Inside JEB 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

PIRANHAS COMMUNICATE ACOUSTICALLY


Sandie Millot

Thanks to Hollywood, piranhas have a bad reputation and it would be a brave scientist that chose to plunge their hand into a tank full of them. But that didn't deter Sandie Millot, Pierre Vandewalle and Eric Parmentier from the University of Liège, Belgium. 'You just have to pick them up and they make sounds,' says Parmentier. However, it wasn't clear when and why piranhas produce sounds naturally. Intrigued by fish acoustic communication and the mechanisms that they use to generate sound, the team monitored the behaviour of small groups of captive red-bellied piranhas to find out more about the curious sound (p. 3613).

Suspending a hydrophone in the piranhas' tank, Millot and Parmentier recorded the fish's sounds and filmed them as they cruised around and competed for food. According to Parmentier, the well-fed fish were relatively peaceful – attacking each other occasionally – although they were not averse to nipping near-by fingers. 'We both visited the hospital because we were bitten and Sandie's finger was nearly cut in half,' recalls Parmentier.

Comparing the soundtrack with the movie, the team found that the fish were generally silent. However, they became quite vocal as soon as they entered into a confrontation – producing the same barking sound that they had produced when held in the scientists' hands. 'At first we thought there was only one sound,' admits Parmentier, but then it became apparent that the piranhas produce two more: a short percussive drum-like sound when fighting for food and circling an opponent; and a softer 'croaking' sound produced by their jaws when they snap at each other.

Having convinced themselves that the fish had a wider acoustic repertoire than they had initially thought, the team decided to find out how the fish produce the sounds.

Parmentier explains that piranhas were already known to produce noises using muscles attached to their swim bladders; however, it wasn't clear how the swim bladder was involved in sound production. So, the team stimulated the muscles to contract, measured the swim bladder's vibration and found that instead of resonating – and continuing to vibrate after the muscles ceased contracting – the swim bladder stopped vibrating as soon as the muscles finished contracting. In other words, the muscles were driving the swim bladder's vibration directly and the frequency (pitch) of the bark and drum sounds was determined by the muscles' contraction frequencies: not the swim bladder's own intrinsic resonant properties. They also found that the rear half of the swim bladder did not vibrate, so only the head portion of the swim bladder contributed to sound production.

Now that they have discovered that piranhas are quite vocal when behaving aggressively, the team is keen to find out whether amorous piranhas are vocal too. However, Parmentier suspects that the team will have to relocate to Brazil to answer that question. 'It is difficult for the fish to reproduce in the tank, so I am sure we have to deploy hydrophones in the field to have the sounds that are produced during mating,' says Parmentier.

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Millot, S., Vandewalle, P. and Parmentier, E. (2011). Sound production in red-bellied piranhas (*Pygocentrus nattereri*, Kner): an acoustical, behavioural and morphofunctional study. *J. Exp. Biol.* **214**, 3613-3618.

Kathryn Knight

NEW VISUAL CONDITIONING PROTOCOL FOR HONEYBEES

For an animal with a brain the size of a microdot, bees are capable of some remarkably sophisticated feats, such as learning the locations, colours and scents of luscious flowers. But it is their brain size that makes them so attractive to scientists. Martin Giurfa from the University of Toulouse, France, explains that scientists investigate the insect's tiny brain to understand more about the neural basis of learning and memory. According to Giurfa, scientists train bees to recognise odours in the lab by teaching them to associate particular scents with a tasty sugar-water reward so that they extend their tongue (proboscis) whenever they catch a whiff of the same scent. However, this appetitive approach didn't work properly for the bee's visual sense. Giurfa and Jean-Christophe Sandoz decided to try a different tactic. Could bees be trained to memorise colours by associating them with a minor shock – a small electric shock to be precise (p. 3577)?

Giurfa explains that years before, his PhD supervisor, Josué Núnez, had discovered that mildly shocked bees extend their stings, so initially he decided to see whether bees could be trained to extend their stings when they recognised an odour if the scent had been delivered with a weak electric shock.

'Some people said that would never work because when a bee stings a person the bee dies,' recalls Giurfa. However, despite the doubts, Giurfa and Sandoz proved that bees could learn that an odour was followed by a mild electric shock and extend their sting. The team also knew that the shock had to be as gentle as possible. 'We wanted the bee to respond reliably and not to be damaged by the stimulation,' he explains, so they settled on a mild 2 s long 7.5 V shock to protect the bees from injury.

Next, Giurfa and Sandoz teamed up with graduate students Theo Mota and Edith Roussel to test whether bees could be trained to extend their stings when they recognised a colour.

Gently strapping a bee to a copper stage, the team showed it a green screen and then applied the gentle shock 3 s later: the bee's sting popped out. The team also showed the bee a blue screen, without applying the mild shock. After repeatedly showing the two screens to the bee in random order (green accompanied by the shock and blue without) they showed the bee the green screen, this time without the gentle shock, to see if she would recognise it and extend her sting. She did. And she never extended the sting when she saw the blue screen. She could distinguish between the two colours.

