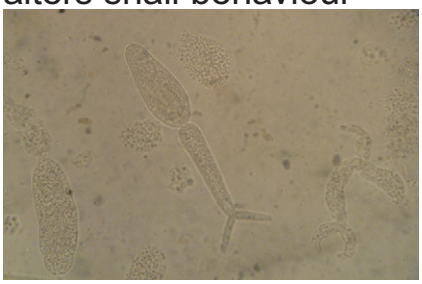
INSIDE JEB

Schistosome parasite alters snail behaviour



Schistosoma mansoni cercaria. Photo credit: Chelsea Wood, University of Michigan.

For such a tiny worm, the schistosome parasite causes a great deal of suffering. Infecting more than 200 million people worldwide and causing liver damage, kidney failure, infertility and cancer, the parasite could be responsible for up to 200,000 deaths annually. 'Some estimates suggest that human schistosomiasis imposes a greater global disease burden than either malaria or tuberculosis', says Susanne Sokolow from Stanford University. Snails carry the parasite and release its larvae into fresh water; the larvae then burrow into the skin of humans contacting the water before migrating through the victim's body and transforming into adult worms, ready to release eggs back into the water to infect the next snail generation. And other factors in the environment could alter schistosome infection rates. Sokolow explains that the exotic Louisiana crayfish may have reduced schistosome infection rates in Egypt by consuming the parasite's intermediate snail hosts. Knowing this, Sokolow wondered whether the parasite could contribute to its own demise. Could the infection alter the snail's behaviour, in a bid to improve the chances of schistosome transmission while inadvertently placing the snail at greater risk?

Teaming up with Scott Schwarz, Giulio De Leo and Chelsea Wood, Sokolow collected two species of snail that are known to transmit schistosome infections from the Schistosomiasis Resource Center in the US and obtained a colony of predatory *Macrobrachium vollenhovenii* prawns, originally from Cameroon, to find out how infected and parasite-free snails reacted to the threatening crustaceans.

First, the team tested the prawns' preferences by isolating individuals with two infected and eight uninfected snails, and found that the prawns were twice as likely to devour the infected snails as the parasite-free animals.

Then the team tested whether the parasite impacted the snails' mobility and found that the infected snails were less mobile and moved much more slowly (18.9 mm s^{-1}) than the uninfected snails (25.2 mm s^{-1}). Finally, the team tested the snails' reactions to a threat by placing them in an enclosure with a caged prawn and some crushed snails. After recording how much evasive action the snails took, the team could see that the uninfected snails were much more cautious than the infected animals – heading for high ground out of the water, avoiding open water and seeking shelter where possible – while the infected snails left themselves much more vulnerable to attack in open water.

Sokolow suggests that the parasite is either deliberately manipulating the snails' behaviour in some way – to improve its chances of being passed on to its human hosts – or the parasite debilitates the snail – restricting the snails' mobility – despite the increased risk of predation. She also suspects that the infected snails may be more reluctant to evade predation by climbing out of the water in order to improve their chances of releasing infectious schistosome larvae ready to infect the first bather they encounter.

Either way, schistosome-infected snails suffer a double whammy: not only are they less mobile than uninfected animals, but predatory prawns also find them more appealing. And the aggressive crustaceans could play a significant role in breaking the insidious *Schistosoma* infection cycle by controlling infected snail populations.

10.1242/jeb.135467

Swartz, S. J., De Leo, G. A., Wood, C. L. and Sokolow, S. H. (2015). Infection with schistosome parasites in snails leads to increased predation by prawns: implications for human schistosomiasis control. *J. Exp. Biol.* **218**, 3962-3967.

Kathryn Knight

Tree snakes' keel gets a grip

Brown tree snake crawling on the underside of a perch. Photo credit: Bruce Jayne.

