Anhydrobiosis

Drying Without Dying

T Ramakrishna Rao

In aquatic and moist soil environments subjected to periodic drying, a diverse group of organisms (mostly microscopic) have evolved extreme desiccation tolerance through a physiological adaptation called 'anhydrobiosis'. Notable among such organisms are tardigrades, bdelloid rotifers, and nematodes. Anhydrobiosis also allows these organisms to tolerate extreme temperatures and ionizing radiation. In this article, the significance and some mechanisms of anhydrobiosis are described.

After retiring in 2004 from the University of Delhi where he was Professor and Director of the School of Environmental Studies, T Ramakrishna Rao served as a visiting Professor for six years (2010–2016) at the Indian Institute of Science Education and Research (IISER), Mohali. He received his PhD from the University of California and conducted postdoctoral research in British Columbia (Canada) and Hawaii (USA). His research interests are in the roles of competition and predation in the life of freshwater zooplankton.

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Introduction

Life on Earth originated in water and continues to be supported and sustained by it. The familiar adage 'water is life' attests to the paramount importance of water for living organisms. The human body is 60% water by weight and humans are notoriously intolerant to the loss of their body water. Any loss exceeding 5% has serious physiological consequences while a loss of >15% may prove fatal. It is reported that every year, dozens of Mexican immigrants illegally crossing the border into Arizona perish due to severe dehydration in the vast and extremely hostile Sonoran desert. Dehydration is also the major cause of high mortalities during a cholera epidemic.

Against this background of the critical importance of water, it is amazing that there are many groups of organisms that survive extreme levels of desiccation. These organisms can survive months to years without any ill effects by entering into an extraordinary state of dryness called 'anhydrobiosis'. Anton van Leeuwenhoek, the inventor of the microscope, was probably the first to observe this unusual phenomenon in 1772. He found that wetting a sam-

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ple of dry sediment collected from his rooftop gutter brought to life many microscopic animals. Many organisms are capable of undergoing 'dormancy', a state characterized by suspended activity and reduced level of metabolism. When dormancy is under endogenous control, it is termed 'diapause', whereas if dormancy is brought about by external factors, it is termed 'quiescence'. An extreme state of quiescence, characterized by nearly undetectable levels (0.01% of normal) of metabolism is called 'cryptobiosis'. The environmental factor inducing cryptobiosis may be lack of oxygen (anoxybiosis), extreme cooling (cryobiosis), or desiccation (anhydrobiosis). During anhydrobiosis, the organism's body water content decreases to <10% of the normal (it is as low as 3% in tardigrades). For comparison, seeds of cereal crops like wheat and rice have a moisture content exceeding 15%.

Anhydrobiosis is likely to have evolved as an adaptation to seasonal or episodic drying in moist habitats. Such habitats include very shallow lakes, ponds, puddles, moist soil, and microhabitats such as a mat of moist moss or lichens. Anhydrobiosis has evolved in a wide variety of taxa including bacteria and protists, mosses, lichens, nematodes, rotifers, tardigrades, insects, and crustaceans. The most extensively studied anhydrobiotic species are shown in *Figure* 1. Of these, the microscopic tardigrades (popularly known as water bears) have received a lot of attention lately in the media (see *Box* 1). Bdelloid rotifers (*Adineta*, *Macrotrachela* in *Figure* 1), tardigrades (*Ramazzottius*), and nematodes (*Aphelenchus*) are species that can go into an anhydrobiotic state at any stage in their life while the anhydrobiotic response is restricted to a specific stage of life in other species – larval stage in the case of African sleeping chironomid, and the cyst stage in the brine shrimp *Artemia*. Cysts (which are thick-shelled, dormant eggs) of *Artemia* are a commercially important resource in aquaculture because the larvae (called nauplii) that hatch from the cysts makes an ideal first food for reared fish larvae. Since *Artemia* cysts are in an anhydrobiotic state, they remain viable for years and can be rehydrated when required, to obtain nauplii within 24–48 hours.

