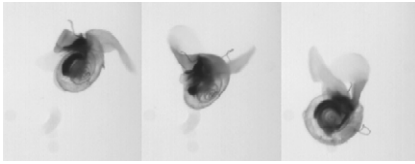


INSIDE JEB

Bizarre snail that swims like a flying insect



A sea butterfly at different stages of a wing beat. Photo credit: David Murphy.

Snails usually lumber along on their single fleshy foot, but not sea butterflies (*Limacina helicina*). These tiny marine molluscs gently flit around their Arctic water homes propelled by fleshy wings that protrude out of the shell opening. But little was known about how they move through water. ‘Most zooplankton swim with a drag-based paddling technique,’ explains David Murphy from the Georgia Institute of Technology, USA, and even though one of Murphy’s thesis advisors – Jeannette Yen – had filmed one of the enigmatic snails swimming while it was attached to a wire in 2003, it had not been possible to observe how fluid flowed around the animals to explain how they move. So, when Murphy built a new 3D system to visualise fluid movements around minute animals, Yen and Don Webster were keen to test more sea butterflies as they swam freely to discover more about their exotic mode of propulsion.

However, working with the delicate animals in land-locked Atlanta posed a unique set of challenges. Murphy explains that sea butterflies are scarce at the best of times, and that transporting the fragile gelatinous creatures across the continent from their ocean home was tricky. ‘You have to ship them overnight in an insulated cooler to keep them cold and if the water is too dirty, particles will stick to them, so the water has to be very clean’, he says. Yen then devised a cunning V-shaped structure at the bottom of the tank to ensure that the freely swimming snails repeatedly ascended through the middle portion of the tank – where four high-speed cameras were focused to capture every detail of the wings’ movements. Even then, Murphy admits that it was a miracle that they were able to collect any data, and he recalls that

the team only had a few short hours to film the molluscs, explaining that they could only be sure that the swimming conditions were ideal then. However, by the end of the afternoon the snails had serendipitously crossed the path of the cameras on four occasions. ‘In this sort of free-swimming experiment, it’s normal to take 30 passes to get three usable ones, but we got really lucky! The animals even cooperated by swimming in different orientations, so we could see different perspectives’, chuckles Murphy, who then began visualising the snails’ wing beats and the fluid movements around their bodies with Deepak Adhikari.

After months of painstaking analysis – interrupted by Murphy’s relocation from Georgia to Johns Hopkins University in Baltimore, Maryland – the team was astonished when they realised that the snails were swimming just like fruit flies fly. ‘I said to myself, “Its wing stroke is just like what an insect is doing”’, recalls Murphy, describing the snails’ characteristic figure-of-eight wing beat that was only apparent after he took account of the molluscs’ extraordinary bobbing motion. And when he investigated how water flowed around the snails’ wings, he was impressed to see that the molluscs generated the same low-pressure system that produces lift in flying fruit flies. Murphy explains that the snails (and fruit flies) clap their wings together at the top of a wing beat before peeling them apart, sucking fluid into the V-shaped gap between the wings to create low-pressure vortices at the wing tips that generate lift. He says, ‘No one has actually been able to measure the flow around an insect doing this while it is flying, and so that was kind of the holy grail of this area of research’. And he adds, ‘It really surprised me that sea butterflies turned out to be honorary insects’.

10.1242/jeb.138230

Murphy, D. W., Adhikari, D., Webster, D. R. and Yen, J. (2016). Underwater flight by the planktonic sea butterfly. *J. Exp. Biol.* **219**, 535-543.

Kathryn Knight

Zebrafish larvae learn to hunt using lateral line in the dark



A zebrafish larva. Photo credit: Krystle Talbot.

Few animals are born fully equipped to deal with the world. Most continue developing after birth, and for a zebrafish larva, one of the key structures that is thought to undergo massive changes during the earliest weeks of life is the lateral line system. ‘The lateral line is how fish sense water flow’, explains Matt McHenry, from the University of California Irvine, USA, who adds that the sensory system may allow peckish larvae to detect swirls in the water produced by juicy water fleas sweeping past. ‘If you have the ability to feed in the dark or in dim, turbid conditions... then you have a great advantage’, says McHenry. Wondering if the ability of zebrafish larvae to forage in the dark improved as their flow-sensitive lateral line system developed, McHenry and graduate student Andres Carrillo began testing how zebrafish youngsters coped with catching live meals when the lights went out.

Carrillo fed young larvae on live rotifers before upgrading them to a larval brine shrimp diet as they grew, and then filmed the tiny fish foraging for food in light and dark conditions at various stages of development. ‘The fish got much better at foraging as they grew, and they also had the ability to forage in the dark’, recalls McHenry. Then Carrillo temporarily knocked out the sensory cells in the lateral line using neomycin sulphate, and this time the zebrafish larvae’s success rate plummeted when the lights were switched off. So the lateral line was essential for the larvae’s success, but could changes in the lateral line sensory cells explain the larvae’s improvements with age?

