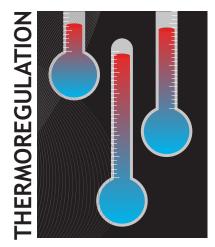


### **OUTSIDE JEB**

## Lazy thermoregulators take body temperature regulation shortcuts



Sloths are a physiological conundrum tiptoeing a fine, physiological line. As herbivores, sloths get all of their nutrients and calories from a diet of low-nutritional leaves, leaving them with relatively few calories to burn for bodily processes after foraging. (Imagine how much energy you would have after eating nothing but unflavoured rice cakes.) Because sloths do not obtain many nutrients or calories from their diet and they use what little energy they have to gather and digest that poor-quality food, it would make sense that they cut corners on other physiological processes, such as body temperature regulation. Daisuke Muramatsu, with collaborators from Kyoto University, Nara University of Education, Nagoya University and Hokkaido Research Center, Japan, and Universidade Federal do Amazonas, Brazil, investigated the body temperature patterns of wild sloths in the Amazon rainforest of Brazil by monitoring the heart rate and skin temperature in freeranging pale-throated sloths (Bradypus tridactylus) to find out how cheaply sloths regulate their body temperature on their low energy budget.

The researchers attached heart rate monitors and temperature data loggers to 34 free-ranging sloths. They caught the animals near the Federal University of

Amazonas in Brazil by climbing trees and gently taking the relatively undisturbed and unprotesting individuals back to the field lab. There, they used a custom-made harness to attach a modified sloth-friendly Fitbit to monitor each animal's heart rate, a temperature data logger for skin temperature measurements and a VHF transmitter to locate the individual animal to retrieve the precious data loggers. After attaching the harnesses, they released the sloths back to the trees where they had been captured and installed a temperature data logger on the trunk of the tree to measure the temperatures that each sloth experienced. Five days later, the scientists returned to the area and normally the animals had not moved too far.

Analysing the information stored by the skin temperature data loggers, the team discovered that the sloths were always slightly warmer than the trunk temperatures, but those elevated skin temperatures did not appear to be very costly for the animals, as their heart rates did not increase with the temperature. Because the researchers don't know exactly where the sloths were hanging out during each temperature measurement, they assumed that perhaps the sloths were basking in sunny spots when their body temperatures were warmer. The sloths seem to be taking an easy option for maintaining their body temperature. Instead of increasing heart rate or using up expensive fuel to increase or maintain their body temperature, the animals were probably relying more on the sun and environment to do that work for them. This dependence on basking could help explain how sloths are able to maintain their body temperature cheaply instead of constantly burning expensive fuel when on such a tight energy budget.

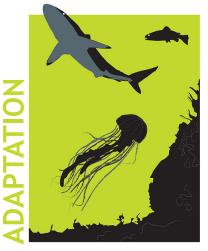
However, this study, posted online as a preprint at Social Science Research Network, has not yet been peer reviewed, so we should be cautious about some of the researchers' conclusions. Referring to the sloths' skin temperatures as body temperatures could lead to some incorrect conclusions about body temperature regulation, as the skin temperatures recorded by Muramatsu and colleagues are likely very different from core body temperatures. Skin temperatures tend to overestimate core temperature during warm environmental temperatures and underestimate it in cool conditions. Regardless, sloths are an exciting study species in which to study body temperature regulation, as they must strike a delicate balance between staying warm enough to keep their fermenting digestive microbiome happy, but doing so in a slow and measured way.

