

OUTSIDE JEB

Fish fitness is on the rocks



We owe a lot of our modern technology to biological concepts adapted from the animal kingdom, but sometimes we miss useful solutions that are right in front of our noses – or rather, our ears. Otoliths are calcified structures that build up in the inner ear of vertebrates and are very sensitive to horizontal and vertical movement. Because of this, otoliths play an important role in the interpretation of speed, balance and direction, but much like the seasonal growth rings of a tree, otoliths also preserve information about the growth rate of fish throughout their life. However, recent work published by an international team of researchers from Norway, Denmark and the UK reveals that these otoliths can also record long-term metabolic activity in the manner of a personal fitness tracker, allowing scientists to monitor and predict how environmental change may affect the metabolism of wild fish populations.

The cornerstone of this impressive new technique is in measuring the relative concentration of two distinct variations of carbon with different atomic masses (isotopes) that comprise the calcium carbonate in the otoliths. Each isotope is acquired from a different source, with the slightly heavier isotope diffusing into the fish directly from the surrounding water and the slightly lighter isotope being released from the respiration of food for energy. As rates of respiration increase

due to physical activity or to the presence of environmental stressors, the concentration of the lighter isotope in the blood increases relative to the concentration of the heavier isotope, which then becomes deposited in the otoliths as carbonate and provides a permanent record of the change in metabolism. While this carbon isotope–metabolism relationship seemed to be a theoretically valid proxy for measuring metabolic rate, it had not been experimentally tested until now.

Through a series of experiments, the team recovered and analysed the carbon isotopes from the otoliths of Atlantic cod (*Gadus morhua*) reared in captivity at temperatures ranging from 4 to 14°C and then investigated how the ratio of the heavy and light carbon isotopes in the otoliths varied compared with their metabolic rates. They found that the relative amount of light carbon isotopes produced by respiration and deposited in the otoliths increased as the temperatures rose from 4 to 14°C in line with the increase in the fish's metabolic rates. So, the proportion of light carbon isotopes in the otoliths is a good indicator of the fish's metabolic rate at the time that the calcium carbonate was laid down in the otolith. The team then collected and analysed the calcium carbonate content in the otoliths of wild Atlantic cod and four deep-water fish species (Baird's slickhead, blue antimora, roundnose grenadier and orange roughy). The ratios of the carbon isotopes in the wild fish otoliths agreed well with measurements made by other scientists of these fishes' metabolic rates, suggesting that this new technique could provide a reliable estimate of the metabolic rates of wild fish – at least for the range of fish species tested so far.

Measuring metabolic rate in free-roaming wild animals is an important yet challenging task; as such, this new technique marks a significant step forward for the field, especially for those invested in monitoring today's rapidly changing aquatic ecosystems. Perhaps most interestingly, this technique allows for the collection of metabolic data not

only from recently deceased fish, but also from the remains of much older fish. Researchers as far back as the 1890s have been collecting fish otoliths as a means of measuring their growth rates without knowing the new significance that their specimens would hold today. To this end, the team have started to analyse the otoliths of Greenlandic cod from time periods dating back to the 1920s. They hope that by looking to the past, they might be able to predict the effects of future environmental change on fish populations thanks to these historic metabolic records that have been, quite literally, etched in stone.

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Alex Evans (0000-0002-5655-012X)
University of Leeds
alexevans91@gmail.com

Small zooplankton rings the alarm for oxygen loss in big oceans



Hypoxia, a low level of oxygen that limits the physiological functions of animals, is a topic that fascinates many biologists. As climate change progresses, the frequency of hypoxic episodes in aquatic environments is increasing, putting fish species under stress and even reducing

populations in some cases. But it is not only fish that suffer the ill effects of hypoxia. Karen Wishner and colleagues from the University of Rhode Island, USA, and the University of Washington, USA, have discovered that some zooplankton species (such as copepods) are now living close to their physiological bottleneck. Zooplankton that live in regions of the ocean with very low dissolved oxygen, known as oxygen minimum zones, are remarkably susceptible to even the tiniest dip in oxygen. Their numbers decline with even the smallest ($\leq 1\%$) decrease in dissolved oxygen levels. Even though these creatures are minute, they are a crucial component of oceanic food webs – providing nutrition for larger creatures further up the network – so understanding their distribution has far-reaching implications for the entire oceanic ecosystem.

