

Liquid Assets

Capital ways to save water

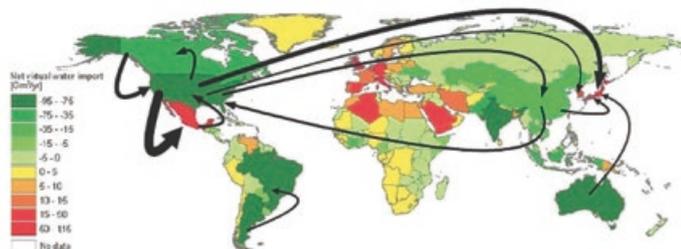
“Water, water, everywhere,
And all the boards did shrink;
Water, water, everywhere,
Nor any drop to drink. “

Samuel Taylor Coleridge
The Rime of the Ancient Mariner [1798]

By **Cornelia Eisenach**

We might not be quite as desperate as our ancient mariner yet, but those fresh drops are running low. And not only in arid regions of the world that are plagued by droughts and restricted access to fresh drinking water.

“Water scarcity and therefore access to clean drinking water is particularly important in semi-arid regions such as the Mediterranean area, but also in other regions where water demand approaches, or even exceeds, water availability. This includes large areas of Europe”, says Dr. Nick Voulvoulis, whose research focuses on water and environmental management at Imperial College London, UK. Certainly, as our ancient mariner experienced, the majority of our planet’s water is too salty for us to drink, or feed our plants and animals. In addition, over-use and water pollution “not only threaten our water security but nature itself, with the removal or impairment of aquatic ecosystems and the services that they can provide to us”, explains Voulvoulis, adding that more integrated sustainable solutions are needed.



Virtual water balance per country and direction of gross virtual water flows related to trade in agricultural and industrial products over the period 1996-2005. Only the biggest gross flows (> 15 Gm³/yr) are shown; the fatter the arrow, the bigger the virtual water flow.¹

Saltier bacteria – fresher water

Water is a limited resource only as fresh water so tapping into the oceans would help address our rising fresh water demands. Countries with access to sea water have been employing a technique called reverse osmosis to desalinate water for use in agriculture and, although this technique has been developed and improved over the decades, it has one major flaw: it consumes a lot of energy. “This is where biology comes in, because there is nothing like biology when it comes to exploiting energy”, says Professor Anna Amtmann, who leads an EPSRC-funded, multi-disciplinary research team in the UK to tackle the problem of fresh water availability.

She and her team have come up with a biology-based idea to desalinate without the need for large energy input using photosynthetic cyanobacteria. These bacteria live and grow on sunlight, they survive in seawater and they can grow to large densities. “Sodium salt is toxic to many organisms, even those

that live and thrive in sea water”, says Amtmann. However, cyanobacteria possess plasma membrane proteins (an ATP-powered proton pump and a Na⁺/H⁺ exchanger that exploits the pump’s proton gradient) that force out salt and so allow the bacteria to grow and reproduce in salt water. Once the cyanobacteria grow to high densities they run out of the ATP necessary to extrude the salt and, as a result, begin to take up sodium and chloride. The team intend to enhance this biological process further using a relatively new synthetic biology technique called ‘optogenetics’, in which a light-powered transport system enhances salt uptake by the bacteria.

This optogenetic technique uses halorhodopsin, a specialist protein found in halobacteria that pumps chloride ions inside the cell when it is stimulated by light. Amtmann’s team set out to genetically manipulate the cyanobacteria to express halorhodopsin: the pump will use the sun’s natural energy to drive chloride accumulation inside the cyanobacteria and the chloride-concentration gradient will draw in sodium. “We have already made progress in a proof-of-principle approach, where we were able to manipulate membrane potential by expressing halorhodopsin”, says Amtmann.

The team has also shown that simply growing and removing cyanobacteria from sea water already reduces the water’s salt content considerably. Sodium chloride adsorbs to cyanobacteria, possibly due to so-called extracellular polymeric substances that are contained within the cell wall and that act like salt scavengers. “We have characterised the chemical and physical properties of these substances and found that they change depending on the salt concentration”, says Amtmann. “Now it’s a matter of identifying the genes in the pathway that underlie this whole metabolism so as to exploit it further”².