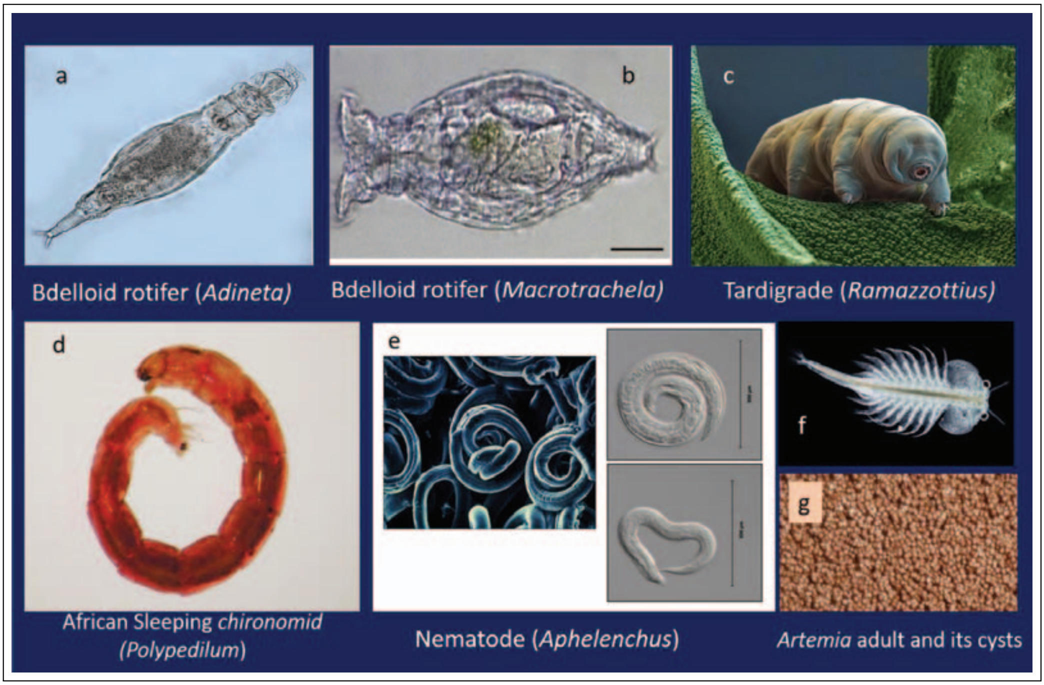

Figure 1. The most extensively studied anhydrobiotic species.

Among plants, desiccation tolerance in seeds is nearly ubiquitous, but the same in vegetative tissues is very uncommon. However, there are a handful of species called 'resurrection plants' which, even after losing 95% of their water content, remain 'resurrectable' (able to come back to life upon moistening) for years. The club moss *Selaginella* and the Jericho rose *Anastatica* are well-known resurrection plants (you can order and get these plants in their dry state from Amazon India and try for yourself!).

The evolution of anhydrobiosis appears to be accompanied by certain collateral benefits also to the organism. Most anhydrobiotes are not only desiccation-tolerant but are able to survive extremes of temperature and ionizing radiation. It is possible that the underlying molecular mechanisms are basically the same for all these extraordinary abilities. Anhydrobiosis also offers an efficient dispersal mechanism for bdelloid rotifers and tardigrades that live in ephemeral water bodies; these animals are so light in the dry state that even winds can easily transport them to other habitats that may be more hospitable.

