OUTSIDE JEB

How summer sleepers stave off desiccation

Have you ever left your house on a sweltering day and immediately gone back inside because it is too hot to even think? Not all creatures have the luxury of escaping torrid heat, so some have found ways to survive even when their homes become as dry as a bone. They can either deal with water shortages and scorching temperatures or use a hibernation-like ‘summer sleep’ called aestivation, when they become inactive and shut off most biological processes except those necessary for survival. Aestivating animals such as earthworms can alter which genes they turn off and on to survive until the rains return. These stoic burrowers are also true unsung heroes in our battle against the effects of climate change because they help store carbon in the ground and help other smaller animals by providing shelter while they undergo aestivation. Since scientists predict climate change will increase the number of droughts, figuring out how these climate change mitigators aestivate during an exceptionally dry summer is critical. Natasha Tilikj and Marta Novo from the Complutense University of Madrid, Spain, investigated which genes earthworms (*Carpetania matritensis*) from the Iberian Peninsula activate and switch off during aestivation.

The duo collected earthworms from El Molar, Spain, and returned with them to the lab. Once there, the team needed the right soil conditions to produce both active and aestivating worms – when they tie themselves into mucus-covered knots – to compare the two. To reproduce the correct conditions, they placed some worms in dry soil to encourage them to begin aestivating and maintained the remaining worms in moist soil to keep them active. After a month, the team checked whether or not the worms were aestivating and then analyzed how the animals changed the patterns of gene expression – which genes they upregulated and which they downregulated – after slipping into a summer sleep.

Unsurprisingly, the team found that the aestivating worms reduced expression of most genes. Other aestivating animals conserve energy by destroying and producing fewer proteins, to extend their state of suspended animation until conditions improve, and worms could be using the same strategy. The aestivating worms reduced expression of genes involved in the production of proteins and other macromolecules in addition to genes involved in protein breakdown and digestion. As worms go into this state of dormancy, they shut down processes that are not vital for survival. The team also found evidence that the worms were under stress and increased the levels of toxic oxygen by-products, known as reactive oxygen species, which can damage DNA after aestivation. In addition, there was an increase in the expression of genes that typically help combat and repair the DNA damage caused by these harmful products, and the stress experienced by the worms also activated the worms’ immune response for protection from possible infection.

Finally, the team discovered that aestivating worms may stave off water loss using an innovative mechanism that researchers have yet to study in earthworms: the worms increased the expression of genes involved in the production of the amino acid arginine. The duo suggest that an increase in arginine, or a build-up of nitrogenous waste, might reduce the amount of water lost by the worms.

Aestivating earthworms employ various strategies to survive desiccation. With increasing drought in the future, whether these protective mechanisms will be sufficient is unknown. The researchers recommend further investigation into the role of digestion, excretion and the central nervous system in aestivation to help scientists design better drought mitigation strategies to protect these uncelebrated champions of the subterranean world.

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A cozy, grizzly superpower

Grizzly bears can hibernate, and I’m jealous. Grizzlies eat to excess and fatten themselves up without health problems and, for all their gluttony, they’re rewarded with half a year of rest. I’d love to use hibernation as an excuse for my overeating. While I barter with my landlord to keep my apartment warm, grizzly bears bask in the cold: they

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reduce their metabolism and temperature so they can save energy throughout the winter. They've turned being lazy into a superpower, so of course I want to know how they do it. Luckily, a recent study by Hannah Hapner Hogan and colleagues at Washington State University, USA, sought to answer this very question. When grizzly bears hibernate, how do their cells know to slow down and avoid disease? Is their metabolism slowed because of something in the blood?

Hogan and colleagues started by taking fat cells from grizzly bears (*Ursus arctos horribilis*) – some hibernating, some awake – and growing them in dishes. Then, they sampled blood from bears in and out of hibernation. This allowed the researchers to mix and match the fat cells with different blood serum samples from awake or hibernating bears to grow 'awake' or 'hibernating' cells. Was serum from hibernating animals enough to make awake cells burn less energy, like the cells of hibernating animals? It turns out that it is. When fat cells that had been collected from active bears were grown in serum from hibernating animals, they had lower metabolisms; they consumed less glucose, they respired less oxygen and they produced less energy (ATP). Serum from active animals had the opposite effect: it kick-started the metabolism of hibernators, increasing glucose and oxygen consumption to make more energy. It turns out that blood serum had a strong effect on cellular metabolism.

Blood serum also influences where hibernators get their energy from. Cells have two favourite foods: glucose and fatty acids. Hogan and her team found that active cells favoured glucose much more than hibernating cells; hibernating cells preferred fat. This makes sense; bears fatten themselves up before hibernating, so it follows they would use this fat.

