

Labelled water in desert survival patterns

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Interest in the survival of desert plants and animals dates back to the early Greek philosophers. It was, however, only at the turn of the last century that this interest was translated into an experimental and analytical approach. The central aim of most of these investigations was to explain how living organisms could survive under conditions of extreme water deprivation and high ambient temperatures. Under these conditions of extreme stress many organisms develop unusual if not unique physiological, morphological and behavioural adaptations. The desert therefore became the natural laboratory and hunting ground of the imaginative biologist seeking bizarre evolutionary adaptations. Studies on water storage, water balance, water metabolism and evaporative water losses understandably occupied a central position in these studies but the methodology remained fairly simple and traditional until after the Second World War. In the Sixties isotopically-labelled water, particularly tritiated water (^3HOH), became freely available at a relatively low cost and this tracer has proved a very powerful but simple tool for following the flow of water through living organisms in the desert. In this article we describe how we have used ^3HOH as a tracer to explain, at least in part, the survival of some interesting plants and animals in the Namib Desert.

One of the first impressions that we gained from our earliest journeys across the massive dunes in the Namib dune sea was of the ability of a tall tufted grass to survive on the dune slopes for many years without rain. This grass, *Stipagrostis sabulicola*, provides food and shelter for a surprisingly large variety of animals in the dune ecosystem and we felt that it was of key importance to our general understanding of the dune ecosystem to explain how it manages to survive, flower and seed under these extremely arid conditions. Our hypothesis was that the plant's extensive and very shallow lateral root system was able to absorb condensed fog water from the top 10 mm of dune sand. This layer reaches field capacity in the early hours of those mornings when advective fog from the cold Atlantic Ocean condenses on the dune slopes, but is rapidly evaporated by solar radiation after daybreak. To test this hypothesis we applied a predetermined concentration and volume of

^3HOH to a segment of sand adjacent to several plants. The amount of tritiated water was just sufficient to dampen the top 10 mm of sand, thereby simulating a typical fog-precipitation event. The tritiated water was applied at 04h00 when low temperatures and high humidity prevailed (15.5 °C and 92 % r.h.), typical of the time and conditions under which fog naturally occurs. Twenty-six hours later samples of plant material were removed from each plant at the following positions: lateral roots, vertical roots beneath the main plant and the green stems plus leaves. After suitable processing in the laboratory the samples were counted in a scintillation counter and identical sampling of the same plants was repeated after seven weeks.

The results of this field trial clearly showed that the plants were able to absorb fog water from the top 10 mm of dune sand very efficiently. Within one day this water was distributed throughout the plant including the leaves, stems and roots. Of perhaps greater interest, however, was the finding that after seven weeks, although most of the water had been incorporated through photosynthesis into photosynthates, there was still a large amount of free water stored in the root systems of the plants. This means that these unusual plants have not only adapted to exploit the minimal precipitation provided by a condensing fog event, but also that the water turnover rate within the plant is very slow. These adaptations have a chain reaction throughout the dune ecosystem because the ability of this important species to survive provides both nutrients and refuge for a host of dependent or satellite organisms living in the dune system. To name but one interesting example, we can consider the scale insects which feed on the plant sap of *Stipagrostis* plants. These scale insects produce honeydew which is ingested by a large and aggressive dune ant which husbands the scale insects and forages in large numbers on the grass tufts. The foraging ants return to their nest and regurgitate the honeydew for the benefit of their nest mates, a process known as trophallaxis. In this way the condensing fog water, originating over the cold Benguela Current in the Atlantic Ocean, passes through the sand then through the *Stipagrostis* leaves into the scale insect's digestive tract and eventually

arrives in the underground ant nests in the form of minute droplets of honeydew. The availability of tritiated water makes it a relatively simple matter to trace this pattern of water flux through a segment of the dune ecosystem.