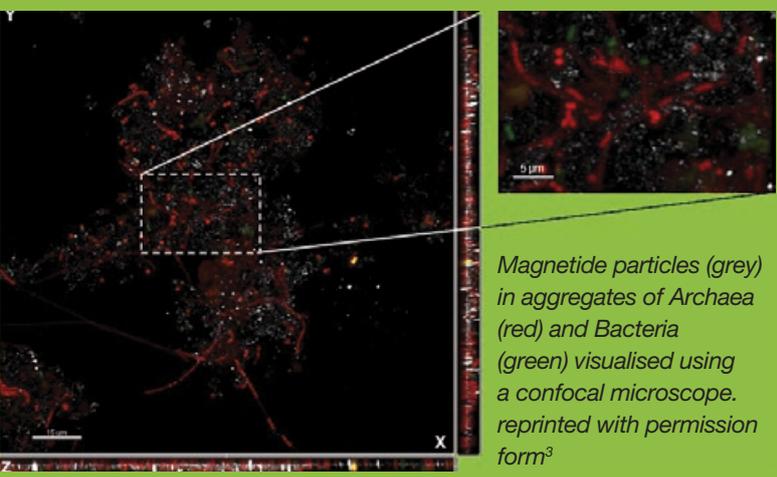


Cyanobacterial cultures being grown in the Amtmann lab at the University of Glasgow, UK.

Photo: Annegret Honsbein.

Wired up bacteria

The power of bacteria has been harnessed in the waste-water treatment industry for a long time. However, as with conventional desalination plants, waste-water treatment plants consume a lot



Magnetite particles (grey) in aggregates of Archaea (red) and Bacteria (green) visualised using a confocal microscope. reprinted with permission form³

of energy. So water engineers have been looking to biology to clean water without a large expense of energy. “Many of these energy-efficiency treatments rely on anaerobic processes involving complex microbial communities”, says Dr. Federico Aulenta of the Water Research Institute, IRSA-CNR in Monterotondo, Italy. He explains that one biological approach to clean waste water is indeed called anaerobic digestion. If you have ever seen images of bubbles arising from lake floors or from water-logged boggy land, you have an idea of what this process looks like. Anaerobic digestion involves distinct metabolic steps and each step is carried out by a different class of microbe such as acetogenic or methanogenic bacteria. These different microbes live in what is called syntrophic association. That is they link up their specialised metabolisms to form a conversion chain that breaks down the organic matter all the way to methane. The electrons arising from the initial oxidation of organic matter are passed on between microbes through a process called interspecies electron transfer. Ultimately, these electrons are used to reduce carbon dioxide to methane.

Aulenta and his team have recently discovered that this process can be boosted by the addition of conductive nanoparticles. These nanoparticles, made up of the natural mineral magnetite, enhance the transformation of organic matter to methane by boosting interspecies electron transfer, essentially ‘wiring up’ the different bacteria involved in the process. Reported in a recent publication, the researchers demonstrated that if they added the magnetite nanoparticles to anaerobic sludge, the production rate of methane from a model substrate could be increased by up to 33%³.

Hungry for electrons

As well as microorganisms being able to transfer electrons from the oxidation of organic matter to other microbes, they can also transfer them to extracellular electron acceptors, for example, an

electrode. This means they can generate electric current from fuel, e.g. organic matter, a principle exploited in microbial fuel cells. However, Aulenta is actually interested in reversing this process to tackle the problem of ground water contamination by industrial pollutants. “Chlorinated solvents are priority pollutants”, he says. “It is estimated that 80% of our contaminated ground water contains these solvents, due to improper storage, handling and accidental spills”.

This is where ‘electron transfer’ comes in: The electrodes donate electrons to the microbes, which, in turn, pass them on to chlorinated solvents, as they use them as a substrate for their respiration. “These anaerobic microorganisms, such as Dehalococcoides, ‘respire’ the solvent, reducing it to ethylene, which is non-hazardous”, explains Aulenta.