Wishner and her colleagues undertook an expedition to one of the major oxygen minimum zones in the eastern tropical North Pacific. They surveyed the region between 325 and 650 m, to measure the amount of oxygen in the water and the numbers of zooplankton in the same area to determine the population size. The team discovered that regions with relatively high oxygen levels ($8\text{--}10\ \mu\text{mol l}^{-1}$) had remarkably high zooplankton levels relative to regions where the oxygen concentration was $5\ \mu\text{mol l}^{-1}$ or less; and they found no zooplankton at all in the zones with the least oxygen. They suspected that these zooplankton species are living close to their physiological limit, where they can just maintain their minimum metabolic needs in regions where oxygen is very low, making them very vulnerable to even the smallest decline in oxygen.

Aiming to understand the physiological mechanisms that potentially dictate the distribution of zooplankton, the team conducted a shipboard metabolic experiment to measure the oxygen levels at which these zooplankton species cannot sustain the minimum metabolic needs. They then calculated the ratio of the amount of oxygen recorded in regions where zooplankton live in to the amount of oxygen required to sustain the zooplankton, known as the metabolic index, which estimates the amount of excess oxygen available to sustain life at a specific location in the ocean. When the metabolic index is less than 1, animals

have insufficient oxygen to metabolize aerobically and have to initiate anaerobic metabolism, and life becomes unsustainable. Despite the challenges of measuring the metabolic rates of these vanishingly small animals on their ship, the authors discovered that there is only sufficient oxygen in the oxygen minimum zone for the zooplankton to sustain a metabolic rate that is barely twice their resting metabolic rate. Putting this into context, fish typically double their resting metabolic rate when digesting a meal.

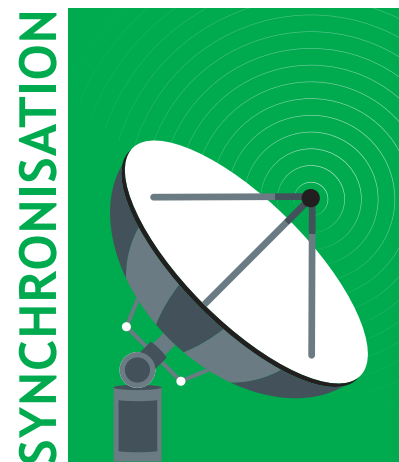
This discovery is alarming, although not entirely surprising. The vertebrate population has crashed by 60% since 1970, likely as a result of human activity. Many species, terrestrial and aquatic, are currently experiencing serious ecological stress. It appears that the zooplankton living in the Pacific oxygen minimum zone are also suffering, which could potentially trigger the collapse of the ocean ecosystem that they underpin.

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Yangfan Zhang (0000-0001-5625-6409)
The University of British Columbia
yangfan@zoology.ubc.ca

Insects that stink and hatch in sync



If you're the kind of person who enjoys watching the perfectly choreographed singing of the von Trapp children in *The Sound of Music*, then the sight of translucent stink bugs crawling forth

simultaneously from their eggs might be the stuff of nightmares. Other people, such as scientists interested in biological timing, are genuinely fascinated by synchronized hatching, since it is quite common throughout the animal kingdom (think of all the egg-laying species, from insects to spiders to turtles). Yet, the mechanisms initiating synchronized hatching are not well studied. Jun Endo and colleagues from Kyoto University, Japan, wanted to find out more about one of these mechanisms, after observing the synchronized birth of brown marmorated stink bugs (*Halyomorpha halys*). Stink bugs lay clumps of about 28 sesame-seed-sized eggs all at once, which hatch later in virtual unison. It all starts with a single embryo cracking open its shell (there is a mesmerizing video of this online), which somehow signals to its other siblings to crack open their own eggs, too.

The researchers first sought to identify the cue that initiates hatching. To do this, they took pairs of eggs from the same clutch and placed them on paper. They then allocated the paired eggs to a group of naturally attached eggs and another four groups of separated eggs that were either reattached by the researchers; kept physically apart, but connected by a thin piece of carbon; kept physically apart; or isolated by different pieces of paper. Nearly all of the naturally attached eggs and the eggs that had been reunited hatched together. However, only half of the eggs connected by the carbon hatched at the same time, followed by 20–25% of the eggs separated on the same sheet of paper, while even fewer of the isolated eggs successfully hatched in sync. This meant the hatching cue was vibrational – ruling out sound or chemical signals – as it required a physical connection for transmission of the signal. The ability of the naturally attached and reattached eggs to hatch together also demonstrated that embryos transmit the hatching vibration to an adjacent sibling's egg.

