OUTSIDE JEB

Intertidal mussels survive icy Canadian winters

For many biologists, the intertidal zone is a captivating place. At low tide, seawater collects in depressions and crevasses along rocky shorelines, leaving behind tidepools teeming with life. Tidepools are often packed wall-to-wall with anemones, barnacles and snails, among other intriguing marine taxa trying to stay wet long after the tide recedes. However, not all intertidal residents are fortunate enough to secure a tidepool refuge during low tide, which is particularly problematic in temperate regions that experience bitter, cold winters. When intertidal animals are immersed in seawater, they are protected from sub-zero air temperatures. Without a tide pool refuge, however, air-exposed creatures can freeze when the tide goes out, thawing only upon immersion when the tide returns. Remarkably, many intertidal residents can survive the formation of ice crystals in their body, but how do they tolerate repeated freeze-thaw cycles? In a fascinating new study led by Lauren Gill at the University of British Columbia, a team of researchers discovered how bay mussels (*Mytilus trossulus*) survive repeated freezing and thawing during harsh Canadian winters.

Gill and her team first collected mussels from the rugged coastline in Vancouver, Canada, to investigate their survival rate under different freezing regimes: one continuous freeze lasting 8 h, two separate freezes lasting 4 h each or four separate freezes lasting 2 h each. The researchers induced freezing in the mussels by exposing them to air at –8°C. For mussels that underwent multiple freezing bouts, the team provided a 24 h recovery in 7°C seawater between each freeze to mimic the relief that they would experience with natural tides. The researchers predicted that repeated freezing and thawing would increase mussel mortality. However, much to their surprise, survival was almost 100% in mussels frozen four separate times (4×2 h) and a mere 25% in mussels frozen only once (1×8 h).

At this point, the researchers were keen to uncover the physiological mechanisms that led to such high survival in their repeatedly frozen mussels. They suspected that two different proteins could be playing an important role. The first, heat-shock protein 70 (HSP70), is known to help the body deal with extreme temperature stress. Extremely hot and cold temperatures can denature (i.e. damage) proteins in the body, but HSP70 can help protect proteins from denaturation. Sure enough, the team discovered that in repeatedly frozen mussels, the expression of HSP70 increased considerably after freezing, thereby providing mussels with protection against subsequent freezing events. The second protein is ubiquitin, which tracks down any damaged proteins in the body and flags them for disposal. Interestingly, the researchers found that in repeatedly frozen mussels, the number of proteins flagged by ubiquitin increased after freezing, indicating that the mussels were working hard to repair any molecular damage caused by freezing.

Taken together, Gill and her team found that mussels have a higher survival rate when they experience repeated freeze-thaw cycles compared with a single lengthy freeze of the same total duration. Furthermore, periods of thawing are critical for surviving sub-zero temperatures because they offer mussels an opportunity to make important proteins that protect against, and repair, molecular damage caused by freezing. So, the next time you're out exploring the captivating tidepools of the intertidal, be sure to remember the creatures outside of these pools and the great lengths that they must go to in order to survive.

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Gill, L. T., Kennedy, J. R. and Marshall, K. E. (2023). Proteostasis in ice: the role of heat shock proteins and ubiquitin in the freeze tolerance of the intertidal mussel. *Mytilus trossulus. J. Comp. Physiol. B* doi:10.1007/s00360-022-01473-2

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Dopamine modulates odour learning in mosquitoes

Researchers have long been fascinated with figuring out how mosquitoes choose a host to feed on and how they can change their preferences. One important missing piece of the puzzle may be the way that mosquitoes learn different smells. Gabriella Wolff and colleagues from the University of Washington, USA, had previously found that yellow fever mosquitoes could form memories associated with odours related to their favourite food – blood – but not with odours that had nothing to do with their life history. These mosquitoes could be trained to avoid those odours by associating them with swatting. However, it was still unknown whether other mosquito species could learn to avoid the same odours and whether dopamine, a chemical messenger important for learning, could be responsible for this olfactory learning. To figure this out,

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the team set out to train four different species of mosquitoes.

Wolff and colleagues exposed the mosquitoes to the scents of human skin, chicken feathers and rose oil. Two of the mosquito species feed on humans [the yellow fever mosquito (*Aedes aegypti*) and the Asian malaria mosquito (*Anopheles stephensi*)], another, the southern house mosquito (*Culex quinquefasciatus*), feeds on birds, and the elephant mosquito (*Toxorhynchites amboinensis*) dines on flowers. The team found that all the mosquito species had the strongest attraction to the scent of the hosts that they preferred to feed on.