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Box 1. The Amazing Tardigrades

'The toughest animals on Earth', 'the last survivors on Earth', 'world's most indestructible species', are some of the headlines appearing lately in the media, bringing to light the amazing abilities of microscopic (50–2000 μm) organisms belonging to Tardigrada, a phylum related to Arthropods. They occur in every conceivable habitat from mountain tops to deep sea trenches and in microhabitats like the thin layer of water associated mosses and lichens. They are perfect anhydrobiotes with the ability to go into an anhydrobiotic state at any stage in their life and survive in a dry state for 20–30 years. They can survive for a few minutes at −253 °C (liquid nitrogen) or at 150 °C at the other extreme, and for decades at −20 °C. They can comfortably tolerate atmospheric pressures ranging from zero (space) to 1200 atm (deepest Pacific Ocean trenches). They are resistant to radiation levels of 5000–6200 GY (for comparison, absorption of just 10–20 GY of ionizing radiation by the human body is fatal).

Their resistance to extreme atmospheric pressures and radiation naturally qualified tardigrades as ideal candidates for space research. In 2011, under Endeavour Space Mission, two species – *Milnesium tardigradum* and *Richtersis coronifer* – were sent into space to understand the effects of space stress on DNA integrity. Surprisingly, microgravity and cosmic radiation had no significant effect on their survival rates during space travel.

Are these creatures really the 'world's most indestructible species'? It appeared so to three physicists [1] who, recently in a thought experiment, examined scenarios of major astrophysical events such as supernova explosion, gamma ray bursts, and asteroid impact that could potentially wipe out life from planet Earth. While ruling out the possibility of any cosmic event totally sterilizing the Earth, the authors proclaim that nothing short of vaporizing all the oceans can make tardigrades extinct. However, caution is needed in accepting such an incredible pronouncement. The researchers lumped together all the tardigrades in this study. Certainly, all the amazing defenses attributed to these organisms are present in tardigrades as a group (the phylum has more than 1200 species), but no one species is known to possess all of them. Deep ocean species are adapted to the crushing pressures of the depths but probably not to desiccation or radiation while species living among moist lichens on land may not be resistant to high atmospheric pressures.

Mechanisms of Anhydrobiosis

It is reasonable to assume that anhydrobiotic organisms have evolved the ability to sense the water level or the humidity and the temperature levels of their microhabitat. As declining ambient water levels (often coupled with very high temperatures) signal imminent dehydration, the organism sets in motion a series of steps leading to anhydrobiosis. Its first line of defense is a set of structural or morphological changes. The organism's projecting appendages are withdrawn into the abdomen and the skin shrinks considerably to a structure (called 'tun' in tardigrades and bdelloid rotifers) bearing no resemblance to the active animal. These changes lead to reduced evaporative water loss by reducing the surface area. As dehydration proceeds further, the animal's biochemical and molecular defenses come into play. Laboratory studies show that water loss must be slow and gradual (as it happens in the natural habitat) for the organism to transform successfully into a totally anhydrobiotic state.

At the cellular level, water plays a vital role in maintaining the functional integrity of cytoplasmic and membrane proteins. Every protein is composed of a long chain of amino acids which folds into a particular three-dimensional structure. Water is the source of hydrogen bonds that are so essential for the proper folding of a protein and stabilization of its correct configuration. Stressful conditions such as heat shock and dehydration cause denaturation of proteins. Denatured proteins fold improperly (misfolding) or start clumping (aggregation) and consequently become dysfunctional. In response to such stresses, the cell produces special proteins called heat shock proteins which act as molecular shields or chaperones and help restore the functional configuration of denatured proteins.

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Anhydrobiotes have evolved in addition to heat shock proteins, a variety of special molecules for a more efficient and longer-lasting protection against desiccation-induced damage. The most important among them is a complex sugar called trehalose which is present in diverse taxa of desiccation-tolerant species. It is claimed that trehalose is essential for the long-term survival of anhydrobiotes because it is a more stable and more versatile protective agent than protein chaperones. Trehalose takes over the role of water and prevents protein aggregation and membrane damage. It is also able to vitrify[1] and act as a protective shield. Another class of protective proteins, called 'Late Embryogenesis Abundant' (LEA) proteins[2] are found in anhydrobiotic nematodes and *Artemia* cysts, and also in drought-resistant plants. These proteins are shown to be novel molecular chaperones, often working synergistically with trehalose, to prevent the formation of damaging

[1] Form a glassy matrix.

