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INSIDE JEB

Sneaker male athletic sperm outstrips harem male sperm to get egg

Seba's short-tailed bat. Photo credit: Emmanuel Rey.

When Nicolas Fasel, from the University of Bern, Switzerland, talks about bat reproduction, it sounds more like war than a love story. Having established their supremacy over a territory, dominant 'harem' males then have the right to mate with the females that shelter under their protection; but their battle to father offspring is far from done. Fasel explains that males that do not guard a harem creep around in a bid to mate sneakily with females while their guardian's back is turned. 'So males still have to continue to struggle after copulation to obtain offspring as females mate with several males when they are fertile', says Fasel. And it appears that these sneaky males are better prepared to vanquish their more successful roost-mates as they have more vigorous sperm. Intrigued by the subordinate males' apparently superior fertility, Fasel and his colleagues Charlotte Wesseling, Heinz Richner and Fabrice Helfenstein wondered whether frequent mating could compromise the fertility of harem males, providing sneaker males with an opportunity to outmanoeuvre more successful males at the final hurdle.

Fortunately, Fasel had access to a captive colony of Seba's short-tailed bats at the nearby Papiliorama zoo where he could investigate the question; 'Visitors can enter a dome, the Nocturama, where you have the bats flying freely', he explains. Initially, Fasel, Wesseling and their colleagues Ahana Fernandez, Felizia Koch, Alvaro Sobrino and Laura Panchione gently trapped males as they emerged from their cave roost before carefully collecting blood and semen samples. Investigating the condition of the sperm as soon as it was collected, the scientists could clearly see that the sneaker males' sperm moved faster than the harem males' sperm, potentially beating it to the egg. Then, having individually identified each bat with a unique set of rings attached to the bats' forearms, the team released the bats before monitoring their behaviour over a 13 month period to determine which males were defending a harem and which animals were sneaking around behind their backs.

Having established each male's role, Fasel and Wesseling returned to impose a period of sexual abstinence on the harem males. 'We wanted to control the copulation rate and monitor the sperm quality afterward', says Fasel, who recalls that the males were surprisingly cooperative: 'Inside the cage, they rapidly settled down, and in the absence of females, all of their competitive behaviours disappeared and they grouped together like old friends'. Collecting new blood and semen samples from the bats at the end of 3 days of seclusion, the team was impressed to see that the sperm from the abstinent harem males was as agile and healthy as that of the sneaker males.

But what had facilitated the recovery of the harem males' sperm? Fasel and his colleagues wondered whether the period of abstinence had allowed the quality of the bats' seminal fluid to improve and produce well-nourished sperm that could compete better with that of the sneaker males. However, when they tested for signs of an increase in a component (superoxide dismutase) that could protect sperm from damage in the abstaining males, there was none, so the males are using other mechanisms to improve sperm quality.

Fasel suspects that the sneaker males do not invest more in semen to increase their odds of producing offspring; instead, he suggests that harem males may be the victims of their own success. He says, 'The high copulation rate of harem males may reduce their sperm quality and actually offer sneaker males the opportunity to reproduce'.

10.1242/jeb.141887

Wesseling, C., Fasel, N., Richner, H. and Helfenstein, F. (2016). Modification of sperm quality after sexual abstinence in Seba's short-tailed bat, *Carollia perspicillata*. *J. Exp. Biol.* **219**, 1363-1368.

Kathryn Knight

Croc eyes fine-tuned to lurk at water surface

Juvenile saltwater crocodile. Photo credit: Nicolas Nagloo.

Crocodiles and alligators have perched at the water's edge waiting for the perfect dining opportunity to pass for millions of years. Explaining how the animals also spend much of their time immersed in water with only their eyes and nostrils protruding above the surface, Nicolas Nagloo from The University of Western Australia adds that this posture provides them with a unique perspective on the world. 'The water surface makes up the majority of the bottom of the visual field and the visual horizon occurs along the riverbanks where the crocodile sees best', he says. However, while the visual worlds of saltwater and freshwater crocodiles are essentially identical above the water's surface, the light conditions are significantly different when the animals are submerged: 'In freshwater habitats, there is an abundance of long wavelength [red] light… In contrast, saltwater habitats transmit a broader range of wavelengths, providing a greater amount of short wavelength [blue] light', Nagloo explains. Knowing that crocodiles cannot see clearly when submerged but they have a fantastic assortment of adaptations that help them to see and hunt in dim conditions, Nagloo and his colleagues Shaun Collin, Jan Hemmi and Nathan Hart decided to gaze into the eyes of two Australian crocodile species to

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find out more about their colour vision and visual clarity.

