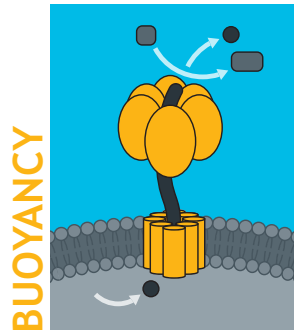


## OUTSIDE JEB

### Aquatic midges take an acid trip



Many years ago in Copenhagen, future Nobel Prize winner August Krogh did what he did best: study a peculiar animal for a very particular reason. He focused on the gas-filled sacs of *Chaoborus* midges, which he found curiously reminiscent of fish swim bladders. Through a series of experiments, Krogh demonstrated that the midges used the sacs as ballasts, expanding them to float and contracting them to sink, but he couldn't figure out how they accomplished this feat and eventually moved on to other interests. Now published in *Current Biology*, graduate student Evan McKenzie of the University of British Columbia and colleagues solve Krogh's century old mystery, revealing that it all came down to a common insect protein used in a unique way.

*Chaoborus* air sacs are unusual: they are completely cut off from the respiratory system and enclosed by a wall made of alternating bands of chitin, a hard polymer found in insect shells, and an unidentified protein. Curious about these mysterious bands and their potential role in regulating buoyancy, the authors examined the sacs under a microscope. Surprisingly, the sacs glowed bright blue under UV light. As zoologists well-versed in the idiosyncrasies of insects, they knew that this could only mean one thing: the unknown protein was resilin; a stretchy, biological rubber found in the elastic tendons of locusts, fleas and other arthropods. An unusual quirk of resilin is that it swells under certain conditions such as high pH. The scientists reasoned

that reversible swelling or shrivelling of the resilin bands within the air sac wall could alter the sac's volume and, therefore, adjust the midge's buoyancy. They tested this idea by exposing freshly dissected air sacs to acidic or basic conditions, finding that the air sacs shrunk up to 20% in acid and expanded by 45% in base, confirming their hunch.

The midges only have to change the pH around their air sacs to float or to sink. Like many organisms, insects control the pH of their guts, mitochondria and other compartments with specialized proteins called proton pumps that move hydrogen ions across cell membranes. The researchers tested if these ubiquitous proteins also controlled the pH of the air sac by exposing them to drugs that directly or indirectly interfered with proton pumps. When the team did so, the sacs expanded like they did in basic conditions, confirming that pH controlled the air sac volume and that a specific type of proton pump called vacuolar-type H<sup>+</sup> ATPase controlled the pH.

Other insect tissues turn this proton pumping ATPase on or off using cellular signalling pathways involving compounds like cyclic AMP or cyclic GMP. To find out whether the midges use either of these pathways to regulate their buoyancy, the researchers exposed dissected air sacs to synthetic versions of AMP or GMP, each attuned to different branches of the signalling pathway, and observed which compounds caused the sacs to expand. This experiment revealed not only that cyclic AMP co-ordinated the expansion of the air sac, but also identified two more classes of proteins, protein kinase A and cyclic AMP-activated exchange proteins, which are involved in the pH-control signalling pathway, setting the foundation for deeper dives into the unusual physiology of these insects.

*Chaoborus* midges are the only planktonic insects – in fact the only non-fish – that regulate their buoyancy using a gas bladder and they are the only organism to use resilin as a pH-activated

means of motion. Unique on three counts, it is perhaps not surprising that they caught the attention of August Krogh or that it took scientists over 100 years to understand how their unique organ works.

doi:10.1242/jeb.243484

McKenzie, E. K. G., Kwan, G. T., Tresguerres, M. and Matthews, P. G. D. (2022). A pH-powered mechanochemical engine regulates the buoyancy of *Chaoborus* midge larvae. *Curr. Biol.* **32**, 927-933. doi:10.1016/j.cub.2022.01.018

**Brittney G. Borowiec (0000-0003-0087-7577)**

**Wilfrid Laurier University**  
bborowiec@wlu.ca

### Spring showers put a dampener on reproduction success



Bat mothers invest incredible amounts of energy in raising a pup. Typically, bats mate in the autumn and hibernate over winter; however, the females do not become pregnant until the following spring and give birth in the summer. To sustain pregnancy, bats feed on insects, such as midges and flies that can become rarer when poor weather sweeps in. In addition, younger bats need to hone their insect-catching skills which can also impair their ability to raise pups. Bianca Stapelfeldt from the University of Greifswald, and colleagues from the Bat Research Project, Germany and the Nossentiner/Schwinzer Nature Park, Germany, set out to determine how weather affects the reproductive success of adult female Natterer's bats (*Myotis nattereri*) of different ages.