But the researchers also found that hibernating cells were picky eaters. They gave cells insulin, a molecule which tells cells to absorb and use glucose. Active cells readily responded to insulin by eating more glucose. But when given insulin, hibernating cells stubbornly continued prioritizing fats. Moreover, serum from the opposite season could flip these results: hibernating cells responded more to insulin when they were kept in awake serum, and awake cells resisted insulin while in hibernating serum. The Washington State team noted just how fascinatingly different bears are from humans. Bears fatten themselves up and ignore insulin without issue. But if we humans gained so much fat within a few months and developed insulin resistance, we would suffer diabetes.

The team's work tells us that the profound drop in metabolism during hibernation is due at least in part to the blood instead of some intrinsic mechanism inside cells. As a next step, Hogan and colleagues are analyzing blood proteins to learn exactly what causes the drop in metabolism and insulin sensitivity. Imagine what secrets bear blood could contain! I can picture an EpiPen-like autoinjector – a SleepiPen – capable of inducing hibernation on command, whether to buy time during medical crises or to extend the shelf life of organs for transplant. But for now, I'm left to wonder what magic bear blood contains, and to dream of a cozy, grizzly future when science will help me rest through winter.

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Baby birds beat the heat thanks to singing parents

Baby zebra finches hatch into one of Earth's most inhospitable environments, the Australian outback, where summer temperatures can exceed 40°C. Fortunately, zebra finch parents can prepare their young for this dangerously hot climate by singing to them before they hatch. When temperatures rise above 26°C, parents emit 'heat calls' that warn their embryos of high environmental temperatures. Unlike their normal vocalizations, heat calls have a high-pitched frequency, a fast rhythm and can prompt embryos to develop differently in preparation for the heat. Notably, birds that are serenaded by heat calls as embryos tend to be relatively small, a characteristic thought to help them lose heat more efficiently and produce more young throughout adulthood. Despite the lasting influence of heat calls on zebra finch physiology, the mechanism through which they act remains a mystery.

Led by Eve Udino (Deakin University, Australia), a team of researchers also from Deakin University, Clemson University, USA, and the Doñana Biological Station, Spain, questioned whether the energy-generating units inside cells (mitochondria) are the missing piece to this musical puzzle. Mitochondria use oxygen to convert food into cellular energy (ATP), while a portion of this oxygen is also diverted to produce heat. Since changes in mitochondrial function during development can have long-term effects on animal physiology, Udino and colleagues suggested that zebra finch mitochondria may be responding to heat calls produced by their parents. The team collected eggs from zebra finches maintained at an outdoor aviary in Geelong, Australia, and incubated the eggs at a blistering 37.5°C. A few days before they were due to hatch, the team played recordings of heat calls to the developing embryos. The newly hatched chicks were then raised in nests at various temperatures, ranging from 22°C to 34°C. Once they turned 13 days old, the team obtained some blood from the chicks to assess the function of the mitochondria in their red blood cells.

The researchers first discovered that nest temperature can alter how well zebra finch mitochondria work. The birds raised in hotter nests had less

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efficient mitochondria, meaning that their mitochondria produced less ATP and consequently more heat than the mitochondria of birds raised at cooler temperatures. Remarkably, embryonic exposure to heat calls improved mitochondrial efficiency across all nest temperatures (22–34°C), suggesting that heat calls re-program how zebra finch mitochondria balance ATP versus heat production.

While a nest temperature of 34°C may seem hot, zebra finches can experience temperatures that are much, much higher. Therefore, Udina and colleagues took the study one step further and exposed a group of the 13-day-old birds to a brief heatwave, warming the youngsters' nests to 44°C over the course of 2.5 hours before checking the mitochondria again. This time, the mitochondria of birds that had listened to heat calls as embryos tended to be less efficient. Although this may seem disadvantageous, the team suspects that there may be benefits to reducing mitochondrial efficiency under extreme heat conditions. High rates of mitochondrial ATP production can produce toxic reactive oxygen species, compounds that can wreak havoc on proteins, lipids and DNA. Making mitochondria less efficient at extremely high temperatures may help birds reduce the damaging effects of reactive oxygen species.

We often think that a bird's life begins once it hatches, but birds can sense and respond to events going on in the outside world before they enter it. As zebra finch parents sound the heat alarm, their embryos get to work altering their physiology. So, next time you hear zebra finches calling in the Australian heat, don't think of their sweet songs as 'music to the ears', but rather, as 'music to the mitochondria'.

