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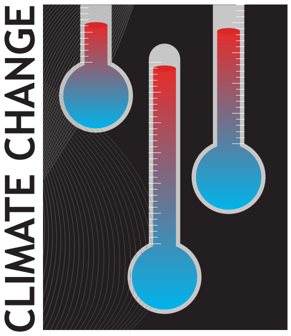
OUTSIDE JEB

Diapausing insects don't like it hot



Many insects undergo phases when they become inactive and dormant – known as diapause – to survive harsh seasons, such as winter or dry periods. They switch off or dramatically slow growth and metabolism, which ultimately reduces their energy demands. Diapausing insects mostly rely on daylength and temperature to anticipate and get properly geared up for the arrival of harsh conditions. Whether or not climate change is driven by human activity, there is little doubt that global warming represents a new hurdle for many seasonal organisms to overcome. Research has already shown that warm winters can reduce the mass, survival and fecundity of several diapausing insects. However, organisms need to enter diapause some time before the onset of inhospitable conditions and a much less explored question is what happens when they cannot best figure out when winter will actually begin.

Matthew Nielsen from the University of Stockholm, Sweden, with colleagues from Stockholm University and Greifswald University, Germany, was interested in finding out whether warmer or longer pre-winter conditions have an impact on the survival of diapausing insects. To test this, they exposed diapausing pupae of the green-veined white butterfly (*Pieris napis*) – which is widespread throughout much of Europe and Asia – to different pre-winter temperatures (ranging from a mild 15°C to what would be considered an extremely warm 25°C in their natural environment) for different durations (from 1 to 16 weeks). Then all of the butterflies experienced the same standardised winter conditions (2°C) for 24 weeks, followed by two consecutive weeks at 17°C to simulate spring and induce the emergence of the adults from their pupal cases. The researchers tracked how the pupae's masses varied at regular intervals for up to 42 weeks, as well as their survival. In addition, they also assessed changes in the metabolic rates of a group of the insects during the pre-winter period by placing each pupa in a sealed small syringe to measure how much CO_2 they exhaled.

The team found that warmth in the run up to winter led to a substantial loss of mass in diapausing butterflies and this loss became greater as the pre-winter periods grew longer. The leaner insects also experienced higher metabolic rates during pre-winter, likely caused by an increase in their energy expenditures to cope with the heat. Yet, although these conditions did not lead to higher death rates in the pupae in the run up to winter, their impact became clear after winter, with fewer of the pupae that had experienced longer and warmer pre-winters surviving to become adults. However, the butterflies that managed to survive winter after experiencing a long warmer pre-winter were leaner, suggesting that these adults were probably less well prepared for life in the future.

In summary, Nielsen's study suggests that a warmer climate may challenge the fate of seasonal organisms and that longer warmer pre-winters could reduce the survival of overwintering insects. The study also highlights the importance of studying the impact of climate change over the entire life cycle of an organism as conditions experienced at one point in life can have delayed effects at later stages.

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Nielsen, M. E., Lehmann, P. and Gotthard, K. (2022). Longer and warmer prewinter periods reduce post-winter fitness in a diapausing insect. *Funct. Ecol.* **36**, 1151-1162. doi:10.1111/1365-2435.14037.

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Hibernating bats may use protein stores for water balance

All hibernating bats need to eat enormous amounts of food during autumn to build up a substantial fat store for hibernation. These large fat stores supply the immense amount of energy needed to survive without access to food and water. However, little is known about how the breakdown of proteins from muscles and organs contribute to the hibernating animal's energy stores and possibly their water budget. Liam McGuire from Texas Tech University, USA (now at the University of Waterloo, Canada), and colleagues from Montanan State University, Texas Tech University and the Wildlife Conservation Society, all in the USA, set out to investigate how proteins from the muscles and organs of two species of bats are used for energy and water during hibernation.

In October 2016, before the bats entered hibernation, the team observed 184 cave myotis (*Myotis velifer*) in a cave in Oklahoma, taking measurements of the animals' forearm length (a measurement of body size), determining their sex and using a mobile laboratory to measure the amount of fat, muscle and organ masses. Next, the team collected the same measurements from 65 Townsend's big-eared bats (*Corynorhinus townsendii*) at two caves in eastern Nevada during both the fall and winters of 2017–2019 to track changes in the fat, muscle and organ masses as the animals hibernated. Then, the team calculated the amount of energy

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and water generated by the Townsends' big-eared bats from the decreases in fat, muscle and organ mass.

