

INSIDE JEB

Alcoholic pupfish kick breathing oxygen



Cyprinodon nevadensis mionectes from Ash Meadows: Photo credit: Frank van Breukelen.

Deep in a warm pool in a limestone cavern in the Mojave Desert lives one of the rarest species on earth: the Devils Hole pupfish (*Cyprinodon diabolis*). With a current population of just 131 adults, the race is on to secure the fish's future. 'One possible cause for the small population size of *C. diabolis* is limited food availability', says Frank van Breukelen from the University of Nevada Las Vegas, USA. Realising that the dark conditions dramatically limit the amount of food that grows in the cavern to support the fish, van Breukelen and his colleague, Stanley Hillyard, were curious to know how many pupfish the restricted ecosystem could sustain. However, before they could do the calculation, the duo needed to measure the scarce fish's metabolic rate. As the native Devils Hole pupfish population is too endangered to study directly, van Breukelen and Hillyard measured the metabolic rate of a refuge population of the fish that had been established as an insurance policy should the population ever be wiped out. But when they performed the measurement, the duo stumbled across an unexpected paradox.

Recording the fish's oxygen consumption at temperatures ranging from 25 to 38°C, Matt Heuton found that they consumed oxygen at a stable rate of around 300 $\mu\text{l h}^{-1}$ when they came from a population that had been raised at 28°C. However, when Heuton measured the oxygen consumption rate of fish raised at 33°C – the temperature in the Devils Hole spring – something went wrong. The fish appeared to stop consuming oxygen. 'My initial reaction was that there was a malfunction of the electrode', laughs van

Breukelen. However, after testing 295 animals, van Breukelen and Hillyard had to concede that the phenomenon was real. The fish were somehow switching from aerobic metabolism to anaerobic metabolism, which was perplexing as anaerobic metabolism is nowhere near as effective as aerobic metabolism – only yielding 1/15 the amount of ATP per glucose molecule generated by aerobic metabolism. Why were the fish indulging in such a profligate practice?

Intrigued, the team tested whether the phenomenon was the result of the refuge *C. diabolis* population breeding with another closely related species, *Cyprinodon nevadensis mionectes*. van Breukelen explains that it has been suggested that interbreeding with other pupfish could have compromised the function of the mitochondria that generate ATP. However, when the team measured the oxygen consumption patterns of the two species, they both performed paradoxical anaerobism, so there was no mitochondrial disruption. And when the team tested whether *C. diabolis* were switching to paradoxical anaerobism because they had depressed metabolism, they found that the fish were still wafting their gill covers to take in oxygen, so they weren't depressing their metabolism. Finally, the team checked whether *C. diabolis* were switching to paradoxical anaerobism because there simply was not enough oxygen to sustain aerobic metabolism in the warm conditions. However, even that theory did not hold water.

'We were going nuts trying to figure out what triggers paradoxical anaerobism', admits van Breukelen, until he and Heuton embarked on an animated brainstorming session one night. It occurred to them that the fish may be switching to paradoxical anaerobism to avoid the toxic side-effects of aerobic metabolism, producing ethanol instead. They knew that the 33°C-acclimated fish naturally produced high levels of ethanol – 7.3 times more than the fish from cooler water: could the ethanol be switching off aerobic energy production and stalling oxygen consumption? 'I said, "Let's get

the fish drunk"', recalls van Breukelen. And when the team added ethanol to the fish's water, even the fish that were adapted to cooler water switched to paradoxical anaerobism. So Devils Hole pupfish switch off aerobic metabolism to avoid the toxic side-effect of an aerobic lifestyle, despite the expense.

10.1242/jeb.134619

Heuton, M., Ayala, L., Burg, C., Dayton, K., McKenna, K., Morante, A., Puentedura, G., Urbina, N., Hillyard, S., Steinberg, S. and van Breukelen, F. (2015). Paradoxical anaerobism in desert pupfish. *J. Exp. Biol.* **218**, 3739–3745.

