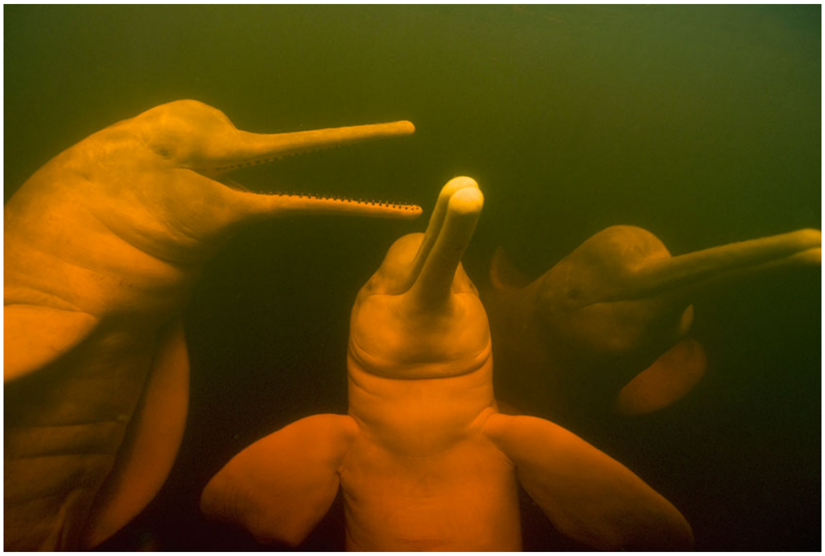
INSIDE JEB

River dolphins fine tune clicks to confined lifestyle



Amazon river dolphins in the Rio Negro, Brazil. Photo credit: kevinschafer.com.

Whales and dolphins cruising the high seas encounter few obstacles to confound their echolocation systems. However, a select group of cetaceans that have made their homes in rivers and estuaries must contend with the bewildering array of confusing echoes produced by their confined environment. And for Amazon river dolphins – also known as botos – the situation could become even more perplexing when their river home bursts its banks and floods the surrounding forest. Michael Ladegaard from Aarhus University, Denmark, explains that bats suffer similar challenges when echolocating in caves and forests, so he and his colleagues, Frants Havmand Jensen, Mafalda de Freitas, Vera Ferreira da Silva and Peter Madsen decided to find out more about the echolocation clicks to see how well they are adapted to the confined conditions.

The team travelled to three locations in the Amazon river basin to record the echolocation clicks in the white silty water of the Rio Solimões and then to São Tomé to record in the dark waters of the Rio Negro and where the waters mingle at the confluence of the two rivers. As the botos had to be perfectly in line with the underwater microphones (hydrophones), Ladegaard and his colleagues recorded almost 35,000 clicks with a string of seven hydrophones suspended vertically in the water beneath a boat before identifying 268 clicks that had been produced when the animals were head-on and within 21 m of the hydrophone array. Analysing the click frequency spectra and intensity, and the width of the beam of sound, the team found that the animals produce narrow (half-power beamwidth of 10.2 deg) soft (peak–peak source level of 191 dB re. 1 µPa) high-pitched (101 kHz) echolocation clicks that lasted 14 ms with a brief interval of 35 ms between clicks.

Comparing the click's acoustic parameters with those of marine dolphins, the team explains that the botos' echolocation system is well adapted to their cluttered surroundings. They click softly so that echoes only return from nearby objects. This in turn means that all of the echoes that they need to interpret return within milliseconds, allowing the animals to produce high click rates of around 30 clicks per second while limiting the reverberations produced in the confined space of shallow rivers. The team also noticed that the botos could change the width of the beam of sound, which may allow them to adjust their acoustic view depending on the complexity of their surroundings.

However, the river dolphins' soft echolocation clicks buck the relationship between click frequency and intensity that has been found for marine cetaceans. According to Ladegaard, large marine cetaceans produce softer clicks with more low-frequency vibrations than smaller species. He also explains that the increase in the low-frequency spectrum means that the softer clicks are more diffuse and the beam of sound is wider, which is fine when echolocating for lunch in the open ocean. However, botos live in a far more complex environment, necessitating better-directed echolocation clicks, so the river dolphins adopted a different tactic. They raised the pitch of their echolocation clicks, allowing the freshwater animals to produce soft echolocation clicks that can be well directed in their cluttered homes.

So, Amazon river dolphins' echolocation clicks are well suited to their surroundings, and Ladegaard concludes, 'Habitat in addition to size may play an important role in the evolution of toothed whale echolocation'.

10.1242/jeb.131979

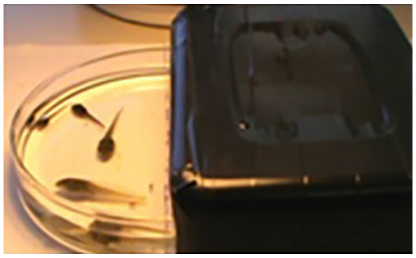
Ladegaard, M., Jensen, F. H., de Freitas, M., Ferreira da Silva, V. M. and Madsen, P. T. (2015). Amazon river dolphins (*Inia geoffrensis*) use a high-frequency, short-range biosonar. *J. Exp. Biol.* **218**, 3091-3101.

