Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

ARE MOTHS JAMMING OR WARNING?


Picture by William E. Conner

It's an everyday scene, which only happens at night. A hungry bat pins its acoustic gaze on a flitting moth, engages its high-precision echolocation system and closes in for the kill. But something goes wrong at the last moment. The bat unexpectedly pulls back within millimetres of its target, and the tiger moth goes free. What triggered the bat to abort its attack? There are several possible explanations according to Jesse Barber. Tiger moths react to bat attacks with trains of ultrasound clicks, which could possibly jam the bat's echolocation cries, startle the airborne attacker into retreat or even warn the hungry mammal that it could be in for a foul tasting surprise. But each type of stalling-call would need very different characteristics; warning calls would need to be delivered early in the attack for the bat to get the message, while jamming signals would need to be targeted late in the bat's approach, milliseconds before the bat strikes. Curious to know when tiger moths respond to bat attacks, Jesse Barber and Bill Conner began recording ultrasound responses from hundreds of tiger moth species as they reacted to an incoming bat (p. 2637).

With few tiger moth species available in North Carolina, Conner directed Barber to the fringes of the Ecuadorian rainforest on the slopes of the Andes, where thousands of species flourish. Setting up a temporary lab at a ranch near Santo Domingo de los Colorados, Barber trekked out into the forest every night to attract tiger moths with a UV lamp. He was inundated. Thousands of insects swamped the lamp. Collecting tiger moths in convenient plastic shot-glasses, Barber photographed each insect, for later identification, before placing them in a sound proof box and playing a 2.1 s ultrasonic bat attack while recording the insect's responding clicks.

But before Barber analysed the recordings, he made a short visit to Becky Simmons at the Smithsonian Institute to identify the 130 tiger moth species he'd captured in South America. After days of rooting through dusty specimen drawers, the pair had identified about 80% of the insects, but many of the moths were new to science.

Returning to Conner's Wake Forest lab, Barber began the colossal task of scrutinising the 700 ultrasonic recordings from 36 species. First Barber timed the onset of the insect's reply relative to the incoming bat's echolocation signal, to see if he could distinguish late jamming signals from early warning messages. He also calculated the percentage of time during a 100 ms window when the insect was clicking, to see what the probability was that the clicks could jam the bat's guidance system.

After months of painstaking analysis, Barber was amazed to see that on average, all of the moths began clicking early in the bat's attack, 960 ms before the bat struck, too early for the clicks to jam the approaching bat's echolocation signals. And when he plotted the percentage of time that each insect clicked during a 100 ms window against the point in the bat's attack when the insect began responding, he realised that clicks probably couldn't jam the incoming mammal's guidance system.

Barber emphasises that his recordings don't rule out that the possibility that the moths have evolved effective jamming, but points out that knowing when the moth begins reacting to the bat attack isn't sufficient to distinguish a warning from a jamming signal.

10.1242/jeb.02391

Barber, J. R. and Conner, W. E. (2006). Tiger moth responses to a simulated bat attack: timing and duty cycle. *J. Exp. Biol.* **209**, 2637-2650.

KEEPING SHARKS WARM IN THE COLD

Packed into our tissues, microscopic mitochondria are the body's power-houses, consuming oxygen to generate the ATP that powers our every move. However, warm-blooded creatures (endotherms) also benefit from one of the organelle's by-products, heat, generated when the organelles leak protons. In fact, up to 25% of the basal metabolic rate of most warm-blooded creatures can be attributed to energy consumed topping up the mitochondrial leak. In contrast, metabolically active tissues in cold-blooded creatures (ectotherms) contain far fewer mitochondria, and they are proportionately smaller than those in gas-guzzling endotherms. As a result, it has been suggested that mitochondrial proton leak could be a key factor in the evolution of a warm-blooded lifestyle. But what about species that seem to straddle both warm and cold camps; have their mitochondria become specialised so that they too benefit from warming proton leak? Kathryn Dickson, Jeff Graham and their colleagues in southern California explain that some shark species are endothermic, while the rest are ectotherms. Could the mitochondria of these endothermic fish contribute to their warmth? To find out, Dickson and her colleagues measured proton leak rates from the tissues of warm shortfin makos and two ectothermic species (p. 2678).

But before the team could go fishing, Dickson set off for a summer in England, to join Martin Brand's Cambridge lab and master the technically challenging assays used to measure mitochondrial proton leak. Having returned to California, Dickson explains that proton leak rates can only be measured on freshly caught animals, so the team could only work on days when Chugey Sepulveda returned from fishing trips in the Pacific Ocean with a catch of endothermic shortfin makos and ectothermic blue sharks and leopard sharks. Knowing that makos maintain their liver and red muscle temperatures well above ambient temperatures, and that both tissues are metabolically active, the team isolated mitochondria from both tissues before measuring the organelle's respiration rates and membrane potential, and calculating the proton leak rates.

