OUTSIDE JEB

Bubble breathing is skin deep for anoles

The underwater world is alien to most land animals, a place we only visit briefly before our breath runs out. A few terrestrial animals extend their stay by bringing air with them, such as diving beetles that smuggle bubbles under their wings, or human divers with compressed air tanks. The newest member of the terrestrial SCUBA club are several species of semi-aquatic *Anolis* lizards, as described by Christopher Boccia, a researcher from the University of Toronto, Canada, and more than a dozen colleagues in their recent study.

Spurred on by their own natural history observations of tropical anoles serenely resting at the bottom of streams, the authors rounded up 20 species of anoles (including five considered to be semi-aquatic) at field sites across the Americas and the Caribbean. Using buckets, aquaria or even kiddie pools, they let the anoles dive voluntarily or gently submerged them, paying very close attention to what happened next.

When submerged, the anoles took on a silvery sheen, caused by a thin layer of air (a 'plastron') forming between the water and their skin. The quicksilver-like coating also invariably connected to a large air bubble around their head to their nostrils. Most species at least occasionally inhaled and exhaled – effectively re-breathing – the bubble while submerged. And when the team compared how many times the different species resorted to re-breathing, the semi-aquatic anoles re-breathed more often and for longer stretches than their non-aquatic cousins. They also stayed underwater longer, with the champion diver of the group staying under for 18 min. Perhaps, the authors mused, the exaggerated re-breathing response in semi-aquatic anoles was an adaptation to their waterfront lifestyle.

But that's a hypothesis that only holds if re-breathing serves a physiological function, such as providing oxygen for respiration. To test this idea, the researchers started jabbing an oxygen-measuring probe into the bubbles, looking for evidence that the anoles sucked oxygen out of their bubbles during their dives. They found that the oxygen levels decreased over time in the bubbles and larger anoles consumed oxygen more slowly than smaller ones, which is exactly what they expected from respiring lizards on land. It seems that diving anoles are probably re-breathing the air they take down with them from the surface.

There are a few ways that breathing and re-breathing the same bubble of air could help anoles make longer dives. The first is that re-breathing mixed the air inside the anole's body, helping them to extract as much oxygen as possible from the air already in their lungs and throat. The bubble may also help anoles dump carbon dioxide into the water around them, preventing the waste from building up in their bodies. Finally, and most intriguing, the anole might take a page from the book of diving beetles and use bubbles like a physical gill. As the oxygen in the bubble declines, more oxygen is pulled from the water into the bubble, which could allow these air breathers to supplement their breath holding with water breathing!

The key to the anole's bubble breathing strategy is probably their waxy skin and the plastron that forms around it, connecting the bubble with their internal respiratory system via the bubble around their nostrils. All anoles get a silvery coating when dunked in water, regardless of their habitat preferences, and so their peculiar skin likely evolved for something other than SCUBA diving, such as keeping clean or dry, and semi-aquatic anoles then co-opted it to create the plumbing of their bespoke underwater breathing apparatus. Anoles may dive deep to keep away from predators or find food, but the trick they use to do it is only skin deep.

doi:10.1242/jeb.236919

Boccia, C. K., Swierk, L., Ayala-Varela, F. P., Boccia, J., Borges, I. L., Estupiñán, C. A., Martin, A. M., Martínez-Grimaldo, R. E., Ovalle, S., Senthivasan, S., Toyama, K. S., Del Rosario Castañeda, M., García, A., Glor, R. E. and Mahler, D.L. (2021). Repeated evolution of underwater rebreathing in diving *Anolis* lizards. *Current Biology* **31**, 1-8. doi:10.1016/j.cub.2021.04.040

Brittney G. Borowiec (0000-0003-0087-7577)
Wilfrid Laurier University
bborowiec@wlu.ca

An agile tail is a boon in trees

Monkeys get around in trees with a remarkable grace that belies the fact that any false moves can lead to a fatal plummet to the ground below. Sure, they have four limbs to orchestrate the balancing act, but in addition they boast a nimble tail. Jesse Young and a team of researchers from universities in Cleveland, Meridian and Austin, USA, wanted to take a closer look at what the tail does to ensure safe passage in trees. Previous studies have noted that monkeys

Outside JEB reports on the most exciting developments in experimental biology. The articles are written by a team of active research scientists highlighting the papers that JEB readers can't afford to miss.

that live and move in trees have longer and heavier tails than monkeys that mainly live on the ground – this led the researchers to believe that bigger tails may have evolved to work as a counterweight, a bit like a tightrope walker's balance pole, to save them from toppling when they overbalance and ensure an overall smooth journey through the canopy.

