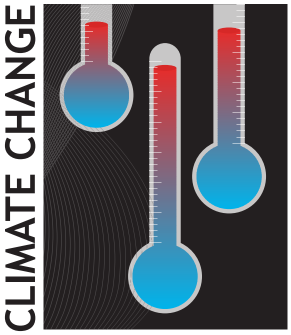
OUTSIDE JEB

Heat-stressed lizards slow their metabolism, but at a cost



As we race to understand how increased heat caused by climate change will affect animals, the potential ‘winners and losers’ of this climate change marathon are beginning to emerge. Many of these struggling animals cannot generate body heat on their own, relying on the temperature of their surroundings to warm themselves. Lizards, for example, must move in and out of shade to maintain a body temperature that allows them to move quickly and helps them digest their food. But hotter is not always better. Heating up lizards speeds up their metabolism, forcing them to burn through energy faster and producing more harmful by-products that damage tissues. But when the environment heats up to the point that even shade temperatures become too hot, lizards must find another way to slow down their metabolism. Xingzhi Han, Baojun Sun and Qiong Zhang at the Chinese Academy of Sciences, China, along with Fushun Zhang at the Chinese Academy of Agricultural Sciences, China, and Liwei Teng and Zhensheng Liu at the Northeast Forestry University, China, wanted to see whether a desert-living lizard could adjust its metabolism and reduce tissue damage when the temperature gets too warm.

To do this, Han and colleagues caught female and male toad-headed lizards (*Phrynocephalus przewalskii*) from Shierliancheng in Inner Mongolia, China in July 2021 and brought them back to their lab in Beijing. Normally, the lizards live in a habitat that is cooler in the morning and hotter in the afternoon (21–39°C). After 2 weeks, the researchers changed the daily temperatures of half of the cages to mimic the heatwaves in China during 2016 and 2018 (21–58.7°C). On the third day of the artificial heatwave, Han and colleagues measured the body temperature of each lizard every hour between 08:00 h and 18:00 h. Unsurprisingly, lizards living under heatwave conditions were hotter than those experiencing normal temperature conditions. Five days later, the researchers measured the resting metabolism of each lizard at 34°C and 38°C. The team found that the lizards that experienced the heatwave had lower metabolic rates at both temperatures, suggesting the heat-stressed lizards could slow their energy use. The team also recorded how fast each lizard could run at 34°C and 38°C. The heat-stressed lizards only ran at about a third of the speed of the lizards from cooler conditions when running at the higher temperature. Although having a lower metabolism when resting can be a good way to save energy on hot days, lizards that have lived through heatwaves do not have as much energy available for running, making them slower when the weather is too warm. This means that these lizards will have a harder time catching their insect prey and avoiding being eaten themselves, but having a slower metabolism also means their tissues are less damaged by turning food into energy.

So, after the 14 day heatwave, the team measured how much damage their metabolic rate caused the lizards’ livers. The researchers found that heat-stressed lizards not only slowed their metabolism, which reduced the amount of overall tissue damage, but their bodies also produced more antioxidants – molecules that help get rid of the harmful by-products of metabolism. The team concluded that making more of the protective antioxidants further helps lizards deal with the heat by shrinking the negative consequences of having a faster metabolism.

Han and colleagues show that toad-headed lizards can adjust their metabolism when it is too hot, but at the cost of being easier to catch. Although these lizards can adjust to living in the heat, the impacts these adjustments have on their survival will be crucial for predicting how these – and other – reptiles will endure the climate change marathon.

doi:10.1242/jeb.246572

Han X., Sun B., Zhang Q., Teng L., Zhang F. and Liu Z. (2023). Metabolic regulation reduces the oxidative damage of arid lizards in response to moderate heat events. *Integr. Zool.* doi:10.1111/1749-4877.12784

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Monarchs that fly long, live long