Surprisingly, the mitochondrial proton leak rates at the same membrane potential were essentially identical in all three sharks; that is that all three species pumped the same number of protons per milligram of protein at the same electric driving force. Dickson says 'this suggests that mitochondria from endothermic tissues of the mako shark are not specialised for thermogenesis'.

However, the team noticed that the mako shark's red muscle oxygen consumption rates were much higher than their ectothermic cousins, suggesting that even though the mitochondria are not adapted for heat production, the increased respiration rate could increase mitochondrial proton leak sufficiently to contribute to the fish's endothermy. And when the team measured the mitochondrial density in all three fishes' livers and calculated the proton leak per gram of tissue, they realised that the endothermic shark's was almost twice that of the ectothermic sharks.

Having found that both red muscle and liver could contribute to mako's endothermy, despite their lack of specialised mitochondria, Dickson and Graham are curious to know whether the mitochondria of other endothermic fish contribute to the challenge of keeping them warm.

10.1242/jeb.02390

Duong, C. A., Sepulveda, C. A., Graham, J. B. and Dickson, K. A. (2006). Mitochondrial proton leak rates in the slow, oxidative myotomal muscle and liver of the endothermic shortfin mako shark (*Isurus oxyrinchus*) and the ectothermic blue shark (*Prionace glauca*) and leopard shark (*Triakis semifasciata*). *J. Exp. Biol.* **209**, 2678-2685.

SWITCHING ON CORAL BLEACHING

Growing up on the Venezuelan coast, Santiago Perez spent much of his childhood camping on the keyes and visiting the coral reefs of Morrocoy Park. But over the years, Perez realised that the reefs were failing. Abused by uncontrolled tourism, and vulnerable to coral bleaching by ocean warming, the reefs were crumbling before his eyes. Perez was fascinated, and determined to learn more about the fragile organisms. Moving to the USA to continue his education, Perez became intrigued by the molecular mechanisms of coral bleaching, as the corals shed their symbiotic algae. Perez and Virginia Weis soon began to suspect that the signalling molecule, nitric oxide, which can be a key player in programmed cell death, could play a role in coral bleaching. But instead of working on delicate corals, the pair turned to a relatively robust symbiotic partnership, the sea anemone *Aiptasia pallida* and its dinoflagellate lodger, to look for nitric oxide (p. 2804).

At this point Perez had a stroke of luck. Thanks to nitric oxide expert Joe Beckman, Perez came across a nitric oxide sensitive fluorescent dye used to detect the molecule in mammalian tissue. Could this dye reveal nitric oxide production in heat shocked sea anemones too? Perez collected the symbiotic animals and bathed them in dye before exposing them to warm seawater. After carefully transferring them to a confocal microscope Perez focused laser beams on the tiny symbiont's tentacles to stimulate fluorescence; the whole tentacle lit up. The heat shocked symbiotic sea anemone was producing nitric oxide.

But was the signalling molecule delivering the algae's eviction notice? Exposing the intact sea anemone to sodium nitroprusside, which stimulates nitric oxide production, Perez reproduced the mass algal ejection; nitric oxide was the messenger.

Curious to know which half of the partnership generated the eviction notice, Perez gently separating the algae and its animal host, heat shocked the isolated partners and checked for nitric oxide production; but found none. The duo needed to be united for heat shock to stimulate nitric oxide production.

