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Inside JEB

FIRST PILOT WHALE HEARING MEASUREMENTS

L. Kloepper

Echolocating animals rely on their hearing to navigate and hunt. Bat's hearing is finely tuned to their high-pitched squeaks but what about echolocation toothed whales' (odontocetes) hearing? How sensitive is it and what can they hear precisely? Aude Pacini from the University of Hawaii says, 'We don't have a lot of information about the hearing abilities of whales, so whenever we get the opportunity to test a new species, wherever it is in the world we go to it.' So, when Pacini's supervisor, Paul Nachtigall, heard about a young long-finned pilot whale called Nazaré at Lisbon Zoo that had been saved after stranding as a baby, he struck up a collaboration with the Zoo's chief scientist, Arlete Sogorb, and her assistant, Sonia Matias, with the hope of measuring the whale's hearing (p. 3138).

Months before Nachtigall, Laura Kloepper and Pacini got on a flight to Lisbon, Sogorb's team in Portugal trained Nazaré to familiarise him with the equipment that the team would use to measure his hearing. Arriving in Europe, the team were joined by Meike Linnenschmidt from the University of southern Denmark, ready to test Nazaré's hearing.

Stationing the pilot whale at the side of his enclosure, the team attached a suction cup with an electrical sensor just behind his blowhole to measure Nazare's brain activity when he heard the sounds, and two other electrodes on the animal's back and dorsal fin to keep track of all of the other electrical activity in the whale's body. Playing beeping sounds at various intensities and pitches ranging from 4 kHz up to a high pitched 100 kHz, the team gradually built up a picture of the Nazaré's hearing and were surprised to find that the whale hardly responded to frequencies above 50 kHz.

'For toothed whales 100–150 kHz is usually the high-frequency cut-off,' says Pacini. 'Even though it was a new species and it was a stranded animal, I would have expected more sensitive hearing.'

Wondering what could account for Nazaré's relatively limited hearing range, the team asked for Nazaré's medical records and found that he was prescribed antibiotics during his recovery – some of which are known to damage the high-frequency hearing of humans and could explain Nazaré's relatively limited hearing range.

Alternatively, Nazaré's hearing could be at the weaker end of the pilot whale hearing spectrum or it could even be perfectly normal for pilot whales. Pacini explains that false killer whales have a similar hearing range to Nazaré.

'The next step is really important: what do other animals of the same species hear? Is Nazaré an exception or representative?' says Pacini, who is keen to measure the hearing abilities of other long-finned pilot whales soon.

Pacini adds that our current understanding of odontocete hearing is relatively limited and it is important to learn more about whale hearing to design the best conservation strategies to protect the delicate hearing of these sensitive echolocating animals and possibly even prevent other pilot whales from stranding like Nazaré.

10.1242/jeb.050765

Pacini, A. F., Nachtigall, P. E., Kloepper, L. N., Linnenschmidt, M., Sogorb, A. and Matias, S. (2010). Audiogram of a formerly stranded long-finned pilot whale (*Globicephala melas*) measured using auditory evoked potentials. *J. Exp. Biol.* **213**, 3138-3143.

DUNG BEETLES' CHOICE HARD WIRED, NOT LEARNED

Dung beetles are possibly the most unsung of eco-heroes. Spending all of their lives, from egg to adult, in and around piles of dung, they hasten its decomposition, either by transporting it into the ground or consuming it. But which dungs are the most attractive to dung beetles and what attracts a beetle to its favourite deposit? 'Many authors thought that these insects are just attracted by many kinds of faecal volatile compounds and that they were not selective, but when I was in the field I could see that I only found certain insects in certain kinds of dung, so I thought that this insect can choose or select dung probably by volatile compounds,' explains Laurent Dormont from the CNRS Centre d'Ecologie Fonctionnelle et Evolutive, France. Curious to find out whether a dung beetle's dung preferences are hard wired or set by the environment they encounter during development, Dormont, Pierre Jay-Robert and Jean-Pierre Lumaret teamed up

to test the French scarab beetle's dung preferences (p. 3177).

Collecting freshly laid *Agrilinus constans* eggs from cowpats in fields around Montpellier, Dormont and Jay-Robert brought the eggs back to the lab and settled them either in fresh cowpats, horse, sheep or wild boar dung. After allowing the eggs to hatch and the larvae to develop into adults, Dormont and Jay-Robert tested the insect's preferences. Offering them a choice between cowpat and horse dung or cowpat and wild boar dung, the team were surprised to see that the beetles always chose to settle in the cowpat, regardless of which type of dung that they had been raised in. And the beetles also preferred sheep dung, even when they had developed in horse and wild boar dung.

'This was a surprising result. We thought that the insect would prefer the dung volatiles in which they had developed, but when we did the experiment the insect preferred cattle or sheep dung even if they came from wild boar or horse dung,' says Dormont. And when the team tested how the presence of other dung residents affected *A. constans*' responses, they found that the beetles avoided dung that had been colonised by other species but happily settled in dung occupied by their own kind.