Kathryn Knight

UVB damages treefrog tadpole DNA

Ever since the blanket of ozone that protects the planet from hazardous UV radiation began to dwindle, there have been concerns about the risks of increased UV exposure. And humans are not the only animals that are likely to suffer. André Schuch from the Federal University of Santa Maria, Brazil, explains that animals that are at the larval life stage are particularly vulnerable to the damaging effects of the radiation. 'The global increases in UVB

Inside JEB highlights the key developments in Journal of Experimental Biology. Written by science journalists, the short reports give the inside view of the science in JEB.



Montevideo treefrog tadpoles under UV-free visible light. Photo credit: André Schuch.

radiation associated with stratospheric ozone depletion have been considered a leading hypothesis for amphibian decline', says Schuch. Yet, little was known about the effects of UV radiation on DNA in developing tadpoles. 'It has been demonstrated that exposure to UVB radiation can cause spinal curvature and reduced growth and development', says Schuch, so he and his colleagues Sonia Cechin and Elgion Loreto set about finding out just how damaging UV radiation is for tadpole DNA.

Explaining that UV radiation directly attacks genes by linking adjacent thymine or cytosine bases in the DNA sequence – causing the gene to be miscopied the next time that the cell divides or altering the way that the gene is converted into a protein during development – Schuch and his colleagues decided to find out how well Montevideo treefrog (*Hypsiboas pulchellus*) tadpoles could repair UV damage to their genes. First, Schuch, Victor Lipinski, Mauricio Santos and Caroline Santos went fishing for tadpoles in a pond on the university campus. Returning to the lab with approximately 1000 recently hatched tadpoles, Schuch exposed some of the animals to UVA radiation and another group to UVB for different lengths of time. Next, knowing that light activates one specific DNA repair mechanism – where a protein called photolyase repairs the damage by detaching the link between the DNA bases – Schuch bathed the animals in light. Finally, Sinara Jardim extracted DNA from the tadpoles' tails, ready for Schuch to measure the amount of damage that the animals had sustained.

Analysing the DNA, Schuch could see that the tadpoles were very sensitive to UVB: in addition to sustaining higher levels of damage, the tadpoles' DNA repair rate was slower. 'The [photolyase] enzymes left almost 40% of UVB-generated DNA lesions in the genome even after 3 h of photoreaction treatment', says Schuch. And when he measured the effects of the radiation on the cell death rates, it was clear that the radiation was extremely toxic. 'Taken together, the results show that these DNA repair pathways are less efficient than we expected, indicating that treefrog tadpoles could be severely affected when exposed to agents that induce large distorting DNA lesions'.

However, the news was not all bad for the tiny developing animals. Wondering whether the tadpoles could detect and evade the damaging radiation, Schuch shaded one half of the tadpoles' Petri dish home with a black-plastic cover and filmed the tadpoles' reactions as he bathed the other half of the Petri dish with light of different wavelengths. Noticing that the tadpoles were content to bask in light that lacked UV radiation, Schuch was impressed to see the animals surge for the shade when he first turned on the UVB and then UVA wavelengths. 'This behaviour should complement the low DNA repair efficiency of the tadpoles', says Schuch, who is keen to learn more about the impact of deforestation at the treefrog's breeding sites on the tadpoles' UV exposure.

10.1242/jeb.131995

Schuch, A. P., Lipinski, V. M., Santos, M. B., Santos, C. P., Jardim, S. S., Cechin, S. Z. and Loreto, E. L. S. (2015). Molecular and sensory mechanisms to mitigate sunlight-induced DNA damage in treefrog tadpoles. *J. Exp. Biol.* **218**, 3059-3067.

Kathryn Knight

Sparrows are better waterproofed in winter than in summer

Female house sparrow. Photo credit: Alex Champagne.

Given that most animals are essentially big bags of water, it would seem to make sense that they have a well-sealed skin to retain precious fluid. But evaporative water loss through the skin has its uses keeping animals cool and no biological membrane can ever be completely water tight. Alex Champagne from the University of Southern Indiana, USA, explains that evaporation through the skin accounts for 65% of the water lost by birds as vapour. However, little was known about the interactions between water and the waterproofing fats (lipids) in the skin's outer layer – the stratum corneum. Champagne was also curious to know how the proportions of the different waterproofing lipids may vary through the skin's depth and how they change with the seasons, so he and his colleagues, Heather Allen and Joseph Williams, embarked on an ambitious series of experiments to get to the bottom of the house sparrow's waterproofing mechanism.