Variety of beetles

Another striking feature of life in the Namib dunes is the large variety of tenebrionid beetles that survive largely on wind-blown plant detritus which accumulates in the dunes. These beetles, more familiarly known as *toktokkies*, exhibit a wide range of adaptations which allow them to survive in this unpredictable environment. Some of the more unusual of these include the ability to collect water from even the lightest precipitation of fog water on the dunes. For example, one species will adopt a head-down posture on the crests of the dunes facing into the fog wind on cold foggy mornings. The fog condenses on the dorsal surface of these 'fog-basking' beetles and the droplets run down to the mouthparts where they are imbibed. Another species, weighing only 80 mg, constructs narrow sand trenches on the dune slope facing into the fog wind. The raised crests of these trenches collect more precipitated fog than the surrounding sand surface and the beetles return along the trench extracting the water from these crests with their specialised mouthparts. We have, however, been equally interested in the fate of this collected water and guessed that the beetles were able to conserve this water very efficiently and had a high degree of resistance to desiccation.

To quantify this resistance to desiccation we could choose from several traditional experimental procedures but these all suffer from one or more shortcomings. The use of ^3HOH again provided the solution and together with our colleagues Sue Nicolson and Eric Edney we designed the following experiment. The beetles (*Onymacris plana*) were each injected with 20 μl of tritiated water which slowly equilibrated with the water pool in all the tissues of the body. The equilibration time allowed was approximately four hours. Thereafter the beetle was immobilised and a small plastic capsule was glued to the cuticle on the dorsal surface. Air was then pumped slowly through the capsule and therefore over the cuticle before passing direct-

