Outside JEB

Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

SUPERFAST MUSCLES

SINGING WITH SUPERFAST MUSCLES

Throughout the animal kingdom, different muscles are specialized for different tasks. Among the most striking examples of extreme specialization are muscles that can contract and relax many times in the span of a single second. While a typical human limb muscle has a maximum contraction frequency of only 3–5 Hz, rattlesnake tailshaker muscles can contract at rates of 90 Hz, and toadfish swim bladder muscles produce a mating call by oscillating at 200 Hz. The rattlesnake and toadfish muscles turn on and off more rapidly than all others examined to date and, therefore, have been dubbed 'superfast' muscles by the researchers who study them.

Although the superfast club is a rather exclusive one, researchers from Wageningen University (The Netherlands) and the University of Utah (USA) propose in the 9 September issue of *Nature* that the syrinx muscles of the ring dove (*Steptopelia risoria*) also belong in this elite group. The syrinx is birds' equivalent of the human larynx; whereas humans speak by moving the vocal cords in their larynx, birds vocalize by moving the membranes lining their syrinx. Two pairs of muscles are thought to adjust the membranes' position and tension, thus controlling sound production. Since doves' trademark cooing songs include trills of brief sounds repeated rapidly (up to 30 Hz), Coen Elemans and his co-workers reasoned that the syrinx muscles might have superfast properties.

To study these muscles further, the researchers attached electrodes to them and measured their electrical activity during cooing. Changes in electrical activity correlated with changes in the sounds emitted by the doves, suggesting that these muscles do indeed control the sounds.

Elemans and his colleagues then removed the muscles to measure their contraction speed in response to individual pulses of electrical stimulation. On average, it only took the muscles about 4 ms to ramp up from 10% to 90% of their maximum twitch force and another 10 ms to drop from 90% back down to 10%. These extremely rapid changes put the dove syrinx muscles in the 'superfast' category and are similar to those of rattlesnake tailshaker muscles.

Interestingly, the dove's superfast muscles are also super-weak; the need for speed apparently limits their ability to exert force. This trade-off between strength and speed is also seen with the rattlesnake tailshaker and toadfish swim bladder muscles, both of which are also quite weak. The low forces of the rattlesnake and toadfish muscles are due, in part, to the composition of the muscle cells, which include: relatively few force-generating proteins (actin and myosin) but lots of sarcoplasmic reticulum (which turns the contractions on and off) and mitochondria (which generate ATP aerobically so that the contractions can continue for many minutes). Presumably the dove syrinx muscle cells have a similar composition, although this was not addressed by the current study.

Are superfast syrinx muscles unique to ring doves? Elemans and co-workers suspect not. Since dove coos are relatively simple as bird songs go, they hypothesize that many other birds with more complex songs will also be found to have superfast syrinx muscles. With more data surely on the way, it is clear that Elemans' study is no swan song.

10.1242/jeb.01335

Elemans, C. P. S., Spierts, I. L. Y., Muller, U. K., van Leeuwen, J. L. and Goller, F. (2004). Superfast muscles control dove's trill. *Nature* **431**, 146.

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MUSSEL ATTACHMENT

MUSSELS PULLING OFF NOT GETTING PULLED OFF

One of the reasons why mussels often dominate wave-swept rocky intertidal zones is that they can securely attach themselves to rocks where food is abundant, and predators and competitors are simply swept away. Mussels manage to hang on where others can't via their byssal apparatus, which is a collection of stretchy and tough connective tissue threads that mussels use to glue themselves to the substrate. One particularly important aspect of byssal thread mechanics is their ability to nearly double in length without breaking. This allows for the recruitment of other byssal threads into tension, which increases the ultimate strength of the entire byssal apparatus. While much is known about the mechanical properties of byssal threads and the fibrous proteins that make them up, little is known about how the structure of the byssal thread proteins gives rise to the macroscopic threads' mechanical properties. In a recent paper in *Biomacromolecules*, Tue Hassenkam and colleagues report the results of their attempts to understand the stretchiness of byssal threads at the molecular level using a technique called atomic force microscopy, or AFM. AFM is a unique microscopy technique in that it constructs images with near atomic resolution by literally tracing the surface of a sample via direct contact with an ultra-fine stylus.