Understanding the differences in how living beings grow, reproduce and survive is one of the key goals of evolutionary biologists. For example, animals often have to choose between living longer and voyaging over great distances or producing future generations, which can take its toll. To explain how animals trade off life expectancy against the cost of living, biologists have proposed many theories. One idea is that toxic chemicals, known as reactive oxygen species – produced as a by-product of energy-intensive activities – damage cells, decreasing lifespans. However, an alternative theory suggests that these damaging toxins actually activate cellular mechanisms to enhance survival. To

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resolve which of the two strategies animals may use in practice to enhance their longevity, Alexander Shephard, Amanda Hund and Emilie Snell-Rood at the University of Minnesota, USA, tested the ideas on monarch butterflies (*Danaus plexippus*), which undertake an immense autumn migration each year from North America to return to their winter roosts in Mexico. During this extraordinary voyage the insects that live eight times longer than their predecessor generations, which made the outbound journey, endure enormous amounts of stress caused by their extreme exertions, yet must conserve sufficient energy to allow them to reproduce when they reach their destination.

To assess whether migration stress affects monarch lifespan, the scientists conducted two experiments. In the initial set, they compelled the monarchs to fly in a confined space for 5 min each day over a 4-day period. This limited space made the butterflies take off more frequently, inducing stress and causing them to use more energy than they would use normally if flying uninterrupted in an open environment. Then they monitored the insects for the rest of their lives, keeping track of how long they survived and how many offspring they produced, while also checking for evidence of damage caused by stress in the males' testes.

The team discovered that flying more improved the butterflies' chances of staying alive. They lived longer, averaging 55 days instead of 46, and when the team looked for evidence of the cellular damage that you would expect as a result the insects' exertions, they found none. However, the levels of stress-fighting proteins that the team found in the flight muscles was high. The insects were prioritizing cell maintenance to protect themselves from the damaging effects of flight. And when the team looked to find out how the period of intense flight had affected the butterflies' ability to reproduce, they found that although the males' testes were unaffected, females produced an average of ∼54 eggs, compared with ∼97 eggs when the females were flying normally. This suggests that flying for long periods stresses the insects, reducing the number of eggs and offspring down the line.

In the next set of experiments, the researchers applied a drug called methoprene to the abdomen of the butterflies to cause the females to produce eggs in early adulthood. Then the team kept track of the insects' longevity, the number of offspring they produced and looked for evidence of physical stress, to test whether reproduction increased physical damage and impacted the insect's life expectancy. On average, the methoprene-treated butterflies produced ∼180 eggs; however, they had the same lifespan (42 days) as untreated females that produced no eggs when young. So, early reproduction did not seem to affect the butterflies' longevity. In addition, the methoprene-treated females that flew more in early adulthood had fewer offspring because they had redirected internal resources towards survival.

This study by Shephard and colleagues suggests that investing energy in flight helps migratory monarchs to stay alive for longer by enhancing the cellular mechanisms that protect them from the damaging effects of intense exercise. It seems that switching to a self-care mode is the secret to a long life for daring monarch butterflies when embarking on their epic migration to their southern winter homes.

doi:10.1242/jeb.246599

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Contracting differences: unifying flight muscle modes

Ever wondered how a mosquito can flap their wings fast enough to produce that high-pitched whine? Some insects are capable of beating their wings up to 1000 times a second. This remarkable ability is due to a specialised form of muscle known as 'asynchronous' flight muscle. Instead of being triggered to contract directly by individual signals from the brain – like the muscle contractions of birds and slower flapping insects – the high frequency contractions of asynchronous muscle are triggered by the initial downbeat muscle contraction. In turn, this stretches the upbeat muscle to drive the upward movement of the wing, starting a self-perpetuating cycle of rapid muscular contractions that are independent of input from the brain. Scientists believe that this kind of asynchronous muscle evolved from regular flight muscle that is controlled directly by nerve signals and that the two muscle types are distinct from one another. However, researchers at the Georgia Institute of Technology, USA, and the University of California, San Diego, USA, led by Simon Sponberg disagreed. They test this assumption and show in their new paper that fast asynchronous and slow regular flight muscle might be two versions of the same underlying flight muscle architecture.