But how easy is it to take what works in the laboratory to a real-life problem? Professor Mark Riley, who heads the Biological Systems Engineering Department at the University of Lincoln-Nebraska, U.S.A., answers: “To develop a technology for a broad application, the process has to be taken from a laboratory scale, via a small pilot scale to full scale. However, a system that works well on a small scale does not necessarily perform with the same efficiency or outcome when scaled-up. This challenge is often seen in the pharmaceutical industry which by necessity must put much effort into this scale-up process”. This is an issue for Aulenta who says: “The key challenges of these technologies is loss of efficiency when you scale up electrochemical reactors. The larger the reactor, the poorer the performance”. To address this challenge Aulenta and his collaborators are going to take a small step towards scaling up their chlorinated solvent decontamination and will soon run a demonstration test at a real contaminated site in northern Italy.



Integrated Constructed Wetland located near Waterford, Ireland. Photo: Dr Rory Harrington.

Wastewater treatment lagoon in Kerepehi, New Zealand





Left: BioHaven® being used to remove algae and create wildlife habitat in a stormwater pond. Barrington, Illinois, USA. Right: Influent (left) vs effluent (right) following BioHaven® treatment of landfill leachate at a waste water treatment site. McLeans Pit, Town of Greymouth, South Island, New Zealand. Images provided by Leela O'Dea.

Safe haven for microbes

Wetlands, which are a natural habitat for water-cleaning biodegrading bacteria, are fast disappearing from the planet. In Ireland, for example, 90% of the wetlands have been transformed into agricultural land since the industrial revolution. Professor Miklas Scholz from the University of Salford, UK, explains: “The transformation of wetlands into agricultural land is having a detrimental effect on the environment and climate change as carbon that had been locked away has now been released as CO₂ and methane.” Scholz chairs the Civil Engineering Research Group at Salford and focuses on the use of so-called ‘Integrated Constructed Wetlands’ in waste water treatment. The principle of this technique is simple: you need a large area of land, divide it into wetland areas, apply a substrate, and seed or allow plants to settle on it and flood it with waste water. The surface will be covered by about 20 cm of waste water. The natural degradation processes performed partly by plants and mostly by microbes remove contaminants from farmland run-off, agricultural or domestic waste water⁴.

Surprisingly, it is even possible to build wetlands in the form of artificial floating islands. “BioHaven® is a commercially available floating wetland”, explains Leela O’Dea, an ecologist and founding partner at the aquatic environmental consulting firm, frog environmental. “It is about six inches deep and made up of a recycled plastic matrix, which allows plants to grow on it”. The floating island allows plants to grow with their roots dangling down into the water. “The advantage of this system is that the roots offer a really high surface area for microbial and sedimentation processes to take place”, says O’Dea. “There are a lot of microscopic particles or colloids suspended in the water and the dangling roots, which are covered with a sticky biofilm of microbes, help the sedimentation of these colloids”. Once the roots become heavy they drop off and carry the colloid contaminants away into the sediment at the bottom of the water. “The sedimentation process is especially important for the removal of phosphorus”, says O’Dea, “because phosphorous often adsorbs to soil particles and so gets trapped in colloids.”

Microbial processes such as nitrification, denitrification and ammonification contribute the lion’s share to a floating wetland’s efficiency. “We estimate that plants manage 6–8% of the treatment efficiency, either through direct uptake or transformation processes. From microbes, however, we get a 61–63% treatment value”, says O’Dea. Despite the huge workload on the microbes, plants can be used in integrated or floating wetland systems depending on their ability to accumulate certain contaminants, for example heavy metals. “We do look at the phytoremediation a plant can offer. For example, we can add Iris to the island which aids treatment of water with lead contamination”, says O’Dea.

Miklas Scholz has been involved in a project examining the use of constructed wetlands in arsenic removal⁵. Scholz says: “If you use plants known to hyperaccumulate certain contaminants in a freshly set-up wetland system, you can actually see differences in

treatment efficiency. But as the system matures, this effect lessens as other natural species out compete the hyperaccumulators”. Scholz believes that other benefits of constructed wetlands, such as attracting natural flora and fauna, creating biodiversity and offering room for recreational activities, should be factored into the equation of cost and energy efficiency when considering treating and decontaminating our water: “Research should not only compare and contrast the most advanced treatment methods but also consider alternative techniques”. ●



Treatment of landfill leachate using BioHaven® technology. McLeans Pit, Town of Greymouth, South Island, New Zealand. Image provided by Leela O’Dea.

