

OUTSIDE JEB

Injured giraffes are at higher risk of dying

GIRAFFE LOCOMOTION



Animals in the wild can suffer from diseases that can then affect their ability to move. Wildlife is also directly impacted by humans hunting animals for food. In particular, scientists have reported that skin diseases and injuries caused by wire snares, set by people to trap animals in the wild for bush meat, can affect giraffes and their ability to move normally. Most previous research has only been done on captive animals in zoos and with giraffes that suffer from a skin disease that affects their mobility. Recently, an international collaboration between researchers based at universities and zoos in the USA and Namibia recorded videos of giraffes in the wild in Uganda with skin diseases and/or wounds from wire snares.

But first, Laura Bernstein-Kurtycz and colleagues collected video of four Masai giraffes (*Giraffa tippelskirchi*) walking at the Cleveland Metroparks Zoo and used it to quantify the movements of healthy giraffes by measuring how long their stride was, the footfall timing between the back limb and the front limb, how fast the animals were walking, and how long each foot remained in contact with the ground. Additionally, they measured the giraffe's neck movements, up and down as well as back and forth during each stride. After completing their pilot study at the zoo, Michael Brown (Smithsonian National Zoo and Conservation Biology Institute,

USA) and J. Evenhuis (Case Western Reserve University, USA) filmed 52 male Nubian giraffes (*G. camelopardalis camelopardalis*) in the Murchison Falls National Park, Uganda, for more than a year. Bernstein-Kurtycz and colleagues then classified the 52 giraffes into a healthy group, a group with skin disease, a group with wounds from wire snares and a group that had both skin disease and wounds from snares, and analysed their walking movements from the movies.

They discovered that skin disease found on the Nubian giraffes did not affect their walking. This might be partially because the disease was found only around the neck region. In contrast, the giraffes that had wounds caused by wire traps had a shorter stride length and slower walking speeds, the limb that was wounded spent less time in contact with the ground bearing weight, and they tended to keep both limbs on the same side of the body in contact with the ground almost simultaneously, possibly to spare the wounded limb from bearing too much weight. In contrast, there were no meaningful differences in the neck movements between the healthy group and any of the groups that had incurred wounds from snares and those that had the skin disease.

Traps set by humans have a damaging effect on animals, even if they are not the intended targets. Bernstein-Kurtycz and colleagues have demonstrated that the injuries to these majestic animals caused by wire traps force the giraffes to walk more slowly and unevenly, leaving them vulnerable to attack and predation by lions. Such injuries can also affect their ability to migrate efficiently, locate and compete for mates, and dominant males that are wounded may be unable to protect their mates from males competing to mate with them. Describing how a colleague reported that 257 live giraffes were freed from wire snares in the Murchison Falls National Park between February 2019 and December 2021, the team state that the risk posed by human activity to this endangered population is clearly significant, and conclude that 'identifying and quantifying potential threats to this

population is of global consequence for their conservation'.

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More than one way to thaw a frog

FREEZE TOLERANCE



Freezing temperatures pose a challenge for cold-blood animals such as frogs. Ice can damage cells from within, like soda rupturing a can left too long in the freezer. But even if it forms outside a cell, a chunk of ice leaves the remaining body fluid saltier, more concentrated and therefore less hospitable for the cells it bathes. To minimize cellular damage in the winter, some animals produce antifreeze – molecules that disrupt ice formation. For instance, the Cope's gray treefrog (*Dryophytes chrysoscelis*) makes glycerol, which scientists often use to preserve frozen lab samples. But whereas scientists avoid thawing their samples more than once for fear of damaging them, Cope's gray treefrogs must survive winters in the eastern United States, where they are

thawed and refrozen repeatedly as temperatures regularly fluctuate above and below freezing. Elizabeth Yokum, Matthew Wascher and Carissa Krane from University of Dayton, USA, with David Goldstein from Wright State University, USA, tested these frogs by freezing and thawing them multiple times to understand how they survive the winter.

Previous research on another antifreeze-producing frog – the wood frog (*Lithobates sylvaticus*) – had shown that repeated cycles of freezing and thawing led that species to increase antifreeze production, consistent with studies on antifreeze-producing insects. However, the repeated freeze–thaw cycles took their toll on the insects, which accumulated more damage as they went through more cycles. Thus, Yokum, Krane and their colleagues suspected that freezing and thawing Cope’s gray treefrogs multiple times would cause them to produce more antifreeze and sustain more injuries than frogs that were only frozen and thawed once.