The researchers initially examined the evolutionary history of all flying insects to see how many times asynchronous flight muscle evolved. They demonstrated that the most likely scenario is that this muscle evolved once but at some points in the history reverted back to the regular form of flight. One such reversion likely occurred in the hawkmoth species *Manduca sexta*. When the team looked closer at the hawkmoth's flight muscle, which is controlled directly by slower brain signals leading to slower wing beats, they found it retains the physiological properties of the fast-beating asynchronous flight muscle. This suggests that although hawkmoths reverted from the asynchronous muscle back to controlling each wingbeat directly, the physiological scaffolding that allows the muscle to be stretch activated has stuck around, even though it is now redundant. This transition from asynchronous flight muscle back to regular flight muscle, and vice versa, raises the question: what does flight look like when an organism is in between the two types of muscle? Showing that this muscle transition still allows for smooth flight is essential because otherwise organisms with 'in between' muscle types would not have been able to fly, effectively grounding the evolutionary argument. To show that this transition was possible, the researchers employed two techniques.

Firstly, they developed a computational simulation of their hawkmoths that contained all the features of the insects' muscle and body movements during flight. This allowed them to show that given the right movement and muscle inputs – such as rate of flapping and the correct muscle structure – simulated insects with transitioning muscle types could still fly. To prove this, they modified a dragonfly-sized robot to be able to transition mechanically between asynchronous and regular flight modes. Using this robot, they were also able to achieve a smooth flight while switching between the asynchronous muscle powered flight and flight powered by regular muscle. The robot even remained airborne at the transition when the muscle contractions stopped being stretch activated and switched to being directly controlled by signals from the brain. Taken together, the results suggest that the two types of muscle are not so different after all, changing our understanding of evolution of flight in insects and opening up the possibility of building robots that better mimic insect flight muscle.

doi:10.1242/jeb.246569

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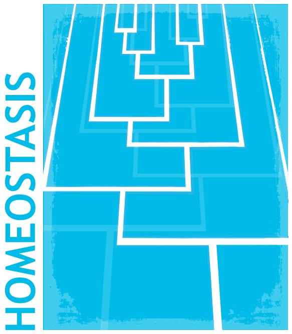
A secret gill…or a problem solved?



While slugs are known for being slow and slimy, they've found success in living all over the world. Slugs and snails (otherwise known as gastropods) are found in freshwater, saltwater and on land, and are probably a great group to teach us how animals adapt to different lifestyles. One group of slugs (called *Acochlidium*) possesses a strange net of branched tubules that extend from the open cavity surrounding the heart, known as the pericardium. When these structures, called 'dorsal vessels' were first discovered, it was assumed that they were connected to the heart, thus replacing gills. To determine whether the *Acochlidium* slugs have a secret gill, or if the tubules function in some other way, Timea Neusser from Ludwig Maximilian University of Munich and colleagues from Bavarian State Collection of Zoology in Germany, studied the anatomy and function of four freshwater slug species to better understand the adaptations that make it possible for these animals to shift from life in the salty ocean to freshwater.

The researchers gathered four species of bottom-dwelling *Acochlidium* freshwater slugs from streams and rivers on various Indo-Pacific islands. Then, they collected tiny slices of tissue samples from the slugs' excretory and circulatory systems, viewed them under a microscope and stitched together the images to form 3D digital versions of the organs. With the reconstructions in hand, the team examined and compared the organs of the circulatory and excretory systems of these freshwater species with the organs of other slugs that live in saltwater.

Contrary to what was believed previously, the team discovered that the net of branched tubules close to the heart of *Acochlidium* is not actually homologous to gills. Instead, it has an entirely different physiological function. Freshwater and marine animals face very different challenges to maintain a healthy water balance in their bodies. Where ocean water is dense with salt (and animals have to prioritize their water intake and excrete concentrated, salty urine), freshwater does not contain much salt, so animals must excrete a lot of dilute urine. It turns out that the dorsal vessels that connect to the cavity that surrounds the heart work to pull salts from the freshwater that the slug ingests. The tubules are lined with a thin membrane of special cells that helps to retain valuable salts in the body and also increases the surface area of this cavity, which increases pressure in the cavity surrounding the heart. This helps to move lots of haemolymph (slug blood) to the kidney to produce urine, an adaptation that allows these freshwater slugs to expel large volumes of water from the body while keeping the salts that their bodies need to stay healthy. Interestingly, the tubule structure found in a related nudibranch (*Elysia*) that lives in salt water does not have a thin membrane lining increasing its surface area; therefore, the structure is not capable of filtering fluid or moving it quickly to the kidney. This is a great example of how similar-looking structures can function very differently in related animals.