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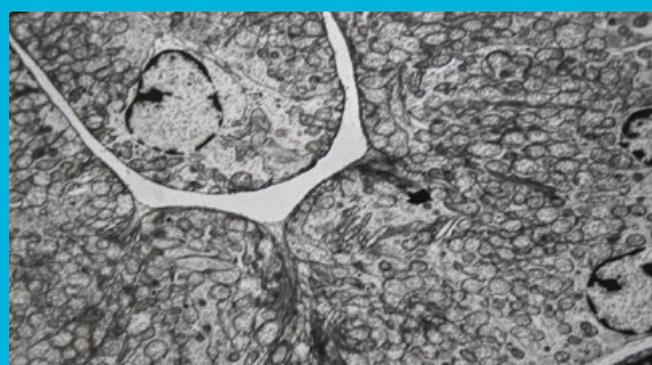
Accounting for every drop

by Caroline Wood

“Necessity is the mother of invention”, so the saying goes. In a world where water can be critically limiting, plants and animals have evolved some amazing innovations to make the most of every drop. Cutting off limbs, making protein from muscles and organs, not urinating for several months...there is no end to the lengths some species will go to, when it comes to overcoming water deficit.

Water from the Air

When you live far from oceans, rivers and any form of accessible water, there is still one source left: the water vapour in the air all around you. But it is surprising just how few animals and plants have evolved the ability to exploit this limitless resource. One success story is the Firebrat (*Thermobia domestica*), a primitive wingless insect similar in size and morphology to Silverfish. Although thought to have originated in hot, dry deserts, their name refers to their common occurrence around domestic fireplaces and boilers. Their ability to tolerate the dry conditions at these heat sources is granted by a mechanism which uses energy to absorb water directly from air. Dr John Noble-Nesbitt (University of East Anglia, UEA) was the first to discover that this process specifically occurs at the rectum rather than over the entire body surface, as

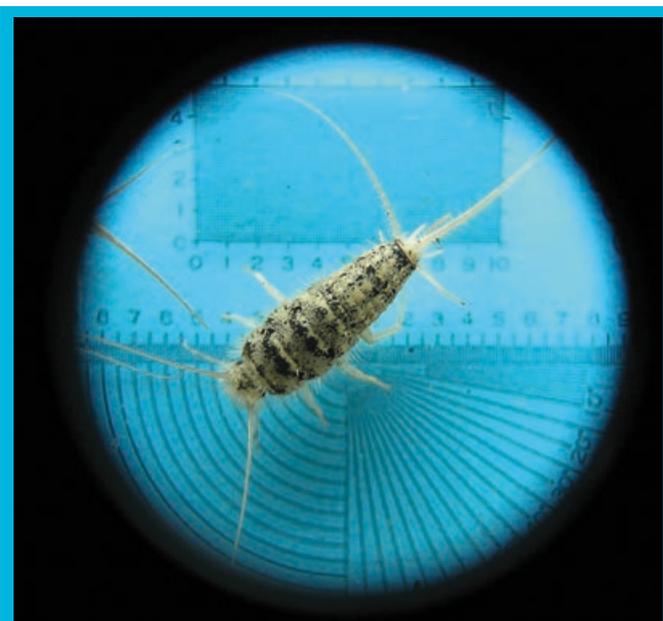


Epithelial layer of a water-absorbing sacculus belonging to the Firebrat *Thermobia domestica*
Photo: John Noble-Nesbitt

had been previously thought. “My idea that vapour absorption takes place on the rectum came from the observation that certain insects produce powder-dry faeces”, Noble-Nesbitt explains. “If the anus is sealed off using a beeswax-resin mixture, vapour absorption cannot occur but when the firebrat moults, the seal is removed and the process recommences”.