Beginning in 1990 and continuing every year since, researchers have collected Natterer's bats from bat and bird boxes in the Nossentiner/Schwinzer Nature Park in Mecklenburg-Pomerania, Germany, between July and September. The researchers measured the body mass, forearm length (measurement of body size) and identified the bat's age (whether they were adult or juvenile), before marking the bats with forearm bands to keep track of individuals through the years. In addition, the scientists noted whether the adult females had given birth that year by assessing the fur around the nipple; if there was fur, then the bat likely did not have a pup, while a lack of fur meant that she had given birth to a pup that season. Over the decades, the team also monitored archived climate data from a nearby meteorological station in Goldberg, Germany, and identified the average and lowest temperatures, the daily precipitation levels and the overnight wind speeds and total precipitation levels from 2003 to 2020.

The team found that over the 17-year period, the younger adult female bats had less success in birthing and rearing baby bats, compared with older adult females, likely because the younger mothers may be unable to provide themselves with the immense amount of energy required during pregnancy and when feeding their pups. The researchers speculate that younger adult bats may forgo pregnancy to avoid investing energy into a pup that they may be unable to raise. Interestingly, wetter springs also resulted in lower birth rates in younger adult bats, which was not seen with the older mothers. The researchers suspect that rainy periods make it difficult for the bats to echolocate and hunt for food and to stay warm. Therefore, poor weather may cause the bats' body weight to reach a critical low, so that younger bats may forgo pregnancy altogether.

In addition, the researchers identified a 2-week window (from the end of April to 11 May) where poor weather had the greatest negative impact on the females' pregnancies. It is unknown when Natterer's bats become pregnant, but this 2-week critical period is the same time pregnancies begin for other bats across Europe. Since pups have little time to grow and prepare for hibernation, adult females cannot delay their pregnancies any later, and so may abandon

pregnancies if they experience adverse weather during this pivotal period.

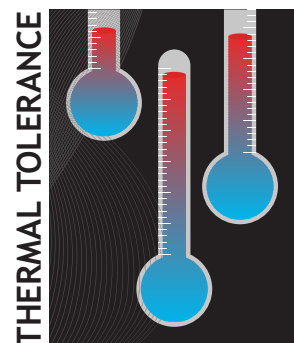
Stapelfeldt and colleagues have successfully shown the impact of poor weather on the pregnancy and baby rearing by bat mothers using long-term monitoring of a bat population and the weather conditions they encounter. Climate change is an imminent threat worldwide and many European countries are likely to become increasingly wet during this critical period for bat pregnancies, which could dramatically affect the ability of bat populations to reproduce. Increases in rainfall during the spring may be a particular threat for European bat species at risk of extinction.

doi:10.1242/jeb.243482

**Stapelfeldt, B., Scheuerlein, A., Tress, C., Koch, R., Tress, J. and Kerth, G. (2022).** Precipitation during two weeks in spring influences reproductive success of first-year females in the long-lived Natterer's bat. *R. Soc. Open Sci.* **9**, 211881. doi:10.1098/rsos.211881

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

## Aussie trout handle the heat



Some fishes can make themselves at home anywhere, with several species thriving in subzero Antarctica while others live in temporary tide pools. Within a single species, some populations are locally adapted to particular habitats based on what they've become accustomed to over the years. However, not all species show this flexibility. To make predictions about how fish populations will change with global warming, scientists are trying to understand which fish can adapt to the heat and why.

While rainbow trout are generally thought of as a cold-water fish of the Pacific Northwest, a specific population has thrived in the desert of western Australia for almost 50 years. Isolated from their original population of the San Francisco Bay area, these fish have been used to stock natural lakes, and have undergone a few traumatic heat events during their time in Australia. More than once, summer temperature extremes have killed many fish that couldn't handle the heat, unintentionally selecting for fish with impressive upper temperature limits. Olivia Adams from the University of British Columbia, Canada, with colleagues from the same university, Harvard University, USA, the University of Western Australia and Aquatic Life Industries in Perth, Australia, wanted to investigate if a lengthy exposure to warm temperatures would improve this population's already remarkable ability to withstand extreme heat.