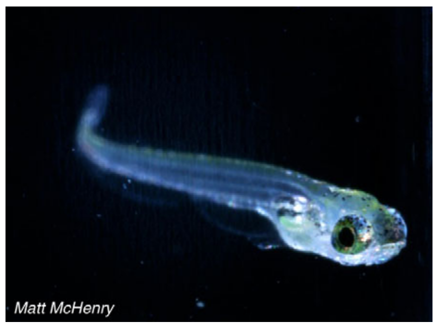
Finally, Dormont teamed up with chemists Jean-Marie Bessière and Sylvie Rapior to analyse the volatile components from each type of dung to see if they could identify the compounds that the beetles found so irresistible. Dormont collected samples of each odour and analysed them with combinations of gas chromatography and mass spectrometry. 'Jean-Marie Bessière is a flower scent specialist, so for him it was really new and he was very excited to identify dung odours. He knows plant odours well; however, the volatiles in dung have been transformed by digestion, so it was difficult for him to analyse and identify these compounds,' explains Dormont. However, the team eventually found that all four odours shared nine components. Cow dung turned out to have the most complex odour, with 36 volatile compounds, while the wild boar was the least complex, with only 25 compounds.

Dormont and his colleagues are now keen to identify the volatile components that the beetles respond to with electrophysiology to understand why sheep and cow dung are the French scarab beetles' dungs of choice.

10.1242/jeb.050757

Dormont, L., Jay-Robert, P., Bessière, J.-M., Rapior, S. and Lumaret, J.-P. (2010). Innate olfactory preferences in dung beetles. *J. Exp. Biol.* **213**, 3177-3186.

STATIONARY FISH MORE SLURP SENSITIVE THAN MOVING FISH



Knowing when a hungry fish is about to slurp you up is a good skill if you're a 5-day-old zebrafish larva. Karla Feitl from the University of California, Irvine, explains that she and Matt McHenry knew that the tiny larvae use flow sensors (mechanosensors known as superficial neuromasts) along the fish's lateral line to detect threatening fluid flows, and if they inactivated the flow sensors with antibiotics the youngsters became 'deaf' to the water's motion and vulnerable to predation. Feitl and McHenry also noticed that stationary fish might be more sensitive than moving fish to fluid flows generated by suction, potentially leaving active fish at risk from hungry predators. Realising that moving fish are always surrounded by apparently 'flowing' water, the duo decided to take a closer look to find out if the fluid flows generated by a fish's own movements reduced it sensitivity to threatening gulps (p. 3131).

First, the duo built a tank attached to a computer-driven piston so that they could 'suck' water through the tank to produce fluid flows similar to the suction produced by a slurping fish predator. Having encouraged the larvae to swim in the dark by switching the lights off and on, Feitl filmed their responses before and immediately after a computer-generated slurp with high-speed video in infrared light. 'We had no say over what the larvae did or how they oriented within the tank,' says Feitl, 'so we ran huge numbers of larvae until we got the necessary sample sizes in each category to allow us to statistically evaluate whether or not there were differences in responsiveness between the stationary and swimming larvae,' she explains.

Next, Victoria Ngo analysed the high-speed videos, determining each larva's orientation toward the fluid flow as the piston sucked water toward it, and then painstakingly checked each frame of video to see if the larva was unperturbed or startled into a C-shaped escape response. The team saw that 76% of the stationary fish twisted into a 'C' ready to beat a hasty retreat; however, only 40% of the moving fish picked up the warning. The moving larvae were less sensitive to suction.