Active absorption is thought to take place on the three posterior anal sacs, each made up of numerous smaller sacculi. Inhalation along the anal canal allows a continuous flow of moisture-containing air over the sacs and ultrastructural studies show that the surface is perfectly adapted for extracting water. The outermost layer lining the sac is just one cell deep, but this is optimised to aid water absorption. The plasma membrane facing the lumen is considerably enlarged and forms deeply pleated infolds, providing a large surface area for water uptake. Vapour absorption is a highly active process, so these cells also have the highest known concentration of mitochondria in the animal kingdom, which provide the energy for active absorption. Noble-Nesbitt concludes: “Active water vapour absorption is of enormous benefit to those species that have the ability to carry it out. Understanding the cellular mechanisms involved could potentially benefit humans, for instance in developing improved dialysis for treating kidney failure”.

Some plant species are also capable of absorbing water from the air, which can aid survival in the most arid regions on the planet, including the Namib Desert on the West Coast of Southern Africa. “The Namib Desert receives on average only 50 mm of rainfall each year with some years not seeing a drop”, says Paul Rees, Horticulturalist of the Dry Tropics Unit at the Royal Botanic Gardens, Kew. “Yet plants can grow here due to a life-giving fog belt which forms when cold currents coming up from Antarctica collide with warm air circulations in the tropics.” *Welwitschia mirabilis*, a bizarre-looking gymnosperm closely related to primitive cycads and conifers, is one such plant.



Firebrat female, *Thermobia domestica*
Photo: John Noble-Nesbitt



Welwitschia
Photo: Paul Rees

These plants have a simple structure: a short, one-two foot trunk with two long, leathery leaves which are kept throughout its lifetime. “The surface of these leaves is covered in a bloom of white hairs which reflect light, helping the plant to cool and therefore conserve water”, says Rees. They also reduce air movement on the plant surface, creating a microclimate with increased humidity around the stomata (the microscopic pores on plant leaves that allow exchange of gas and water vapour). When the difference in water-potential between the inside and outside of the leaf is minimised, less water is lost through evaporative transpiration. “These hairs also help moisture from the fogs to condense on the leaf surface, forming water droplets that run off to the soil below”, says Rees. “Interestingly, when these plants are wet, the hairs become translucent, revealing more of the green pigment below, meaning that photosynthesis is maximised when there is available moisture.”

Unlike most dry tropical plants, *Welwitschia* has stomata on both the upper and lower surfaces of the leaf, also making it possible for water to be absorbed directly by the leaves. Collected water is likely to be stored in the large tap root, which can extend up to nine feet long underground. Such adaptations allow these curious plants to endure extreme conditions for a lifespan of up to a thousand years, giving rise to their alternative name of ‘living fossils’.

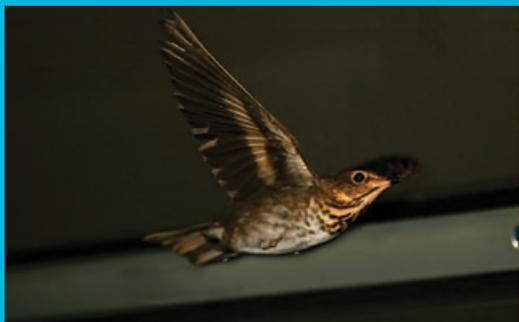
Making water on the wing

Even when animals don’t live permanently in a desert, they may have to cross one, with no chance of a drink along the way.

Migrating birds make such journeys, which can be more than 11,000 km at a time, crossing deserts or oceans. Yet their intense level of activity puts them at risk of dehydration as Dr Alexander Gerson (University of Massachusetts) explains: “Flight has a considerable aerobic demand, so flying birds have very high breathing frequencies to provide oxygen to the working muscles. As a consequence of this, they experience very high water losses. Birds can’t really do much to reduce these water losses whilst flying so, instead, their strategy is to offset these losses by increasing the amount of water they produce endogenously”. Catabolism of protein, fats and carbohydrates all produce endogenous metabolic water but the yield varies according to the substrate. Migrating birds, however, can switch between substrates to provide the optimum water gain. Burning fat yields a higher amount of metabolic water per gram than carbohydrates or protein, but when metabolic water is expressed per kilojoule of energy produced, protein catabolism produces over five times more water than fat.

