

INSIDE JEB

Type I cod haemoglobin no better than type II in warm water



Head of an Atlantic cod. © Hans Hillewaert, via Wikimedia Commons.

Not all cod are created equal. Scratch beneath the surface and an essential difference in their blood appears. Michael Berenbrink from the University of Liverpool, UK, explains that most cod found at the southern extent of their range tend to produce a specific form (genotype) of the oxygen carrying protein haemoglobin in their red blood cells (type I haemoglobin), while individuals from the population further north tend to carry a second form of the protein (type II). And when fish with one or other of the two haemoglobin forms are offered a choice of temperatures, cod carrying the first prefer warmer conditions, while those carrying the second select the chillier water. 'There is something that translates from the genotype to how the whole organism behaves in a thermal gradient, so there must be something that is different, but what it is we don't know', says Berenbrink.

Evidence suggested that the different haemoglobins could lie at the heart of the population distributions, if the first form of haemoglobin allowed the fish to transport more oxygen in warm conditions while the second haemoglobin was optimised for oxygen transport in cold conditions. 'But nobody has ever checked this carefully', says Berenbrink. With the possibility that climate change may eradicate cod from the Irish Sea by the end of this century, Berenbrink and his colleagues, Samantha Barlow, Julian

Metcalf and David Righton, decided to test how tightly red blood cells from fish in the Irish Sea bind oxygen.

Knowing that she could catch type I and type II individuals in the Liverpool bay, Barlow headed out into the ferocious sea conditions in the dead of winter on charter boats to fish for blood samples. 'We chose that time of year because there was no difference in the water column temperature and we took all of the fish from a certain spot', says Berenbrink, explaining that different temperature experiences and stress can dramatically affect how tightly fish red blood cells bind oxygen. And when Barlow returned to the lab with the precious vials of blood, she had to work fast, running the red blood cell proteins on a gel to identify which form of haemoglobin each individual fish carried before selecting the blood group she would test the following day. She then cautiously isolated the red blood cells and stabilised them at three different temperatures (5, 12.5 and 20°C) and three different pHs (7.9, 7.65 and 7.4), before painstakingly adjusting the oxygen concentration in the red blood cells (ranging from 0–100% air) and measuring the subtle colour change as the haemoglobin in the red blood cells absorbed the gas, to determine how tightly the blood cells bound oxygen.

However, when Barlow plotted the characteristic S-shaped oxygen binding curves and adjusted for the internal pH change that occurs naturally as blood cells warm, she and Berenbrink could see that all of the curves essentially overlapped. The different forms of the oxygen carrying haemoglobin did not affect the red blood cells' ability to bind oxygen at different temperatures, despite the fish's temperature preferences: 'We are back to the drawing board to figure out what limits cod thermal tolerance', says Berenbrink.

Realising that the red blood cell oxygen binding was essentially identical, the duo then pooled the data from all 16 samples and calculated how well the fish could absorb oxygen and deliver it to tissues as

their temperature and metabolic rates increase under future climate scenarios. However, their calculations suggest that the fish's red blood cells will struggle to meet their increasing oxygen demands at temperatures around 20°C and above. 'This does not necessarily mean the animal is doomed, just that any improvements in oxygen supply must come from other sources', Berenbrink concludes.

10.1242/jeb.154930

Barlow, S. L., Metcalfe, J., Righton, D. A. and Berenbrink, M. (2017). Life on the edge: O₂ binding in Atlantic cod red blood cells near their southern distribution limit is not sensitive to temperature or haemoglobin genotype. *J. Exp. Biol.* **220**, 414-424.

Kathryn Knight
kathryn.knight@biologists.com

Hedgehogs driven from countryside by landscape of fear



A juvenile hedgehog. Photo credit: Carly Pettett.

Armoured in a coat of apparently impenetrable spines, hedgehogs should be almost invincible. Yet, the rapid loss of their hedgerow homes and changes in agricultural practice may be forcing these iconic creatures out of their rural homes into more urban settings. And badgers, one of the hedgehog's main predators and competitors for food, might also be restricting the small mammal's ability to forage and roam. Explaining that the decline in the hedgehog population appears to be most severe in rural locations, Carly Pettett from the

University of Oxford, UK, and her colleagues, Paul Johnson, Tom Moorhouse and David Macdonald, began tracking the enigmatic mammals and measuring their daily energy expenditure to learn more about the impact of modern environmental factors and predation on hedgehog populations.

Selecting four sites in Yorkshire and North Norfolk, UK – including one site in each county that was occupied by badgers – Pettett and her field assistants roamed the countryside at night in search of the spiky mammals. Pettett recalls, ‘The hedgehogs were initially hard to track down’, but adds, ‘Luckily, some of the inhabitants of the villages who regularly fed the hedgehogs gave us access to their gardens and allowed us to wait close by the food until the hedgehogs appeared’.