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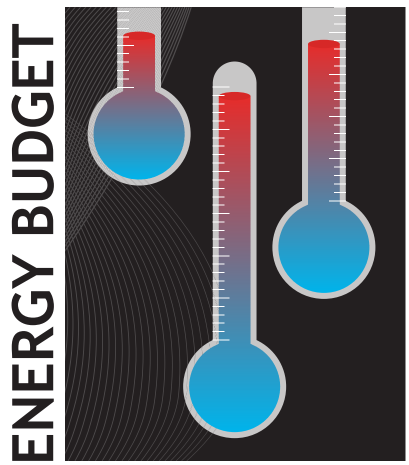
Migrating hummingbirds put a new twist on torpor



Hummingbirds are some of the most metabolically active birds, beating their wings 50 times per second while sipping nectar from flowers and, as a consequence, they live their lives on a metabolic knife edge. For some species – such as ruby-throated hummingbirds (*Archilochus colubris*) – these energetic challenges are made worse by the need to engage in other taxing activities such as a 5000 km autumn migration from Canada to Costa Rica. So it makes sense that these birds would take advantage of any physiological tools available to ensure that they have sufficient metabolic fuel in their daily struggle to survive. One important tool that hummingbirds use is torpor, an energy-saving strategy where metabolism, activity and body temperature are suppressed for short periods of time. These birds generally use torpor to make it through cold nights when their fat stores are low. Although this behaviour is well understood in many contexts, Erich Eberts from the University of Toronto, Canada, and colleagues from the same institution and the University of Western Ontario, Canada, wanted to understand if these birds could alternatively use torpor as a way to maximize their fat stores as they entered their migration season.

The scientists caught hummingbirds in London, Canada, and housed them at the University of Western Ontario, simulating the daylight conditions that the birds would experience during the transition from the midsummer breeding season (15 h light:9 h dark) to the migratory season (12 h light:12 h dark). During this period, the researchers tracked changes in fat content and body mass of individual birds to determine when they began preparing for migration. The team also measured the metabolic rate of these same birds as they slept to determine when and if these birds reduced their metabolism and switched into torpor.

During the midsummer, the thinner hummingbirds with lower fat stores dipped into torpor earlier and remained torpid for longer – confirming that these birds use torpor when they had the least energy available. But as these birds readied themselves for migration – as days became shorter – their entry into torpor became more predictable and fatter birds went into torpor more often. By abandoning the 'old rules' surrounding the use of torpor as a survival strategy, hummingbirds are able to reduce their metabolism to stock up on fuel to ensure that they can maximize their energy stores for migration.

Life for hummingbirds involves a constant battle to ensure that they have plenty of metabolic fuel. Eberts and colleagues have shown that these birds can change the rules that govern the use of torpor based on their environmental conditions. This means that torpor is something of a metabolic Swiss Army knife – a multifunctional tool that animals can repurpose to increase their odds of survival. This research opens up new and exciting questions about how hummingbirds control this switch in torpor use and whether other animals are similarly capable of bending the rules.

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Macaques get their tongues in a twist

Primates chew their food on one side of their mouth. Try it with a sandwich: take a bite, and immediately the piece of food will be ushered to the left or right cheek teeth. This action occurs when the jaws open up and happens because of one mysterious and versatile structure – the tongue. Whilst it is easy to see the action of the upper and lower jaw when we eat, it is far harder to know how the motion of the tongue simultaneously pushes food around, as it's encased by the flesh and bone of our heads. So far, some have theorised what might be happening, but no one has yet been able to see the motion of the tongue in three dimensions.

Kara Feilich and Callum Ross from the University of Chicago, USA, wanted to know how primates use their tongue during eating. Together with their colleagues Jeffrey Laurence-Chasen (University of Chicago, USA), Courteny Orsbon (University of Vermont Medical Center, USA) and Nicholas Gidmark (Knox College, USA), Feilich and Ross set out to shed light on how the tongue moves during the ingestion of food. They trained four rhesus macaques to sit and eat grapes whilst being filmed using a three-dimensional x-ray system. This allowed the researchers to see inside the mouth whilst the macaques ate their grapes, to figure out how the tongue makes its twists and turns.

Feilich found that the bending of the tongue was in synchrony with the opening–closing motion of the jaws. After biting down on the grapes, the jaws opened whilst the body of the tongue pushed the food sideways by rolling to one side, feeding it between the upper and lower cheek teeth – all ready to be chewed. You'll be aware of doing the same thing, perhaps even more so when it goes wrong. If you've ever bitten your tongue during eating, chances are you've over-enthusiastically squashed your own tongue into your gnashing teeth.

Analysing the tongue's manoeuvres, Feilich and colleagues realised that the tongue bending came about by asymmetrical changes along its length. Whilst the tongue rolled towards the chewing side, it simultaneously bent itself away, so that the tip pointed to the opposite side – perhaps to avoid the pain of being chewed on. Feilich pondered how the tongue contorts itself into these complex shapes. Assuming that the tongue keeps a constant volume, asymmetrical muscle contractions could produce the tongue-twisting shapes demonstrated by the macaques.

Primates' lingual dexterity assists in other functions too, such as vocalisation and grooming. But despite this varied repertoire, Feilich and colleagues found that tongue motion during eating was remarkably consistent, which hints that this pattern is important for primates in general, you and I included. Irrespective of the occasional masticatory mistake, Feilich and colleagues revealed the tongue's twisting role in shifting food, which can be further studied by medical colleagues to help patients recovering from tongue damage and neuromuscular disorders.

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