Inside JEB highlights the key developments in *The Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

SEEING RED: BLOOD FLOW IN A SNAKE'S SPECTACLE

When Kevin van Doorn found himself setting up an experiment to investigate how snakes' eyes are able to focus at the beginning of his PhD in Jacob Sivak's lab at the University of Waterloo, Canada, he noticed something rather unusual: the bright light beam from the equipment lit up the eye from behind and revealed an array of tiny blood vessels branching their way across the transparent skin covering the snake's eye. This see-through skin, called the spectacle, is found in all snakes and is analogous to our own eyelids, except in the case of the snake, the two halves are fused together and have become transparent. Although snake spectacles are well known, van Doorn recalls: 'I never knew that snakes had blood vessels in their spectacle. In fact, it seems like most vision researchers and even snake researchers, herpetologists, in general had no idea.' So, although van Doorn had initially set out to study how snakes' eyes focus, he decided to turn his attention to these blood vessels instead (p. 4190).

First van Doorn wondered who else knew about these blood vessels and did some investigative work. Delving into the scientific literature, he found one brief report on the eye's vasculature dating back to 1852. With the help of Google Translate, he also found an Italian scientist describing the vessels in 1888, and then with the help of some German-speaking friends, he unearthed Manfred Lüdicke's manuscripts from the 1940s–1970s. But by and large, these vessels had fallen under the radar and were a mere curiosity.

Being interested in vision, van Doorn next wondered what implications these blood vessels might have for the snake's eyesight. 'Evolution has done a pretty good job of keeping blood vessels out of the optically transparent portion of the eye, and so from that we can infer that blood vessels in the eye generally have a negative effect on vision', says van Doorn. What's more, Lüdicke had seen that in a snake with foveas (regions of the eye responsible for sharp vision), the overlying spectacle was less densely packed with blood vessels, suggesting that these vessels do affect vision.

To investigate in more detail, van Doorn put coachwhip snakes into a small thin box made of Perspex glass. Luckily, unlike in most snakes, this small enclosure made the coachwhips feel safe enough to hunker down and stay still so that van Doorn could use a slit lamp to illuminate and magnify their eyes. van Doorn then hid behind a curtain for half an hour, while a camera attached to the slit lamp recorded the capillaries periodically dilating and contracting to allow and inhibit blood flow, respectively. On average, van Doorn found that periods of contraction lasted for 115 s, before the vessels dilated to allow blood flow to re-oxygenate the eye. Next, van Doorn crept out from his hiding place to perform some routine tasks in the background. However, given their twitchy nature, the coachwhips regarded his presence as a potential threat, and during the 8 min van Doorn spent in the lab, the coachwhips decreased the period of dilation and blood flow down from 57 s to 33.5 s. When van Doorn returned to his hiding spot, the rhythm of contraction and dilation returned to normal.

Whether the snakes actively reduce blood flow in the eye or do so as a side effect of a sympathetic response induced by a threat, van Doorn doesn't know. However, either way, by reducing the amount of blood in the vessels by prolonging periods of contraction, van Doorn thinks this would allow the snake to see better, which would certainly be advantageous had a real threat been in the room.

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van Doorn, K. and Sivak, J. G. (2013). Blood flow dynamics in the snake spectacle. *J. Exp. Biol.* **216**, 4190-4195.

Nicola Stead

NO FUEL REQUIRED, ALBATROSSES FLY FOR FREE

Long before any jumbo jets had taken to the air, albatrosses were navigating the global skies, travelling up to 15,200 km in any one trip. However, undertaking such long journeys requires a lot of energy, and calculations suggest that albatrosses cannot rely on flapping their wings unless they sacrifice half of their body mass as fuel. To overcome this problem, albatrosses have learnt to harness the energy from the winds just above the sea's surface. But how exactly do they do this? Some researchers have suggested they use gusts of winds caused by breaking waves and others think that using the shear wind gradient, where wind speed increases with altitude, may be sufficient. With so many theories, Gottfried

Sachs, from the Technische Universität München, Germany, decided to investigate in more detail with the help of his aerospace engineering background (p. 4222).

As GPS technology has become much smaller and more advanced in recent years, Sachs and his PhD student at the time, Johannes Traugott, realised they could use GPS loggers to investigate the albatrosses' movements in fine detail. By manipulating the loggers to increase data recording to 10 times per second and developing a special computational algorithm, Sachs and Traugott could track both horizontal and vertical movements to within decimeters. Equipped with 20 GPS loggers, Traugott then made the long journey to the remote Kerguelen Islands in the Indian Ocean. With the help of biologist Anna Nesterova, a post-doc in Francesco Bonadonna's lab at the CNRS Centre d'Ecologie Fonctionnelle et Evolutive, France, he was then able to attach his loggers to albatrosses just about to depart the islands on a long foraging trip.

From the logged data, the team could then characterise the small-scale soaring and diving movements the birds made, into four distinct phases: (1) a climbing upward phase against the wind, (2) a leeward turn, (3) a downward descent with the wind and (4) a windward turn just above the sea to reorient themselves against the wind for their next climb. The GPS data also provided the team with information of the albatrosses' speed and altitude so they could calculate where and exactly how most of the energy was gained. While some energy was gained because of an increase in altitude, most of the harnessed energy was kinetic energy, gained after the albatrosses had made their leeward turn and were heading downwards with the wind behind them.