### doi:10.1242/jeb.243507

Muramatsu, D., Vidal, L. V., Costa, E. R., Yoda, K., Yabe, T. and Gordo, M. (2022). Low-cost thermoregulation of wild sloths revealed by heart rate and temperature loggers. SSRN doi:10.2139/ ssrn.4070573

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### Wild fish beat the heat



Wild animals can adapt to a wide range of temperatures. The ability to perform well in a constantly changing environment is called physiological plasticity. Physiological plasticity allows ectothermic animals, such as fishes, to maintain their metabolism even when the water that the fish is in heats up or cools down wildly. Fishes living in laboratories are less likely to have experienced temperature changes, so will their metabolism still perform well if there is a

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big temperature shift in their tank? Or have they lost their physiological plasticity because they have only experienced steady temperatures for more than 150 generations? Can living in steady environments affect a fish's behavior, metabolism or gene expression compared with living in a more turbulent environment? To study these questions, Rachael Morgan and colleagues from the Norwegian University of Science and Technology and the University of Greenwich, UK, compared the physiological plasticity of two populations of zebrafish: a wild population collected from India and a population raised in the lab. They aimed to understand whether living in a stable environment can change a fish's ability to perform when there is a big change in temperature.

The researchers collected 300 zebrafish from the two populations and exposed each group to a variety of temperatures (from 10 to 38°C) for 1 month. They then measured the fish's swimming ability, escape response, growth rate, metabolic rate and gene expression and compared the measurements of the two groups to learn whether the lab-raised group had more difficulty in adapting to large temperature shifts than the wild group because they have less physiological plasticity.

Sure enough, the lab-raised fish were less able to adjust to temperature change than the fish that had come from India. The lab-raised zebrafish were slower swimmers than wild zebrafish at all temperatures, and when escaping, labraised zebrafish responded more slowly than wild zebrafish, especially when in colder temperatures. In addition, the labraised zebrafish grew faster than the wild zebrafish, likely because domesticated animals are selected for faster growth rates. When comparing metabolism between the groups, the researchers measured the range of temperatures at which the fish could reach their maximum metabolic rates. They found that labraised fish could only reach their maximum metabolic rate in water temperatures between 26 and 36°C, but wild fish could reach their maximum metabolic rate over a much broader temperature range (21–38°C). Gene expression was also different among the two groups. The lab fish had greater levels of proteins that deal with stress compared with the wild fish. Interestingly, very cold

temperatures were especially difficult for both groups, which may suggest that physiological plasticity is limited at these extreme temperatures.

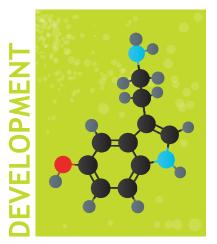
This study tells us that lab domestication affects the ability of animals to adjust to changes in their environment across several biological levels, from behaviour to gene expression. With this knowledge, scientists should take into account that domestication can change animals' responses to an experiment when choosing study animals. Morgan and colleagues also suggest that there may be a trade-off between high levels of physiological plasticity and growth rates and warn that domestication can quickly lead to a loss of physiological plasticity.

doi:10.1242/jeb.243506

Morgan, R., Andreassen, A. H., Åsheim, E. R., Finnøen, M. H., Dresler, G., Brembu, T., Loh, A., Miest, J. J. and Jutfelt, F. (2022). Reduced physiological plasticity in a fish adapted to stable conditions. *Proc. Natl. Acad. Sci. USA* 119, e2201919119. doi:10.1073/pnas.2201919119.

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# Ground squirrels hibernate through puberty



Comparative physiologists love studying trade-offs, the compromises that animals make to balance competing needs in challenging environments. If there is barely enough food to survive, then extracurriculars such as growth, reproduction or even routine activity will take a back seat. Thirteen-lined ground squirrels take this trade-off to the extreme, hibernating through much of the year in a coma-like state known as torpor. After observing several young squirrels become fathers almost immediately upon emerging from torpor, Rafael Dai Pra from Yale University, USA, and colleagues hypothesized that juvenile squirrels became sexually mature during their maiden hibernation season. Recently published in *Current Biology*, their research used a combination of endocrinology and neurophysiology to show that thirteen-lined ground squirrels approach trade-offs a little bit differently than other animals.

Dai Pra and colleagues monitored juvenile males going through their first ever hibernation season and took tissue samples to track how their reproductive system changed over time. Not surprisingly for animals that do not eat or drink, the squirrels lost 48% of their body mass during hibernation, largely from the depletion of fat reserves. Their body temperature plummeted to a chilly 4°C, only warming for brief periods of interbout arousal when the squirrels briefly regained consciousness. Losing mass, cold and inactive, hibernating squirrels clearly experienced a profound negative energy imbalance akin to starvation.