To test whether multiple mosquito species could learn to recognize the same odours, the team trained female mosquitoes from each species to avoid different scents by exposing them to scent components from human odours (octenol and hexanoic acid) or a flower scent component (linalool), regardless of whether they naturally feed on humans or flowers, and then they mimicked swatting at the insects to make the scent unattractive. The team then investigated each mosquito's preference by putting them into a maze that provided them with a choice between either a host odour (octenol, hexanoic acid or linalool) or no scent. The team found that the mosquitoes learned to avoid the odours associated with their favourite food, but not other odours. For example, the yellow fever mosquito, which dines on people, learned to avoid octenol and hexanoic acid, but not linalool. Meanwhile, nectar-feeding elephant mosquitoes only learned to avoid linalool, but not octenol and hexanoic acid.

To see whether these differences in species learning were due to the effects of dopamine, the researchers investigated where dopamine was localized in the brain of each of these species. Wolff and colleagues found that dopamine was localized in the antennal lobe and mushroom bodies – structures in the brain that are important for learning and memory. However, the dopamine was localized in different areas of these structures from species to species, which could be important for differences in their learning abilities. To see whether dopamine was necessary for this olfactory learning, the team also trained yellow fever mosquitoes and the Asian malaria mosquitoes, which feed on mammals, to avoid an odour associated with their preferred diets, and found that when they blocked the dopamine receptor, they eliminated their ability to learn to avoid the odour. Last, the team found that areas in the antennal lobe that had an increased response to these scents also had a raised level of dopamine.

Learning the basis of how mosquitoes smell and the impact on their host preference is vital, as the insects can be responsible for the spread of some terrible diseases. The researchers highlight that being able to change dopamine inputs without having to change brain structure may give mosquitoes an evolutionary advantage and could be a way for mosquitoes to change their host preferences quickly.

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Winter leaves Alaskan blackfish breathless

Most fish breathe water with their gills, but a surprising number of fish can inhale air directly. Even common household pets such as Siamese fighting fish (betta fish, *Betta splendens*) breathe at the surface, so they can live in tiny bowls without filters or aeration. The Alaskan blackfish (*Dallia pectoralis*) shares this superpower. A modified esophagus lets them 'surface breathe', so they can get oxygen even in the stagnant and muddy waters that pepper the Arctic tundra. Most of the year, this ability gives blackfish a great advantage – they can live where most fish cannot, in a niche of their own. But in the winter, blackfish can no longer breathe air. The rough Alaskan winters bring ice and snow, which cover the water surface and lock out fresh air. Yet even after losing access to oxygen, blackfish remain active throughout the long winter season. For Gina Galli (University of Manchester, UK) and a team led by Jonathan Stecyk (University of Alaska Fairbanks, USA), this was a fascinating question. How can an animal survive for a whole season without air?

To investigate, the team visited Palmer, Alaska, in the summer and caught blackfish with a minnow trap. Back in the lab, they housed the fish in aquaria and mimicked the conditions they would experience in the wild: over several weeks, they reduced the temperature to 5°C and reduced dissolved oxygen levels in the water. They also installed a grate just underneath the water surface to simulate the arrival of snow and stop the fish from breathing air. Then, they collected mitochondria – which are ideal to understand how oxygen is used, because they use oxygen to make energy – from the blackfish and analyzed how they were using oxygen. Do blackfish mitochondria work overtime to keep cells energized throughout the winter? It's not unheard of, and Stecyk's team noted that as many fish species acclimate to the cold, their mitochondria become more productive.

But it turned out that cool temperatures did not energize blackfish. Their mitochondria didn't fight any harder for oxygen. Likewise, they had the same appetite for a chemical needed to make energy (adenosine diphosphate, ADP), which means they weren't making extra energy or working more efficiently. Unfazed, the blackfish mitochondria just chugged along at the same tempo.