Anticipating that they would find dramatic changes in the structure of the lateral line's sensory cells (neuromasts), McHenry and Carrillo measured the diameter of each neuromast and the number of hair cells associated with each structure as the larvae grew, but they were in for a shock. 'We had this big behavioural change; we knew it was mediated by this sensory system but we didn't see anything in the sensory system that reflected that change', says McHenry. 'We were pretty puzzled,' he admits.

Carrillo presented the disturbing results to colleagues during a group meeting in Irvine and McHenry recalls the discussion, saying, 'Tim Bradley suggested that maybe it's an effect of learning; that they basically have the same receptors at all ages, but they are just getting more attuned to using them as they grow'. Realising that they would have to raise fish that had never experienced the 'whoosh' feeling as lunch swam past in order to test Bradley's theory, Carrillo suggested temporarily disabling the fish larvae's lateral lines with neomycin sulphate every time that they were fed, so that they were unable to associate fluid movements with the presence of food.

Painstakingly bathing the youngsters in the chemical just before they were fed every day for a month, Carrillo then allowed the fish's lateral lines to recover before testing their responses to lively brine shrimp larvae at the age of one-month. And this time the zebrafish failed to grab a morsel. They had not learned to interpret the tell-tale swirls in the water associated with live food. And when Carrillo fed the developing fish on a diet of dead brine shrimp, these fish larvae were equally inept at catching a meal.

So, the zebrafish larvae with uncompromised lateral lines had learned to associate disturbances in the water with the presence of dinner and Carrillo and McHenry are now eager to learn more about the factors associated with fluid movements that trigger zebrafish larvae to snap at passing prey.

10.1242/jeb.138248

Carrillo, A. and McHenry, M. J. (2016). Zebrafish learn to forage in the dark. *J. Exp. Biol.* **219**, 582–589.

Kathryn Knight

Plant defences wear vole teeth



Tundra vole (*Microtus oeconomus*). Photo credit: Karol Zub.

In the fields of Northern Europe, a tundra vole (*Microtus oeconomus*) population explosion could be going on right now. As they hit the reproductive jackpot, their numbers rocket. But with most booms there comes a bust, and the same is true for vole populations, which can collapse suddenly and unexpectedly. Ivan Calandra, from the University of Reims Champagne-Ardenne, France, explains that the cause of these dramatic population crashes was not clear, although there was one candidate that could account for the dramatic fall from grace: the vegetation upon which the voles feast. Plants are not as vulnerable as they may at first appear. 'It has been known for at least 30 years that plants build up silica bodies (phytoliths) in response to intense grazing,' says Calandra. But it was not clear whether these plant defences were directly responsible for the vole's demise, and if they were, how? Were the abrasive silica structures that accumulate in plant cells wrecking the rodents' teeth or damaging their intestines, leading to starvation and population collapse?

Karol Zub, Andrzej Zalewski and Paulina Szafrńska, from the Polish Academy of Sciences, decided to feed voles on two diets – one composed of pellets containing high levels of sedge, which is reinforced with silica, and another in which the sedge was replaced with lower-silica grasses – to find out how different levels of silica affected the rodents' teeth. Then Zub and Szafrńska shipped the voles' teeth to Gildas Merceron, a CNRS Researcher at the University of Poitiers, France, where he and Calandra could look for any damage using one of only four profilometers – confocal microscopes that

scan surfaces to produce 3D representations of structures – that can analyse tooth wear.

However, when the duo compared the damage on the surface of teeth that the voles had used to grind their food, they were surprised to see little difference between the patterns of wear in animals on the different diets, possibly because the lab diets were not optimal. However, Calandra's Polish colleagues also knew that voles switch diet from season to season, dining on high-silica sedges in the winter and spring before supplementing the diet with softer, low-silica leafy vegetation in the summer and autumn. In addition, Calandra explains that sedges retaliate as the vole population expands by arming themselves with increased silica levels. Could the team find differences in tooth wear if they investigated the wear patterns in different populations of voles across the years and seasons as the silica levels increased in response to the expanding populations?

This time the team investigated vole teeth from animals that had been trapped in the wild from the late 1970s to 2001, including a period when the population crashed in 2000 after peaking the previous year. And this time, having scanned the surface of 47 teeth, they found evidence of changes in wear pattern as the voles shifted their diet through the seasons and as the sedges became more impregnated with silica.

