

OUTSIDE JEB

Catfish give ‘holding your breath’ a whole new meaning



Oxygen sustains virtually all animal life. Yet, many animals live in environments where oxygen is limited. The striped catfish (*Pangasionodon hypophthalmus*) is native to waterways in Southeast Asia where heavy pollution lowers oxygen levels significantly. Fortunately, the striped catfish is one of a few fish species with a unique superpower – they have the ability to breathe air. When oxygen levels in the water fall too low, these catfish swim to the surface and take big gulps of air. Oxygen from the air then diffuses into the blood, allowing the fish to survive where other species would perish. However, molecules that are carried in solution tend to diffuse from an area of high concentration to low concentration, placing the gulped oxygen – now at high concentrations in the fish’s blood – at risk of being lost to the oxygen-depleted waters in their environment through the fish’s disproportionately large gills. In a new study led by Magnus Aaskov from Aarhus University, Denmark, a team of scientists from across the globe (including from University of North Texas, USA, Can Tao University, Vietnam, and Nagasaki University, Japan) aimed to understand the fate of the freshly gulped oxygen carried in the striped catfish’s blood.

To measure the extent of oxygen loss across the gills, the team used a technique called bimodal respirometry, in which the oxygen consumption of an air-breathing fish can be measured both underwater and

above the water’s surface. Aaskov and the team discovered that when the water was well oxygenated, only 7% of the oxygen that the catfish consumed came from its above-water gulping routine. But hold your breath for the twist – catfish in poorly oxygenated water obtained 105% of their oxygen from air. The researchers explained that aerial oxygen uptake exceeds 100% because fish take up enough oxygen to meet the body’s needs, plus extra to account for oxygen lost across the gills in water. Contrary to what we may have learned in biology class, striped catfish lose a mere 5% of gulped oxygen across the gills.

The researchers then decided to take their study a step further and determine whether unique anatomical features in the striped catfish were responsible for minimizing oxygen loss across the gills. Based on a hunch that the catfish would shunt blood away from their gills in low-oxygen water to minimize oxygen loss, the team made delicate casts of the bloodstream in the fish’s gills and body. Then, they scrutinized the casts with micro-computed tomography (micro-CT) to reveal minute details in the blood flow pathways in the fish’s bodies. The researchers also used ultrasound scans of living catfish in well-oxygenated and oxygen-depleted water to better visualize the movement of blood.

Interestingly, the researchers found a ‘shunt’ in the catfish’s circulatory system that could theoretically redirect blood away from the gills, potentially reducing oxygen loss in oxygen-depleted waters. However, much to their surprise, the team discovered that catfish were losing significant amounts of carbon dioxide in oxygen-depleted water, indicating that blood was not being shunted away from the gills – which would prevent oxygen from being lost the oxygen-depleted water – because blood needs to flow through the gills to eliminate carbon dioxide from their bodies. This discovery deepens the mystery surrounding how air-breathing fish manage to retain precious oxygen in aquatic environments in which the life-giving gas is remarkably scarce.

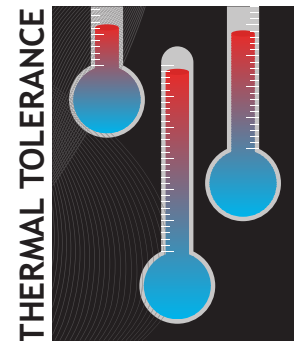
Once again, scientists are left puzzled by the wonders of life concealed in the depths – and at the surface – of the underwater world.

doi:10.1242/jeb.245038

Aaskov, M. L., Nelson, D., Lauridsen, H., Huong, D. T. T., Ishimatsu, A., Crossley II, D. A., Malte, H. and Bayley, M. (2023). Do air-breathing fish suffer branchial oxygen loss in hypoxic water? *Proc. R. Soc. B* **290**, 20231353. doi:10.1098/rspb.2023.1353

Giulia Rossi (0000-0002-4812-8869)
McMaster University
rossig1@mcmaster.ca

Cell self-cleaning helps fruit flies handle the heat



As climate change threatens various species, researchers are particularly interested in how animals can deal with heat, particularly coldblooded (ectothermic) critters whose temperatures fluctuate with the environment. One of the many damaging effects of toasty temperatures starts in the cell. As conditions get hotter, proteins and other molecules in the cell can unfurl, become sticky and form hazardous protein bundles that can damage the rest of the cell. Thankfully, animals have a built-in protective mechanism that can overcome these perils. As damage occurs, animals can activate autophagy – where cell components consume these menacing protein clumps, broken small structures and old proteins to clean up – along with other protective mechanisms. To figure out whether autophagy can also help insects handle the heat, Quentin Willot and colleagues from Stellenbosch

University in South Africa were interested in seeing whether an increase in autophagy led to an increase in the fruit flies' heat tolerance.