Figure 5

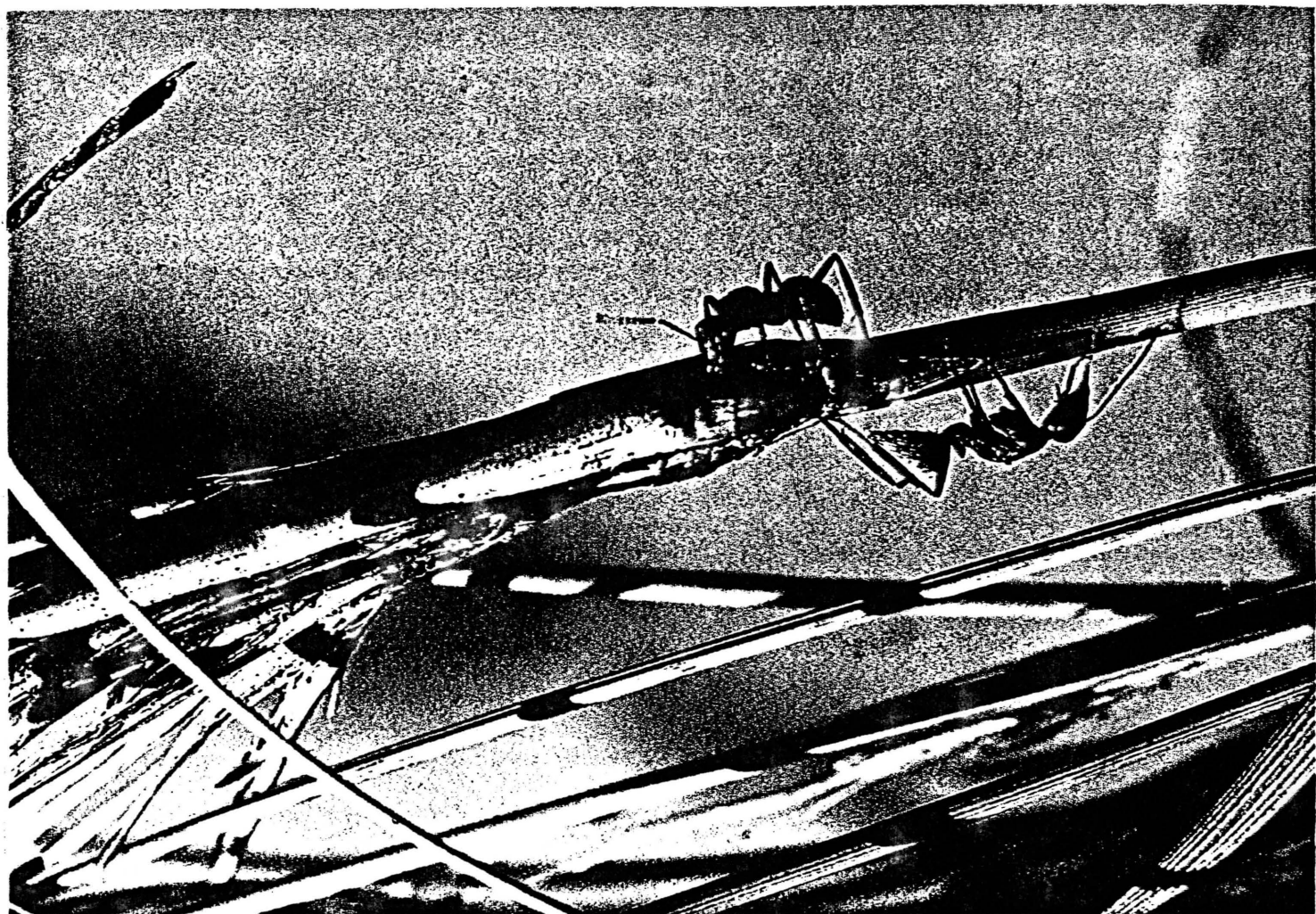


Figure 3

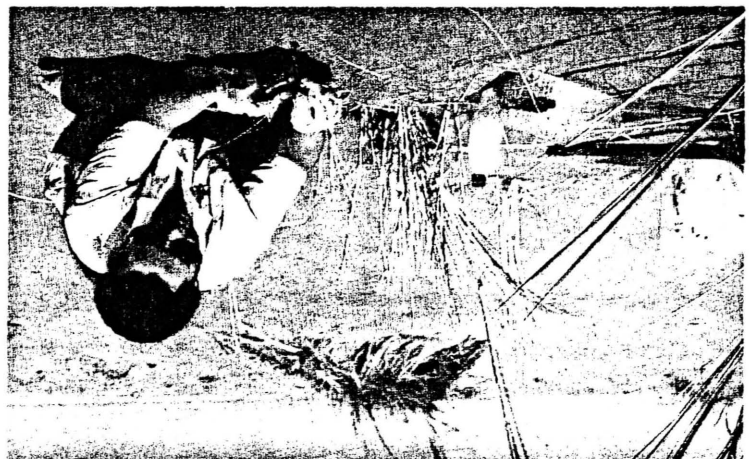


Figure 1

Figure 4



Figure 2

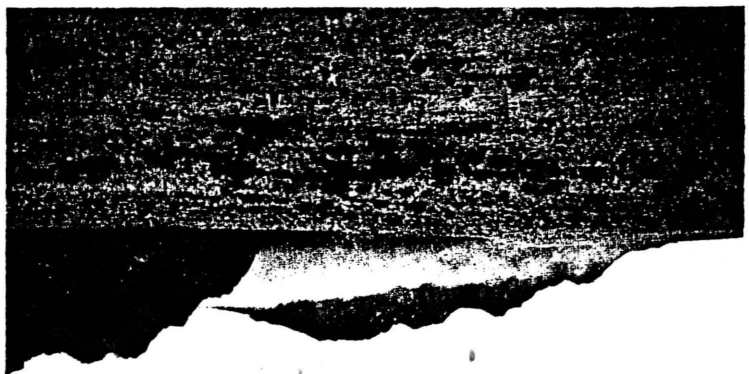


Figure 1 Advective fog is a very important source of moisture for plants and animals in the Namib Desert.