When Gerson and his colleagues flew Swainson’s Thrushes (*Catharus ustulatus*) in a wind tunnel under conditions of high and low humidity, they found that drier conditions increased the rate of protein catabolism. This gave an approximate 21% increase in endogenous water production relative to energy expenditure. “Shifting the proportions of fat and protein catabolism allows birds in flight to modify the rate of metabolic water production to match losses” says Gerson. This may explain why trans-Saharan migrants often fly under conditions which would incur high water losses so that they can benefit from favourable winds. A major

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Swainson's Thrush in wind tunnel.
Photo: Alexander Gerson

drawback to this strategy, however, is that the protein being catabolised is supplied by the muscles and organs, leading to functional decline. "After a long flight, migratory birds can have pectoralis muscles that are 20-30% smaller than before and the intestines, liver and kidney can be up to 50% smaller. As a result, each bird has to re-build all those organs and muscles and deposit a lot of fat before it can continue on its journey", explains Gerson. If climate change causes migrants to experience even higher water losses during their flights, this could result in greater breakdown of lean protein tissues. This may affect how quickly birds can refuel during stopovers and influence their arrival time at breeding grounds.

But water challenges are not just restricted to global migrants. As Professor Marcel Klaassen (Centre for Integrative Ecology, Deakin University) explains: "Here in Australia, we have very irregular rainfall and a lot of nomadic species, which seize opportunities when they can, flying back and forth across the country". An example is the Banded Stilt (*Cladorhynchus leucocephalus*), which breeds exclusively in inland lakes. Satellite tracking by Reece Pedler and his group, also at the Centre for Integrative Ecology, has recently revealed that these birds have a remarkable ability to locate rainfall events, even when these are hundreds of kilometres apart. "If there is a lot of rainfall in Southern Australia, we see Banded Stilts congregating there and using the opportunity to breed", says Klaassen. "But if there is a lot of rainfall in Western Australia, these birds will travel to that region." It is unknown how these birds know when and where rainfall events occur; a mystery that is of great interest to the weather forecasters.

Recycle, Recycle!

Meanwhile, even some of the most inactive animals can face water challenges, including hibernating bears. "Black and Grizzly bears hibernate for three to five months in winter dens with no access to free water or food during this time" says bear expert, Professor Hank Harlow (University of Wyoming). "From what we

know, a combination of many physiological processes helps these bears to conserve water and prevent dehydration". Firstly, the bears use bradycardia, a reduction of the heart rate to as low as five beats per minute, to reduce renal blood flow and the filtration rate of the kidneys, helping to conserve water. Secondly, and more remarkably, hibernating bears are completely anuric - they do not urinate at all during the winter months. However, this poses a problem; breaking down protein to provide endogenous water through metabolism produces toxic nitrogen waste, which must be converted to urea and eliminated from the body. In mammals, this is done by diluting urea with copious amounts of water in urine, whereas in the case of migrating birds, nitrogen waste is excreted as uric acid, which requires less water.

Previously, the bear's large fat reserves were thought to provide enough endogenous water, sparing the need for protein catabolism. However, fat is a high-energy substrate, so burning fat to produce water has the effect of increasing the metabolic rate and respiratory water loss. Besides this, converting fat into Acetyl-CoA and operating the Krebs cycle requires products of amino acid catabolism, hence a minimum level of protein catabolism must occur to keep these animals in water balance. But despite the inevitable production of nitrogenous waste, electrolyte measurements indicate that hibernating bears do not experience urea toxicity. So how do they manage this without urinating?



Grizzly bear

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“Post-renal urea nitrogen salvaging allows bears to be anuric and ensures that blood urea levels do not elevate during the winter”, explains Harlow.