The team's first step was chemically turning on the cell's self-cleaning system in the flies. To do this, Willot and colleagues fed the fruit flies rapamycin, a chemical that triggers autophagy. They also knew that rapamycin limits growth, so they could tell whether they had successfully activated autophagy because the flies whose self-cleaning process had been triggered would grow more slowly. Additionally, they predicted that there would be an increase in the number of lysosomes – structures within the cell that break down damaged or old cellular components. Sure enough, the researchers found that flies fed rapamycin took 4–5 days longer to develop than flies that were not fed the drug. They also found that flies fed rapamycin had more lysosomes containing damaged cell parts in midgut cells. The researchers had successfully chemically activated the cell's autophagic self-cleaning process.

To determine the relationship between the cell's self-cleaning system and heat stress, Willot and the team then measured the ability of flies that had been fed rapamycin for 2 days to withstand heat in two different scenarios. First, they put the flies into glass vials and immersed these vials in a hot water bath at 37°C. Every 15 minutes, the team gently shook the vials, looked for flies that had fallen and could not get up or move, and recorded the time it took them to lose this balance. In a second set of experiments, the team measured how long the rapamycin-fed flies took to recover after they lost balance as a result of the stressful effects of the heat. In this case, the team immersed the flies in a hot water bath at 41°C until they fell and could not get up after a gentle shake. Then, they were immediately put at room temperature and the team monitored how long it took the flies to stand on their legs without toppling or falling over after gentle shaking.

Impressively, the team found that the fruit flies that had been fed rapamycin took longer to fall and lose control as a result of heat stress compared with those not fed rapamycin. This suggests that activating autophagy protects the flies, allowing them to handle higher temperatures without losing coordination. Moreover,

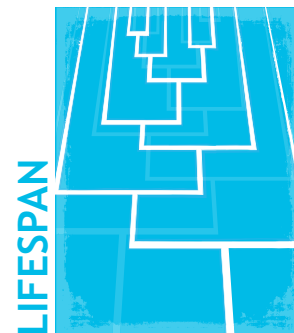
the researchers found that when the flies were fed rapamycin, they recovered from the ill effects of heat faster, showing that the cells' self-cleaning defended them from the heat. This is extremely important as it highlights autophagy as a vital process for surviving future environmental stresses, as insects have little control over their internal temperatures.

doi:10.1242/jeb.245037

Willot, Q., du Toit, A., de Wet, S., Huisamen, E. J., Loos, B. and Terblanche, J. S. (2023). Exploring the connection between autophagy and heat-stress tolerance in *Drosophila melanogaster*. *Proc. R. Soc. B.* **290**, 20231305. doi:10.1098/rspb.2023.1305

Andrea Murillo (0000-0003-4793-708X)
McMaster University
murilloa@mcmaster.ca

Underground anti-aging secrets from burrowing rodents



Naked mole rats (*Heterocephalus glaber*) are not pretty rodents; their yellow teeth and pale, wrinkly skin make them look like a hairless gopher that has sat in a bath far too long. But while they don't look beautiful, they age gracefully. They survive past 30 years old, whereas laboratory mice rarely have a third birthday. And unlike mice and humans, age doesn't curse naked mole rats with cancer, dementia or heart disease. So, what's the secret to their longevity? Scientists previously found a few factors that keep them young: their DNA repairs itself well when damaged and their cells have loads of antioxidants. But their most interesting anti-aging strategy may be outside their cells altogether. Most cells live in a blanket of molecules and minerals called an 'extracellular matrix'. In naked mole rats, this blanket is woven from a thicker fabric: naked mole rats produce a heavier and larger version of the

molecule hyaluronan, which is the backbone of this extracellular matrix. As Andrei Seluanov and Vera Gorbunova's team at the University of Rochester, USA, show, this extra padding protects cells from inflammation and early death.

To better understand this anti-aging strategy, Seluanov and Gorbunova's team genetically modified mice to produce this naked mole rat version of hyaluronan throughout their skin, muscles and internal organs. Then, the researchers recorded how long they lived, and whether they developed cancer with age – as older mice tend to do.

The naked mole rat gene was a powerful anti-aging treatment. Mice lived up to 12% longer lives with this bulkier hyaluronan molecule. If these results were translated directly to humans, that would mean an extra precious decade of life. The mice also developed less cancer, even when exposed to tumour-producing chemicals.

The scientists then asked, as the mice are living longer, does that mean they live stronger? They gave the mice an array of tests to measure their 'frailty index' – a measure of health and vitality. These tests include things like hearing and vision tests, coat condition, body temperature and weight. Mice with the naked mole rat molecule showed a lower frailty index, meaning they were healthier and more robust. They had denser bones, produced a stronger grip and were more capable in coordination tests. As the team put it, the mice not only had a longer lifespan but also had 'an improved health span'.

