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INSIDE JEB

Air sacs insufficient for penguin pressure protection

Emperor penguin at Cape Washington, Antarctica. Photo credit: Paul Ponganis.

Anyone who has tried diving will know the uncomfortable feeling of water pressing on their ears. For divers, the knack of holding their noses and exhaling hard to relieve the pressure is second nature. But other gas-filled chambers in the body are also at increasing risk of damage as air compresses at depth, and it may not be as easy as blowing your nose to equalise the pressure within. Paul Ponganis from the Scripps Institution of Oceanography, USA, explains that the lungs of birds are rigid, so as they dive and air in their lungs is squeezed, the delicate tissue is at risk of rupture. However, no one seems to have told penguins about the risk; they routinely plummet to depths of hundreds of metres. 'The question, "How does the avian lung avoid damage at high pressure?" has never been addressed', says Ponganis, so he teamed up with Judy St Leger and Miriam Scadeng to find out more about the champion divers' survival strategy (p. 720).

Ponganis explains that in addition to their lungs, birds are equipped with compressible air sacs that function as bellows to drive air through the lungs. Could the air sacs provide a reservoir of air that can be injected into the lungs to equalise the pressure as penguins plumb the depths? The only way to find out was to CT scan emperor, king and Adélie penguins to measure the size of their lungs and fully inflated air sacs. However, before Ponganis and Scadeng could begin investigating the penguins' bodies, they had to make sure that the polar birds were comfortable, so St Leger arranged a lift in SeaWorld's refrigerated truck to keep the penguins cool in transit to the local veterinary hospital and the University of California San Diego. Then, after carefully anaesthetising the birds, Ponganis and St Leger gently inflated their air sacs and scanned the sedated animals to find out how much air they could hold. 'We even put little cooling packs on their [the emperor penguins'] wings and feet during anaesthesia to manage temperature', adds Ponganis.

However, after analysing the images of the penguin's bodies, it was clear that the air sacs did not carry enough air to compress into the lungs and protect them from damage. 'We were surprised by how large the air sac volumes were', says Ponganis, but adds, 'Despite the large maximal air volume, that volume is not enough to prevent barotrauma [pressure damage]'.

The team suspects that the animals use other strategies to keep them safe. Ponganis suggests that the lung and trachea may not be as rigid as previously thought, allowing the tissue to compress and avoid ruptures. He also suggests that the birds may be able to contract muscles in the walls of the lung airways to reduce the volume as the pressure rises, or inflate blood vessels to fill the volume as the air compresses. Ponganis also suspects that by fully inflating their air sacs the birds are able to carry down significantly larger oxygen reserves, allowing them to swim longer and go deeper before resorting to less effective anaerobic metabolism to fuel the dive.

10.1242/jeb.121038

Ponganis, P. J., St Leger, J. and Scadeng, M. (2015). Penguin lungs and air sacs: implications for baroprotection, oxygen stores and buoyancy. *J. Exp. Biol.* **218**, 720-730.

Kathryn Knight

Ammonia excretion: *C. elegans* style

For such a tiny animal, *Caenorhabditis elegans* must be one of the best understood creatures on the planet. Its development has been painstakingly scrutinized cell by cell and its 100 million base pair genome has been deconstructed in fine detail; yet little is known about the physiology of these microscopic soil dwellers that reside in the water film surrounding soil particles. 'It's not just a worm in a fridge', chuckles Dirk Weihrauch from the University of Manitoba, Canada, who adds that he is always keen to learn about how animals from exotic environments dispose of toxic nitrogenous waste. Yet, it wasn't even clear whether the nematodes excrete ammonia or urea. So, Weihrauch and his student Aida Adlimoghaddam rolled up their sleeves and decided to get to grips with nitrogen excretion in this super-star model organism (p. 675).

Caenorhabditis elegans usually dine on bacteria in soil, so Adlimoghaddam and Ann-Karen Brassinga made sure that the lab worms were well fed with nutritious *E. coli* before collecting the nematodes' waste for 1 day. Analysing the media that was the worms' home, the duo found that instead of producing costly urea, they were excreting ammonia.