Investigating the structure of the retina at the back of the eyes of juvenile saltwater crocodiles (*Crocodylus porosus*) and freshwater crocodiles (*Crocodylus johnstoni*), Nagloo discovered that instead of having a compact fovea (a depression in the retina where there is a high density of photoreceptors that provide a high-resolution view of the world), the foveae of saltwater and freshwater crocodiles are stretched across the back of the eye in line with the horizon. Describing the elongated structure as a foveal streak, Nagloo says, '[it] gives greater visual clarity', adding that the elongated shape of the structure allows both species to keep a close eye on events on the riverbank without moving their heads.

However, when Nagloo investigated the sensitivity of the different photoreceptors in both species' eyes, he was surprised to find that the crocodiles have relatively sophisticated colour vision, provided by three colour-sensitive cones that are tuned to violet, green and red wavelengths. Also, the sensitivity of the saltwater crocodiles' colour photoreceptors was slightly shifted to shorter (bluer) wavelengths compared with that of the photoreceptors of the freshwater crocodiles, even though neither species can focus underwater, suggesting that they may use their vision underwater more than had been thought.

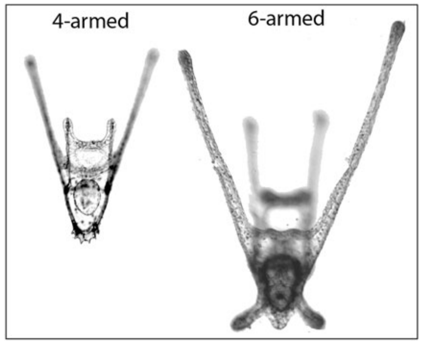
Considering the subtle differences between the visual systems of the two Australian species, Nagloo says that the crocodiles have finely tuned visual systems that give each species an advantage while in their own environments. And he is now keen to find out how the vision of both species adapts as they grow. 'We know that eye size has a big effect on visual clarity and we also know that saltwater crocodiles reach much greater maximum body sizes than freshwater crocodiles, so it would be interesting to find out whether the eye of saltwater crocodiles also gets much bigger than the freshwaters' and what impact that would have on visual clarity,' he says.

10.1242/jeb.141929

Nagloo, N., Collin, S. P., Hemmi, J. M. and Hart, N. S. (2016). Spatial resolving power and spectral sensitivity of the saltwater crocodile, *Crocodylus porosus*, and the freshwater crocodile, *Crocodylus johnstoni*. *J. Exp. Biol.* **219**, 1394-1404.

Kathryn Knight

Extra arms destabilise sea urchin larvae



4- and 6-armed sea urchin larvae. Photo credit: Karen Chan.

The ocean is a big place, especially for minute larvae searching for a permanent home to settle in. 'Many marine organisms have planktonic early stages and rely on them for dispersal', explains Karen Chan from the Hong Kong University of Science and Technology, adding that the minute pioneers have to navigate the constantly fluctuating water column while interpreting their surroundings until they find their final resting place. 'If we can figure out how various cues are used by larvae, it would help us better understand their abundance and distribution', says Chan, who was a postdoc at the Woods Hole Oceanographic Institution in the USA when she decided to find out how larvae of the Atlantic purple sea urchin (*Arbacia punctulata*) cope with water turbulence during these early stages of development.