With some further analysis, Sachs found that albatrosses will climb to different altitude levels: in one case this was 9 m above sea level and in another it was 15 m, suggesting that they will fly high enough to enable them to the gain sufficient energy to sustain continuous non-flapping flight. Next, Sachs calculated the maximum propulsive force generated from this wind and found that it was more than 10 times higher than anything the albatross could create by merely flapping its wings. This conclusively showed, for the first time, that at no point during their four-step routine do the albatrosses resort to this energy-draining mode of flying.

What's more, with his calculations, Sachs was also able to rule out a number of other theories. He shows that the energy gain cannot be explained by the wind gradient alone, which predicts that energy would be gained on the upward stage of flight. He also ruled out that gusts caused by crashing waves helped, as several albatrosses started their acrobatic manoeuvres whilst still over land and there were no sudden gains in energy. All in all, the albatrosses seem to have mastered a very complicated flight manoeuvre that allows them to fly for 'free'.

10.1242/jeb.097212

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Nicola Stead

LIVING THE HIGH LIFE, HOW GROUND SQUIRRELS COPE

Danielle Tufts

If you've ever experienced altitude sickness, you'll know that living at high altitude isn't easy. However, the lower oxygen levels at higher altitudes don't cause problems for everyone, and in the mountain ranges of western North America some species of ground squirrels can live quite happily at elevations of up to 4300 m above sea level. What's more, these small mammals are used to low oxygen levels found in their poorly ventilated burrows during hibernation. So what makes them so resilient to poor oxygen conditions? Angela Fago, from the Aarhus University, Denmark, and Jay F. Storz, from the University of Nebraska, USA, joined forces to find out (p. 4264).

Fago and Storz began by assembling a team that travelled from Nebraska to the Rocky Mountains of Colorado and then onto Alaska to collect blood samples from six different species of ground squirrel living at different altitudes ranging from 200 to 4300 m. Back in the lab, Fago's group began their experiments by measuring the effects of two allosteric effectors, 2,3-diphosphoglycerate (DPG) and chloride anions, on the ability of the oxygen-carrying protein haemoglobin to bind oxygen. 'These small molecules and ions normally bind to the haemoglobin and they tend to decrease the oxygen affinity of the haemoglobin', explains Fago. 'This is favourable for species living at sea level, but in species living at high altitude, these co-factors normally don't bind so strongly, so that the haemoglobin remains in a high affinity form.' In fact, in the case of the ground squirrels, Fago's team found that neither allosteric effector affected oxygen uptake whatsoever.

The team then went on to investigate the effect of lowering pH on blood samples from two ground squirrel species inhabiting different altitudes. Fago explains that lowering pH stabilises the haemoglobin in a low-affinity conformation, in what is known as the Bohr effect, and this helps to release oxygen where it is needed in the tissues. Animals living at high altitude often show a bigger Bohr effect to compensate for the fact that their haemoglobin generally already has a higher affinity for oxygen, and indeed both samples were strongly affected by lowering the pH. However, unusually, both the highland inhabitant (2000–4300 m) and the lowland inhabitant (200–2000 m) performed as well as each other and Fago believes that 'it shows that subterranean life puts a kind of stress on these animals so they are able to withstand different conditions and already had what it takes to be able to colonise high-altitude environments.'

Meanwhile, Storz's team focused on analysing the haemoglobin proteins in more detail. Looking at the amino acid residues that are crucial for binding H^+ ions and aiding the Bohr effect in human haemoglobin, the team found amino acid substitutions that would in theory reduce, not increase, the Bohr effect. To add to the riddle, Storz's group found that all the amino acids that bind allosteric effectors in human haemoglobin remained unchanged in the ground squirrel's haemoglobin, even though Fago's results suggested that neither DPG nor chloride could bind the haemoglobin to an appreciable extent. It appears that, because of some subtle differences in structure, identical amino acids do not have identical effects in human and ground squirrel haemoglobins. Fago concludes, 'We still have a lot to learn from doing comparative measurements and although the human haemoglobin is one solution to the problem, it's not the only solution.'