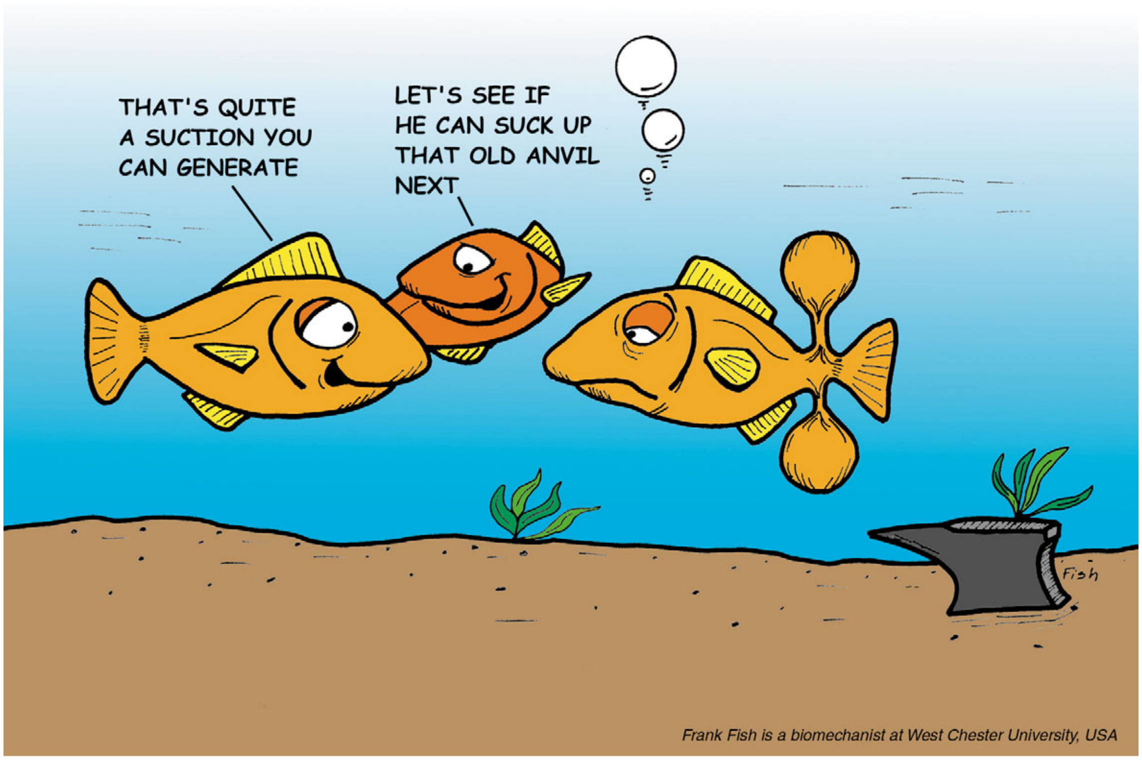
Changing his approach, Perez looked at nitric oxide production stimulated by another means in the intact symbiotic partnership to find which half of the duo ejected the lodger. Testing the effects of lipopolysaccharide, a compound that stimulates nitric oxide production in mammalian immune systems, on intact sea anemones and aposymbiotic sea anemones (where the algae had been gently removed), Perez returned to the confocal microscope and looked for nitric oxide production. Amazingly, the aposymbiotic sea anemone produced nitric oxide. The host was the source of the algae's eviction notice. More surprisingly, Perez realised that the animal host probably uses the same cellular mechanisms as the mammalian immune system to generate nitric oxide and trigger cell death.

But why are the sea anemones evicting their humble lodger when the algae are such valuable roommates? Perez explains that at times of stress, the algae switch off photosynthesis, and instead switch to generating toxic reactive oxygen species. Which is probably why their hosts are quick to kick them out, but Perez points out that the eviction comes at a high price, 'it's like an immune reaction gone too far' says Perez, and can ultimately kill the anemone that remains.

10.1242/jeb.02388

Perez, S. and Weis, V. (2006). Nitric oxide and cnidarian bleaching: an eviction notice mediates breakdown of a symbiosis. *J. Exp. Biol.* **209**, 2804-2810.

SHAPING SUCKERS' MOUTHS



HAROLD NEVER SHOULD HAVE MADE THE BET THAT HE COULD LIFT A BARBELL BY SUCKING

No matter what their favourite diet, most fish tackle a snack by the same approach; they suck it up. Despite these unvarying tactics, almost every species seems to have come up with a remarkably different mouth shape. Given that all of these structures have evolved to achieve the same goal, is it possible to divine any unifying principles of mouth structure evolution that might explain their extraordinarily diverse morphology? Timothy Higham from the University of California, Davis, thinks so, and outlines in this issue of *The Journal of Experimental Biology* how two suction feeders have honed their suck to their own tastes (p. 2713).

Visualising fluid flow with digital particle image velocimetry, Higham and his colleagues, Peter Wainwright and mechanical engineer Steven Day, tempted largemouth bass and bluegill sunfish with their favourite snacks. Having filmed each animal's lunge and calculated the fluid flow as they gulped down a meal, the team was able to calculate the trajectory of each gulp to find out how each species had optimised their chances of catching their favourite meal.

According to Higham and his colleagues, the bluegill sunfish's mouth generated high fluid speeds, relative to their surroundings, with high accuracy; both of which are ideal for picking off their preferred diet of zooplankton and larvae. However, the largemouth bass gulped down huge mouthfuls of water while closing in fast on their prey, generating high flows relative to their bodies, which is ideal for predators intent on trapping evasive fish and larger crustaceans.

So what overarching principles have the team been able to glean from analysing the feeding fish? They suspect that species generating high flow speeds with high accuracy will have small mouths, while fish gulping down huge mouthfuls to engulf elusive prey will be able to throw their mouths wide open, but with relatively low force.

10.1242/jeb.02389

Higham, T. E., Day, S. W. and Wainwright, P. C. (2006). Multidimensional analysis of suction feeding performance in fishes: fluid speed, acceleration, strike accuracy and the ingested volume of water. *J. Exp. Biol.* **209**, 2713-2725

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