Describing how the shuttlecock-shaped larvae acquire additional pairs of arms over the course of their development, Chan explains that the limbs are covered in beating hairs, which propel them through the water. Collecting larvae as they were produced by two pairs of sea urchins, Chan, Jeanette Wheeler and Lauren Mullineaux then monitored the larvae's development until they acquired four arms (8 days) and then six arms (23 days), although Chan admits that it was a challenge to rear sufficient larvae to fill their tank, which was over 100 l in volume. Then, the team generated turbulence in the water by oscillating a pair of vertical grids up and down to simulate calm nearshore waters and open ocean conditions while filming the larvae as they swam in the gently churning water. 'We had to complete all the trials for one larval stage in as little time as possible, to minimise any between-trial differences in growth', says Chan, who admits that running the experiments was exhausting. And, she also recalls that tracking the orientation of the larvae's arms, with Erik Anderson's help, was challenging. 'We explored various automated algorithms and continue to refine these methods with Erik, but we realised at the time that the human eye was one of the most efficient pattern-recognition machines to identify a complex 3-dimensional shape, when observing a 2-dimensional slice through it', she explains.

Interpreting the larvae's tumbling motions, the team discovered that all of the animals swam harder as the turbulence increased. But when she focused on their vertical motion, Chan noticed that the 4-armed larvae swam vertically most strongly in the still water, while the older 6-armed larvae swam upward hardest in the gently swirling nearshore-like water. The team also analysed the larvae's orientation in the eddying water and observed that the larger 6-armed larvae tumbled more in the turbulent conditions than their smaller 4-armed younger siblings, which surprised Chan. 'We initially expected the opposite', she says, adding that she had expected the larger larvae to be more stable because they have a heavier skeleton, which she thought would act as ballast to stabilise their position.

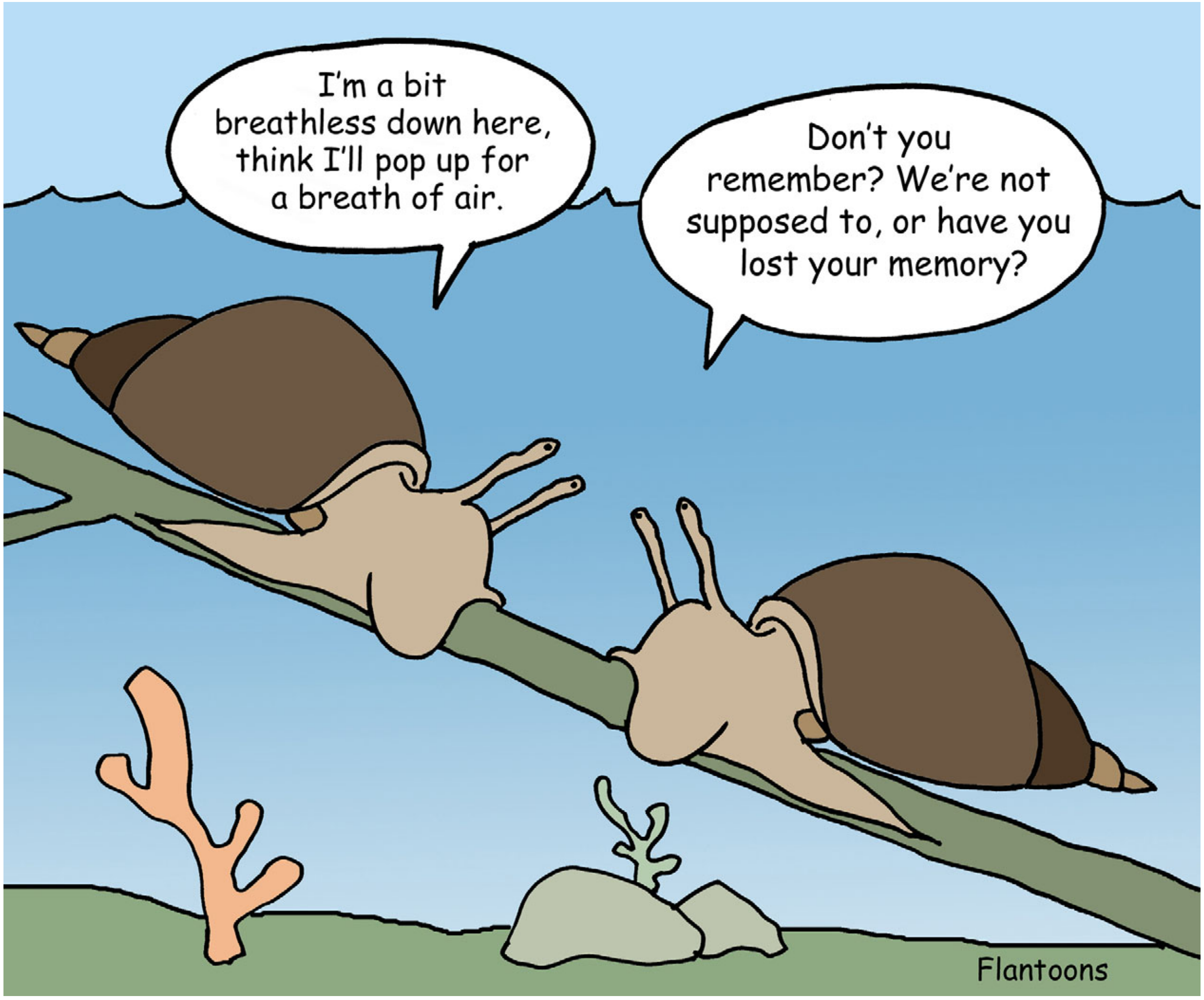
Chan suggests that the reduction in stability as the larvae acquire more arms could trigger them to swim harder than the younger 4-armed larvae, resulting in more of the 6-armed larvae migrating into shallower water. However, it is unclear how the sea urchin larvae sense the movements in the water that trigger their swimming actions. 'They lack the sensory organs for rotation and acceleration that other larvae and plankton possess', she says, adding that she is keen to identify the sensory mechanisms that help the larvae to respond as they wash around in ocean currents.