McGuire and colleagues found that prior to entering hibernation, muscle and organ masses in the cave myotis was ~25–38% of the animals' total body mass. The team suspect that during this period, the bats build up their digestive organs to allow them to binge and build up their fat reserves in addition to bulking up their muscles to carry the large fat store. However, larger muscles and organs also provide bats with access to protein throughout hibernation. In addition, the males had proportionally larger muscles and organs, whereas the females had more body fat, which the researchers suggested may be due to differences in the animals' reproduction strategies. Females need to conserve energy reserves throughout hibernation in preparation for possible pregnancies following hibernation. With their larger protein stores, the males have greater flexibility when arousing from hibernation to burn through organs and muscle to fuel their energy and water requirements, as protein breakdown produces more water compared to fat. But how did the hibernating big-eared bat reserves hold during their long hibernations?

The males consumed approximately twice the amount of muscle and organ tissue than the females, accounting for 35% of the males' total body mass loss and contributing 7% of the energy they used during hibernation. The females, however, had more body fat and were able to generate more energy as fat produces larger amounts of energy relative to protein. The researchers calculated how much water the animals generate as they both broke down muscle and organs and discovered that, after they subtracted the additional water that the animals lost excreting the toxic waste products generated by consuming protein, the female bats benefited by retaining four times more of the water they generated than the males. The team argues that bats could potentially use protein breakdown for hydration during hibernation.

McGuire and colleagues have successfully shown that bats build up their muscles and organs prior to hibernation, which could be used as a minor source of energy but could also play a major role in the production of water. Relatively little is known about how hibernating bats using protein as a source of water, but this new study opens the flood gates to new avenues in bat hibernation research.

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McGuire, L., Fuller, N., Haase, C., Silas, K. and Olson, S. (2022). Lean mass dynamics in hibernating bats and implications for energy and water budgets. *Physiol. Biochem. Zool.* **95**, 317-325. doi:10.1086/720160

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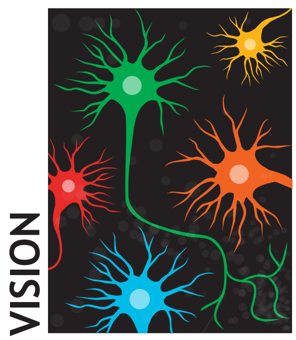
Mayflies see the world in a different light



Everyone gains new perspectives as they grow up, but insects that go through metamorphosis see with a completely different set of eyes as adults. Mayflies spend most of their lives under water as larvae before emerging as adults for one final day of speed dating in a last-ditch effort to produce the next generation. In order to compare how mayflies navigate the two different visual landscapes in which they live, Ádám Egri with Ádám Mészáros and György Kriska at the Eötvös Loránd Research Network Centre for Ecological Research, Hungary, measured how mayflies' eyes respond to different wavelengths of light and compared what they found to the light levels where the insects tend to congregate.

Adult mayflies don't have much time to travel so they spend most of their airborne life mingling around the local neighborhood. By reviewing footage from webcam videos, the researchers determined that *Ephoron virgo* mayflies typically swarm in the evening when the sun elevation is low, about –15 to –7 deg below the horizon. They then measured the visual environment when the sun is at different elevations with a spectrophotometer, which measures the strength of light across a range of wavelengths. It turned out that light levels when the adults congregate at dusk are low and the majority of the light is made up of short wavelength colours, closer to ultraviolet. In contrast, the water where the larvae grow up is murky, so longer wavelengths like visible green light are better able to permeate the darkness. These differences suggest that the insects' eyes may be adapted to distinct environments throughout their life cycle.

In order to measure what the insects can see, the team collected larval and adult wild mayflies to test their vision in the lab. Light is sensed when photons are absorbed by specialized cells in the eye that communicate with the brain via electrical signals. The researchers measured these electrical signals with a fine electrode probe while shining lights of different wavelengths onto the insects' eyes. The adult mayfly's eyes were less sensitive to light that is visible to humans and instead picked up on short ultraviolet wavelengths that are invisible to us. Given that the adults emerge in the evening and die the following day, the reduced sensitivity to visual light suits the insects. However, the larvae living in muddy riverbeds sense light and darkness through simple eyes called ocelli, which absorb photons and send electrical signals to the brain, but can't resolve shapes. The researchers found that larval eyes were also sensitive to UV light, but they were most sensitive to light with longer wavelengths closer to green, which is fitting given the murky world they grow up in.