Figure 2 The extensive, superficial roots of *Stipagrostis sabulicola* allow this dune grass to obtain moisture from condensing fog.

ly into scintillation fluid contained in three scintillation vials arranged in series. At the end of the experiment a blood (haemolymph) sample was taken from the beetle and the radioactivity of this sample as well as the scintillation fluid, receiving the excurrent air from the ventilated capsule was measured in a scintillation counter. This relatively simple procedure allowed us to compare the radioactivity of the blood with the activity of the water vapour moving through the cuticle and being trapped in the scintillation fluid. From these data and a measurement of the surface area of the cuticle enclosed by the capsule, we were able to measure water loss from the cuticle of the beetle precisely and express it in terms of $\text{mg cm}^{-2} \text{ h}^{-1}$. A further advantage of this technique was that it allowed us to place the ventilated capsule on any selected area of the surface of the insect in order to compare rates of water loss from various parts of the body. In this way it is, for example, possible for the first time to partition respiratory and cuticular water losses in a living insect. We were not surprised to find that our results showed that evaporative water loss from the cuticle of our desert beetle *Onymacris plana* was among the very lowest measured to date in any animal, namely $0.7 \mu\text{g cm}^{-2} \text{ h}^{-1} \text{ mm Hg}^{-1}$.

Small desert mammals

We turn now to more familiar animals in the shape of small desert mammals. Many of these animals are highly successful in deserts because of their small size and burrowing behaviour. This enables them to retreat to the favourable micro-environment of the burrow during the hottest and driest periods of the day. In addition, many species of small desert mammals possess extraordinarily efficient kidney function to minimise water loss in the urine. Urine concentrations as high as $9000 \text{ mOsm kg}^{-1}$, or nine times the concentration of sea water, have been measured in certain species. Although we were well aware of these adaptations in the small rodents in our study area because we had investigated both their behaviour and kidney function in the laboratory, we were nevertheless keen to study their water turnover rates under natural conditions, while they were free-ranging in the field. To do this we worked with Philip Withers near an

Figure 3 After application of tritiated water to the sand dune surface in the root zone of *Stipagrostis sabulicola*, samples of the leaves are taken for analysis.

Figure 4 *Stipagrostis sabulicola* being studied in the Namib desert dunes.

island mountain (*Inselberg*) on the desolate gravel plains of the central Namib. Again ^3HOH provided a simple yet highly sensitive technique for our studies. We first laid out a grid of live traps baited with peanut butter around the periphery of the *Inselberg*. Immediately after the animals were trapped they were injected intraperitoneally with $10 \mu\text{Ci}$ of ^3HOH and retained in captivity for three hours while the isotope equilibrated with the total body water in all the tissue fluids. This equilibration time had been previously determined in the laboratory. After three hours a blood sample was collected from the suborbital eye sinus in $100 \mu\text{l}$ heparinised haemato-

Figure 5 The dune ant, *Camponotus detritus*, obtains its moisture from the honeydew of scale insects which, in turn, obtain moisture from the dune grass *Stipagrostis sabulicola*, and ultimately from the Namib fogs.

Figure 6 A track of the small insectivore, the golden mole, which feeds on beetle larvae and other animals obtaining water from fog.

crit tubes and centrifuged immediately in the field. The animals were then released to return to their normal activities under natural conditions and, depending upon subsequent retrapping success, blood samples were removed at intervals of two to four days for a period of three weeks. The rate of disappearance of the ^3HOH from the body fluids naturally provided us with the data to calculate daily water turnover rates. Our results showed that in the case of one species, *Petromyscus*, the water turnover rate was as low as 0.8 ml day^{-1} . This is among the very lowest values measured for mammals and it is also significant that the values were