The researchers divided Cope’s gray treefrogs into three groups: one that remained unfrozen, one that was frozen and thawed once, and one that was frozen and thawed three times. During each cycle, the scientists kept the frogs frozen for a day at a time before warming the animals and monitoring them for signs of coordinated movements that would indicate successful thawing. Although all of the frozen frogs recovered, the time it took for the frogs to recover from thawing after their second or third freeze was nearly double that of frogs thawing after their first freeze. Furthermore, the scientists took blood and tissue samples from frogs in each group at the end of the experiment and analyzed them for molecules that would indicate damage to the frogs’ blood cells. They found the lowest levels of damage indicators in frogs that were never frozen and the highest levels in frogs that were frozen repeatedly. Together, these results suggest the animals do sustain more damage each time they are frozen and thawed.

However, the results were more complicated when the scientists looked for antifreeze in the blood and tissue samples they collected. All the frogs that were frozen once or frozen repeatedly had higher levels of antifreeze than frogs that were never frozen, but the levels were not always highest in the frogs that were frozen

repeatedly. In fact, individual frogs showed a lot of variability in the amount of antifreeze they accumulated after being frozen, regardless of the frequency. The scientists did find a pattern though, when they compared the levels of antifreeze in the frogs with how much damage the frogs’ blood cells had sustained and how quickly the frogs recovered upon thawing. Frogs with more antifreeze in their system recovered more quickly, but also sustained more damage to their blood cells. Frogs with less antifreeze showed the opposite trends. In other words, fast recoveries were more costly, in terms of both antifreeze production and damage accumulation.

The researchers concluded that individual Cope’s gray treefrogs employ different strategies to survive the winter and that variation may be a key asset for the species in this era of volatile and extreme weather.

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Yokum, E. E., Wascher, M., Goldstein, D. L. and Krane, C. M. (2023). Repeated freeze–thaw in freeze-tolerant treefrogs: novel interindividual variation of integrative biochemical, cellular and organismal responses. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **324**, R196–R206. doi:10.1152/ajpregu.00211.2022

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Murray cod waterhole woes



During bouts of prolonged drought and little to no river flow, waterholes are a haven for freshwater fishes. However, as humans divert more water for their own use and as global temperatures rise, these small bodies of water are increasingly vulnerable to climbing temperatures and

low oxygen levels. Fortunately, some fishes are able to change quickly to cope with high temperatures, and occasionally these changes also help them do better with low oxygen levels. But size matters when it comes to dealing with these stressors. Big fish are thought to deal with low oxygen better than small fish, and small fish are thought to deal better with high temperatures, but all are likely to be vulnerable when both stressful circumstances occur simultaneously. To investigate how body size influences a fish’s ability to tolerate both high heat and low oxygen, Darren McPhee, with researchers from the University of Queensland and the Queensland Department of Regional Development, Australia, turned to massive Murray cod – which can grow to the size of a giant panda (over 100 kg) – to find out how they deal with the combined threat.

Working with fish ranging from 0.2 g to 3 kg, the team transferred the animals to water at temperatures mimicking a hot summer (28°C). After 4 weeks, all of the cod, regardless of size, increased the temperature at which they lose their balance (known as the upper thermal limit), indicating that the cod are able to deal with persistent high temperatures. But when the team tested the fish’s abilities to remain upright at high temperatures as the water oxygen levels decreased, their ability to tolerate heat declined. Surprisingly, the fish’s body sizes had an unusual impact on their ability to withstand high temperatures when their oxygen supply was restricted. As expected, when the fish had access to well-oxygenated water (100% and 50% oxygen saturation), the smaller animals coped better with high temperatures than the big fish. But this pattern flipped when the oxygen levels in the water were low (30% and 16% oxygen saturation), where the largest fish tolerated the high temperatures better than the smaller fish. Intriguingly, this didn’t mean that the big fish were thermal tolerance champions. At the lowest oxygen level (16% oxygen saturation), the mid-sized fish coped best with the high temperatures.