10.1242/jeb.141895

Wheeler, J. D., Chan, K. Y. K., Anderson, E. J. and Mullineaux, L. S. (2016). Ontogenetic changes in larval swimming and orientation of pre-competent sea urchin *Arbacia punctulata* in turbulence. *J. Exp. Biol.* **219**, 1303-1310.

Kathryn Knight

Hot snails methylate DNA to keep memories



For animals that regulate their body temperature, 30°C can be the perfect temperature to relax and sit back in, but for pond snails (*Lymnaea stagnalis*), the pressure is on. Their immunity suffers and the snails begin to reproduce frantically as mortality beckons. However, there does seem to be an up side to a blast of heat for the diminutive molluscs. Ken Lukowiak from the University of Calgary, Canada, explains that snails that have experienced a heatwave form long-term memories under circumstances where chilled-out snails wouldn't recall a thing. In addition, a whole suite of genes that produce protective 'heat shock' proteins are activated when animals experience heat stress, and these proteins help the molluscs to consolidate long-term memories. Lukowiak and his colleagues wondered whether these memory-promoting changes in gene expression are caused by DNA changes that occur when the temperature rises.

To test their theory, Lukowiak and his colleagues injected the snails with a drug (5-AZA) that prevents a specific type of DNA modification (methylation) that is known to alter gene expression patterns. Then, the team warmed the snails up to 30°C for 1 h before training them to remember to keep their breathing tubes (pneumostomes) closed when they were placed in de-oxygenated water 24 h later.

Snails that had not experienced the heatwave had no memory of the lesson and extended their breathing tube to the surface to gulp air, whereas snails that had experienced the heatwave remembered to keep their breathing tubes closed in the hypoxic water. However, when the team tested the memories of the snails that had received an injection of 5-AZA before the heatwave, they had no recollection of the lesson that they had been taught the day before. So, changes in the methylation of the genes that are activated when pond snails get warm are essential if pond snails are to recall memories formed when they get hot under the collar.

10.1242/jeb.141903

Sunada, H., Riaz, H., de Freitas, E., Lukowiak, K., Swinton, C., Swinton, E., Protheroe, A., Shymansky, T., Komatsuzaki, Y. and Lukowiak, K. (2016). Heat stress enhances LTM formation in *Lymnaea*: role of HSPs and DNA methylation. *J. Exp. Biol.* **219**, 1337-1345.

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