Comparing the structure and function of the body parts and organs of animals that are closely related but live in very different environments can give us important insights. We can understand more about the adaptations that allow animals to shift to these different habitats, like how these slugs use very different organs to deal with the pressures of living in the salty ocean or in a freshwater stream. Studies such as this one also show us that we have only scratched the surface in discovering the diverse ways that animals function – even in the possession of unique organs.

doi:10.1242/jeb.246570

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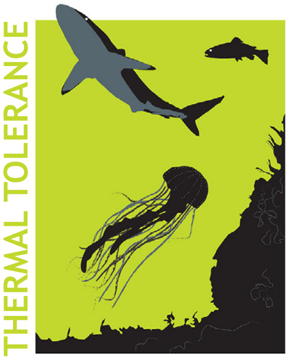
Small but mighty: juvenile sturgeon beat the heat



Sturgeon are giant, ancient fishes that can grow up to 600 kg and live upwards of 100 years. Although these iconic fish have existed for more than 100 million years, they now face significant threats from humans – including habitat loss and climate change – making them some of the most endangered species on Earth. For example, one population of white sturgeon (*Acipenser transmontanus*) in British Colombia has not produced offspring that have survived to adulthood since the 1960s. To assist the dwindling population, humans are currently raising white sturgeon in a lab for over a year before releasing them into the wild to increase their chance of survival during this vulnerable life stage. One possible explanation for why the developing fish are struggling in the wild is the increasing occurrence of climate change-induced heatwaves in their river habitat, which simultaneously warms the water and lowers the amount of oxygen in it. Researcher Madison Earhart worked with colleagues at the University of British Columbia, Canada, the University of Glasgow, UK, and Vancouver Island University, Canada to investigate how sensitive juvenile sturgeon are to the stressful changes in river conditions caused by heatwaves.

To start, the scientists measured the hottest water temperature and the lowest amount of oxygen that the fish could tolerate before warming the water in their tanks from 13 to 20°C to simulate a heatwave for 20 days. Because every species of fish can react differently to heatwaves, the team was not sure how the sturgeon would respond. However, they figured two things could happen: either the heatwave conditions would lower the fish's ability to deal with extreme heat and low oxygen, or the heatwave could increase their ability to handle the harsher conditions. So, Earhart and colleagues repeated their tests after the heatwave, and to the team's delight, the young fish dealt with the heatwave remarkably well. In fact, the fish were more resilient to both the extreme heat and the low oxygen in the water after they had experienced the heatwave, suggesting they were better prepared to respond to future stress.

Delving deeper, the team wanted to uncover the secret behind the fish's high resilience after the heatwave. To do so, the researchers measured the activity of genes associated with resilience to high temperature and oxygen deprivation in the young fish. What Earhart and colleagues found was fascinating. In response to the heatwave, the sturgeon lowered the amount of methylation on their DNA, which enabled rapid increases in gene expression, meaning that the fish could quickly turn on their heat and oxygen stress tolerance genes when needed. By examining the sturgeon's response to the heatwave at the molecular level, the researchers revealed a mechanism facilitating the survival of these fish in harsh environments.

It appears that juvenile white sturgeon have a heatwave superpower, which could explain how these ancient and long-lived fish have endured the test of time. Their surprising toughness when faced with hot waters and low oxygen will be especially important as they continue to navigate the challenges of a changing climate. Despite other threats to their survival in the wild, this discovery offers a glimmer of hope for the future of white sturgeon and their conservation.

doi:10.1242/jeb.246571

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