Byssal threads consist primarily of fibrous proteins made up of a long, central collagen-like domain that is flanked by globular terminal domains. These proteins form collagen-like triple helix trimers that are mostly straight, but that also possess a prominent kink due to a disruption of the collagen repeat. Previous work using transmission electron microscopy has shown that these trimers associate in the byssal gland into higher-order rod-like structures, termed 'mesogens,' which consist of seven trimers. Mesogens then self-assemble into liquid crystalline arrays that are eventually locked into place via a poorly understood cross-linking process. Molecular models predict that the mesogens can adopt either a 'flower' configuration, in which the kinked ends flay out in different directions, or a more compact 'banana' configuration in which the kinked ends all point in the same general direction. Hassenkam hoped to distinguish which configuration mesogens adopt in the byssal apparatus using AFM, and by imaging threads stretched and held at a strain of 100%, he wished to find out which structures are responsible for the impressive extensibility of byssal threads.

AFM proved to be useful for answering all of these questions. The team only found mesogens in the banana form, joined end to end to form liquid crystalline arrays resembling crimped sheets of paper. They also found that stretching the threads correlated with three major structural changes that could account for the extensibility of the threads at the molecular scale. First, the banana-shaped mesogens straightened out, resulting in a flattening of the crimps in the liquid crystal arrays. Second, the mesogens adopted an orientation more in line with the axis of the thread, whereas before they had been off to an angle. And thirdly, the globular terminal domains that link the individual proteins end-to-end lengthened. Together, these results provide a satisfying picture of the structure of byssal thread proteins at several levels of organization, as well as how those structures contribute to the ability of the threads to stretch as far as they do without breaking. By probing the mechanics of byssal threads at the molecular scale, Hassenkam and colleagues are beginning to reveal some of the essential details of how mussels pull off not getting pulled off.

10.1242/jeb.01337

Hassenkam, T., Gutsmann, T., Hansma, P., Sagert, J. and Waite, J. H. (2004). Giant bent-core mesogens in the thread forming process of marine mussels. *Biomacromolecules* **5**, 1351-1355.

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ECHOLOCATION

RETURN TO SENDER

Reports of chemical pollution, oil slicks and the resulting habitat destruction are an all too familiar story in the press these days. However, an often overlooked, but no less-damaging form of pollution is noise; we're all familiar with the inconvenience of noisy neighbours. No matter how irritating these noises are for us, for animals that rely on echolocation to sense their environment, noise pollution could be deadly: whales and dolphins have been found stranded on beaches following sonar testing by the military. Examination of some of these whales revealed that they had gas lesions in their liver and kidneys due to rapid decompression similar to 'the bends' that human divers suffer when they return to the surface too fast. Could the sonar used by the military lead to these mass strandings and, if so, how can they be prevented in the future? Intrigued by the possible threat posed to cetaceans by man's acoustic activities, Mark Johnson and a team of researchers from the USA, Italy and Spain decided to study echolocation in two species of beaked whale, *Mesoplodon densirostris* and *Ziphius cavirostris*.

A key step in understanding the dangers posed to whales by noise pollution is to study how echolocation works in some of the whale species that become stranded. However, many stranded whales belong to the beaked whale family, which are so elusive that new species have been discovered as recently as 2002. Fortunately these creatures' elusiveness did not deter Mark Johnson and his colleagues. By attaching a non-invasive acoustic recording tag to individuals from both species in the wild they were able to record the sounds generated and the echoes received by the whales for long periods of time (up to 15 hours) while the whales were behaving normally. These extended recordings revealed that although neither whale species produced clicks (short bursts of sound) when they were close to the surface (within 200 m) when the whales made deep dives (up to 1200 m) they clicked continuously and only stopped when they started their ascent to the surface. These clicks closely resembled those of dolphins during echolocation, which have been much more extensively studied. The clicks were also followed by echoes (that were also recorded by the tags) suggesting that they may be used in echolocation.