Starved and cold mammals rarely reproduce, as poor conditions inhibit the release of sex hormones, thereby delaying sexual maturation and activity. Not so for the squirrels. The serum levels of luteinizing hormone, which tells the testicles to make testosterone, and testosterone itself climbed over the hibernation period. Changes in the young squirrels' brains caused these hormonal changes: the hypothalamus of hibernating squirrels had far more cells expressing kisspeptin, a protein that triggers the release of luteinizing hormone from the pituitary gland, compared with active squirrels. Swimming in sex hormones, the young hibernating males' reproductive systems kicked into high gear: their testicles doubled in size between the time that they entered hibernation and the time when they emerged.

With clear evidence that thirteen-lined ground squirrels completed puberty during their first hibernation, the researchers turned their attention to understanding how they co-ordinated this process without light, food or warmth to stimulate them. To do this, they brought in a new set of juvenile males, this time holding them in comfortable conditions to prevent them from hibernating and monitored their body mass, fat stores and hormones, like the hibernating squirrels. The non-hibernating squirrels experienced similar changes in testosterone and testicle size as hibernators. They also gained lean mass and lost fat, both of which consistent with the effects of elevated testosterone. This means that thirteen-lined ground squirrels are capable of going through puberty regardless of whether they are fed or starving, cold or warm, hibernating or active.

As a species, thirteen-lined ground squirrels live an extreme life, having as little as 5 months to get all of their business done, such as mating, digging burrows and fattening up, before waiting out the rest of the year in suspended animation. Motivated by this tight deadline, they have figured out a way to prioritize reproduction when resources are scarce and, in doing so, show us that trade-offs are not as predictable as we might think.

#### doi:10.1242/jeb.243508

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Predator fishes are more intelligent than you might think



If you have ever snorkelled before, you would know that it is hard to catch a fish

in the ocean. Equally, trying to collect a fish from a tank is very difficult, sometimes almost impossible. You may think that being unpredictable makes it hard to catch fish, and in the marine world, avoiding being caught can mean staying alive. Even though behaving unpredictably may appear to be crucial for survival, a recent study in *Proceedings of the National Academy of Sciences* has shown that predators may be able to overcome the unpredictability of their prey and adapt to outwit them.

Although it is well established that unpredictable behaviour is common among a variety of species, only few studies have been able to recreate wildlife responses in a laboratory setting. Knowing this motivated Andrew Szopa-Comley and Christos Ioannou from the University of Bristol, UK, to find out how the predatory bright blue acara cichlid fish (Andinoacara pulcher) pursue a Bluetooth-controlled robot prey determine how they reacted to the robot victim's escape attempts. First, the researchers trained the elegant blue fish to chase the roboprey in a tank. Then, once the cichlids were happy to pursue the robot, the researchers divided them into two groups: one in which the roboprey always fled along the same departure angle, selected depending on the initial direction the roboprey fled during the fish's first successful trial; and a second group where the roboprey's initial escape angle varied from trial to trial. After recording a total of 117 trials over a 3week period, filming the fish to analyse their responses, Szopa-Comley and Ioannou determined the time the predators took to reach the roboprey, the predators' behaviour during the approach phase and their behaviour while pursuing the fleeing victim.

Despite the roboprey's sometimes unpredictable behaviour, Szopa-Comley and Ioannou determined that the blue cichlid predator took the same amount of time to capture its target, regardless of the roboprey's escape strategy. When the roboprey consistently took the same departure angle, the fastest fish always pursued the roboprey as it fled from the fish along its forward line of sight, which is the best direction for getting furthest from the attacking predator. Conversely, when the fish were presented with a robot that darted off in various directions, they had no preferred angle at which they hit a top pursuit speed. Instead, the fish that never had the chance to learn in which direction the roboprey would take off accelerated the hardest and hit their fastest speeds when they were beside the roboprey. In addition, the attacking fish slowed as the victim moved toward them. No matter how unpredictable the roboprey's evasive strategy, the predatory fish were always able to outmanoeuvre it.