Endo and colleagues then investigated the source of the vibrations. In a scene reminiscent of *Alien*, they saw that the first embryo to hatch wielded an egg burster (a T-shaped structure protruding from the forehead of all embryos) to crack open its shell. This cracking generated the precise vibrations needed to transmit the hatching cue to neighbouring eggs, which the researchers recorded using a sensitive laser. Next, the researchers placed

individual eggs on a vibration-producing platform and played the laser recordings back to the eggs. Excitingly, the eggs began to crack immediately. Now that they knew that the egg-cracking vibrations trigger hatching, the researchers wanted to know how efficiently they travel. They noted that as many as three inactive eggs can lie between two live embryos, before the eggs fail to hatch simultaneously, indicating that the vibrations can travel through the eggs of immature embryos.

A synchronized birth provides stink bugs with a survival advantage, as it ensures that only fully developed embryos hatch. Hatchlings will then cannibalize any unhatched, underdeveloped siblings. This gruesome, but essential, behaviour prevents the killing of viable embryos and provides a readily available source of food. Furthermore, although you might believe stink bug hatching to be horrifying and smelly (it is), studying this behaviour has far-reaching implications for biotremology (the study of vibrations). Research like this informs us more about the widespread use and transmission of vibrations by animals to communicate social information.

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Ilan Ruhr (0000-0001-9243-7055)
The University of Manchester
ilanruhr@manchester.ac.uk

A spider's decision depends on its thirst



Have you ever thought about how your preference for food changes with the weather? In winter, we often fancy something warm and highly calorific, like roasts or cheese-loaded pasta, while we might prefer something fresh and crunchy, like a salad, in summer. This decision is not just influenced by our desire to fit into our swimwear. On days when the sun is burning and the air is dry, it is important to keep our body hydrated, and this is often reflected in our decisions of what to eat. So, why should this only be the case for humans?

Israel Leinbach, Kevin McCluney and John Sabo from Arizona State University, USA, wondered whether the feeding decisions of predators might also vary in response to water availability and how these changes might influence the lives of their prey. The team went to the area surrounding the San Pedro River in Southeast Arizona during the dry season and conducted feeding experiments in which they varied the amount of water available to dining spiders. After capturing large wolf spiders (*Hogna antelucana*), the team offered the arachnids a choice of meals: small spiders (*Pardosa* sp.) or crickets. The tiny spiders are low in water content, but rich in energy, whereas the crickets provide a low-energy snack that is relatively moist. The scientists then supplemented the diets of some of the spiders with additional water, and left other spiders to cope with the water that is naturally available, which is usually quite low during the dry season.

Not surprisingly, the researchers found that the spiders' decision on whether to feed on moist prey with a high water content or a delicacy with high energy content depended on the amount of water that was available to them. When their water supply was supplemented, the spider predators fed primarily on smaller arachnids and less on crickets. Moreover, the availability of additional water not only reduced the wolf spider's appetite for crickets, but the crickets' survival rates were even higher than when the wolf spiders were completely absent. While the researchers are not entirely sure why the crickets' survival chances are better when well-hydrated wolf spiders are around, they assume that the wolf spiders' appetite for smaller spiders reduces the overall spider population, which is a good thing

for cricket wellbeing. However, the team also found that male crickets were more likely to be preserved by water supplementation. Looking into the differences between the water content of male and female crickets, this makes sense as Leinbach and colleagues showed that male crickets have a higher water content than females.

Importantly, the decisions made by thirsty spiders influence the surrounding animal and plant communities. Animal food webs can be described as a cascade; large predators at the top of the cascade feed on smaller predators or plant eaters beneath, while plants are right at the bottom. If wolf spiders feed more on plant-eating crickets, the plants that are beneath them in the food chain thrive. However, if wolf spiders are not thirsty and fill up on smaller spiders instead of crickets, this leads to an increase in the numbers of plant eaters and, therefore, fewer plants. Hence, a thirsty wolf spider can have far-reaching implications for their neighbours.

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Julia Nowack (0000-0002-4512-5160)
Liverpool John Moores University
J.Nowack@ljmu.ac.uk