But could this echolocation be important during normal behaviour? The team calculated the time between the clicks and the echoes returning to the whales from an object and converted these into distances to the object just as the whales must do. By aligning the responses to successive clicks, their recordings of the beaked whales show the echoes returning more rapidly, suggesting that the distance to the object was getting smaller. As the whales get close to the object they switch from the distinctive pattern of clicks to a buzz and the whale starts to accelerate. This was interpreted by the team as showing individuals tracking and closing in on a prey item, suggesting that the whales use echolocation during hunting.

This study provides the first evidence that beaked whales, like dolphins and other whale species, hunt using echolocation. One possibility is that military sonar exercises could interfere with their echolocation, causing the whales to ascend rapidly to the surface and, therefore, depressurise. This work may provide information that could be crucial in future efforts to protect these whales against noise pollution.

10.1242/jeb.01338

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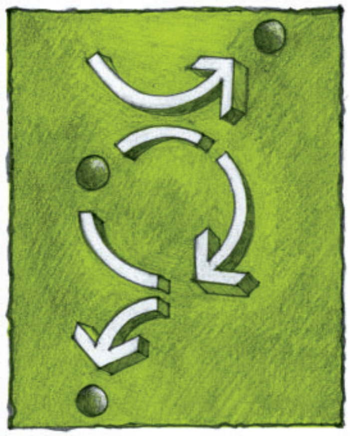
FREEZE TOLERANCE



LACTATE LIMITS LIFE

Most North American freshwater turtle neonates typically hatch in late summer or early fall and move from their subterranean nests to nearby lakes and ponds, avoiding winter's subzero temperatures in the water's depths. However, painted turtle (*Chrysemys picta*) hatchlings remain entombed in their shallow subterranean nest throughout their first winter, frequently experiencing ice and body temperatures as low as –10°C. Nevertheless, the hatchlings emerge unscathed when the ground thaws in the spring.

Although two mechanisms could explain the painted turtle hatchling's remarkable cold tolerance (a capacity for supercooling and an ability to tolerate freezing), supercooling is thought to play a significant role in the neonate's ability to overwinter. However, the importance of freeze tolerance in survival continues to be debated. Gary and Mary Packard of Colorado State wondered why hatchling painted turtles can only recover from a few days of freezing under relatively mild conditions (–2 to –2.5°C) and decided to investigate whether lactate accumulation in the hatchling's tissues affects the youngster's freeze tolerance.

The Packards explain that when hatchlings freeze, much of the extracellular water forms ice, preventing the circulatory system from functioning and the delivery of oxygen for mitochondrial ATP production. Cells are instead forced to meet their ATP demands through anaerobic glycolysis, which causes an increase in tissue lactic acid levels, known as anoxic lactic acidosis. Normally, unfrozen turtles counteract the dangerous drop in tissue pH that accompanies lactate accumulation by the release of carbonate buffers into the blood from the shell, and lactate is also transported to and sequestered in the shell itself. However, these buffering processes may not be available to frozen hatchlings due to their arrested circulation.

Assessing lactate's role in freeze tolerance, the Packards exposed recently hatched painted turtles to freezing conditions at –2°C for periods between zero and eight days. Every second day, the Packards thawed a group of animals and recorded the mortality percentage. They also measured whole-body lactate levels from another group that was not allowed to thaw. The team found that the frozen hatchlings' mortality levels were correlated with the amount of whole-body lactate. They also found that the frozen turtles' whole-body lactate levels were greater than those previously measured from comparable supercooled turtles, which maintain a functional circulatory system. Thus, freezing compromises the hatchling's ability to effectively deal with the anoxic lactic acidosis that accompanies anaerobic metabolism, a phenomenon the Packards suspect is due to the cessation of the circulation that occurs with freezing.