To the untrained eye, most snakes look alike, but when Bruce Jayne observes a serpent, he sees many different features. 'Sea snakes have paddle-shaped tails and gliding snakes are vertically flattened,' he explains, adding that many species of tree snake have flattened bellies and sharp ridges running along both belly edges, known as keels. Jayne is also fascinated by how animals interact with their surroundings and is particularly intrigued by how tree snakes stop themselves from slipping on rugged bark surfaces. Reasoning that more rotund species would have problems getting a purchase on rough surfaces, as they would simply roll over bumps and ridges, while flat bellied species may be able to lodge the keel ridge against protrusions and lock themselves in place, Jayne recruited a team of students to test the tree-worthiness of three species, ranging from stout boa constrictors to corn snakes and agile brown tree snakes.

First, Jayne, Steven Newman, Michele Zentkovich and Matthew Berns simulating the rugged surfaces encountered by snakes in the wild. 'Variation in the roughness of the bark on natural vegetation is very complex and the spacing between various ridges and grooves can be quite irregular', explains Jayne. So, instead of trying to mimic every detail on a branch, he focused on one aspect of bark structure – the height of ridges on the surface – by embedding 8 mm wide screws (at lengths of 1, 2, 10, 40 mm), spaced at 10 cm intervals, into the top face of long narrow poles that he

inclined over a range of angles from horizontal to vertical. 'The sheer number of trials was a significant challenge', chuckles Jayne, estimating that the student trio must have videoed almost 10,000 movies of the snakes ascending poles over the course of the study.

Analysing the snakes' techniques, Jayne saw that on the steepest branches the boa constrictors and corn snakes preferred to grip the pole with one portion of the body and straighten other portions to slide the animal along using a concertina-like motion. However, the tree snake only resorted to this approach when presented with a smooth vertical pole, switching predominantly to sliding sinuously as soon as they were provided with the shortest pegs to lean on. As the pegs became longer the tree snakes dispatched with the concertina style of ascent entirely, while the boa constrictors and corn snakes continue shuffling until the pegs were quite long. Even at the shallowest pole angles, the boa constrictors seemed to struggle, preferring to shuffle along slowly; meanwhile the tree snakes slithered at higher speeds as the pegs became longer and the corn snakes' performance fell somewhere in between. Even when the team set the tree snakes an additional challenge of negotiating a pole angled at 60 deg with the pegs positioned along the bottom face of the pole, the tree snakes seemed completely unfazed.

But how were the tree snakes clinging on? '[They were] catching their keel on surface projections that were only 1 mm high', Jayne says. Where the boa constrictors' rotund bodies simply rolled over the shortest pegs – forcing them to grip the pole in their coils – the tree snakes were able to lock themselves onto the pole by running their keel ridges against the pegs like a rope passing through a pulley. And Jayne hopes that a better understanding of how tree snakes get a grip could help us design snake unfriendly surfaces, to prevent invasive species – such as the brown tree snake in Guam – from getting into places where they are not wanted.

10.1242/jeb.135434

Jayne, B. C., Newman, S. J., Zentkovich, M. M. and Berns, H. M. (2015).Why arboreal snakes should not be cylindrical: body shape, incline and surface roughness have interactive effects on locomotion. *J. Exp. Biol.* **218**, 3978-3986.

Kathryn Knight

Gas movement through aquaporins is significant

Zebrafish larva stained with o-dianisidine to show red blood cells. Photo credit: Krystle Talbot.

The movement of gases across biological structures is essential for life from the instant of conception to the last gasp. However, Katie Gilmour from the University of Ottawa, Canada, explains that there is much debate about the mechanisms by which gas molecules pass across membranes to move into and out of cells. Although gases can simply diffuse across membranes, certain membrane-embedded pore proteins – such as aquaporin water channels and Rhesus proteins – also allow gas molecules to pass through membranes. She says, 'Arguing that membrane proteins are physiologically important for gas movement when membrane proteins are relatively limited (in comparison to overall membrane area) becomes challenging.' So, in a bid to resolve the mystery, Gilmour and her colleagues turned to 4-day-old zebrafish larvae to find out just how significant aquaporins are in the transfer of gases across cell membranes.

