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OUTSIDE JEB

Mitochondria on demand for overwintering beetles

There's a rhythm to life in temperate and sub-polar areas due to seasonal changes in warmth and food availability. The Colorado potato beetle waits out winter in a low-energy state of suspended animation known as diapause before emerging in the spring to look for food and a mate. Metabolic suppression is the hallmark of diapause, but exactly how animals turn it on and off remains an open question. In an integrative study, Jackie Lebenzon and colleagues from the University of Western Ontario, Canada, used respirometry, high-powered microscopes and a cutting-edge gene silencing technique to show how diapausing beetles use their mitochondria like a metabolic thermostat.

During diapause, the Colorado potato beetle's flight muscles waste away, only to be completely rebuilt in time for spring. As 40% of insect flight muscle is made up of mitochondria, Lebenzon and colleagues hypothesized that these powerhouse organelles have an essential role in controlling diapause. Checking mitochondria freshly extracted from diapausing beetles, the team found that they consumed 90% less oxygen, matching the decrease in metabolic rate observed in living beetles. That the beetle and its isolated mitochondria had such similar metabolic responses confirmed that the mitochondria had a big role in setting the beetles' low metabolic rate during diapause. But how were the mitochondria keeping the metabolic rate so low?

One clue was that diapausing beetles had a very low activity for citrate synthase, a protein only found deep within the mitochondria that prepares metabolites for the mitochondria to use. Wondering whether low citrate synthase activity translated into fewer mitochondria, the researchers viewed the muscle under a high-powered microscope: it was nearly bereft of the familiar bean-shaped organelles. In addition, the cell's disposal system was bustling, with lysosomes, autophagosomes and autolysosomes – which break down old and damaged parts of the cell – dotted around the field of view. Despite this, diapause muscle had the same number of cells and oxygen-delivery tubes as active muscle; all signs pointed to beetles selectively breaking down their mitochondria, a process known as mitophagy, as a way of bringing their metabolic rate down during diapause.

Mitophagy is a complicated process. Key proteins include Parkin, which tags proteins on damaged mitochondria, and ATG5, which aids uptake of tagged proteins into lysosomes for disposal. Both the Parkin and ATG5 genes were much more active during diapause. Mitophagy is one of two complementary processes, the other being the production of new mitochondria, which animals use to balance mitochondria levels. During diapause, the beetles also bumped up the levels of two transcription factors, PGC1α and NRF1, that promote mitochondria production. Together, these observations told the researchers that a complex interplay between mitochondrial breakdown and production was responsible for the start, maintenance and end of diapause. Early mitochondrial breakdown brought metabolic rates down in the winter and kept them low, only to give way to mitochondrial production as winter thawed to spring.

Finally, to connect gene activation in the muscle with metabolic suppression in the beetle, the researchers injected beetle muscle with custom RNA sequences that turned off the Parkin gene. The injected beetles had 80% less Parkin protein and didn't lose their mitochondria to mitophagy, showing that all the pieces of the pathway worked together in living animals. Interestingly, the injected beetles only recovered about 40% of their metabolic rate during diapause, suggesting that while Parkin is important, it isn't the only regulator of metabolism during diapause.

Colorado potato beetles destroy and regrow their own mitochondria on demand to suppress their metabolism and, in doing so, expertly weather the seasonal rhythms of their environment.

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Bigger is not better in warm water

Oxygen is essential for life, providing the energy needed for surviving and thriving in our world. However, animals that live in aquatic habitats have their work cut out for them. Not only is oxygen less freely available in water than in terrestrial environments but also, with climate change, bouts of low oxygen (hypoxia) are becoming more and more common in these habitats. For decades, scientists have been studying and categorizing how

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well fish can handle low oxygen (or not) to understand who the 'winners' and 'losers' would be in an oxygen-limited world. The ultimate goal for many scientists is to create rules that allow us to predict which species are more vulnerable to changing environments and whether this vulnerability will change their current distributions around the globe.

Until recently, scientists have been studying how one or two environmental factors in isolation can affect oxygen limitation – but many factors can conspire to impact a fish's oxygen supply or demand. For example, low oxygen levels in water frequently occur when temperatures are elevated. This increases a fish's demand for oxygen and effectively makes it worse at dealing with limited oxygen availability. However, there's conflicting evidence for how other factors that influence oxygen demand, such as body size, affect a fish's tolerance of low-oxygen scenarios within and across species.

To approach this in a more holistic manner, Wilco Verberk from Radbound University, The Netherlands, and colleagues from Chile, the UK and France, searched the literature and an existing database of limiting oxygen levels for what factors might influence fishes' ability to withstand low oxygen. Compiling traits for 195 fish species, they took into account the many physical factors that relate to the fishes' oxygen supply or demand and investigated whether these factors work separately or together to explain why some fishes are better than others at dealing with low oxygen.