10.1242/jeb.097030

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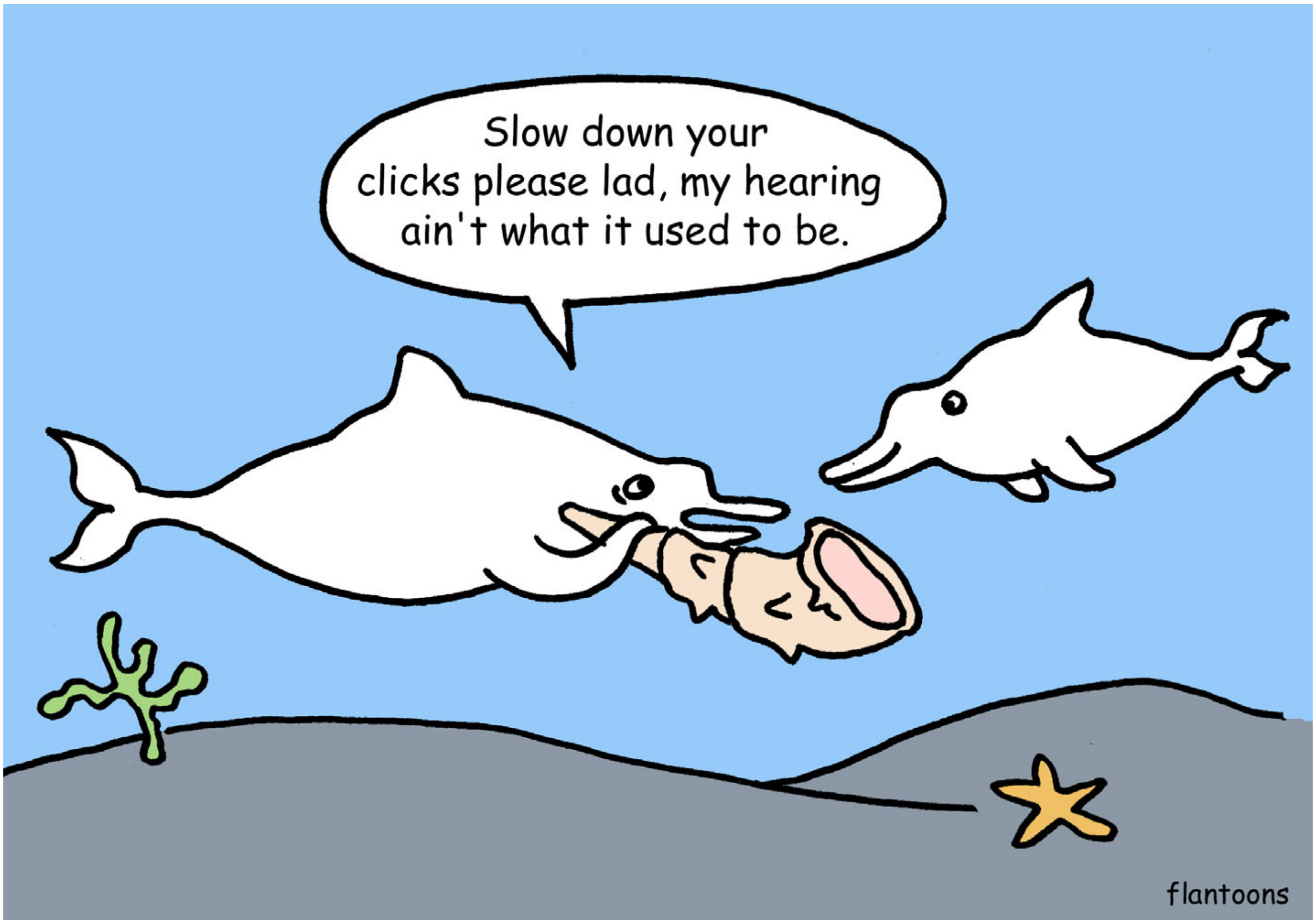
Nicola Stead

PARDON ME? HEARING LOSS IN WILD DOLPHINS



At some point in our lives most of us will have resorted to shouting during a conversation with an elderly relative. Unfortunately, as we age, sounds have to be louder to be heard and high-pitched sounds are no longer perceived. But are humans alone in suffering auditory decline with age? Certainly in captive dolphins, older members of the family do show signs of age-related hearing loss, but the same has never been observed in the wild. However, when a male Indo-Pacific humpback dolphin approximately 40 years old was rescued after it had stranded itself in an inland river near Foshan, China, in March 2012, Songhai Li, from Sanya Institute of Deep-sea Science and Engineering of the Chinese Academy of Sciences, China, and his colleagues seized the opportunity to find out (p. 4144).

The team began by giving the rescued dolphin a hearing test. To do this, three EEG (electroencephalograph) suction-cup electrodes were placed on the dolphin's head and back, to measure electrical activity in response to a range of different sounds. The researchers varied both the frequency (kHz) of the sounds and the loudness to find the lowest threshold of hearing, namely the frequency of the quietest detected sound. For the 40-year-old dolphin this corresponded to a sound at 38 kHz. By comparing this with a hearing test from a younger 13-year-old male dolphin that had been stranded in a similar spot back in August 2007, the team could see that in the younger dolphin this threshold was at a higher frequency. Overall, the results showed that the older dolphin had more difficulty hearing high-frequency sounds, with a cut-off about 30–40 kHz lower than for the younger dolphin.

The team next went on to record the dolphin's echolocation clicks using a hydrophone. Overall, compared with the younger dolphin, the older dolphin's peak and centre click frequencies were about 16 kHz lower. This shift suggests that the older dolphin shifted its echolocation clicks towards frequencies that it is able to hear. Both the decreased sensitivity to high-frequency calls and the compensation in vocalisations strongly suggest that wild dolphins also suffer from hearing loss.

10.1242/jeb.096933

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