Blue acara cichlid fish are intelligent enough to adapt to any unpredictable behaviour that their prey may have, although they pursue at slower speeds than fish chasing prey that always escaped at the same angle. However, acceleration rather than approach speed allows the predatory blue fish to capture their prey. This means that small guppies might be unable to evade their big bright blue nemeses. Who knows, maybe blue acara fish are the guppy's equivalent of our great white shark under-the-sea nightmare.

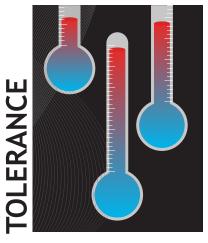
#### doi:10.1242/jeb.243505

Szopa-Comley, A. W. and Ioannou, C. C. (2022). Responsive robotic prey reveal how predators adapt to predictability in escape tactics. *Proc. Natl. Acad. Sci. USA* **119**, e2117858119. doi:10.1073/pnas.2117858119

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## Fishes working on their fitness



We all know that the world is getting warmer. Global temperatures are rising and extreme heat events are happening more often, which is particularly bad news for ectotherms (cold-blooded animals) that adapt to the temperature of their environment. For these organisms to survive extreme heat, some scientists say the ability to take up and use oxygen during these events is key. The absolute hottest temperature an ectotherm can tolerate is thought to be limited by how well they can get oxygen to the tissues (where it is needed in crisis mode). Factors such as heart condition, breathing rate and even blood chemistry could help (or hinder) how well oxygen is moved through the body. But what if you could train to improve your oxygen transport abilities? Could you then handle the heat?

These are the questions Daniel Gomez Isaza from Murdoch University, Australia, and Essie Rodgers from the University of Canterbury, New Zealand, asked. Exercise has been shown to increase heart size and the ability of blood to carry oxygen, so the scientists predicted that fish could improve their thermal tolerance by increasing exercise. As salmon are active fish, migrating long distances and often living in fast-flowing water, the duo trained young Chinook salmon every day for 1 h by taking them for a 'group run' in a swim tunnel, which acts as a fish treadmill. The researchers set the water flow rate so that fish were swimming at about 60% of their maximum sustainable speed; think easy recovery runs instead of sprint workouts. Then, after 3 weeks the researchers put the salmon to the test.

Would exercise training improve the fish's oxygen transport and their thermal tolerance in turn?

To find a fish's upper thermal limit, Gomez Isaza and Rogers warmed the fish's tank gradually until it reached a temperature where the fish toppled over and couldn't right itself again. Immediately following this, they took a blood sample to see whether the combination of the high temperature stress with the exercise regime had affected the blood's ability to carry oxygen, and they also collected samples of the fish's heart and spleen (a red blood cell factory).

However, the duo found that exercise training did not actually impact the fish's ability to transport oxygen around their bodies. There were no changes in the amount of red blood cells or the protein that carries oxygen (haemoglobin), nor were there increases in heart or spleen size. Only immediately after the thermal tolerance test did they see an increase in the blood's oxygen carrying abilities, which was similar in exercised fish and fish that simply relaxed in the tunnel each day. With no differences in oxygen transport between the exercised and control fish, it was not surprising to find that the thermal tolerances of both groups were similar.

That's not to say that exercise might not still be beneficial. The exercised fish were able to tolerate slightly higher temperatures than their unexercised counterparts, managing to remain upright at temperatures up to 28.60°C, while the unexercised fish toppled over at 28.24°C. This might not be ecologically relevant if a heatwave of 29°C occurs, but it gives us some insight into the effects of exercise training. It seems that many factors could affect not only the oxygen transport abilities but also the thermal limits of the fish. And it is possible that exercising at 60% of maximum swim speed for an hour might not have been the ideal training plan for juvenile Chinook salmon, even though it has increased swim performance, active metabolism and energy stores in other species. Perhaps a longer exercise regime might benefit the Chinook's thermal tolerance. Many questions about the impact of exercise remain to be explored.

doi:10.1242/jeb.243509

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