OUTSIDE JEB

Giant trashcan crab also makes creepy noises

Imagine relaxing on an Indo-Pacific tropical island beach under a palm tree with a coconut drink in your hand. You hear a mysterious tapping noise above you, so you look up and see a meter-wide crab at the top of the palm tree, tearing a red-footed booby apart with its big, meaty claws. Sorry for the nightmares, but this is the coconut crab (*Birgus latro*), the largest terrestrial crustacean in the world, which became famous a few years ago after a photo of one climbing a trashcan went viral. These giant, omnivorous hermit crabs are known for a variety of odd behaviours, including producing eerie clicking sounds. However, nobody knew how or why they produce sounds, until a recent study published in Zoology by a team of scientists led by Shin-ichiro Oka of the Okinawa Churashima Foundation in Japan. The researchers figured out the coconut crab's means of sound production, along with providing some explanations for how they use these sounds.

As crustaceans, coconut crabs are covered in hard segments, which show up well in X-rays. Therefore, Oka and his team had a straightforward way of determining how the crabs produce sound: filming the crabs with X-ray videography while recording the sounds they make. By looking at which body parts were moving only when noises were audible, Oka was able to determine the parts used for sound production. Unlike many crustaceans that use their claws and appendages to produce sound, coconut crabs produce sound by beating hard parts of their mouth structures – known as the scaphognathites, which draw water and air over the crab's gills – against hard panels in the gill channels. The work by Oka and his team suggests that rather than developing a new organ, coconut crabs use a behavioural modification of existing organs to produce sound. However, what are these sounds for?

When in the water, crustaceans produce sounds mainly to deter predators, court mates and ward off competitors for resources. The purpose of the sounds made by the crustaceans when on land was largely mysterious, but we now have some possible explanations, thanks to Oka and his team. They recorded sounds from male and female crabs during mating and at other times. Oka found that both sexes produce sounds regardless of whether they are trying to attract the most desirable mate with their devastatingly attractive clicks, suggesting that that they use sound for more than just casual sex. Additionally, both sexes produced a variety of sounds by adjusting the pitch and sound intervals between their clicks, demonstrating a potential 'multi-word vocabulary'. Some of these 'words' likely serve a courtship role to woo mates, as the crabs' sounds changed throughout the mating process. However, the rest of the coconut crab language is still a mystery.

Many crustaceans communicate using odours underwater, but these giant crabs spend most of their lives on land, so some of their aquatically adapted senses may not function so well in the air. While they have evolved a strong sense of aerial smell, odours are carried differently through the atmosphere. Therefore, the coconut crab's diverse acoustic vocabulary may complement their odour repertoire to communicate a variety of messages to other crabs on land. The work by Oka and his team highlights just how little we know about some of the biggest, most conspicuous animals on our planet and why we need to develop a 'Rosetta shell' to understand these overgrown hermit crabs.

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Oka, S., Kobayashi, N., Sato, T., Ueda, K. and Yamagishi, M. (2019). Sound production in the coconut crab, the largest terrestrial crustacean. *Zoology* **137**, 125710. doi:10.1016/j.zool.2019.125710

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Wing swing, not shape, is key to bird flight

Be it finches flapping, hummingbirds hovering or seabirds soaring, birds are capable of achieving all manner of aerobatic approaches to flight. It would not be unreasonable to assume that these specialised flight behaviours would be associated with specific wing types, but, in fact, many birds with similar flight styles possess wings of varying shapes and sizes. One theory for this inconsistency between form and function is that traditional 2D wing shape measurements are a poor representation of the way birds actually use their wings during flight. By focusing instead on the wing's 3D movements, or their 'range of motion', a team of researchers from the University of British Columbia, Canada, led by Doug Altshuler recently revealed that when it comes bird flight behaviour, it's less about the wings you've got and more about how you use them.