Verberk and colleagues found that the relatedness of species heavily influences their ability to tolerate low oxygen. For example, sharks and rays are more sensitive to low oxygen than their distantly related 'cousins' goldfish, which are renowned low-oxygen champions. Salinity is also a predictor of how well fishes deal with limited oxygen availability, wherein freshwater fishes are less sensitive to hypoxia than marine fishes. This is unsurprising as freshwater habitats tend to have more intense fluctuations in oxygen and temperature than marine habitats and so species that live in freshwater need to be better prepared to withstand frequent bouts of low oxygen.

The scientists' most interesting finding was that bigger fishes and fishes with bigger cell sizes are more vulnerable to low oxygen, specifically in warm habitats. At higher temperatures, their demand for oxygen is high but they might be limited by the flow of oxygen into the blood, making them more sensitive to low-oxygen situations. In cooler environments, fishes with larger bodies and bigger cell sizes are actually less vulnerable to low oxygen than their small counterparts. Colder water has a higher viscosity – it's thicker and stickier – which would make it more difficult for small fishes to breathe, giving larger fishes the leg (or fin?) up.

Low oxygen and warmer temperatures are predicted to work together to limit desirable habitats and reshape species distribution. Yet, other environmental and biological factors can impact oxygen supply or demand. By including body size in their considerations, Verberk and colleagues were able to resolve how body mass impacts hypoxia tolerance and add to the growing body of work seeking to predict fishes' vulnerability in a changing world.

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Hot cold-adapted lizards are born 'old'

It is no secret that biodiversity is declining at an alarming pace worldwide. The Earth's average temperature is rising and extreme weather events, such as heat waves, are becoming more frequent. This is particularly bad news for cold-blooded animals (ectotherms), which adapt to the temperature of their environment. Research in terrestrial ectotherms, such as snakes and lizards, has already shown that warmer climates hasten an animal's rate of ageing and increase the risk of population extinction. However, a much less explored question is whether the effect of accelerated ageing due to climate warming is transmitted from one generation to the next and whether this could be linked to the risk of extinction of affected populations.

Andréaz Dupoué from Ifremer, Plouzané, France, together with a large international team of scientists, had in his hands the perfect model system to answer exactly this question. For over a decade, they have studied populations of the common lizard (*Zootoca vivipara*) spread across the Massif Central of France, an area of plateaus and mountains at altitudes ranging from 800 to 1800 m. This small reptile is adapted to live in very cold habitats, but recently some populations, especially across the mountains in southern France and northern Spain, have been repeatedly exposed to summer heat waves and dry spells, which could make them more vulnerable to the risk of extinction. Having previously established that shorter telomeres – the protective structures that cap chromosomes and degrade with age – predict population declines in warmer habitats in this species, Dupoué and colleagues took a step forward and examined whether mothers that had experienced more demanding lives, including exposure to hotter summers, could pass on shorter telomeres to the next generation.

The team caught pregnant females from nine different lizard populations (seven that were stable and two that were declining) and took tiny blood samples from them to estimate their telomere lengths. The female lizards were then brought to the lab where the scientists could track birth date and hatchling survival, and clip off a minute piece of the tip of the hatchlings' tails as soon as they were born to measure their telomere lengths.

They found that mothers from declining populations tended to produce fewer surviving offspring and had shorter telomeres, which they potentially passed on to their young. In addition, they estimated that only 7% of hatchlings in the threatened populations were expected to survive long enough to have young of their own, based on the impact of telomere length on survival, in contrast to 73% in the stable thriving populations. In effect, the baby lizards in the declining populations were basically born in an 'old body'.

This study shows that telomere length is a promising indicator to track the effects of climate warming, not only within one generation but also across multiple generations. As the team proposed, damaged chromosomes of stressed animals may accumulate across generations and drive populations to extinction. These results also suggest that monitoring telomere length could help wildlife ecologists to identify threatened populations before it is too late and to find effective conservation strategies that could mitigate the negative consequences of climate change on biodiversity.

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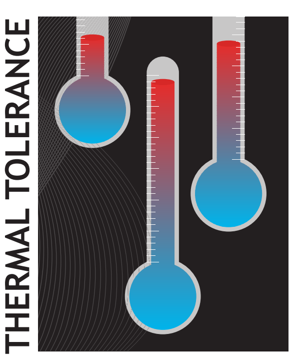
Hot tortoises running out of daytime



Animals try to avoid the hottest and coldest parts of the day and opt to lie low instead. While this seems like a good strategy to avoid heat, it does cost them time when they could be out finding food or a mate. As climate change continues to push environmental temperatures to extreme highs and lows, it's important to understand how much time species will lose sheltering to avoid those extreme temperatures. To quantify how the most drastic prediction of climate change could affect an already vulnerable species, Rafael Lara-Reséndiz from the Universidad Nacional de Córdoba and Centro de Investigaciones Biológicas del Noroeste, Argentina, along with his collaborators, investigated how activity time was affected by the temperatures that Goode's thornscrub tortoises (*Gopherus evgoodei*) can withstand in the tropical dry forests of Northwest Mexico.