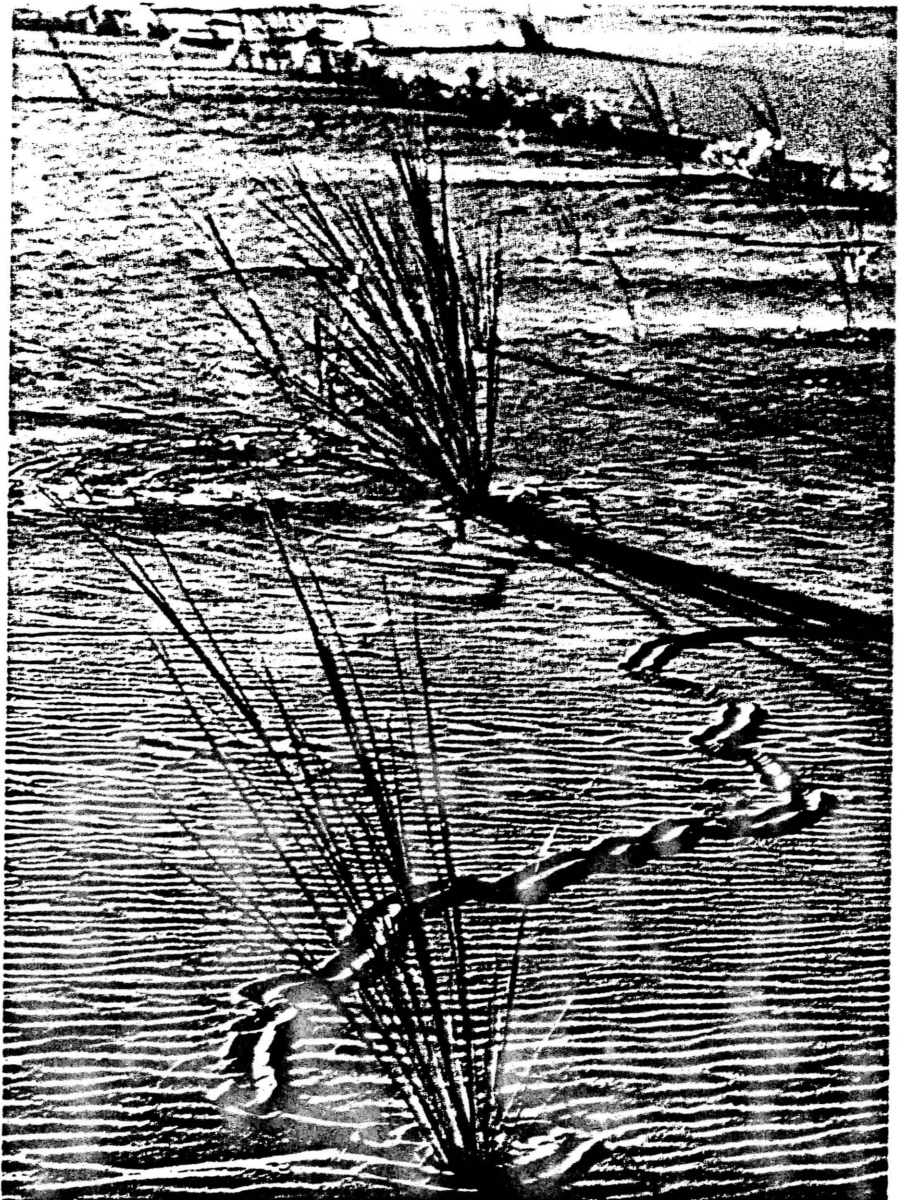


Figure 6

- 14 lower than those obtained under artificial conditions in the laboratory. This result becomes even more impressive when we calculate the ratio of daily water turnover rate to daily energy expenditure in these animals. The lowest theoretical value of this ratio is determined by the stoichiometry of metabolic water production to oxygen consumption when a nutrient is fully oxidised to CO_2 and H_2O . In the case of, say, carbohydrate the value would be $0.034 \text{ m} / \text{kJ}^{-1}$ and our field ratios obtained for *Petromyscus* were $0.04 \text{ m} / \text{kJ}^{-1}$. This again emphasises the extremely efficient use of water by this desert species and it comes as no surprise that they are able to survive indefinitely without drinking.

The desert ostrich

A final example of how we have used ^3HOH to study desert survival is provided by that fascinating bird, the ostrich. This bird, apart from being the largest bird in existence, is also a very successful inhabitant of extreme deserts. It is able to survive for weeks on end without drinking free water but when water is available at isolated water holes its high efficiency of locomotion allows it to range over extensive areas of the desert in search of both food and water. In addition, by manipulating its wings and feathers and orientating its body towards the solar beam it is able to minimise the heat load impinging upon its body, thereby conserving water loss through evaporation. For example, on hot sunny days the birds face into the sun when they are grazing and move the wings away from the thorax and the naked surface of the thorax, which is now in the shade, acts as a thermal window for radiant heat loss. The feathers on the back are erected to provide a thermal shield against direct solar radiation but because of their sparse distribution also allow air to flow across the skin surface from the slightest breeze, thus allowing a maximum amount of convective cooling and minimising the necessity for evaporative cooling.