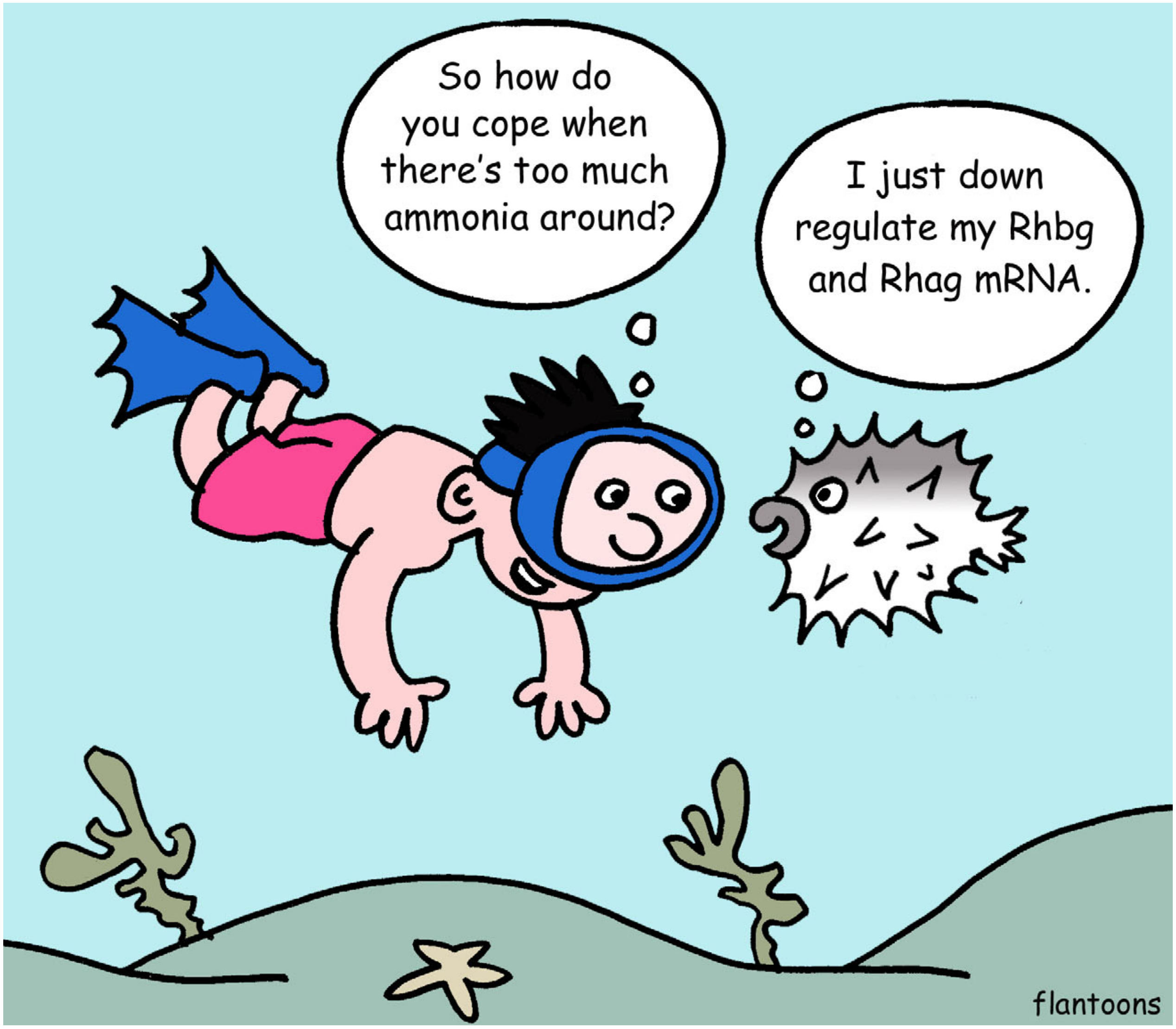
Wondering if the moving larvae were simply less sensitive to the fluid movement than stationary larvae, the team checked how quickly the two groups of larvae responded to the simulated slurp. If the sensitivity of the moving fish's flow detectors was reduced by the relative flow of fluid over their bodies, then the team expected them to respond more slowly than the stationary fish: but they didn't. 'We think it is more complicated than a straight reduction in sensitivity,' says Feitl.

Having found that stationary fish are more sensitive to slurped water than moving fish, Feitl and McHenry suggest that intermittently swimming larvae could benefit from their stop-and-start swimming style by having a higher chance of escaping hungry predators than continuous swimmers. The team is also keen to find out if the larvae escape in a particular direction relative to the fluid flow, to improve their chances of swimming for another day.

10.1242/jeb.050773

Feitl, K. E., Ngo, V. and McHenry, M. J. (2010). Are fish less responsive to a flow stimulus when swimming? *J. Exp. Biol.* **213**, 3131-3137.

RHESUS PROTEINS PUMP PUFFERFISH AMMONIA



Ammonia is an extremely unpleasant and toxic compound, which is very inconvenient for fish; they produce it as the end product of nitrogen metabolism. How fish handle ammonium excretion has long fascinated physiologists. Most fish were thought to dispose of ammonium simply by leaking it out of their gills but when Shigehisa Hirose from the Tokyo Institute of Technology discovered a new family of proteins (Rhesus proteins) in pufferfish with a similar amino acid sequence to other ammonium transport proteins, it became clear that ammonium disposal was more complex. Soon after, Chris Wood found that freshwater trout dispose of ammonia through Rhesus proteins in the membrane of a specialised gill cell, known as a pavement cell, so Hirose and Wood teamed up to find out how saltwater pufferfish handle ammonia excretion (p. 3150).

Exposing pufferfish to high levels of ammonia and analysing the expression patterns of Rhesus proteins and other transporters involved in ammonium excretion, Hirose, Wood and their colleagues, Michele Nawata, Tsutomu Nakada and Akira Kato, found that, in addition to passively leaking ammonium through their pavement cells, pufferfish can actively pump ammonium out of their gills through another cell type, known as a mitochondrion rich cell, with Rhesus proteins on the external surface. The team also found that pufferfish can switch off Rhesus protein expression in their leaky gills if external ammonium levels are high – to prevent the toxin leaking back in – and pufferfish could also use Rhesus proteins to transport carbon dioxide out through their gills.

10.1242/jeb.050781

Nawata, C. M., Hirose, S., Nakada, T., Wood, C. M. and Kato, A. (2010). Rh glycoprotein expression is modulated in pufferfish (*Takifugu rubripes*) during high environmental ammonia exposure. *J. Exp. Biol.* **213**, 3150-3160.

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