To measure the temperatures individual tortoises were actually experiencing on the forest floor, the researchers built hollow copper models of the tortoises with thermometers inside. They then painted the models leaf-green like the tortoises, so the models would absorb the same amount of heat as the animals. They then placed six of these models throughout the landscape in places where tortoises had previously been seen, to record the temperatures that the tortoises could reach every 30 min for 5 years, providing the scientists with a rich set of temperatures experienced naturally by the animals. By comparing the range of environmental temperatures the model tortoises experienced over the five year period with the temperatures when tortoises are known to be active, the researchers could predict how the amount of time the animals can be out and about could change in the future as temperatures rise.

The researchers found that the time when tortoises were able to be active varied across the months and from year to year, which matches the changes during the seasons in the forest; when the trees lost their leaves during the dry season, the conditions the tortoises experienced also became warmer and dryer. After summer rains, the conditions on the forest floor became more humid and cooler, which in turn gave the tortoises more time at comfortable temperatures. The researchers also found that the tortoises were active during two periods of the day, morning and evenings, and would overheat if active during the hot afternoons. This pattern is likely due to the tree canopy shading the ground beneath where the tortoises live but only during the summer months when the trees still have their leaves.

Additionally, the scientists found that the temperatures predicted by climate change models are likely to increase the amount of daytime when temperatures are too hot, limiting the overall time when tortoises can be active. Tortoises need a minimum of 3.85 h per day to forage sufficiently and mate; if a region is too inhospitable for an animal to be sufficiently active, then it is unlikely that these creatures will be able to make it their home. Given the temperature changes that are predicted to strike this region, the researchers suspect that only a few populations would survive to 2070 under the most pessimistic scenarios.

Although this study predicts tortoises will lose time for foraging and mating under alarming climate change scenarios, the researchers also suggest that tortoises may begin foraging earlier in the morning or later in the evenings to make up for lost time during hot afternoons or move to areas more protected from the heat. To learn more about how rainfall and variation in temperature affect other aspects of tortoise activity times, the researchers are conducting additional studies to inform conservation management.

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Birds flap their wings over wider arcs to overcome challenges

Have you ever wondered how it is that birds can fly for a long time without having to take a break and recover their energy? The amount of energy birds expend when moving can be a determining factor for when they finish their migrations. During flight, birds may choose to flap their wings as fast as they can or over as wide an arc as they need to maintain steady flight to make it to a suitable spot at the end of migration without running out of fuel. Researchers are trying to figure out how important these two flying characteristics are for energy savings and how they may change because of the demands of the environment.

While there is existing evidence that birds beat their wings slower as their energy demands decrease, previous work has shown that this might not be entirely true. Knowing this, Krishnamoorthy Krishnan from Swansea University, UK, and a group of more than 20 researchers from around the world were motivated to use the information of wing movement from 14 different species in the wild to determine how different birds behave when they are flying. But first, Krishnan and the team developed a new technique to estimate how wide birds sweep their wings up and down during each wingbeat, in addition to how fast they move, based on the movements detected by a motion sensor mounted on migrating birds. In the lab, they attached a motion sensor and a magnetometer to the back of two pigeons (*Columbia livia*) and a dunlin (*Calidris alpha*) as well as a magnet on one wing of each bird, which the magnetometer detected each time the wing swept up and down, to measure how wide and how fast the wings were sweeping. The team then set the birds the challenge of flying at different speeds in a wind tunnel to compare how well the motion sensor recorded each wingbeat picked up by the magnetometer.

Having confirmed that they could detect wingbeat signatures in the movements recorded by the motion sensors as the birds flew in the wind tunnel, the team then investigated the maneuvers of 12 wild species, including the red-tailed tropicbird (*Phaethon rubricauda*), the western barn owl (*Tyto alba*) and feral pigeons in Germany to learn more about how these birds use their wings during flight across different airspeeds and climbing rates in their natural environment. The tropicbird and barn owl swept their wings with a wider arc as well as faster when the birds were climbing. Airspeed had no effect on any of these three birds in the wild. In contrast, the data from the wind tunnel suggested that pigeons needed wider wing movements when the airspeed increased, indicating that the birds adopt a different flying strategy whenever the wind changes, which could be associated with increased energy demands for a more challenging flight. Lastly, Krishnan and colleagues were curious to know whether the size and shape of the bird can play a role in how fast the birds move their wings. However, after looking at all 14 species, they concluded that all have very similar wing movement patterns, regardless of their different sizes or shapes.

Krishnan and colleagues' lab-based study has revealed how researchers can extract previously unnoticed detail about bird wing movements in the wild from motion sensors mounted on their bodies. Their observations show that birds beat their wings with a wider arc when they need more propulsion under challenging conditions, such as when climbing, or when they encounter winds during long migrations.

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