To clear up the conflicting relationship between wing morphology and flight behaviour, the team started by acquiring examples of 61 bird species and measured the area, shape and the ratio between length and width (known as aspect ratio) of the extended wing. Next, the team identified each wing's full range of motion by marking locations on the wing, such as the elbow and wrist joints, before

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manually flexing and extending the wings – while filming the motion of the markers from multiple angles to reconstruct the motion in 3D. In order to compare these hand-made range of movement measurements with the animal's free movements, the team also filmed two of the 61 species, pigeons (*Columba livia*) and zebra finches (*Taeniopygia guttata*), flying from various angles. After researching in the literature what was already known about bird flight, the team then assigned each of the 61 species to at least one of 12 distinct categories of flight styles, including different combinations of hovering, gliding, soaring and flapping flight. Finally, to investigate how wing shape and flight behaviour varied between related species, the team constructed a family tree from the DNA sequences of 220 bird species and paired each species with their flight styles.

The team's experiments confirmed the theory that the flight style of a bird is much more strongly linked to the wing's range of movement than its static shape, in addition to finding that the wings' range of movements were almost twice as likely to correctly predict a bird's flight behaviour than their wing shape or body mass. These results reveal that bounding and gliding birds tend to have a greater range of wing movement and lower body mass, allowing for a wider adaptability of wing motion, while soaring birds, such as eagles, tend to possess rigid wings with a much more restricted range of movement. The team also report that pigeons and zebra finches rarely fully extend their wings when flying freely, further suggesting that wing shape does not accurately represent a bird's true range of flight styles.

To explain these findings, the team then turned to their evolutionary tree and realised that 2D wing shape is much more similar between related species than the wing's range of movement, suggesting that bird species are more likely to vary their flight behaviours through 3D wing motion than by adapting the shape of their wing. Not only do these results help to improve our understanding of bird flight behaviours and the evolutionary processes behind them, they also show promising applications for our own aeronautical ambitions. For drones and crewed aircraft, overcoming turbulence and strong winds are issues that could be addressed by morphable wing shapes, and maybe one day soon, this area of research will influence the shape of wings to come.

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Guts and microbes: a revolution in aquaculture

Take some guts, a few bacteria species and throw in some brains while you are at it…and what does that give you? One of the most unexplored interactions that has scientists all riled up: how the bacteria population in the gut, known as the gut microbiome, interacts with the brain to allow the animal to grow and adapt to stress in its environment. And this interaction could not be more relevant than it is for farmed fish, where overcrowding, waste generated by the animals and increasing global temperatures are just some of the stressors that the fish can endure. How can fish farmers increase production in a sustainable way, yet minimize the adverse conditions that the fish may encounter? Is it possible to manipulate the diversity of the gut microbiome to improve the overall health and growth of fish in aquaculture? Can we make them bigger, faster, healthier – and do it at a lower cost?

Victor Alfonso Castaneda-Monsalves from Universidad Nacional de Colombia in Medellin and his colleagues set out to identify the species of bacteria living in the gut of the third most cultured fish in Colombia, the white cachama (*Piaractus brachypomus*). For their work, the group compared both juvenile and adult fish. Wanting to differentiate between the anterior (mouth to stomach) and the posterior (intestine) gut, the authors separated the sections and compared the bacteria species in each at both life stages. Initially, the team found that bacteria known as Firmicutes, Spirochaetes and Fosobacteria – which are involved in fermentation, breakdown of plant products and immune protection – occur in both life stages of the white cachama. However, Fusobacteria dominated the front section of juvenile intestines, while Spirochaetes dominated in the posterior. In contrast, Fusobacteria and Firmicutes dominated the population of the entire adult gut. As members of the Fusobacteria family produce vitamin B12 during digestion, the authors suggested that their presence could eliminate the need to supplement the fish diet with vitamin B12, therefore cutting down costs to the fish farmer.