Gilmour explains that zebrafish larvae were great animals for her team to work with because it is possible to directly switch off the production of specific proteins and measure the impact on the amount of gas passing through cell membranes. However, she admits that working with the minute animals was extremely fiddly. 'Measuring CO_2 excretion in tiny aquatic animals requires that very small increases be measured in the CO_2 concentration of the water in which the animals are held', says Gilmour. But Mike Murphy, a talented engineer in the University of Ottawa's electrical workshop, eventually designed and built a bespoke CO_2 analyser to allow Gilmour and her colleagues to make the sensitive measurements.

Then, Krystle Talbot painstakingly injected a molecule specially designed to switch off production of aquaporin protein into newly fertilized zebrafish eggs and allowed them to develop for 4 days before measuring the amount of CO_2 produced by the larvae. Amazingly, the larvae's CO_2 excretion rate fell by 35%, despite consuming the same amount of oxygen as larvae with aquaporin proteins embedded in their cell membranes. The aquaporin proteins were contributing significantly to the movement of gas molecules across cell membranes.

However, it was not clear whether the aquaporin proteins were involved in CO_2 moving across red blood cell membranes or the membrane surrounding the larvae's yolk sac. So Talbot bathed the tiny animals in phenylhydrazine-water, to remove their red blood cells, and measured how much CO_2 they produced. The larvae were unaffected, excreting as much CO_2 as fish with red blood cells. However, when Talbot tested the CO_2 production of larvae that lacked both red blood cells and aquaporin proteins, she found that it fell, so the aquaporin molecules embedded in the yolk sac membrane were responsible for CO_2 excretion.

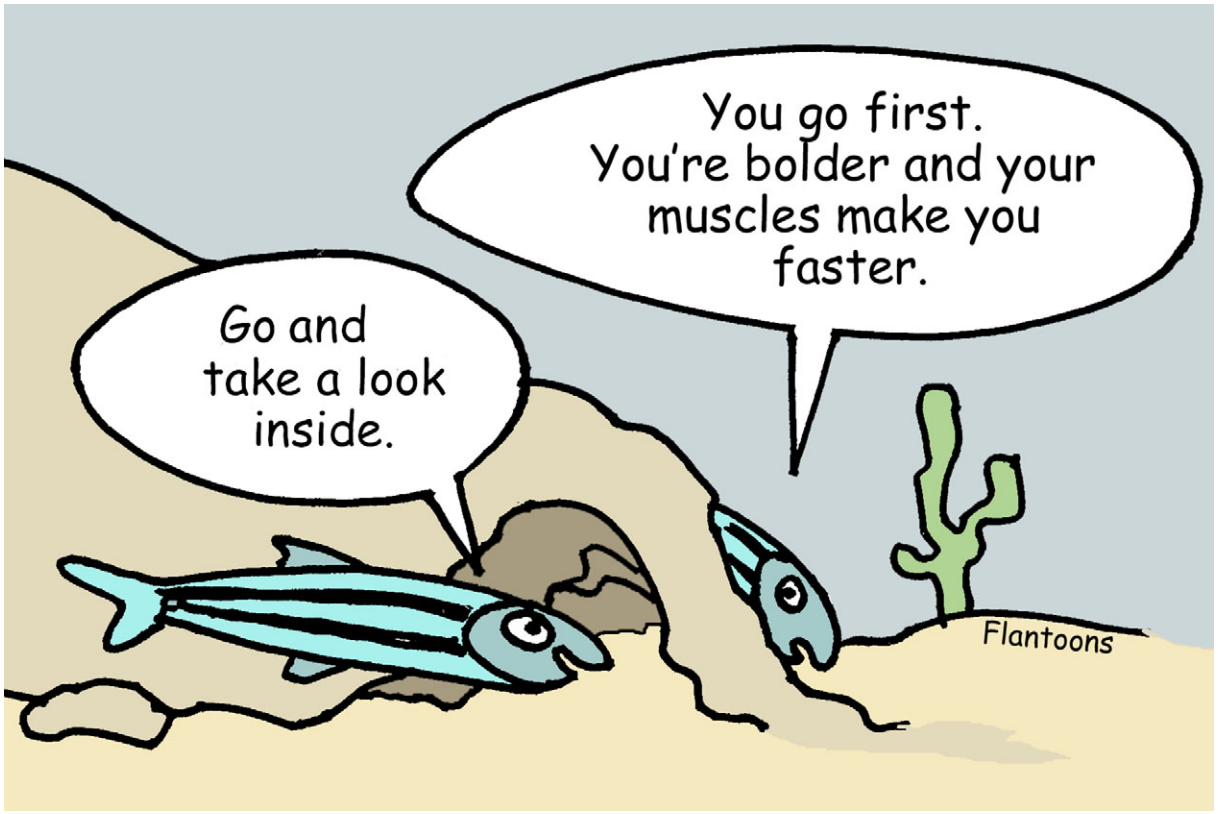
Aquaporins have also been suggested to excrete toxic ammonia gas through cell membranes, so Talbot then measured ammonia excretion in larvae that did not produce aquaporin and in a second group of larvae that did not produce the Rhesus ammonia channel. Not surprisingly, the larvae lacking the Rhesus protein channel had significantly reduced ammonia excretion rates, but so too did the fish lacking aquaporin. And, when Talbot and Raymond Kwong investigated aquaporin gene expression and protein production in larvae that lacked the Rhesus protein when there were high levels of ammonia in the environment, they found that the larvae were mobilising more aquaporin. So, aquaporins could be working together with Rhesus proteins to excrete nitrogenous waste, in addition to helping the animals remove CO_2 from their bodies.