After we had established the above patterns of thermoregulatory behaviour in the desert ostrich we turned our attention to its water balance. One of the first questions we asked was, 'is the ostrich capable of exhaling air which is unsaturated with water vapour?' This had recently been shown to be the case in the camel by

Schmidt-Nielsen, working in the Negev Desert, and we were interested in examining the same phenomenon in the ostrich. To carry out this investigation we placed small electronic sensors in the nostrils of the ostrich which could measure the relative humidity and temperature of both the inhaled and exhaled air. The response time of the instrument was extremely rapid and allowed a breath by breath analysis and, much to our satisfaction, we found that the ostrich is indeed capable of exhaling air unsaturated with water vapour. The next step was to quantify the water savings accruing to the animals as a consequence of this phenomenon. We turned once again to the isotopic dilution technique using ^3HOH . Ostriches were injected with tritiated water and after a suitable equilibration period blood samples were collected serially over a period of 10 days to determine the daily water turnover rate. These studies showed that the daily water turnover rate of a dehydrated ostrich was as low as $2\,000 \text{ cm}^3 \text{ day}^{-1}$ and if we assume an air density of 1.1 kg m^{-3} and a ventilation rate of the respiratory system of the ostrich to be $7\text{--}18 \text{ dm}^3 \text{ min}^{-1}$, the saving amounts to $200\text{--}500 \text{ cm}^3 \text{ day}^{-1}$. The net savings accruing to the ostrich are therefore between 10 and 25 % of the total daily water turnover rate and, consequently, are very significant in the survival of this species in the desert.

Doubly-labelled water

Looking towards our future use of labelled water in our desert research program, we should briefly mention the potential value of doubly-labelled water ($^3\text{HH}^{18}\text{O}$ and $^2\text{HH}^{18}\text{O}$). The rate of CO_2 production by an animal, and therefore indirectly its metabolic rate, can theoretically be measured by following the disappearance of the isotopes of oxygen and hydrogen from the body fluids after a suitable dose of these isotopes has been administered to the animal. Because of the action of carbonic anhydrase, the oxygen of exhaled CO_2 is in isotopic equilibrium with the oxygen of body water in mammals that have been injected with H_2^{18}O . Consequently the decline of ^{18}O in body water over time is correlated to the rate of CO_2 loss as well as body water loss. If the animal's body water is also labelled with an isotope of hydrogen, the kinetics of this isotope are re-

lated primarily to water loss only, while the difference between the turnover rates of the two isotopes is a measure of the rate of CO_2 production. This technique has great potential for measuring the metabolic rate of free-ranging animals under natural conditions as the animals can be injected with doubly-labelled water, released to engage in their normal activities and then recaptured at a later stage for blood sampling to determine the rate of decline in activity of the two isotopes. In this way the energetic or metabolic cost of free existence of the species concerned can be measured. This measurement is of great value to ecologists as it is the most sensitive measurement of the functional role that a species or population plays in the ecosystem as a whole. The technique has one serious disadvantage and that is the high cost of ^{18}O , particularly when large animals are being studied. This disadvantage can, however, be overcome by using the method of charged particle activation based on the nuclear reaction $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}(\text{T}_{1/2}, 110 \text{ min})$ which allows the analysis of water samples as small as $1.5 \mu\text{l}$ for ^{18}O content. With the recent improvement of nuclear facilities in the Western Cape we look forward to testing this method on selected desert species. A critical evaluation of the doubly-labelled water technique has been published by Dr Ken Nagy of UCLA (1980), a pioneer in this field, and someone who has generously assisted us in the past.

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