Stable isotope studies on related species have demonstrated that, during hibernation, urea transporters in the bladder are upregulated to sequester urea from the bladder into the blood. From here, the urea is moved by the urea transporters to the cecum and intestines, where microbes that have the enzyme urease hydrolyse it to ammonia and carbon dioxide. The ammonia is then transported to the liver where it reacts with glycerol released from fats and is synthesised into amino acids. These are used to replenish protein in lean tissues, which explains why skeletal muscle loss over winter is less than would be predicted for these bears. This elegant, symbiotic partnership with invisible gut microbes allows these giants to slumber peacefully until the spring arrives.

Meanwhile, in the plant kingdom, several species of the *Tylecodon* genus also adopt a recycling strategy. The shrub *Tylecodon paniculatus* is a rare example of a plant that is both succulent and deciduous, annually shedding its leaves at the end of the wet winter months and remaining leafless throughout summer. But these shrubs are careful not to let any water go to waste as they drop their leaves. “It’s thought that the leaf water is first drawn back into the stem before the leaves are lost”, says Professor Guy Midgley (Stellenbosch University, South Africa). “This is based on field observations where the bark of the stem cracks and is replaced as the succulent shoot swells. It seems strange – a succulent deciduous plant – but it’s just one of the many adaptations to a semi-arid environment!”

A similar process occurs in ‘pebble plants’ belonging to the *Lithops* genus. Paul Rees (Royal Botanic Gardens, Kew) explains: “In the wild, these plants only ever have two leaves per stem and grow a new leaf pair during the dry summer period, after flowering. During this process, water in the old leaves is drawn out into the xylem to be used for new growth. Cultivated plants, however, will keep their older leaves if watered, giving an unnatural appearance of a small pile of pebbles. When water is so hard to come by, it makes sense to reuse as much as you can”.

Damage limitation

Plants that don’t cast off their leaves can face a severe problem when it is very hot and dry; they may lose water from their leaves so rapidly that they place the xylem under extreme tension. Xylem carries an unbroken column of water up from the roots, so if this tension becomes too great, the water column may break,



Quiver tree, *Aloe dichotoma*
Photo: Wendy Foden

resulting in the death of the plant. However, stockpiling reserves of sugar may be a strategy to avoid this. Dr Michael O’Brien, a researcher at the University of Zurich, found that tropical tree species in Sabah, Borneo, with higher levels of soluble and storage carbohydrates – called non-structural carbohydrates – consistently survived for longer under drought conditions. “These species, from the family Dipterocarpaceae, can experience very severe irregular or inter-annual droughts that are often associated with El Niño events”, O’Brien explains. These species do not close their stomata during drought, hence they continue to transpire. However, this runs the risk of hydraulic failure if the negative tension forms air bubbles that lead to embolisms or cavitation of the vascular tissue. However, according to O’Brien: “Our work shows that having greater reserves of non-structural carbohydrates – both osmotically active sugars such as glucose and storage compounds such as starch – prolongs the point at which this hydraulic failure occurs”.

O’Brien’s team used an ingenious ‘alternating light environment strategy’ to experimentally manipulate the levels of the carbohydrates in individual trees: “If you grow one seedling in high light and one in low light, the seedling in the low light will have fewer sugars but will also not grow. But if you swap the light environments after a period of time, the seedlings that were originally in the low light environment increase their sugar content and catch up in height, whereas the seedlings that were

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Banded Stilts

Photo: Ben Parkhurst

originally in the high light environment stop growing and decrease their sugar content. At the end, you have a group of seedlings at a similar size but with altered sugar contents". When deprived of water, the seedlings with the higher non-structural carbohydrate reserves survived 10% longer than their counterparts. It is not yet clear why this is the case but O'Brien has a few suggestions: "Possibly, sugars are used to create osmotic gradients which cause water to refill embolisms. Besides this, the sugars could also be used to maintain the turgor pressure of the cells to support basic life functions. We plan to investigate this further using stable isotope labelling to track sugar use and transport during drought".