Next, Adlimoghaddam tested whether the worms were expressing the genes encoding proteins that are known to participate in nitrogen excretion in other animals. As Weihrauch explains, the Na^+/K^+-ATPase enzyme pumps ammonium ions across the cell membranes of various animals, while the V-type H^+-ATPase produces a pH gradient across membranes that ammonia can diffuse along, and Rhesus proteins form channels that ammonia gas travels through. When Adlimoghaddam looked for evidence of expression of each of these proteins by measuring mRNA expression of the *C. elegans* equivalents, she found that the worms expressed them all.

However, Weihrauch wanted to know more about the worms' excretion mechanism, even though it was impossible to measure

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.

directly the transport processes in the minute animals. So he and Adlimoghaddam resorted to measuring the ammonia excreted by large numbers of the animals bathed in drugs that targeted specific proteins in ammonia excretion in other organisms to find out which participated in ammonia excretion. The duo discovered that inhibiting the V-ATPase, carbonic anhydrase (which provides protons to fuel the V-ATPase) and the worm's microtubule network all reduced ammonia excretion. The drug targeting the Na^+/K^+-ATPase (ouabain) had no effect on ammonia excretion, suggesting that it was unable to access the pump in the cell membrane. However, when Jason Treberg directly tested whether Na^+/K^+-ATPase could transport ammonium, the protein successfully transported the ions, so it also contributes to excretion.

Next Mélanie Boeckstaens and Anna-Maria Marini tested whether one of the worm Rhesus channel proteins – CeRhr-1 – actually transports ammonia. Inserting the channel into yeast mutants that were unable to import ammonia – which is essential for their survival – the duo found that the mutant yeast carrying the CeRhr-1 protein survived. So CeRhr-1 is an ammonia transporter.

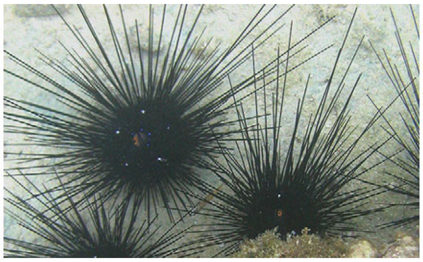
Tying all of their results together, Weihrauch concludes that *C. elegans* excrete ammonia through their skin using two mechanisms. The first – known as ammonia trapping – occurs when the V-ATPase, fuelled by protons provided by the carbonic anhydrase, creates a pH gradient across the cell membrane allowing the gas to diffuse through Rhesus proteins to cross membranes in the skin. In the second mechanism, ammonium is transported in lipid vesicles through the cell's microtubule network to the external membrane, ready for excretion. And Weihrauch is excited to have discovered the ammonia trapping mechanism in such an ancient animal, saying: 'Ammonia excretion mechanisms… evolved early on'.

10.1242/jeb.121012

Adlimoghaddam, A., Boeckstaens, M., Marini, A.-M., Treberg, J. R., Brassinga, A.-K. C. and Weihrauch, D. (2015). Ammonia excretion in *Caenorhabditis elegans*: mechanism and evidence of ammonia transport of the Rhesus protein CeRhr-1 in invertebrates. *J. Exp. Biol.* **218**, 675-683.

Kathryn Knight

Sea urchin catch connective tissue controlled by nerves



Diadema setosum sea urchins. Photo credit: Tatsuo Motokawa.

Most connective tissue is fairly inert, providing padding or holding cells and tissue in place. But when soft starfish want to prise apart a mollusc or a sea urchin wants to hold its spines rigid, they stiffen a unique form of connective tissue, known as catch connective tissue, to hold themselves firm. 'The merit of this tissue is economy', says Tatsuo Motokawa from the Tokyo Institute of Technology, explaining that the tissue can maintain a posture over lengthy periods with very little energy cost, unlike muscle, which requires a constant supply of energy. Motokawa adds that there was plenty of indirect evidence that changes in the material properties of this exotic tissue were controlled by nerves, but there was no definitive proof. So, as he approached retirement, Motokawa set his focus on finally proving that the well-documented dramatic material changes that catch connective tissue undergo to either hold structures rigid or soften are controlled by nerves (p. 703).