Then the team tested to see if the bees could be trained to distinguish between two blue screens (439 nm and 440 nm) that were indistinguishable to human eyes. This time, one of the blues was associated with the shock and the other was not. Again, the bees only extended the stings when they saw the blue that had been associated with the mild shock during training. Finally, the team tested whether the bees could be trained to distinguish between colours with different intensities, and again the insects passed with flying colours.

So, restrained bees in the lab can be trained to distinguish and memorise colours if they are coupled with a gentle shock and this new training regime offers scientists the opportunity to discover how bees use their memories to avoid enemies and other risks in the environment. 'Now we have a protocol in which you can do both [visual and odour cues] so that we can study multimodal learning in the brain,' explains Giurfa.

10.1242/jeb.066290

Mota, T., Roussel, E., Sandoz, J.-C. and Giurfa, M. (2011). Visual conditioning of the sting extension reflex in harnessed honeybees. *J. Exp. Biol.* **214**, 3577-3587.

Kathryn Knight

BLACKBIRDS ADAPT SONGS TO HUMAN NOISE

It's hard to find peace and quiet these days. No matter where you are, you can usually hear the rumble of passing cars or an aeroplane overhead. Humans tackle noisy environments by raising their voices, but how has our continual racket affected the calls of other species? This is the question that puzzled University of Ottawa honours student Dalal Hanna when she struck up a collaboration with David Wilson from the University of Windsor. 'We were both doing fieldwork at the Queen's University Biological Station. My expertise is in animal communication and her interest was in conservation biology, so we joined forces,' recalls Wilson (p. 3549).

Drawing on the experience of Gabriel Blouin-Demers and staff at the Research Station, Hanna and Wilson decided to find out how anthropogenic noise might affect the calls of red-winged blackbirds by comparing the songs of populations living in marshes adjacent to Canadian Provincial Highway no. 15 to the songs of red-winged blackbirds from pristine marshes on the biology station.

Heading into the marshes in the early morning, Hanna and Wilson recorded the calls of birds adjacent to the highway, before the traffic – and noise – levels became too high, and in the relative peace of the wilderness. 'Working in the marshes was a bit of a challenge,' admits Wilson, 'you can't walk into or through them so you are confined to the perimeter and even then it can be very soggy and difficult to move through to record different birds around the edges of the marshes.'

After successfully recording 436 songs from over 60 birds, Wilson teamed up with Daniel Mennill to analyse and compare the birds' calls. 'The challenge was separating the signal from the background noise to make very accurate measurements of the songs,' recalls Wilson. However, they eventually found that the final harsh trill of the song produced by the highway population had become deeper and more whistle-like than the wilderness birds' songs: in other words, the song had become more tonal, allowing the birds to be heard above the road noise. Also, instead of gaining low frequency components, the highway birds had lost the higher frequencies found in their rural cousin's songs, leaving the low frequency sounds that travel further for communication.

Next, Hanna and Wilson wondered how the wilderness red-winged blackbirds would respond if they suddenly encountered noise levels that the highway population endure constantly. Could they adapt and, if so, would they use the same strategy as Highway no. 15's neighbours?

Playing white noise and silence to the wilderness birds and recording their songs, Wilson and Hanna successfully extracted the noise from the recordings and compared the songs. Again, the bird's songs had become more whistle-like as they competed with the noise. So, even though the birds had never experienced traffic noise, they were able to adjust their calls in exactly the same way as birds that had been living with human noise for generations.