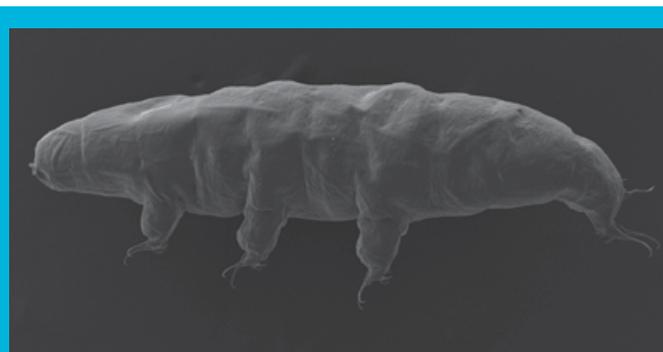
Meanwhile, Quiver trees (*Aloe dichotoma*) have adopted a different solution to this problem: instead of allowing tissues to get damaged by drought, they simply eliminate them! These tree aloes, distributed across arid regions in Southern Africa, can perform a remarkable self-amputation strategy when confronted with drought. The succulent trunk stores water in specialised parenchyma cells and supports branches that terminate in rosettes of succulent leaves. Their common name refers to the practice of the San tribe of hollowing out the branches to use as containers for arrows. It is estimated that Quiver trees can live for 350-500 years, although accuracy is limited as they do not produce tree rings. "During drought, these trees slowly shed a varying number of their leaf rosettes, reducing the canopy area", explains Dr Wendy Foden (IUCN Climate Change Specialist Group). "The branch's apical meristem dies off and the terminal tip closes into a 'stump' that stops growing. By comparing the stump length with that of healthy branches, it's possible to estimate when droughts have occurred and how severe they have been".

It is thought that this method reduces water loss through leaf transpiration, a theory that is supported by the morphological variation observed within Quiver Trees. Large canopies are typically found in wetter regions whereas in the drier north the canopies are much smaller. It is unknown what signalling pathways initiate amputation, although one possibility is that the process is triggered by hydraulic failure of xylem vessels in the branch tips. To compensate for losing their leaves, Quiver trees also have photosynthetic tissue underneath translucent bark on the trunk, enabling them to continue photosynthesising even when leaf area is low. So whilst some trees avoid damage at all costs, some species have turned it to their own advantage.

Suspending Life

For the ultimate water conservation strategy, however, look to tardigrades – invertebrates less than a millimetre-long related to Arthropods. These 'cute' animals have also been christened 'water bears', due to their likeness to tiny, eight-legged Paddington bears. Terrestrial species generally occupy damp environments, such as moss, lichen and leaf litter, as their

bodies need to be covered with a thin layer of water to remain active. Should this dry out, however, tardigrades rapidly undergo a remarkable transformation called cryptobiosis – a state of suspended metabolism. First, their bodies contract to a third of their normal size, as they lose up to 97% of their water content. Following this "their metabolism completely stops and they become like a dead animal", describes tardigrade researcher Professor Roberto Guidetti of the University of Modena and Reggio Emilia. "Within this state, tardigrades can survive being frozen to absolute zero (-273°C), heated to 150°C and even being exposed to outer-space conditions". Even more remarkably, the process is completely reversible; once water levels have been restored, tardigrades are able to continue as though nothing had happened. "After twenty years of being frozen at minus eighty, they can be taken out to begin life again without any problems", says Guidetti.



Scanning Electron Micrograph of a species of the largest tardigrade genera, *Milnesium cf. tardigradum*.

Photo: Robert Guidetti

The physiological mechanisms supporting this transformation remain largely unknown. "Protective molecules are thought to produce a 'glassy matrix' that maintains cell membranes and cell structure", says Guidetti. "However, it is still a complete mystery how tardigrades survive the increased level of oxidative stress caused by dehydration, as the level of antioxidant enzymes does not increase during cryptobiosis".

The question also remains as to why tardigrades (particularly those living in temperate climates) should evolve such an extreme survival strategy. According to Guidetti, "It is thought that this process helps terrestrial tardigrades survive fluctuations in wind or sunlight that could cause the surrounding water to dry out. However, cryptobiosis has also recently been described in marine species, where the environment is more stable".

Can humans learn anything from what has been described as "the hardest animal on earth"? Yes indeed: this process has already inspired methods for long-term storage of DNA and RNA at room temperature and specialised dermatological products. ●