[2] First discovered accumulating during late embryogenesis of cotton seeds.

protein aggregates.

Interestingly, most recent reports show that tardigrades have evolved a different biochemical solution to the same problem, an example of convergent evolution. In these animals entering the anhydrobiotic stage, protective vitrification is achieved, not by trehalose, but by proteins unique to the taxon – 'Tardigrade-specific intrinsically Disordered Proteins' (TDP). In a hydrated state these proteins instead of folding into any three-dimensional structure, remain in a jelly-like state. But when the animal starts desiccating, these proteins vitrify, forming a glassy cocoon to protect the genetic material against damage.

Last year, researchers isolated a protein unique to tardigrades, which they called the 'Damage Suppressor Protein' (D_{sup}). This DNA-associating protein is shown to suppress DNA damage induced by radiation [2]. D_{sup} actually appears to suppress or repair DNA single-strand and double-strand breaks. The same protein might also be shielding DNA against similar damage caused by desiccation. In the genomic treasure trove of *Ramazzottius*, Hashimoto *et al*., observed two unique phenomena involved in stress tolerance – loss of gene pathways that promote stress damage and expansion of stress-related gene families.

Do Anhydrobiotic Organisms Age During Dormancy?

What happens to the aging process during anhydrobiosis? Is the process suspended or does the animal continue to age during dormancy?

What happens to the aging process during anhydrobiosis? Is the process suspended or does the animal continue to age during dormancy? For example, if the animal enters dormancy at age x and the duration of its dormancy is y time units, is its age $(x + y)$ or only x at the termination of dormancy? It is interesting to note that both modes exist in the anhydrobiote world.

'Picture of Dorian Gray' Model (after the protagonist of Oscar Wilde's famous novel): The animal continues to age during dormancy, and therefore its age is $(x + y)$ when it emerges out of that state. Anhydrobiotic nematode worms exhibit this pattern.

'Sleeping Beauty' Model (after the classic character from the fairy tale by Charles Perrault): In this model, aging is suspended during dormancy and the animal's age is only x at its termination. Bdelloid rotifers like *Macrotrachela* and *Adineta* and tardigrades like *Milnesium* are examples of this model. In one experiment, the rotifer *Adineta ricciae* was kept in anhydrobiotic state for 8 days and then rehydrated for revival. The longevity of the rehydrated individuals was not significantly different from that of the controls. It appears that bdelloid rotifers and tardigrades emerging out of their dormancy simply 'reset' their age to the beginning of dormancy.

Anhydrobiosis and Asexual Reproduction

Anhydrobiotic tardigrades include both sexual and asexual species. Bdelloid rotifers, however, are all anciently asexual; there is no evidence of sexual reproduction in this group at least during the last 40 million years. Could there possibly be an evolutionary link between the two attributes – asexual reproduction and anhydrobiosis in bdelloid rotifers?

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One of the many hypotheses put forward to explain the enigma of sexual reproduction is the 'Red Queen' hypothesis. The hypothesis posits that sexual reproduction evolved to generate sustained genetic variability that organisms needed to combat ever-evolving cellular parasites. Now, suppose an organism could evolve an alternate mechanism to escape from parasites, then why does it need the costly sexual reproduction? That is what bdelloid rotifers may have done and remained asexual since millions of years! They can, at any stage in their life, slip into an anhydrobiotic state which is hostile to internal parasites. Further, in the dry stage, these rotifers can be easily 'blown away' by winds to another wet habitat enabling them to stay ahead in the host-parasite race.

There is another interesting link. It has been hypothesized, and later experimentally demonstrated in bdelloid rotifers that animals having the ability for anhydrobiosis also have an increased ability to incorporate environmental DNA through horizontal gene transfer (HGT). Bdelloid rotifer genomes reveal the presence of 8–14% of acquired and incorporated 'foreign' genes. Frequent anhydrobiotic episodes in bdelloid rotifers can facilitate HGT and thus generate sufficient genetic variability, obviating the need for sexual reproduction.