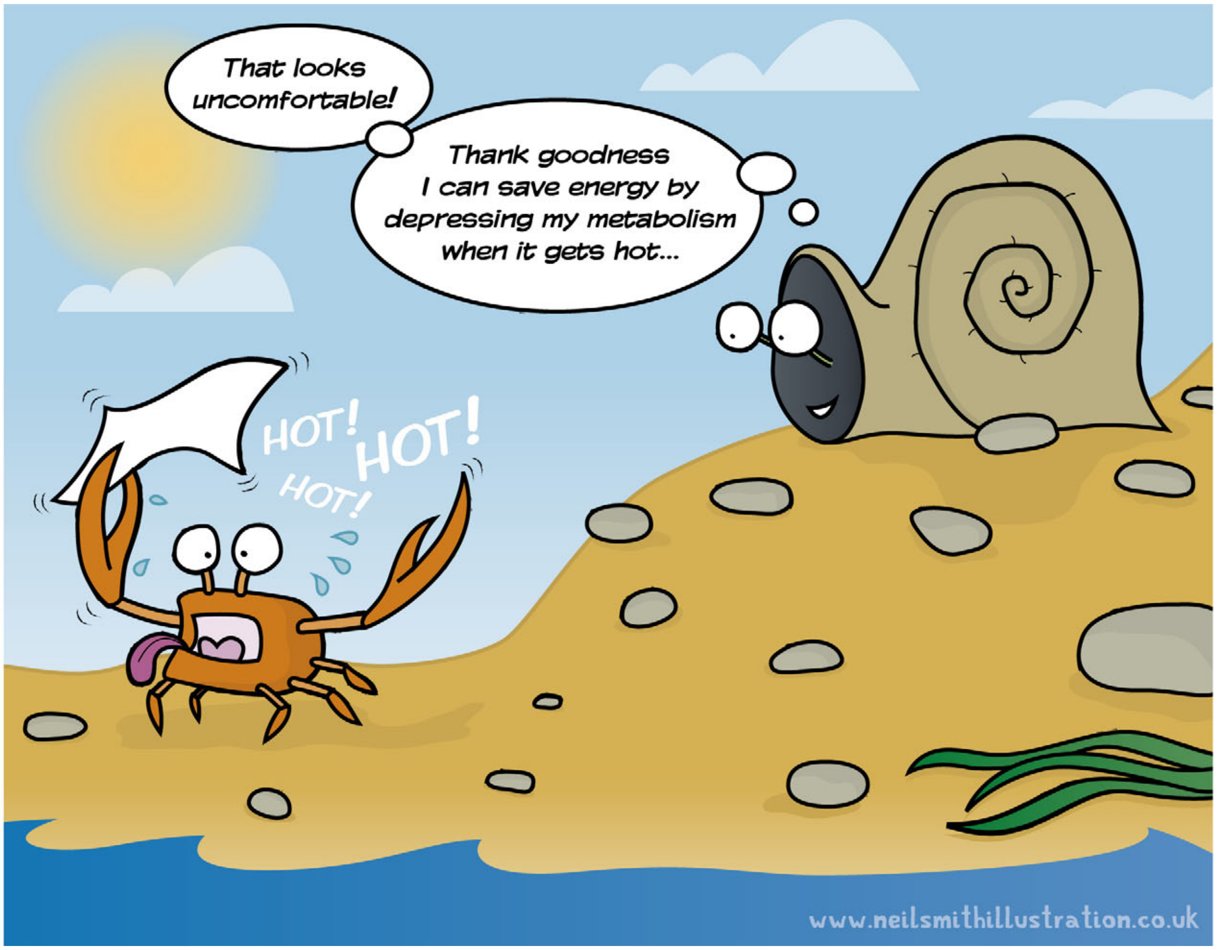
But how could these song changes affect the birds' lifestyles? Wilson says that it would be interesting to find out whether the alteration affects mate selection by females and how males defend their territories. 'Ultimately, we could use this information to identify the real costs of anthropogenic noise in terms of survival and reproduction in birds and use that as a model for gauging the effects on other species, as well as ones that are more endangered,' says Wilson.

10.1242/jeb.066282

Hanna, D., Blouin-Demers, G., Wilson, D. R. and Mennill, D. J. (2011). Anthropogenic noise affects song structure in red-winged blackbirds (*Agelaius phoeniceus*). *J. Exp. Biol.* **214**, 3549-3556.

Kathryn Knight

INTERTIDAL SNAILS ARE THERMALLY INSENSITIVE



Life in the intertidal zone is harsh. As soon as the tide retreats, shore-dwelling species are deprived of food and exposed to potentially life-threatening temperatures, so conserving their energy is probably at a premium. David Marshall from the Universiti Brunei Darussalam and an international team of collaborators suspected that instead of allowing their metabolism to escalate as temperatures rise – like most land-based cold-blooded animals (ectotherms) – shore-dwelling ectotherms might have chosen to conserve resources by reducing their metabolism when environmental temperatures escalate, until the cooling sea returns, when they can begin foraging again (p. 3649).

Collecting *Echinolittorina malaccana* snails from the Hong Kong and Brunei Darussalam coasts, the team measured the molluscs' resting metabolic rate and found that instead of increasing as the mercury rose, it remained almost constant between 35 and 46°C: they were conserving energy. The team also saw that the snails stopped crawling around at 41.5°C and went into a heat coma at 46°C, which is the temperature that activated the snails' protective, but costly, heatshock response. Finally, as the team turned the thermostat up higher, the snails' resting metabolic rate rocketed as they ramped up their heat shock response to combat the heat.

So *E. malaccana* snails have become thermally insensitive from 35 to 46°C, when they maintain a stable resting metabolism. The team points out that this strategy is extremely successful as the temperature that the snails experience naturally in their habitat rarely exceeds 46°C, allowing them to conserve energy rather than fritter it away when resources are scarce.

10.1242/jeb.066266

Marshall, D. J., Dong, Y.-w., McQuaid, C. D. and Williams, G. A. (2011). Thermal adaptation in the intertidal snail *Echinolittorina malaccana* contradicts current theory by revealing the crucial roles of resting metabolism. *J. Exp. Biol.* **214**, 3649-3657.

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