To answer this question, the team raised groups of 1-year-old trout at six different temperatures ranging from 15 to 25°C for at least 1 month. Typically, to understand how fish cope with increased temperature, scientists focus on one or a few measures, such as growth or upper thermal limit, in a single study. But in the most comprehensive study to date, Adams and colleagues were able to look at how temperature affected the growth, energy it takes to digest a meal, physical fitness, upper temperature limit, ability to withstand low oxygen, and heart rate of these animals raised at 15, 17, 19, 21, 23 and 25°C.

The Australian rainbow trout showed off their skills, with just about every aspect of their performance peaking in the groups of fish raised at 17–23°C. At these temperatures, fish had the best growth, used the least energy to digest dinner and, when exercised, were the most physically fit. These fish could even endure temperatures up to 31°C before toppling over (which is how scientists typically measure a fish's upper thermal limits). Impressively, the fish raised at 25°C were still able to grow and could also handle temperatures up to 31°C before losing their balance, but they began to struggle with digestion and fitness, and couldn't tolerate low oxygen as well as the other groups. And when the scientists tested how the fish hearts handle a brief heat exposure, they actually started to fail at

27°C, well below the temperature at which they topple over. While fish raised at 25°C show some remarkable performance in growth and increased upper thermal limits, they are noticeably limited in other ways. By using a variety of tests, Adams and colleagues presented detailed evidence that this population optimally perform at 21–23°C, which is an incredible feat when rainbow trout are thought to do best below 20°C.

While clearly superstars, Australian rainbow trout aren't the only trout population that can handle the heat. Their cousins, redband rainbow trout, are geographically isolated in the desert region of the Pacific Northwest, where they regularly encounter summer temperatures between 19 and 29°C. Adams and colleagues suggest that rainbow trout may naturally carry the genes that they require to tolerate high temperatures. With a combination of artificial and natural selection, populations like the Australian rainbow trout have been able to tap into that latent heat-tolerant talent and give us a bit of hope for the future in a warming climate.

doi:10.1242/jeb.243485

Adams, O. A., Zhang, Y., Gilbert, M. H., Lawrence, C. S., Snow, M. and Farrell, A. P. (2022). An unusually high upper thermal acclimation potential for rainbow trout. *Conserv. Physiol.* **10**, coab101. doi:10.1093/conphys/coab101

Angelina Dichiera (0000-0002-9635-0229)  
The University of British Columbia  
dichiera@zoology.ubc.ca

## Cold, cheap love songs



Songs help birds attract mates by advertising their fitness or territory to potential mates. Because the sexiest songs are assumed to be energetically expensive, or in some way related to a certain attractive attribute, potential mates

can judge the singers to learn more about their qualities and potential as an eligible mate. In much the same way that birds use song, some bat species also use complex songs either to boast about their strength or to lure mates into their territory. The New Zealand lesser short-tailed bat (*Mystacina tuberculata*) is one of only two bat species where courting males congregate together – forming a lek – where they perform serenades within each other's earshot in the hope of attracting females. To be considered sexy, the quality of the bats' songs should be costly or related to the beauty of their territory. Yet, if the songs were expensive to produce – somehow revealing the singer's inner strength – bats might hypothetically try to cut corners in other expensive areas of their lives, such as the body temperature they maintain. Kathleen Collier from the University of Auckland, New Zealand, along with collaborators Zenon Czenze from the University of New England, Australia, and Stuart Parsons from Queensland University of Technology sought to estimate the costs of singing for lek-breeding bats, using skin temperature, to estimate how much energy singing uses.

To study this unique phenomenon of communal singing in bats, the researchers attached temperature-sensitive radio transmitters to the backs of 14 male New Zealand lesser short-tailed bats at their singing roosts. The researchers then recorded the bats' skin temperatures while the mammals quietly went about their usual routine and also when they were singing. The researchers then used the skin temperatures collected by the radio transmitters to estimate the metabolic rate of the bats during both activities.