In addition, the team found that probiotic bacteria, which boost the immune system to fight infections, occurred in the fish guts, although they were more dominant in the adult intestines than in those of the juvenile fish. However, the researchers also discovered Proteobacteria in the guts of all farmed fish, which can cause infections if the animals are stressed. They suggested that manipulating the 'good' probiotic bacteria in the fish digestive systems could allow them to outcompete the 'bad' ones, as well as boosting the immune system to reduce infection and promote growth.

The idea of an interaction between the microbiome and the brain is not new, but how they interact and to what degree bacteria in the gut can influence how the brain responds to stress is still a mystery that we are only now beginning to unravel. Exploring this question in the context of aquaculture opens new possibilities for food production. Can we improve the health of the fish by simply manipulating the population of the bacteria growing in their intestines? Can we improve the taste of the meat? Can we make fish grow faster, reproduce sooner and at high rates, to increase production? These are just some of the questions that are worth exploring, as the human population continues growing and the global demand for fish is ever increasing.

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Castañeda-Monsalve, V. A., Junca, H., García-Bonilla, E., Montoya-Campuzano, O. I. and Moreno-Herrera, C. X. (2019). Characterization of the gastrointestinal bacterial microbiome of farmed juvenile and adult white Cachama (*Piaractus brachypomus*). *Aquaculture* **512**, 734325. doi:10.1016/j.aquaculture.2019.734325

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A consistently performing mitochondrion for every season

Tucked into almost every cell of an animal's body there are fierce little engines that turn food into energy. These little power plants, called mitochondria, fuel animal activities by generating ATP (adenosine triphosphate) that animals use to contract their muscles or send nerve impulses so they can get moving and do things. For any species, some individuals will be high performers – you know the type – they run the fastest or jump the highest, others are more ho-hum and average, and some drag their heels not doing well at all. Scientists already know that this range or variation in animal performance is partly caused by the number of mitochondrial engines animals have and how effective their mitochondria are at turning food into energy. We also know from lab studies that couch potatoes can actually increase their performance when faced with energetically demanding situations. Yet, it is unclear whether the number of mitochondria or how effective they are at pumping out energy can change over time within wild animals.

This is an important question because some stages in life require more energy than others; for example, reproduction is infamous for being energetically draining. Antoine Stier, Pierre Bize, Bin-Yan Hsu and Suvi Ruuskanen from universities in Finland and the UK set out to address this very question by studying pied flycatchers while they were caring for their offspring. The team collected female birds that were keeping their eggs warm in nest-boxes at a field site near Turku, Finland. They took a small blood sample containing cells with mitochondria for later analysis and returned the mother to continue incubating her eggs. The team then returned 10 days later to collect blood once again when the eggs had freshly hatched and the mothers were scrambling to care for their chicks, allowing them to test whether there was a change in mitochondria number or performance given the task at hand. The blood samples allowed the researchers to measure two things. First, they measured the number of mitochondria in each bird's blood cells by comparing the amount of genetic material that came from mitochondria to the amount of genetic material from the rest of the cells. Second, they measured how efficient or high performing a bird's mitochondria were by testing how well they produced ATP given the amount of fuel available.

Stier and colleagues found that the birds had fewer mitochondria while they were tending to their nestlings compared to when they were incubating the unhatched eggs. However, the mitochondria performed more efficiently (produced more ATP with less fuel available) while the birds were busy wrangling their nestlings. A female's 'energy budget' is likely much tighter when she has nestlings, because she has to feed both herself and a nest full of hungry, begging chicks.

The researchers also found the birds were consistent across contexts: those with the most and the highest performing mitochondria during incubation, also had the most and the highest performing mitochondria during the nestling phase. Overall, the team's findings suggest that wild animals may have some wiggle room to modify the amount and performance of their mitochondria across contexts. However, they are also stuck with the cards they were dealt and are consistently either 'high' or 'low' performers in all scenarios.