The Packards hypothesize that without a functional circulatory system, frozen turtles cannot shuttle lactate from individual tissues to the shell to be sequestered or mobilize the shell's buffers. As a result, individual organs may accumulate lactate to lethal levels sooner than if the circulatory system remained operational. As follows, the Packards suggest that a cold tolerance strategy based on freezing is more stressful than a strategy based on supercooling and argue that the inability to deal with lactic acidosis may be one of the reasons why hatchling painted turtles are unable to tolerate freezing for prolonged time periods.

10.1242/jeb.01339

Packard, M. J. and Packard, G. J. (2004). Accumulation of lactate by frozen painted turtles (*Chrysemys picta*) and its relationship to freeze tolerance. *Physiol. Biochem. Zool.* **77**, 433-439.

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PREY TARGETING

WHEN SPITTING IS A MATTER OF LIFE AND DEATH

We can do some pretty remarkable things with our bodies, not all for public retelling. However, even the most mystic yogis among us would probably have trouble immobilising their live food at a distance – and through a highly refractive water–air interface. This, however, is exactly what archer fish (*Toxotes jaculatrix*) must do successfully from a young age, if they are to survive and grow. They are capable of directing a jet of water directly at perching insects up to a metre away, so dislodging them and causing them to fall back into the water, where they are eaten. All this is done from underwater, so that the insect is unaware of the impending attack. How is this proficiency acquired so successfully? And – perhaps more interestingly still – how can experiments be designed to investigate this behaviour? Stefan Schuster and colleagues provide an answer to both questions in their recent *Current Biology* article.

They offered single archer fish a choice of different target sizes (8 black discs, from 2–30 mm diameter, printed on white paper) at a range of distances (200–800 mm) above the water surface. Would they try to match some internal ‘template’ (in which case their preferred size of disc would increase as the paper was moved further away); or would they choose the same absolute size of disc, irrespective of range? The latter would have to imply that the fish were actively calculating absolute size and range through a highly distorting air/water interface.

Intriguingly, the fish went for the same absolute size of disc, irrespective of range. Even more impressively, rather than assessing the targets from directly underneath, or from a precise underwater viewing angle, the fish immediately made a choice of which target to aim for from a wide variety of starting positions in the tank, swam directly to a firing position, and fired at one of the discs. As the fish had already made their choice by the time they took up position, their initial calculation of absolute size and position must have been highly complex. Thus, it is puzzling that the fish seemed to make a clear choice of target; how could they tell the absolute size of their target, when the distortion of refraction at the air/water interface could (for example) make a close small disc appear larger than a larger, more distant, one? The authors’ clear implication is that the fish must be calculating absolute size and range (despite the vagaries of variable refraction through the air/water interface), rather than matching potential targets to some internal ‘template’. Surprisingly, naïve fish were nearly as good as seasoned hunters, although their preferred disc size did seem to increase slightly with distance, to the extent that, at the furthest distances, they were firing at sizes that would have been too large to consume. However, it proved relatively easy to train fish to associate a particular absolute size of target with a reward (of a crane-fly). This was achieved by presenting a range of disc sizes at a range of distances from the target, and only rewarding an immediate attack on the correct target when presented. Remarkably, the fish were able to discriminate differences in diameters of only 1–3 mm over a distance of up to a metre – and all from a variable distance, and from underwater.

The authors interpreted the results as implying that the fish could not possibly be comparing their targets with a range of pre-stored, or hard-wired ‘templates’. Rather they must be able to calculate, over a wide range of conditions, a remarkably accurate absolute size for their potential targets. They are thus necessarily learning the laws of refraction – another remarkable thing we are capable of, although usually only after formal education!

10.1242/jeb.01336

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