Throughout 100 nights of observation, the researchers found that the bats were not significantly warmer or cooler when singing. And when the team compared the bats' estimated metabolic rates, they also were not significantly different. The bats were not exerting themselves while singing out their hearts for a mate, implying that their singing may not be as much of an energetic sacrifice as was previously thought. However, it is possible that using skin temperature to estimate metabolic rate may have missed some other important energy consuming activities – such as panting to keep body temperature close to normal – and may have hidden the animals' actual metabolic

rates while singing. Yet, even though these data are just rough estimates of the bats' actual energy use, they do allow us to compare between serenading and non-serenading behaviours, which had not been possible before.

This study is also the first to have investigated the energetic costs of singing in a mammal and suggests that the costs may be less than previously thought. If these mating rituals are less energetically expensive, then what messages could these displays actually be sending to potential mates? In fact, are potential mates even getting what they thought they were investing in? Even though that isn't clear right now, it isn't deterring male New Zealand lesser short-tailed bats from getting together and serenading in the hope of attracting an eligible date.

doi:10.1242/jeb.243483

Collier, K., Parsons, S., Czenze, Z. (2022). Thermal energetics of male courtship song in a lek-breeding bat. *Behav. Ecol. Sociobiol.* **76**, 36. doi:10.1007/s00265-022-03141-5

Ana M. Breit (0000-0002-4990-3353)  
University of Maine  
ana.breit@maine.edu

## Scientists may have discovered why Speedy Gonzales is always fast



Almost every person has walked or run on the beach at some point in their life. Moving on loose sand feels more challenging than walking on a sidewalk. In general, walking on sand requires more work and energy and this is not unique to humans. Now, you may also be familiar with Speedy Gonzales, a Looney Tunes cartoon mouse who runs super-fast all the time. You may wonder how Speedy can always run so fast across all the different terrains that he encounters, but a very similar looking animal to Speedy

Gonzales in real life, the kangaroo rat (*Dipodomys deserti*), may prove that the cartoon's versatility is not so far off reality.

Some researchers have previously tested different types of animals, such as lizards and ostriches, running over solid surfaces versus sandy surfaces. This line of research motivated Joseph Hall (Washington State University, USA), Craig McGowan (University of Idaho, USA) and David Lin (Washington State University) to study a hopping animal, the kangaroo rat, to expand on our current understanding of the effects of different types of ground on animal movements. These little hopping animals are equally adept moving across rocky and sandy terrains, both of which exist in their natural desert habitat. So, the researchers cleverly developed the very first variable terrain rotatory treadmill and put these animals to the test hopping on a solid and a sandy surface while controlling the speed and recording video of the animals' manoeuvres.

However, before the kangaroo rats were allowed to hop in place, the team had to ensure that the level of sand on the treadmill was sufficient to prevent the animal from touching the solid base beneath. To do this they designed a device that can measure how far the rodents' feet would penetrate the sand and determined that at least 1.5 cm of sand was sufficient.

Although the six desert kangaroo rats had to move at five different speeds, ranging from 1.25 to 2.25 m s<sup>-1</sup>, Hall and colleagues only analysed the motion of the little rodents' hind legs at 1.8 m s<sup>-1</sup>, the speed at which these animals tend to hop in the wild. Comparing the animals' hopping styles on solid and sandy surfaces, they found no significant difference in the amount of time the animals' feet were in contact with the ground or the distance covered during each hop. Also, the researchers found no meaningful difference in the rodent's speed and acceleration between the two different surfaces, although the

animals were more crouched when hopping on sand.

Even though the environment has induced changes in the running styles of some animals, it appears that kangaroo rats have evolved so that changes in the surface they hop around on does not affect their speed. They maintain very similar physical hindlimb movements regardless of the firmness of the surface that they are moving over, suggesting that they are finely tuned to their environment. And even though Speedy Gonzales is a runner and kangaroo rats hop, the discovery that kangaroo rats are perfectly tuned to move on hard and soft surfaces could hold the key to Speedy's super-fast reputation.

doi:10.1242/jeb.243481

Hall, H., McGowan, C. and Lin, D. (2022). Comparison between the kinematics for kangaroo rat hopping on a solid versus sand surface. *R. Soc. Open Sci.* **9**, 211491. doi:10.1098/rsos.211491

**Jonaz Moreno Jaramillo (0000-0003-4640-8573)**

**University of Massachusetts, Amherst**  
jmorenoj@umass.edu