Kathryn Knight

It takes personality for catfish to breathe air

Life is full of risks. Getting the next meal or defending your territory can be hazardous. David McKenzie from the CNRS Montpellier, France, explains that animals gamble with fate for several reasons. Some animals have to place themselves at risk when foraging to satisfy a high metabolic rate, while others are simply bolder: and fish are no exception. McKenzie is also intrigued by air-breathing fish and was puzzled why less than 2% of fish have evolved the ability. 'Air is a rich source of oxygen but breathing it is risky, so it made me wonder if it might be linked to personality', says McKenzie, explaining that fish that breathe at the surface are vulnerable to predation. Knowing that Brazil is gripped by an invasion of air-breathing African sharptooth catfish (*Clarias gariepinus*), McKenzie decided to visit his collaborator Tadeu Rantin at the Federal University of São Carlos, Brazil, to find out whether it takes courage for the catfish to breathe air or whether they simply surface to satisfy a fast metabolism.

Collecting feral fish in São Paulo state and transporting them back to the lab, McKenzie, Rantin and Thiago Belão then developed a bimodal respirometer – with a space to allow individual catfish to breathe air – to measure the animal's air- and water-oxygen consumption

simultaneously. After allowing the fish to rest for 24 h in the respirometer, McKenzie and Belão then recorded the amount of oxygen that they consumed from air and water to determine their metabolic rates. Next, they startled the fish by tapping on the respirometer and recorded how long it took the animals to pluck up the courage to return to the surface. ‘We assumed that how quickly they returned to air breathing was a measure of how bold they were’, says McKenzie.

Initially, they found that the fish in fully oxygenated water did not need to breathe oxygen from the air during the day, although they did so anyway. However, the fish in oxygen-depleted water were clearly struggling to meet their metabolic demands and resorted to air breathing often. And when the team plotted a graph of the fish’s metabolic rate against the time that it took them to resume air breathing after a fright, they could see that the fish that had the highest metabolic rates breathed air the most. ‘There was an element of strong drive of individual metabolic rate to air breathe’, says McKenzie.

But McKenzie also wondered whether boldness could be another factor affecting how swiftly the fish resumed air breathing. Maybe some of the fish were more courageous than others and this was being obscured by the fish’s strong urge to breathe air. Recalling colleagues suggesting a technique to help tease apart the impact of boldness from the metabolic drive to breathe air, McKenzie and Shaun Killen from the University of Glasgow scrutinised the data again and realised that the fish could be divided into two groups – one timid and the other bold – with the bold fish returning to the surface to breathe within 75 min of a fright, while the timid animals remained cowering at the bottom for at least 115 min. Also, the bold animals tended to air breathe more during the day and they chose to breathe air in well-oxygenated water, even though there was no need to place themselves at risk. ‘The bold individuals were apparently just ‘choosing’ to [put themselves at risk]’, says McKenzie. Adding that physiology and personality are not necessarily linked, he says, ‘Some animals take risks because they have to,

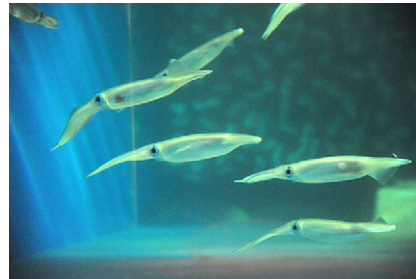
others seem to take risks just because they want to’.

10.1242/jeb.134627

McKenzie, D. J., Belão, T. C., Killen, S. S. and Rantin, F. T. (2015). To boldly gulp: standard metabolic rate and boldness have context-dependent influences on risk-taking to breathe air in a catfish. *J. Exp. Biol.* **218**, 3762–3770.

Kathryn Knight

Thermocline essential for squid egg survival



Japanese flying squid inside an experimental tank. Photo credit: Hae-Kyun Yoo.