Unfortunately, doing neurophysiology on echinoderms is very difficult: 'Action potentials are seldom observed in echinoderm nerves', Motokawa explains, adding that electrical signals caused by stimuli only travel small distances in echinoderm tissue, making it hard to decide whether an electric current was propagated through the nerves or simply spread through the tissue to directly stimulate a target. However, he knew that sea urchins (*Diadema setosum*) wave their spines when a shadow passes over – in a bid to avoid being nibbled by a fish – and that the movement is controlled by the radial nerve. He also reasoned that the catch connective tissue encasing the spine joint must soften in synch with activation of the muscles that move the spines. Motokawa realised that if he could show that shadows caused the catch connective tissue to soften in conjunction with spine waving, then: he says, 'We could provide the definitive evidence for coordination… [that] is mediated by nerves'.

Carefully dissecting sections of the delicate sea urchin shell – complete with spines and intact nerve – and then slowly wiggling one spine (at 0.1 Hz) to measure the catch connective tissue stiffness, Yoshiro Fuchigami began testing the effects on the spine joint of a gentle nudge and plunging the urchin shell into darkness (to simulate a passing shadow). Tapping the spine, Fuchigami found that it became immobile and upright while the connective tissue stiffened 1.5 times within 10 s of the impact. However, when he tapped the adjacent spine, the catch connective tissue in the joint of the spine that he was studying softened, in a bid to lay flat to protect the region of shell that had just been touched (known as the 'convergence response'). And when Fuchigami turned the light off, the spine began waving as the muscles activated while the stiffness of the connective tissue halved. However, when Fuchigami cut the nerve to the spine before turning the lights out, the spine no longer waved and the connective tissue remained stiff. Finally, Fuchigami electrically stimulated the base of the spine and the connective tissue softened again, exactly as it had done when he simulated the effect of a passing shadow.

'The present results clearly showed coordination between the catch apparatus and spine muscles through nerves', says Motokawa, who is pleased to have finally confirmed that catch connective tissue is under neural control, and is optimistic that the next generation of smart materials could benefit from the lessons that sea urchins can teach us.

10.1242/jeb.121053

Motokawa, T. and Fuchigami, Y. (2015). Coordination between catch connective tissue and muscles through nerves in the spine joint of the sea urchin *Diadema setosum*. *J. Exp. Biol.* **218**, 703-710.