10.1242/jeb.135442

Talbot, K., Kwong, R. W. M., Gilmour, K. M. and Perry, S. F. (2015). The water channel aquaporin-1a1 facilitates movement of CO_2 and ammonia in zebrafish (*Danio rerio*) larvae. *J. Exp. Biol.* **218**, 3931-3940.

Kathryn Knight

Strong muscles contribute to fish boldness



Life is full of risk – from the dangers posed by a famished hunter that may be lurking in the shadows to the challenge of negotiating an unpredictable environment – and some animals are simply better at dealing with risk than others. These bold creatures seem more content to explore unfamiliar situations than other, timid creatures that would prefer just to hide. However, Frank Seebacher and his colleagues Alexander Little and Rob James were curious to determine how much of an impact the muscles that give an animal strength might have on their courage.

'Locomotor performance is determined to a large extent by energy metabolism and muscle function', Seebacher says, so the team decided to find out how the strength of zebrafish muscle contractions affects their boldness.

The team first exposed fish to a drug (nifedipine) that reduces the strength of muscle contractions and then filmed the animals as they explored a new territory and an unfamiliar object, assessing the fish's boldness and swimming performance as they became weaker. They then directly measured the strength of each fish's muscular contractions before investigating the contribution of muscle function to the animals' audacity.

Combining various muscle contraction characteristics and analysing the impact of this 'muscle factor' on the fish's swimming performance, the trio showed that muscle contraction has a major impact on the animal's activity levels and that activity, in turn, also significantly influenced their boldness. So muscle contraction does contribute to an animal's boldness, and Seebacher and colleagues suggest that an animals' boldness may simply reflect how fast it can swim, with faster fish appearing bolder simply because they move farther than slower fish, providing them with greater opportunities for exploration.

10.1242/jeb.135483

Seebacher, F., Little, A. G. and James, R. S. (2015). Skeletal muscle contractile function predicts activity and behaviour in zebrafish. *J. Exp. Biol.* **218**, 3878-3884.

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