigrades evolved their anhydrobiotic abilities independently here on earth. Tardigrades' ability to survive in (near-earth) space has been tested in European Space Agency experiments on the International Space Station, which showed that they can survive space vacuum and cosmic radiation, but not well, and not for long.

## Making tardigrades useful

Tardigrades are near the bottom of lists of organisms that threaten our well-being: they do not cause disease in humans or our farmed species. Similarly, apart from claims of a role in ecosystem functioning, they do not provide benefits: we do not eat them, or derive life-saving drugs from them – yet. Their main current contribution to human life appears to be that they are 'cute'. However, the biology of anhydrobiosis offers tantalizing leads for exploitation. If the mechanisms that tardigrades use to survive dehydration could be isolated and deployed elsewhere, their obscurity could be a thing of the past.

Preserving cells and tissues is an important part of modern medicine and agriculture. Important germplasm and cells are frozen away, at expense and sometimes with low recovery rates, and under the ever-present risk of system failure, freezer meltdown and loss. What if cells could be dried and rehydrated at will, simply by infusing them with one of the natively disordered proteins tardigrades have evolved? Personalized tissue banks could be developed and stored cheaply, assuring availability of matched tissues for grafting or therapy. The *R. varieornatus* protein that protects cells against radiation could be targeted to healthy cells surrounding an invasive tumour and the tumour lethally irradiated while the healthy cells survive. A cell bank could be kept securely on a shelf in a lab or medical facility, shipped around the world and deployed where needed. Cellular and live vaccines currently need a chain of (working) fridges and freezers between the production plant and the communities in low- and middle-income countries where they are needed most. Shipping a vaccine costs more than producing it. If the vaccine was dried, stably preserved in a tardigrade anhydrin, it could be posted to the village clinic, kept there ready for use in outbreaks at low cost and high reliability. Tardigrade genes promise to become interesting biotechnology tools.

## Life after death

One last thought. Life is defined as the presence of biochemical activity, the processing of metabolites into other metabolites with the production and consumption of ATP as its core currency. Life involves the maintenance of ion gradients and thus electrical charge across lipid membranes. Life involves the processing of nutrients into reproductive propagules that carry the genetic material of the parent forward in time.

As a tun, a 'living' tardigrade is indistinguishable from a 'dead' one. No biochemistry is happening. There is no calorific output, and no enzyme-driven transformations are taking place. No food is being consumed and no waste produced. Its chemical and isotope signatures will indicate that it is likely the product of a living process, but it is not possible to distinguish a tun from a dead tardigrade, or from dead organic matter. Like Schrödinger's mistreated cat, it is only possible to tell if a tun is alive by rehydrating it, at which point it is no longer a tun. Neither dead nor alive, it exists in a third state of possibility. ■

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