By terrestrial standards, the Japanese flying squid (*Todarodes pacificus*) is rather shy. Little is known about the elusive animals’ reproduction, despite tons of them appearing on dining tables every year. Pandey Puneeta from Hokkaido University, Japan, says, ‘A full understanding of its life history is hampered because observation is difficult at the depths at which spawning occurs’, adding that the squid were assumed to spawn in shallow coastal waters above the continental shelf surrounding Japan. Previous attempts to observe spawning in the field and the lab had led Yasunori Sakurai to suggest that the squid migrated above the layer of water separating the cold deep waters from the warmer surface waters (the thermocline) to spawn, with the egg masses sinking down later into the thermocline where the eggs would develop prior to hatching. However, when the Hakodate Research Centre for Fisheries and Oceans opened in Japan in June 2014, Sakurai finally had access to a 6 m deep, 300 m³ tank where he and Puneeta could test the theory.

Having collected male and female squid by hand jigging and in traps in the waters off Hokkaido, Puneeta pumped warm and cold seawater into the colossal reinforced concrete tank to create a thermocline

between 2.5 and 3.5 m deep, ready to observe the females as they spawned. And then Puneeta repeated the experiment in water where the temperature was a uniform 22°C across the entire depth.

Filming the females in the water with a thermocline, Puneeta and her colleagues Sakurai, Dharmamony Vijai, Hae-Kyun Yoo and Hajime Matsui saw the squid descend into the cool water beneath the thermocline where they produced egg masses ranging in diameter from 17 to 80 cm containing 38,000–200,000 eggs. Explaining that the animals could only use their fins to swim as they produced the egg masses – because the mantles were used to pump water into the jelly surrounding the egg mass – Puneeta describes how each animal cradled the egg mass in their arms and tentacles as it was extruded. In addition, the squid sank gradually to near the bottom of the tank during the 7 min it took for them to produce each egg mass. However, she never saw the squid sit in the posture that Sakurai had suggested they adopt before spawning. Puneeta also noticed that initially the egg masses were elongated, although they eventually became more spherical and then their density changed and they floated up into the thermocline, where the eggs developed and hatched 5–6 days later.

However, the eggs that were laid in the tank with uniform temperature water did not hatch successfully. They failed to become buoyant, remaining at the bottom of the tank until 2–3 days later, when the egg masses collapsed and were consumed by microorganisms.

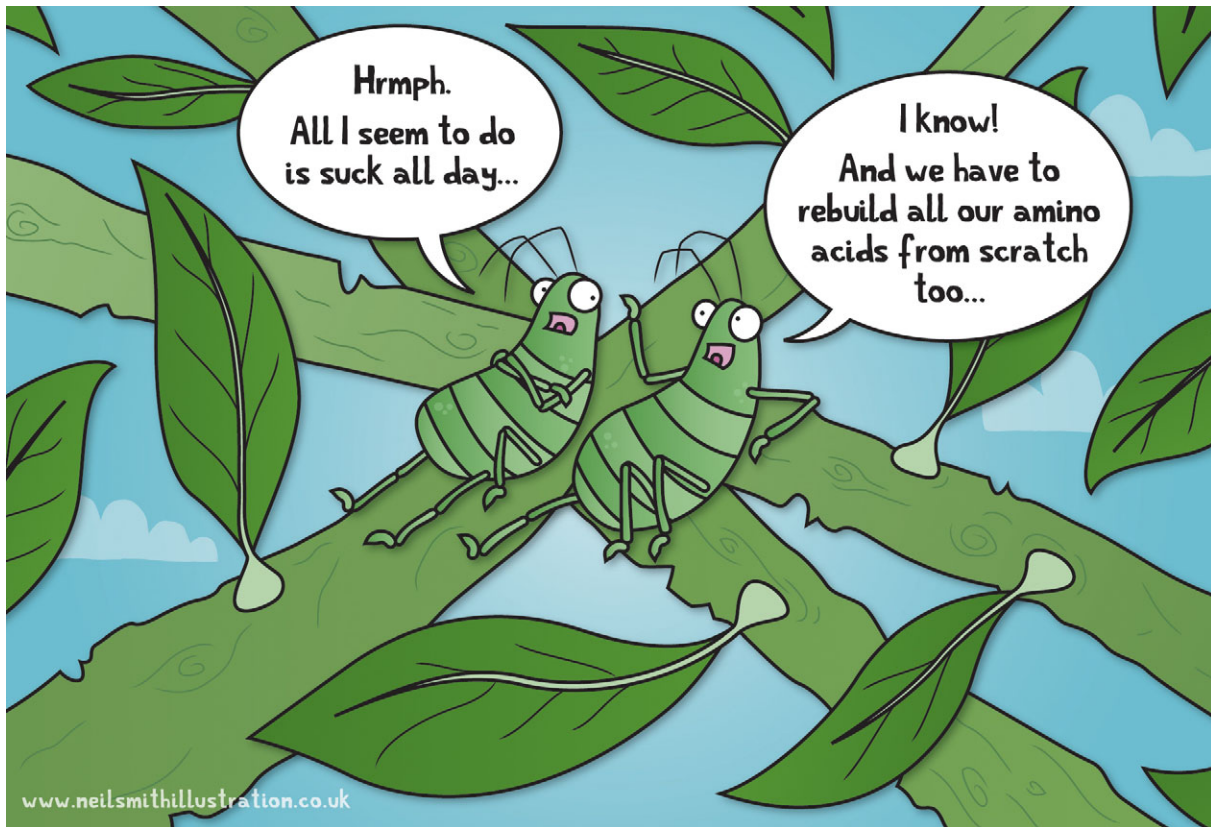
The team suspects that in addition to spawning in shallow coastal waters, the squid may head further offshore to spawn. They also suggest that the thermocline is responsible for maintaining the buoyancy of the egg mass and preserving the outer jelly layer that protects the eggs from microorganism damage.