The scientists also tested how short-term and persistent exposure to different temperatures affected the cod’s ability to survive low oxygen in several different ways. In the short term (less than 24 h), fish exposed to high temperatures breathed faster and lost their balance at an oxygen level twice as high as what they would

normally handle. But when exposed to a high heat for 4 weeks, the responses to low oxygen weren't quite as drastic. Again, the largest cod were the least bothered by decreasing oxygen – not changing their breathing frequency and taking longer to lose their balance (unlike the little fish).

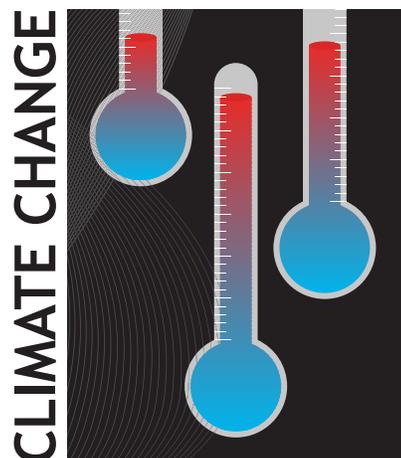
Although Murray cod of all sizes demonstrated an amazing ability to cope with high temperatures in the lab, McPhee and colleagues also went into the wild to check the water quality of three Queensland waterholes that are much loved by the cod. The researchers found that the fish are living dangerously close to the edge – at the limit of the high temperatures and low oxygen levels that they can endure – especially during the summer. And, as the smallest cod are most vulnerable to the combined stress of high temperature and low oxygen, this slow-growing species is at genuine risk from catastrophic loss if their littlest ones succumb in the face of the deadly duo.

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Northern copepod mitochondria can't beat the heat



How hot is too hot and why is it too hot? Unravelling the physiology of how animals cope with warming environments

can tell us a lot about their evolutionary history as well as their current (and future) habitats. One possible mechanism by which an animal's physiology could limit its heat tolerance is if high temperatures interfered with routine but important metabolic processes such as making energy, ATP, by the mitochondria. Copepods, which often live in splash pools above the high tide line, adapt to the peculiarities of their particular pools. The copepod *Tigriopus californicus* has made its home in rock pools from balmy Mexico to chilly northern California, and has adapted its mitochondria to the vastly differing climes. With beakers of these copepods, descended from various populations, available in the lab, Tim Healy and Ron Burton from the Scripps Institution of Oceanography, USA, compared how quickly the mitochondria from different populations of copepods made ATP when in warm water and how that related to each population's natural habitat to find out whether their mitochondria influenced what temperatures they tolerated and, in doing so, their natural habitat ranges.

For each population, the researchers isolated mitochondria from several individuals and provided them with all the materials they needed to make ATP. The team then compared how quickly the mitochondria did this at a range of temperatures from 20°C to a scorching 36°C, and found that the mitochondria from copepods from cold climates generally made ATP faster than those from warm climates, especially at temperatures below 25°C; although that was not surprising, because some animals such as copepods can counteract the natural slowing effect of cold temperatures on their metabolism. However, the northern population's mitochondria were also much more sensitive to high temperatures, as their ability to make ATP declined sharply when the temperature was above the mid-30s. Meanwhile, the mitochondria from southern copepods resisted the heat and happily made ATP at temperatures hot enough to hinder making ATP in the northern populations. Taken together, this suggested that the mitochondria of each population are best adjusted to make ATP at the temperatures that they would naturally encounter in the wild. Healy and Burton then turned their attention to figuring out whether

the differences in mitochondrial physiology uncovered in the lab contributed to differences in heat tolerance in the wild.

The duo compared the temperature at which each population of copepods made ATP half as fast as normal with the temperature at which they stopped swimming, a sign that it was too hot for them, and discovered that the southern populations, whose mitochondria could churn out decent amounts of ATP at relatively high temperatures, also tended to keep swimming at hotter temperatures, supporting the idea that copepods hit their upper temperature limit when their mitochondria stop performing well. This suggested that having 'southern style' or 'northern style' mitochondria not only impacted which temperatures the copepods could make the most ATP, but also which temperatures they could survive in the wild.

Taken together, it is clear that northern populations of copepods like the cold, while southern populations like the heat, and how fast mitochondria can make ATP at these temperatures underlies at least some of this variation. But how copepod mitochondria react to heat does more than distinguish different populations – it also seems to set the maximum temperature where different populations can survive. As animals scramble to respond to the looming threat of climate change, understanding what sets the temperatures an animal can tolerate will be key to understanding why animals live where they do in the present and where they might live in the future.

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