Capturing 11 sparrows during the summer of 2012 and 8 in the following winter, Champagne first measured the skin's evaporative water rates and found that the summer birds' water loss rate (48 mg H_2O cm^{-2} day^{-1}) was almost twice as high as that of the winter birds (26 mg H_2O cm^{-2} day^{-1}). Next, the team measured the absorption of infrared light in micrometre-thick layers of the skin, to calculate the amount of water carried in the layer and how tightly the water interacted with the waterproofing lipids, before carefully measuring the different types of lipids present at different depths through the skin's structure.

Amazingly, the team found the birds were able to vary the degree of skin waterproofing through the seasons, increasing the lipid content of the skin in winter by retaining more lipids near the surface and increasing the amount of cerebrosides and ceramide II deeper in the skin. They also found that the skin was better hydrated at depth than near the surface and that the increased hydration was associated with a greater number of kinks in the long fat molecules that hold water in the lipid layers. They also suggest that water-attracting structures in the deeply buried ceramide and cerebroside molecules could hold on tightly to water molecules in their vicinity, to increase the viscosity of the water and slow evaporation.

So, the deepest layers of bird skins are the most waterproof and best hydrated, and the team adds, 'Birds modify the locations of certain lipid classes seasonally, likely to

Journal of Experimental Biology

Journal of Experimental Biology (2015) 218, 2981-2983

acclimatize to changes in temperature and humidity and thus maintain heat and water balance, even in cold, dry conditions'.

10.1242/jeb.131961

Champagne, A. M., Allen, H. C. and Williams, J. B. (2015). Lipid composition and molecular interactions change with depth in the avian stratum corneum to regulate cutaneous water loss. *J. Exp. Biol.* **218**, 3032-3041.

Kathryn Knight

Athletic humpbacked conches leap to safety

Humpbacked conch inside a respirometer. Photo credit: Sjannie Lefevre.

When a predatory cone snail launches an attack, it is hardly a high-speed encounter. Lumbering towards an unsuspecting humpbacked conch, the cone snail looks likely to outpace its quarry; but then events take an unexpected turn. As soon as the conch gets a whiff of the approaching predator, it leaps out of reach. And Sjannie Lefevre from the University of Oslo, Norway, explains that the athletic conches can continue flipping for as long as 5 min to get clear of danger. Intrigued by the humble mollusc's remarkable escape reflex, Lefevre and her colleagues, Sue-Ann Watson and Philip Munday from James Cook University, Australia, and Göran Nilsson from the University of Oslo, wondered how the animals powered their gymnastics. Lefevre explains that the oxygen consumption of fish rockets when they exert themselves, but she was less sure that the conches – with their relatively simple hearts and circulatory systems – could rise to the challenge in quite the same way.

Collecting conches buried in the sand in the Lizard Island lagoon, the team headed back to the lab to measure the animals' oxygen consumption rate while resting and leaping. However, before Lefevre could entice the conches to exert themselves, she had to collect some of the terrifying odour from the water bathing the predatory snails: '[The humpback conches] react very quickly; almost as soon as you inject the odour into the respirometer, they start jumping', she says. And Lefevre was amazed to see the oxygen levels in the respirometer plummet as soon as the conches began jumping to evade the smell. 'We had some conches increase their oxygen consumption six times', she says, adding that this is similar to the metabolic boost measured in escaping fish. So, despite their unsophisticated circulatory systems, the conches were able to increase their oxygen consumption dramatically as their metabolism soared during an escape bid.

But then the team wondered how well the molluscs will cope as climate change takes hold and the planet's oceans become more acidic. Lefevre explains that the additional physical burden of increased temperature and acidity limits the performance of many ectotherms as they divert energy to cope with the physiological challenges. To test how well the conches may cope in the future, Lefevre turned up the thermometer – first to 33°C and then to 38°C – and measured their oxygen consumption while resting and leaping. However, the conches didn't miss a beat, successfully sustaining their athletic leaps at the highest temperature when other species would have diverted energy from their exertions to combat the heat. And when Lefevre turned up the pressure and increased the CO_2 level of the water from about 450 µatm to nearly 1000 µatm – to simulate conditions in 2100 – she was amazed to see that the conches barely suffered any ill effects and continued leaping. 'These snails have aerobic capacity in excess of current and future needs', she says.

However, this does not mean that the future will be all plain sailing for the athletic conches. Lefevre explains that increased levels of CO_2 in the water affect the ability of many aquatic species to react to predators, making them more vulnerable to predation. She suspects that the nimble conches may suffer the same fate, as she and her colleagues have found that the molluscs are reluctant to jump in high-CO_2 water. But, she is optimistic that enough of them will retain the ability to sniff-out a foe and take evasive action to keep the conches leaping into the future.

10.1242/jeb.131987

Lefevre, S., Watson, S.-A., Munday, P. L. and Nilsson, G. E. (2015). Will jumping snails prevail? Influence of near-future CO_2, temperature and hypoxia on respiratory performance in the tropical conch *Gibberulus gibberulus gibbosus*. *J. Exp. Biol.* **218**, 2991-3001.

Kathryn Knight
kathryn@biologists.com