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Stier, A., Bize, P., Hsu, B. and Ruuskanen, S. (2019). Plastic but repeatable: rapid adjustments of mitochondrial function and density during reproduction in a wild bird species. *Biol. Lett.* **15**, 20190536. doi:10.1098/rsbl.2019.0536

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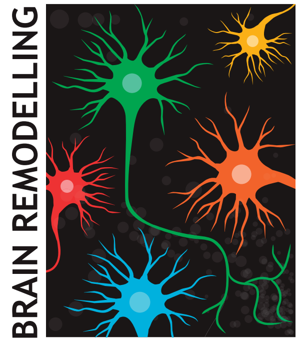
Mouse neurogenous zone doubles during puberty



Puberty is often accompanied by making dumb decisions. Yet, despite its reputation, Michael Brecht and colleagues from Humboldt University of Berlin, Germany, recently discovered that the awkward period of adolescence coincided with parts of the brain getting bigger in rats (doi:10.1016/j.cub.2015.11.041). Appropriately enough, the region that expands during puberty is called the 'genital cortex', a part of the brain that's active when its namesake region is aroused. The scientists made this discovery by comparing snapshots of the brain taken from different rats before, during and after puberty. However, comparing the same brain cells in the same region of the same animal across development would be a more reliable way to compare how puberty remodels the brain.

Following up on this study, Johanna Sigl-Glöckner, also from Brecht's laboratory, turned to another lab animal, the house mouse (*Mus musculus*), for which there is a wide range of fancy molecular tools that allow scientists to flag specific brain cells. Using this technology, she and her colleagues spied on brain cells in the genital cortex of individual mice several times during their development, as recently reported in *Current Biology*.

First, the team determined if, like rats, male and female mice grew more touch-sensitive brain cells in the genital cortex during puberty. While mice have other cell types in their genital cortex, since sex is all about touch, the team focused on tallying

Journal of Experimental Biology

touch-sensing cells as they play a more obvious role. To accomplish this, Sigl-Glöckner brought in specially designed mice that produce glow-in-the-dark tags only within brain cells receiving touch information from the thalamus, the brain's sensory relay station. This allowed the team to peek in on the mice periodically and count how many new touch-sensitive cells were born in the genital cortex as the mice grew up. Following puberty, both male and female mice doubled the size of their pre-pubertal genital cortex and added nearly two new touch-sensitive cells a day across adolescence. Therefore, mice, like rats, expand the size of their genital cortex during sexual maturation. However, while (brain) size matters, it wasn't clear how responsive to touch these new cells were.

In order to measure how genital cortex cells respond to stimulation, Sigl-Glöckner tweaked her designer mice so that the touch-sensitive cells would glow even brighter when they were activated: the brighter the cell, the more responsive to touch. Then, the team turned on a tiny vibrating device, the same one that makes your cell phone buzz, to sexually stimulate mice while measuring how bright their brain cells glowed. Despite having a similar number of cells in the genital cortex, the male mouse cells shone brighter than the female cells – both pre- and post-puberty – suggesting that male mice have a more sensitive erogenous zone across development. However, if female mice mated with a male before puberty or if females were abstinent and just reaching adulthood, their genital cortex cells became more responsive to stimulation compared with pre-puberty. Taken together, the changing response to sexual stimulation depends not only on mouse age, but also their sex and sexual experience.

These findings by Sigl-Glöckner and her colleagues expand our understanding of how different factors drive brain remodelling across development and reveal that just as hormonal pre-teens increase in size and sensitivity during puberty, so too does the mouse genital cortex, thus stimulating more questions to be answered about how puberty changes the brain.

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Sigl-Glöckner, J., Maier, E., Takahashi, N, Sachdev, R., Larkum, M. and Brecht, M. (2019). Effects of sexual experience and puberty on mouse genital cortex revealed by chronic imaging. *Curr. Biol.* doi:10.1016/j.cub.2019.08.062

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