So, why don't blackfish fight harder for oxygen? Stecyk's team pointed out that although breathing sustains life, oxygen has a dark side. In any cell that consumes oxygen, a fraction of that oxygen creates toxic byproducts, called reactive oxygen species, which can damage mitochondria and kill cells. The researchers measured the reactive oxygen species produced by blackfish mitochondria using a fluorescent dye. In most species, cells produce more harmful reactive oxygen species when the temperature falls and oxygen is low – the exact conditions experienced by blackfish while breathless during Alaskan winters. Yet Stecyk's team found the opposite: the blackfish produced fewer reactive oxygen species in the cold and fewer still when oxygen was low. This means the cold Alaskan winters prepare the blackfish to protect themselves from the damaging effects of low temperatures and oxygen levels, preconditioning them so they can hold their breath all season long.

It's fascinating enough that a fish can breathe air. But blackfish take it a step further: they hold their breath for an entire season. Many animals pant, gasp and struggle to get more oxygen when they can't breathe, but blackfish don't struggle. Their mitochondria maintain their pace, yet they work 'cleaner' to avoid polluting cells with toxic oxygen byproducts. In this way, they're like a seasoned marathon runner: they don't foolishly sprint when the race starts, they just maintain their pace and watch their form.

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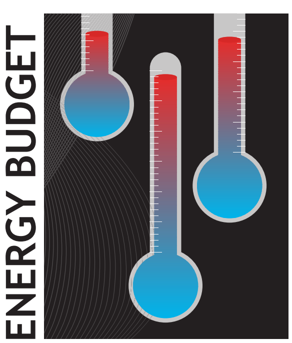
Hummingbirds use insulated nests to save energy for the clutch



All wild animals need to perform a balancing act with the energy they take in and the energy they burn. To save energy, hummingbirds can use torpor, a process where they decrease their body temperature and metabolism. However, mothers only use torpor in an emergency, as their eggs and chicks are sensitive to low temperatures. Erich Eberts, from Loyola Marymount University, USA, and an international team of colleagues from various US and Canadian universities set out to determine how female Allen's hummingbirds (*Selasphorus sasin*) manage their energy budget at night-time while incubating chicks and, importantly, whether their nests provide energy savings.

From January to May in 2017 and 2018, the team located more than 60 Allen's hummingbird nests with eggs or chicks in the suburbs around Loyola Marymount University in Los Angeles, CA, USA. The researchers then monitored 56 of the nests until the chicks fledged (40 nests), were killed by predators (12 nests) or failed for some other unknown reason (4 nests). To measure the temperatures of the hummingbird mothers, the researchers placed thermal cameras 0.5-1 m away from nests, programmed to capture one image every minute, successfully collecting thermal images on 108 nights from 14 of the 56 nests they were monitoring. The researchers then used the thermal images to determine the mother's body temperature, and the temperature of a nearby branch or leaf to measure the local environmental temperature.

For their analysis, the team then defined the thermal states of the hummingbirds as either normal body temperature, deep torpor (low body temperature), shallow torpor (moderately low body temperature) or the transition to or from torpor. Then, in a separate series of theoretical models, the researchers estimated the energetic costs of the hummingbirds under a range of conditions, with either torpid or normal body temperatures for 0, 2, 6, 10 or 12 h, and either nesting or completely exposed to the surrounding air.

The researchers found that on 103 nights (of the total 108), the hummingbird mothers maintained normal body temperatures, and failed to enter torpor to conserve energy. However, one hummingbird did enter deep torpor on two nights in February, decreasing its skin temperature to 14°C, and two other hummingbirds used shallow torpor on three nights, decreasing skin temperatures to approximately 19°C. However, the chicks of the mothers that sporadically used torpor still developed successfully and took flight when they were old enough. The researchers concluded that hummingbird mothers rarely enter torpor to protect their chicks from nocturnal low temperatures, although they do resort to using torpor in exceptional circumstances.

Nevertheless, the team discovered that the temperatures within the nest remained approximately 7.5°C higher than the surrounding air, thanks to the insulation they provided, which allowed the roosting mothers to conserve energy. In one of the teams' energetic models, a nesting mother maintaining a normal body temperature for 12 h could save around 24% of her energy thanks to her nest compared with a mother exposed to the environment. Yet, if nesting hummingbirds used torpor for 6 h, they could save 41% of the energy they would expend maintaining a normal body temperature in the open.

Overall, Eberts and colleagues successfully used a non-invasive method to determine that mother Allen's hummingbirds almost always forgo torpor when nesting. The mothers prioritize the health of their chicks, instead of saving energy for themselves. In addition, hummingbird mothers can depend on their well-constructed, insulated nests to provide a warm home and save energy when raising their young.

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