Next, the researchers asked exactly how this naked mole rat molecule kept mice alive and thriving. First, they looked in the genes for clues, and genetic scans showed that inflammation genes were dialled down throughout multiple organs. This was a good lead as inflammation is a risk factor for age-related diseases such as cancer, arthritis and heart disease. To double-check, they took blood samples to look for signs of inflammation; there were fewer pro-inflammatory molecules in the blood of mice with heavy hyaluronan. Finally, they did a third test, by injecting a bacterial protein that causes inflammation and checking how the mice reacted. There was less inflammation in their tissues, and fewer pro-inflammatory chemicals in their blood. This may be naked mole rats'

secret of youth: this thick hyaluronan coating may protect cells from inflammation, so they live longer and avoid disease.

Perhaps the most interesting thing about this anti-aging chemical is, it's already in each of us. Humans produce heavy hyaluronan too, we just don't make much. For centuries, humans have dreamt of anti-aging elixirs and the mystical Fountain of Youth. Instead, the Fountain of Youth may be embodied in the heavy hyaluronan of naked mole rats – nearly blind rodents, with lots of wrinkles and yellowed teeth.

doi:10.1242/jeb.245035

Zhang, Z., Tian, X., Lu, J., Boit, K., Ablueva, J., Zakusilo, F., Emmrich, S., Firsanov, D., Rydkina, E., Biashad, S., Lu, Q., Tyshkovskiy, A., Gladyshev, V., Horvath, S., Seluanov, A., and Gorbunova, V. (2023). Increased hyaluronan by naked mole-rat HAS2 extends lifespan in mice. *Nature* 6, 196-205. doi:10.1038/s41586-023-06463-0

Michael William Country (0000-0001-5239-6607)

RIKEN Center for Developmental Biology
michael.william.country@gmail.com

Body temperature flexibility in Temminck's pangolins helps beat the heat



The climate of South Africa poses many challenges for its wildlife species. However, an additional challenge for many animals is a changing climate,

which is rapidly shifting to hotter and drier conditions, changing the availability of food for many animals. One of South Africa's current inhabitants – the endangered Temminck's pangolin (*Smutsia temminckii*) – is no exception and must contend with changes in its environment. Given that the animal is also at risk from the illegal wildlife trade, researchers are interested in how this unusual creature alters its physiology and behaviour to cope with changes in the abundance of ants and termites, its primary food source, due to climate change and droughts. This question led Wendy Panaino and colleagues from the University of Witwatersrand, the University of Pretoria and Tswalu Kalahari Reserve (all in South Africa) to find out how wild Temminck's pangolins survive when faced with difficulties finding food in a rapidly changing climate.

Between April 2015 and March 2017, the team gently collected 10 Temminck's pangolins (5 male and 5 female) in Tswalu Kalahari Reserve. They then equipped each pangolin with a tracking transmitter attached to one of its scales, before releasing the animal and allowing it to roam freely. This allowed the researchers to follow the pangolins to their burrows and set up camera traps there to monitor the activity of the pangolins. After a few months, the team successively recaptured seven of the animals and then fitted them with an internal thermometer to measure their core body temperature every 5 min before rereleasing them into the wild. The team also collected information on the climate of Tswalu Kalahari Reserve – measuring the temperature and rainfall throughout the study period. Importantly, the researchers recorded the quantity of ants available in the reserve for the pangolins to consume each month too, using 300 pitfall traps placed into the ground.

The team then analysed the changes in the food available for the pangolins throughout the seasons and years, and the changes in pangolin activity and body

temperature. Throughout most of the year, the pangolins maintained a consistent body temperature (34–36°C); however, during the winter seasons (June–August) and during the year when the pangolins experienced a drought (study year 1), the their body temperature fluctuated more, falling as low as 32°C. This period also coincided with a drop in the ant population (56% lower than the following year). This led the researchers to conclude that the pangolins were able to reduce their body temperature and metabolism, thereby decreasing the amount of energy they burn. Interestingly, during the winter when there were fewer ants, the pangolins shifted from being active at night, to being more active during the day. The team concluded that they did so to reduce the amount of energy they use to stay warm during the colder nights. However, the shift meant the pangolins were being exposed to higher temperatures (up to 37°C) during the day. As pangolins have small bodies and are poorly insulated against the heat by their scales, the hotter temperatures caused them to have higher body temperatures, which could impact their welfare.

Overall, Temminck's pangolins show flexibility in their body temperature and activity patterns when there are fewer ants around, likely reducing the energy they burn. Climate change is rapidly altering the conditions that all wildlife face daily. However, the question remains whether the pangolins' ability to adapt to changing climate conditions is going to be enough to prevent this species from disappearing from South Africa all together.

doi:10.1242/jeb.245034

Panaino, W., Parrini, F., Kamerman, P., Hetem, R., Meyer, L., Smith, D., van Dyke, G. and Fuller, A. (2023). Temminck's pangolins relax precision of body temperature regulation when resources are scarce in a semi-arid environment. *Conserv. Physiol.* 11. doi:10.1093/conphys/coad068

Kristina A. Muise (0000-0002-0497-4086)
Royal Veterinary College
kristinamuise@gmail.com