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INSIDE JEB

Wapiti whistles sound like Ringwraith shrieks

A bugling wapiti. Photo credit: Roland Frey.

For a majestic animal that looks equally at home as the monarch of the glen or astride the great American Plains, the wapiti's call is somewhat disconcerting. Sounding more like the shrieking cries of a Ringwraith from the Lord of the Rings, their haunting high-pitched screeches can carry great distances. 'Larger animals tend to have deeper resonances and lower voices', says David Reby, from the University of Sussex, UK, explaining how the pitch of an animal's voice tends to be a good indicator of its size: animals from larger species have larger larynges that vibrate at a lower pitch than animals from smaller species. However, the wapiti's extraordinary bugle calls would be more at home coming from the body of an animal the fraction of the size of these impressive red deer. The paradox had puzzled scientists for decades, but when Megan Wyman returned from a trip recording deer bellows in New Zealand, Reby knew that they might have a chance to finally lay the mystery to rest.

Visualising the spectrum of the eerie shrieks, Reby could clearly see the unnatural sounding high-pitched shriek at frequencies up to 4000 Hz. However, there was another band of lower pitched sound around 150 Hz, the frequency at which Reby would expect the male deer's vocal folds to vibrate. And when Reby and Daniella Passilongo investigated a series of the calls, they realised that the high- and low-pitched sounds shifted independently: sometimes the high-pitched wail rose and fell while the tone of the lower pitched roar remained constant. So the vocal folds were vibrating and producing a call that matched the animal's size while the deer simultaneously produced a high-pitched, high-volume, wraith-like cry by whistling.

Having realised that the deer were using two mechanisms to produce their eerie calls, Reby and colleague Ben Charlton wanted to confirm that the lower pitched vocalisation was produced by the vibrating vocal folds. So, when Yann Locatelli contacted Reby to let him know that one of the wapiti males in the herd at France's Muséum National d'Histoire Naturelle reserve had died, this was Reby's opportunity to take a close look at the structure of the deer's throat to learn more about their sound production. After CT-scanning the head and throat of the animal in the position that they assume when calling, Reby could see that the vocal folds were ~3.5 cm long, the ideal length for producing the low-pitched component of the call. And when Roland Frey investigated the structures in the deer's throat, he noticed that there were two possible routes for the animals to produce the high-pitched shriek. In the first scenario, the wapiti could whistle through their nostrils and adjust the wailing pitch by flaring and contracting their nostrils. However, he noticed that the deer's soft palate – known as the velum – descended far down in the animal's throat, separating the nasal tract from the oral cavity, possibly allowing the deer to blow air into the nasal cavity through the glottis. And when physicist Joel Gilbert calculated how the air might vibrate in the oral cavity, Gilbert realised that the jet of air from the glottis could hit the velum in much the same way that air in a flute vibrates, to produce a whistle that matched the wapiti's call at frequencies around 2600 Hz.

So the wapiti are being honest about their size. However, the low-pitched roar that accurately reflects their stature is drowned out over longer distances by their strident whistle, and Reby is keen to discover what other messages are communicated by the wraith-like shrieks.

10.1242/jeb.141135

Reby, D., Wyman, M. T., Frey, R., Passilongo, D., Gilbert, J., Locatelli, Y. and Charlton, B.D. (2016). Evidence of biphonation and source–filter interactions in the bugles of male North American wapiti (*Cervus canadensis*). *J. Exp. Biol.* **219**, 1224-1236.

Kathryn Knight

Crab neurons directly modified by predation pressure

Neohelice granulate from the population that experienced predation. Photo credit: Fiorella Magani.

Most crabs defend themselves with a pair of mighty claws when threatened, but *Neohelice granulata* crabs scamper for the nearest burrow. However, when Tomas Luppi, from the University of Mar del Plata, Argentina, told his colleague Daniel Tomsic, from the University of Buenos Aires, about an isolated population of these crabs that appeared to be relatively unbothered by predatory birds, Tomsic realised that he may have a chance to challenge a fundamental biological question. Might it be possible that a riskier lifestyle had directly altered the strength of the crab's visual response to danger, and could Tomsic track those changes to individual neurons in the nervous system? In short, could the team show a direct link between the selection pressure of predation on the crabs from the population that was at greater risk and modifications in their nervous systems?

First, Tomsic, Luppi, Fiorella Magani and Jesus Nuñez had to coordinate collecting the crabs from the two locations and returning them to the lab for testing. 'One beach is 400 km from Buenos Aires and the other is farther south, but we had to collect them and bring them to the lab on the same day', says Tomsic, who was determined to ensure that the crabs' experiences were essentially identical.

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Then Magani and Tomsic placed individual crabs in a tracking device and recorded their reactions to a threatening piece of card moving in a similar way to an attacking gull. The crabs from the high-risk population struggled harder to escape the threat than crabs from the population on the safe beach.