Many insects can see ultraviolet, but these experiments shed light on the green sensitivity of larval ocelli. Although they can't see as well as other animals, the simple eyes of larval mayflies are well matched for the muddy riverbed conditions where they spend most of their lives. As adults, mayflies emerge and face their first and last evening of life above water with a fresh set of eyes that help them find a mate in the dark.

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Egri, Á., Mészáros, Á. and Kriska, G. (2022). Spectral sensitivity transition in the compound eyes of a twilight-swarming mayfly and its visual ecological implications. *Proc. R. Soc. B.* **289**, 20220318. doi:10.1098/rspb.2022.0318

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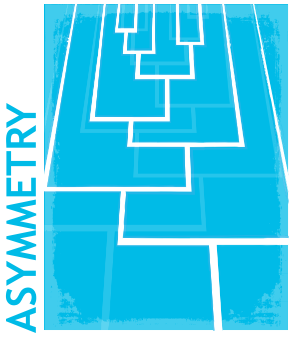
Asymmetric architecture in nests and nature



Satisfying natural symmetries surround us – in snowflakes, starfish and butterfly wings. And our fondness for mirror images is also reflected in the design of exquisitely balanced buildings, such as the Taj Mahal and the White House. Asymmetries, however, also abound across many living things. Male fiddler crabs bear two claws that are starkly different in size, the larger of which they use to fight and flirt. Within flatfish, winter flounder host eyes on their right side while summer flounder have eyes on their left. Perhaps closer to home, differences between our left and right sides are the reason your left-handed friend struggles to operate that can-opener. To examine why asymmetries are so abundant in nature, Nicolas Adreani and colleagues from the Max Planck Institute for Ornithology, Germany, observed the nest architecture of red ovenbirds (*Furnarius rufus*).

Red ovenbirds are native year-round to eastern South America, inhabiting open habitats, including urban areas where they build remarkable domed nests resembling old adobe wood-fired ovens made from mud, straw and dung, featuring entrances that face either left or right. As these birds are cultural icons in each of the five South American countries where they are found, most inhabitants can recognize these large thick clay 'oven' nests, which appear on trees or man-made structures such as fenceposts and buildings. Taking advantage of this widespread recognition, Adreani and colleagues released a free multilingual smartphone app and recruited over 1200 citizen scientists from Argentina, Uruguay, Brazil, Bolivia and Paraguay through social media platforms. This team of citizen scientists – over 400 of whom are listed as co-authors – collected information about <12,500 nests spanning the bird's entire distribution. For each nest, the app provided the volunteers with an 8-step survey, collecting photos and information on nest location (automatic GPS coordinates), height above ground, whether the nest was covered or uncovered, and whether it was built on a natural or artificial structure. They also logged whether the nest was in a natural, rural or urban location, which direction the entrance was pointing, and whether the entrance was on the right or left side of the nest, which they referred to as 'asymmetry type'.

With this information in hand, the authors found that nest asymmetry did not occur randomly, since there were 12% more right-entrance nests than left-handed nests. However, from the environmental data collected by volunteers, the scientists concluded that these factors alone could not explain the occurrence of either right- or left-leaning nests. Next, they set out to determine whether there was a genetic component that could explain the nest asymmetry.

Ovenbirds typically work together for 2–3 months to build their annual nest. Since they tend not to reuse the nests in the following years, it's common to see several nests clustered close together within a single territory, sometimes even one on top of the other! As these birds are monogamous, nests that were in close proximity within a territory and showed up on a single photo were likely to have been produced by one couple. The team measured how often right- and left-sided nests occurred in a territory and used that information to calculate the chance that couples built nests with the same asymmetries. They found that the pairs tended to build nests with the same sidedness, hinting at either a cultural or inherited basis for this behaviour. This discovery opens a whole new line of inquiry that could unravel an underlying genetic component for asymmetric architecture in nests and nature.

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