Anhydrobiotic Genes for Human Welfare?

Recent successes in identifying the specific genes that enable anhydrobiotic animals to survive for long periods without water opens up exciting possibilities of genetic engineering for human welfare. Just imagine being able to incorporate anhydrobiotic genes into drought-sensitive crop plants! When engineered into other organisms these genes are likely to confer not only desiccation tolerance but radiation resistance as well. Recently Tokyo University researchers [1] added the extract of one of the toughest tardigrades – *Ramazzottius varieornatus* – containing the damage-suppressing protein (D_{sup}) to human cell cultures. The protein attached itself to the cell's DNA and promptly went to work the way it does in the tardigrade. The researchers reported that the protein suppressed or repaired single-strand and double-strand breaks by about 40%. These exciting results have implications for human space travel in future. Suppose the gene encoding for D_{sup} protein can be engineered into the human genome, would humans venturing into space be protected from the damaging cosmic radiation? At present, such a scenario is only in the realm of science fiction, but who knows? There was a time when the very idea of humans landing on the moon was quickly relegated to science fiction!

Image Credits

(a) http://www.microscopy-uk.org.uk/mag/imgnov08/6-A.cf.tuberculata-Verolet.jpg

(b) http://rotifera.hausdernatur.at/Rotifer_data/images/addscan/_full-size/Macrotrachela%20quadricornifera%20Milne

(c) https://www.nature.com/polopoly_fs/7.39237.1474377844!/image/WEB_C0089248

(d) https://alchetron.com/Polypedilum-vanderplanki-4022015-W

(e) `http://slideplayer.com/slide/7631592/25/images/9/NE-SARE+Professional+Development+Plant-Parasitic+Nematode+Workshop.jpg`

(f) `https://s-media-cache-ak0.pinimg.com/originals/ef/58/2d/ef582d208de5ef8b8b8e2f5566eab53b.jpg`

(g) `https://sc01.alicdn.com/kf/UT8q6ZRXqBXXXagOFbXf/Artemia-Cysts-best-for-fish.jpg`

Suggested Reading

[1] **D Sloan *et al*., The Resilience of Life to Astrophysical Events, *Scientific Reports*, 7: 5419, 2017, DOI: 7:5419/DO!:10.1038.**

[2] **T Hashimoto *et al*., Extremotolerant Tardigrade Genome and Improved Radiotolerance of Human Cultured Cells by Tardigrade-unique Protein, *Nature Communications*, 7:12808, 2016, DOI: 10.1038/ncomms12808.**

[3] **P Alpert, The Limits and Frontiers of Desiccant-tolerant Life, *Integr. Comp. Biol*., Vol.45, pp.685–695, 2005.**

[4] **K Goyal *et al*., Molecular Anhydrobiology: Identifying Molecules Implicated in Invertebrate Anhydrobiosis, *Integr. Comp. Biol*., Vol.45, pp.702–709, 2005.**

[5] **L Rebecchi, T Altiero and R Guidette, Anhydrobiosis: The Extreme Limit of Desiccation Tolerance, *Invert. Surv. Jour*., Vol.4, pp.65–81, 2007.**

[6] **C Ricci, Bdelloid Rotifers: 'Sleeping Beauties' and 'Evolutionary Scandals', But not Only, *Hydrobiologia*, Vol.796, pp.277–285, 2017.**

[7] **M Watanabe, Anhydrobiosis in Invertebrates, *Appl. Entomol. Zool*., Vol.41, pp.15–31, 2006.**

[8] **E Weronika and K Lukasz, Tardigrades in Space Research – Past and Future, *Orig. Life. Evol. Biosph*., October 2016.**

Address for Correspondence
Prof. T R Rao
19 Shakti Apartments
Phase-III, Ashok Vihar
Delhi-110 052, India.
Email: trrao39@gmail.com