However, when the duo measured the crabs' reactions to a mild electric shock, both populations reacted to the same extent, and when moving images were projected on the enclosure surrounding the crabs, all of the animals moved in time with the images as they tried to hold their view of the world steady. So the differences in the crabs' reactions to the simulated gull visual threat appeared to be a result of the reduced risk of predation at the southern beach. And when Luppi and Nuñez returned to the beaches to monitor the antics of the bird population, they could clearly see that the crab population from the northern beach was far more menaced by the gulls than the southern population.

But would the differences in the crabs' responses to a visual threat correlate with an increase in activity in the motion-sensitive nerves that trigger a response to danger? Gently inserting a microelectrode into the large neurons that trigger an escape response in a crab's brain, Magani and Tomsic recorded the signals in the neuron as they replayed the simulated gull attack; they were delighted to see much stronger signals generated in the neurons of the crabs that live under constant threat than in the crabs from the more peaceful beach. 'In the low-risk population it [the neural response] was approximately half of the spikes (on average) than we recorded from the animals in the high-risk population', says Tomsic.

The increased risk of predation had directly affected the nerves that triggered the animal's escape response, and Tomsic is eager to discover whether the two populations are genetically different, or whether the crabs that live in the riskier situation simply learn to react more strongly to visual threats in order to survive.

10.1242/jeb.141101

Magani, F., Luppi, T., Nuñez, J. and Tomsic, D. (2016). Predation risk modifies behaviour by shaping the response of identified brain neurons. *J. Exp. Biol.* **219**, 1172-1177.

Kathryn Knight

Leeches are primed to feed

A fed and an unfed leech. Photo credit: Sarah Lemke.

If your next meal could be as much as a year off, the pressure is on whenever a tasty snack shambles past. Medicinal leeches (*Hirudo verbana*) only have one shot to pump their victims full of anticoagulant and anti-inflammatory saliva to ensure that their host is none the wiser as the blood is drained from them. Having satisfied their hunger, leeches then have to prepare for whenever the next meal may pass their way by recharging their salivary glands with the proteins that are essential for when they feed. However, it was not clear whether leeches refilled their salivary gland cells straight after finishing sucking on their victim or waited until they had finished digesting the meal. Jan-Peter Hildebrandt, from the Ernst Moritz Arndt University, Germany, explains that the first alternative would leave the leeches better prepared to grasp any opportunities that brushed past, at the expense of maintaining the fully charged salivary glands, whereas recharging the salivary glands after completely digesting the meal would be less costly but prevent the leeches from feeding opportunistically when another meal presented itself.

Intrigued, Hildebrandt and his colleagues Sarah Lemke and Christian Müller began investigating the salivary tissue of recently fed leeches over a 3-week period. The trio observed the salivary cells immediately after the leeches had fed, and saw that the salivary glands collapsed as they emptied. However, within a week the cells had returned to their pre-feeding size. And when the team analysed the proteins in the salivary tissue before and after feeding, they saw a dramatic reduction in one group of proteins – which recovered over the following week – while other proteins remained unaffected over the weeks following feeding. 'This indicates that one portion of the extracted proteins represented secretary proteins, while the other portion represented housekeeping proteins from salivary gland cells and surrounding tissues', says the team. They also investigated the mRNA level of the anticoagulant protein hirudin in the salivary tissue, and found that it peaked 5 days after the leeches' meal.