10.1242/jeb.134601

Puneeta, P., Vijai, D., Yoo, H.-K., Matsui, H. and Sakurai, Y. (2015). Observations on the spawning behavior, egg masses and paralarval development of the ommastrephid squid *Todarodes pacificus* in a laboratory mesocosm. *J. Exp. Biol.* **218**, 3825–3835.

Kathryn Knight

Aphids reconstruct amino acids from scratch



It's a gardener's worst nightmare: your newly planted seedlings become infested by a horde of aphids. With unlimited sap on tap, aphids should have a ticket to the good times, but Meena Haribal and Georg Jander from the Boyce Thompson Institute for Plant Research, USA, explain that the sugary fluid is far from nutritious. Plant roots primarily produce four amino acids – glutamine, glutamate, asparagine and aspartate – which provide the voracious insect's only source of nitrogen. To overcome the restrictions of their diet, many aphid species have struck a cooperative deal with symbiotic bacteria: providing their lodgers with food and security in return for nutritional supplements that the aphids cannot provide for themselves. Haribal and Jander were curious to find out how the pea aphid (*Acyrtosiphon pisum*) and its bacterial lodger

(*Buchnera aphidicola*) convert the four non-essential amino acids into the essential amino acids that are necessary for the aphid's survival.

Feeding the aphids a series of diets laced with amino acids fortified with heavy nitrogen and carbon isotopes, the duo then embarked on a brain-teasing set of gas chromatography–mass spectrometry experiments to untangle the amino acid synthetic web linking the aphid to its symbiotic bacteria. Scrutinising hundreds of mass spectra and tracing the paths of nitrogen and carbon atoms, the duo found that the amine nitrogen of asparagine was incorporated more readily into other amino acids than the amide nitrogen. However, the duo was intrigued to see that most of the amino acids in the aphids' bodies were composed of combinations of normal and heavy carbon, suggesting that

instead of building the amino acids directly from the skeletons of the four plant-derived amino acids, the aphids were breaking down the plant-derived amino acids into small components which they then used to synthesise the essential amino acids that were not provided by their diet. The duo was also surprised that instead of directly using the amino acids that they consumed in sap, the aphids were resynthesising them too. 'Even when there is an excess of an amino acid in the diet, it gets broken down and re-synthesised by the aphids', says Jander.

10.1242/jeb.134635

Haribal, M. and Jander, G. (2015). Stable isotope studies reveal pathways for the incorporation of non-essential amino acids in *Acyrtosiphon pisum* (pea aphids). *J. Exp. Biol.* **218**, 3797-3806.

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