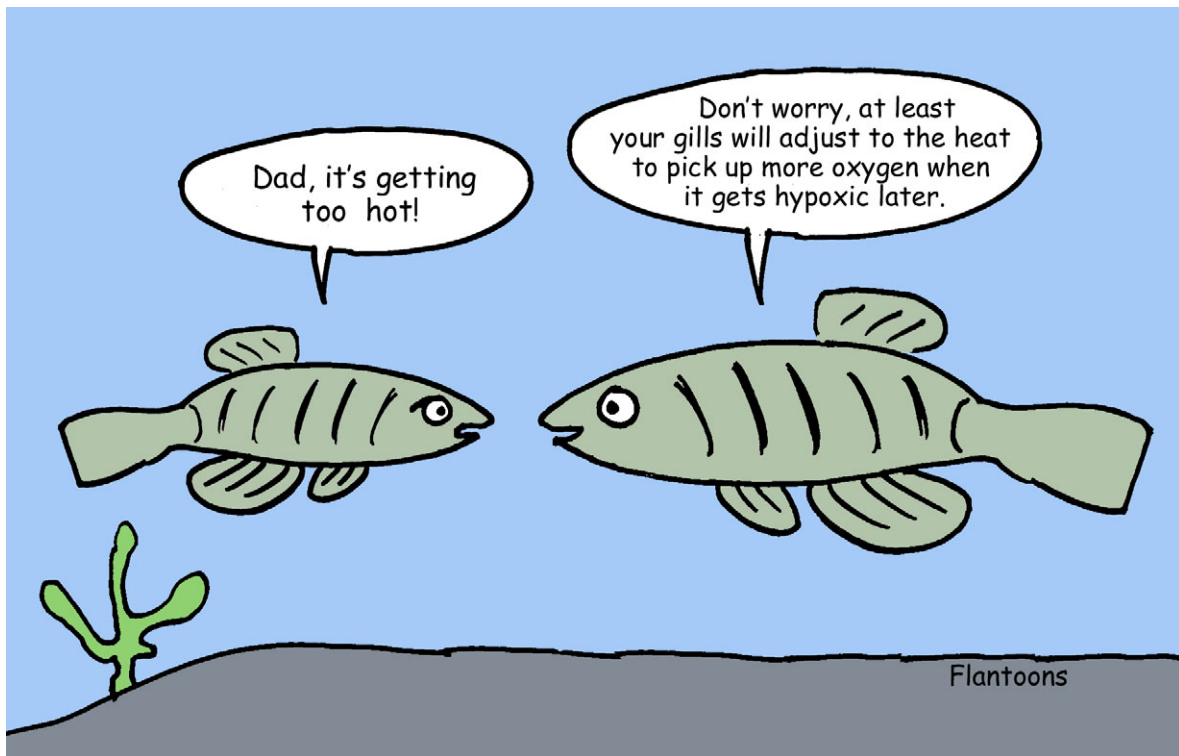
However, the team warns that this evidence of changes in tooth wear may not account for vole population crashes, saying, 'Tooth wear rates might seem unimportant in voles since their molars are ever-growing'. They also add that there is no clear mechanism linking the effects of tooth wear to dramatic reductions in vole population, although they suspect that if the teeth are worn unevenly, the animals may have difficulties feeding and face starvation.

10.1242/jeb.138222

Calandra, I., Zub, K., Szafrńska, P. A., Zalewski, A. and Merceron, G. (2016). Silicon-based plant defences, tooth wear and voles. *J. Exp. Biol.* **219**, 501–507.

Kathryn Knight

Heat helps killifish prepare for hypoxia



We all know that multitasking is challenging, but imagine the multiple physiological obstacles faced by fish as the environments to which they are adapted begin shifting. For example, as CO₂ levels and water temperatures rise, oxygen levels can fall, and pollution often exacerbates the situation. Many aquatic species have to juggle these simultaneous challenges. However, Patricia Schulte and colleagues from the University of British Columbia, Canada, explain that little is known about how fish respond when posed with several simultaneous physical threats. ‘Few studies have considered the possibility that the physiological changes that organisms undergo to cope with chronic exposure to one stressor could alter sensitivity to another’, the team says. Knowing that fish that have adapted to warmer conditions can modify their gills to satisfy the increased oxygen demands associated with a rise in metabolic rate, Schulte and her colleagues wondered whether these warm-acclimated fish may

also be better prepared to cope when oxygen levels fall.

Schulte, Tara McBryan, Timothy Healy and Kristen Haakons turned to their favourite species, the Atlantic killifish (*Fundulus heteroclitus*) – which resides in salt marshes, where water temperature and oxygen saturation can vary wildly from day to day – to find out how they respond to a reduction in oxygen having previously adjusted to warmer conditions. Transporting fish from their Atlantic coast homes to the Vancouver lab, McBryan allowed the animals to acclimate to life at 15°C. Then she warmed some of the fish to 20, 23 and 30°C before testing how well they reacted when she lowered the oxygen levels to 2% air saturation.

The 15°C fish managed reasonably well, appearing to pass out after over an hour, but the fish that had been warmed to 23°C struggled, passing out after just 2.6 min, and the fish in the warmest conditions

collapsed even before the oxygen reached its target. Then McBryan allowed the fish to adjust for 6 weeks to the temperature at which their oxygen response had been measured, before lowering the oxygen again. This time, all of the fish fared much better, holding out significantly longer before they passed out. And when McBryan investigated the fish’s gills, she found a noticeable increase in the surface area that absorbs oxygen. Schulte says, ‘Our experiments show that the changes fish make in their gills at high temperature also help them to tolerate hypoxia. This ability should help them to cope with the complex combination of stressors associated with human-caused climate change.’

10.1242/jeb.138214

McBryan, T. L., Healy, T. M., Haakons, K. L. and Schulte, P. M. (2016). Warm acclimation improves hypoxia tolerance in *Fundulus heteroclitus*. *J. Exp. Biol.* **219**, 474–484.

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