Kathryn Knight

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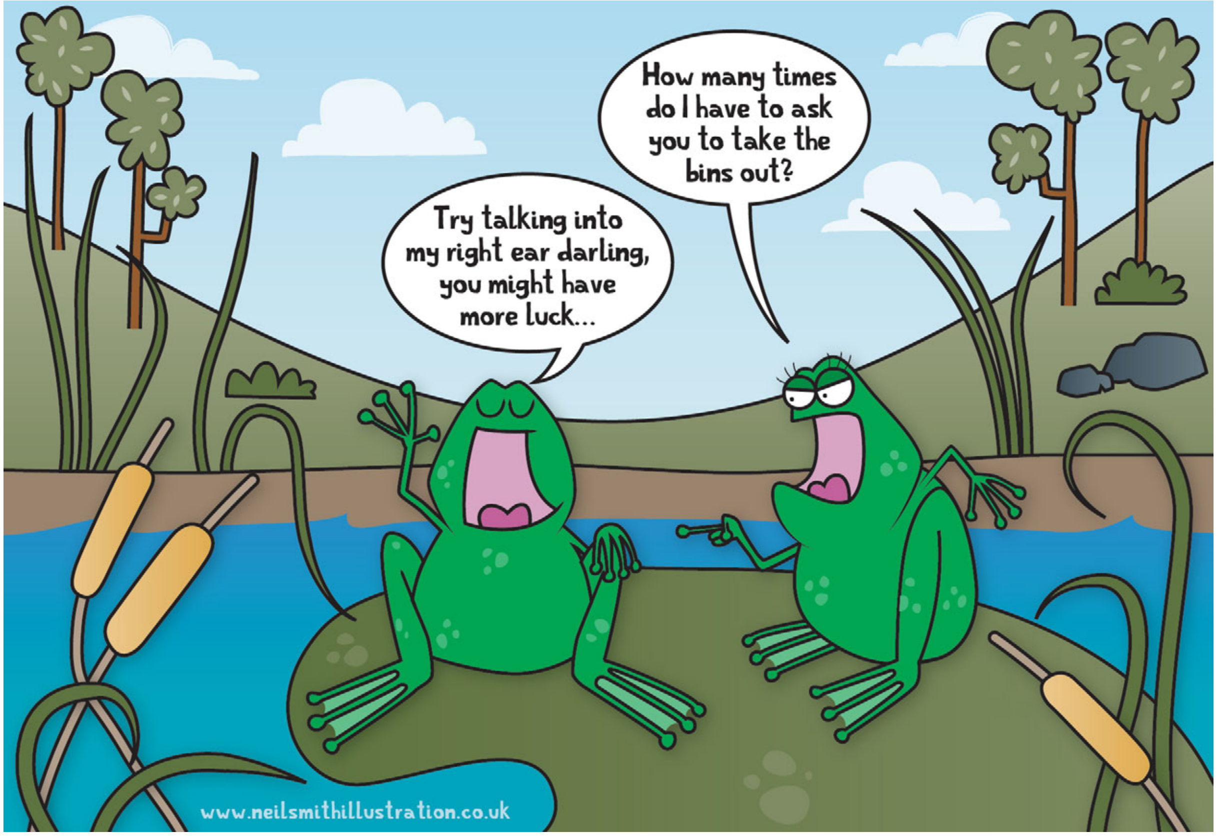
Music frogs listen for each other with right ear



There is handedness everywhere you look. Fish tend to turn to the right when evading a predator, parrots often prefer to grasp with one foot over the other and we all know the human experience from first hand. But handedness doesn't end there. Many animals show a dominance of one ear over the other when it comes to interpreting the sounds made by members of their own species: primates, mice, sea lions and horses all seem to use their right ears in preference to the left to listen to other animals of the same species. And Guangzhan Fang and Yezhong Tang from the Chengdu Institute of Biology, the Chinese Academy of Sciences, explain that electroencephalogram data suggest that music frogs (*Babina daunchina*) preferentially listen to the calls of other music frogs with their right ears. However, they needed behavioural evidence to confirm that the frogs really do listen out for each other with their right ears (p. 740).

Having headed off on the 6 h drive to the Emei Mountains, Fei Xue collected 48 of the vocal animals from ponds in the area, ready to test their auditory responses back in the lab. Xue then played sounds – ranging from males calling from burrows (which are known to be sexually attractive to females) and males calling out in the open (which are less alluring to females) to claps of thunder and the shrieks of music frogs under snake attack – to individual frogs while filming the amphibians' reactions.

Analysing the frogs' movements – they are unable to turn their heads to locate a sound, so they turn their bodies instead – Xue, Fang, Tang, Ping Yang, Ermi Zhao and Steven Brauth found that the frogs tended to present their right ears to the sexually attractive calls of males in burrows, while they turned to the left and moved further away from the speaker playing alarming shrieks and claps of thunder. 'These results support the idea that in anurans [frogs] right ear preference is associated with perception of positive or neutral signals… while a left ear preference is associated with perception of negative signals, such as predatory attack', say Fang, Tang and their colleagues.

10.1242/jeb.121020

Xue, F., Fang, G., Yang, P., Zhao, E., Brauth, S. E. and Tang, Y. (2015). The biological significance of acoustic stimuli determines ear preference in the music frog. *J. Exp. Biol.* **218**, 740-747.

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