The team attached tiny radio transmitters to the animals to track their activity, and returned 3 weeks later to measure how much energy the animals used while roaming around their home patch. Injecting the hedgehogs with 0.6 ml of heavy water (made from the ^{18}O isotope and deuterium [^2H]), the team collected a minute blood sample before releasing the animals to continue rambling. Four days later, Pettett and her assistants relocated each hedgehog and collected a second blood sample, before despatching the samples to John Speakman and Catherine Hambly at the University of Aberdeen, UK, for analysis. Measuring the amount of ^{18}O and deuterium remaining in the hedgehogs’ bodies, Speakman and Hambly then calculated the amount of energy consumed by each animal – based on the amount of ^{18}O that had been lost from the animals’ bodies as carbon dioxide – over the 4 day period.

Pooling together the energy expenditure data with details of the animals’ routes, Pettett could see that energy consumption varied enormously between individuals, with some consuming as little as 227 kJ day^{-1} , while others consumed almost six times as much (1272 kJ day^{-1}). And when she compared the distances covered by the small mammals, the males wandered over $\sim 1.5 \text{ km}$ while the females covered a smaller range of $\sim 1 \text{ km}$.

However, the females tended to consume more energy than the males and the hedgehogs that roamed farthest from buildings had higher energy consumption levels than those that remained near human habitation. However, when Pettett measured the energy expenditure of the hedgehogs that shared their territory with predatory badgers, she was surprised to see that the hedgehogs consumed 30% less energy than the animals that were free to roam without risk.

‘Our findings support the suggestion by other scientists that arable land represents a “landscape of fear” for hedgehogs’, says Pettett, suggesting that hedgehogs may reduce the amount of time that they spend foraging when badgers are on the prowl and may even reduce their body temperatures to conserve resources when unable to forage. She also suspects that hedgehogs that roam further from human structures have to work harder to find nutritious meals in arable land where worms and insects are scarce. Pettett says, ‘These results are of conservation concern for hedgehogs’, and recommends that farmers increase the area given over to hedgerows while reducing the use of pesticides to provide hedgehogs with greater dining opportunities while providing refuge from hungry badgers.

10.1242/jeb.155234

Pettett, C. E., Johnson, P. J., Moorhouse, T. P., Hambly, C., Speakman, J. R. and Macdonald, D. W. (2017). Daily energetic expenditure in the face of predation: hedgehog energetics in rural landscapes. *J. Exp. Biol.* **220**, 460–468.

Kathryn Knight
kathryn.knight@biologists.com

Male mosquitoes sing from the same song sheet

Some close relatives can be hard to distinguish. Take the extensive *Anopheles* family of mosquitoes: until recently, *Anopheles coluzzii* and *A. gambiae* mosquitoes were thought to be members of the same species because they are almost perfectly identical. Yet, despite sharing much of the same geographical range, they rarely, if ever, interbreed – even when mingling in the same habitat. Somehow, members of the two species

manage to select out the correct mates despite their similarities. Patricio Simões from the University of Brighton, UK, wondered whether *A. coluzzii* and *A. gambiae* suitors fine tune their elaborate courtship displays to distinguish themselves from each other in the ears of their intendeds.

When a male *Anopheles* mosquito picks up the distinctive tone of an approaching female, he begins to serenade by speeding up his wing beats before rapidly increasing and decreasing the wing beat frequency (known as rapid frequency modulation) to produce a whining refrain that woos the lady. Would Simões and his colleagues Gabriella Gibson and Ian Russell find differences between the species’ melodies that could help the females select the right suitor? Playing the seductive tone of an approaching female to individual males in an enclosure, the trio recorded the males’ responses. However, the trio found that the love songs from males of the two species were essentially indistinguishable. In both cases, the males accelerated their wing beat frequencies at rates in excess of 1250 Hz s^{-1} before warbling up and down ~ 12 times per second and maintaining the solo for up to 2 s; so *A. coluzzii* and *A. gambiae* females cannot rely on the males’ serenades to select suitors of the correct species.

Referring to a previous study of the mating manoeuvres of more distantly related *Culex quinquefasciatus* mosquitoes, the trio suggests that this courtship behaviour may have evolved over 200 Ma, before the Culicinae and Anophelinae families separated, and they say: ‘Rapid frequency modulation might be found throughout all of the Culicidae [mosquito] family’. They also hope that traps based on the male’s mating melody could be used to ensnare voracious females to reduce the spread of mosquito-borne diseases.

10.1242/jeb.155135

Simões, P. M. V., Gibson, G. and Russell, I. J. (2017). Pre-copula acoustic behaviour of males in the malarial mosquitoes *Anopheles coluzzii* and *Anopheles gambiae* s.s. does not contribute to reproductive isolation. *J. Exp. Biol.* **220**, 379–385.

Kathryn Knight
kathryn.knight@biologists.com