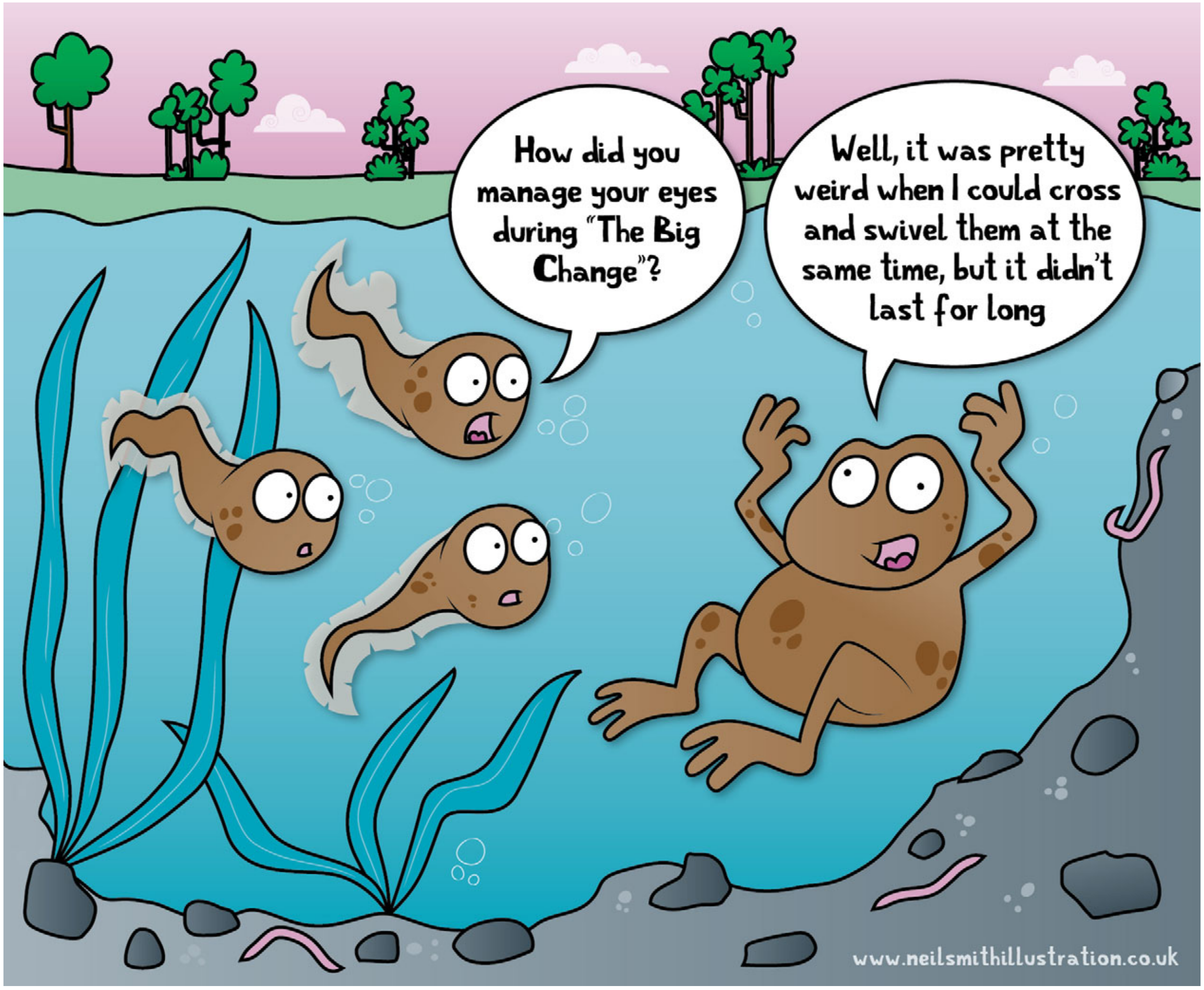
'The results of our study indicate that synthesis of salivary gland proteins starts within days of feeding and results in virtually compete refilling of the gland cell reservoirs within 1 week of feeding', says Hildebrandt and colleagues, adding that this ensures that the leeches are well prepared for any new dining opportunities that pass their way.

10.1242/jeb.141119

Lemke, S., Müller, C. and Hildebrandt, J.-P. (2016). Be ready at any time: postprandial synthesis of salivary proteins in salivary gland cells of the haematophagous leech *Hirudo verbana*. *J. Exp. Biol.* **219**, 1139-1145.

Kathryn Knight

Tadpole eyes swivel and cross during metamorphosis



The changes that most animals experience during the transition from juvenile to adulthood can be disruptive and inconvenient, but pale into insignificance in comparison with the drastic metamorphosis that amphibians undergo. Denis Combes and colleagues from the University of Bordeaux, France, and the Ludwig-Maximilians-University Munich, Germany, describe how metamorphosing tadpoles have to adapt from a wriggling swimming style to a forward thrusting motion with each kick of their newly emerged hind legs. However, this transition is also accompanied by a change in the tadpoles' vision. Wriggling tadpoles swivel their eyes in the opposite direction from their weaving bodies to hold their view of the world steady, while kicking froglets cross their eyes in time with each kick to maintain their view of objects as they appear to loom when the tiny amphibians surge forward. The nerve signals that drive these eye movements are coordinated by the signals that control the respective swimming movement, so Combes and his colleagues Géraldine von Uckermann, François Lambert, Hans Straka and John Simmers wondered how *Xenopus* tadpoles maintain gaze control during the critical transition period from one swimming style to the other.

The team recorded the nerve signals controlling the eye movements and swimming muscles of tadpoles, in addition to filming the motion of the tadpoles' eyes, at various stages of metamorphosis. After analysis of the nerve signal patterns, they discovered that the eye control signals gradually shift from regulating the eyes as they swivel to coordinating both swivelling and looming eye movement patterns simultaneously, until the eye swivel pattern dwindles away and the tadpoles switch exclusively to converging the eyes as they kick-swim at the end of the metamorphosis. The team also realised that the nerve signal pattern that coordinates the eye movements with the swimming action switches gradually from the signals that control the wriggling swim to those driving the adult-style leg kick, allowing the tadpole to transition seamlessly from one lifestyle to the other.

10.1242/jeb.141127

von Uckermann, G., Lambert, F. M., Combes, D., Straka, H. and Simmers, J. (2016). Adaptive plasticity of spino-extraocular motor coupling during locomotion in metamorphosing *Xenopus laevis*. *J. Exp. Biol.* **219**, 1110-1121.

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