To understand how the tail works as a counterbalance, Young and his colleagues filmed squirrel monkeys – a small species about 0.5 kg – as they traversed horizontal poles set up in a laboratory. The poles had decreasing widths of 5, 2.5 and 1.25 cm, mimicking the challenges that the monkeys encounter in their natural habitat. From these videos, the team calculated how much the tail counterbalanced the movement of other body parts, taking into account the tail's weight – a heavy tail is a better balance pole than a light one – and the speed as the monkey snapped the tail from side to side – a faster whip of the tail means more counterbalance.

The team found that the elongated shape of the tail – equivalent to 167% of body length – helps it to counterbalance all the monkey's other limbs despite its meagre size, which only amounts to 5% of the animal's entire body mass. Consistent with their expectation, Young and the team found that the tail worked harder at counterbalancing when negotiating progressively narrower supports. This suggests that the tail is not just a passive appendage but plays an active role in ensuring stability.

Building on this result, the team turned their attention to videos of monkeys recorded in the wild in Ecuador and Costa Rica. These videos showed that monkeys hold their tails more upright and move it more vigorously when they are on narrower branches. This demonstrates the importance of the tail in the real world. The team also found that the counterbalancing movements – perky whips of a tail cocked high – increased with longer tail length, suggesting that the species with longer tails take full advantage to gain the most stability.

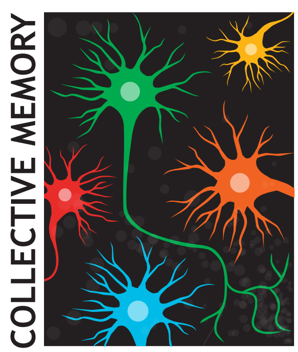
Young and his colleagues have shown that monkeys' tails play a key role in balance when managing treacherous tree branches. The results suggest that the role of long tails in tree-dwelling monkeys is to save them from toppling, which may be considered one of the fundamental adaptations for a life in trees. What is certain is that when you live high up in the trees, evolving a biological balancing pole with the ability to provide a life-saving jolt in response to a bad step is a smart move.

doi:10.1242/jeb.236885

Young, J. W., Chadwell, B. A., Dunham, N. T., McNamara, A., Phelps, T., Hieronymus, T. and Shapiro, L. J. (2021). The stabilizing function of the tail during arboreal quadrupedalism. *Int. Comp. Biol.*, icab096. doi:10.1093/icb/icab096

Jan Stenum (0000-0002-0088-8703)
Johns Hopkins University
jstenum1@jhmi.edu

Fruit flies: together we can remember better



Do you remember your first day of school? Perhaps the smell of a new backpack reminds you of that day? But if you are like me, your long-term memory is fuzzy at best. Fortunately, a quick internet search reveals many ways to enhance memory: sleep better, eat a balanced diet and exercise regularly. But just being around a group of fruit flies can improve a fly's memory recollection, according to a study by Aurélie Muria and colleagues from the University of Toulouse, France, and the University of British Columbia, Canada.

How is that possible? Fruit flies release a tiny quantity of carbon dioxide when they are stressed and as they breathe out. Muria and colleagues thought that fruit flies might improve their ability to recall information from their long-term memory after smelling the carbon dioxide released by other flies in the group, resulting in a form of socially facilitated memory, in addition to their existing individual memory.

To understand whether carbon dioxide can help with the retrieval of fly memories, the researchers trained the insects to associate one smell with a mild electric shock and another smell with no electric shock. The flies form an unpleasant memory of the smell associated with the shock. One day after training, the researchers tested this memory in individual flies and in a group of flies in a maze with two compartments, checking whether the flies avoid the compartment with the smell that they associated with the unpleasant shock. The flies that had been shocked into disliking an odour experienced stress and released carbon dioxide while their recollection of the distressing memory was being tested in the maze. The researchers were thrilled to find that flies tested in a group recalled their shocking experience better than those tested individually by choosing the compartment with the scent that was not associated with an unpleasant surprise. The fly's ability to remember improved as the concentration of carbon dioxide in their surroundings increased.

The researchers then wondered whether the carbon dioxide-evoked social memory and individual memory are processed differently within the insect brain. Carbon dioxide activates neurons called bilateral ventral projection neurons which connect to mushroom bodies, the memory center of the insect brain. The researchers blocked these neurons and found that group-tested flies, but not individually tested flies, had reduced recollection of the smell that was associated with an unpleasant jolt, indicating that bilateral ventral projection neurons are necessary for carbon dioxide-evoked memory. Mushroom bodies are composed of distinct types of neurons called Kenyon cells, so Muria and colleagues silenced the outputs of different types of Kenyon cells one by one to show that individual memory and carbon dioxide-evoked social memory involve distinct Kenyon cells in the mushroom body. So, individual memory and social memory engage different neural pathways within the fly brain and are processed independently.

The presence of other flies can improve the retrieval of a fly's memories via carbon dioxide-mediated social memory. Carbon dioxide released during a stress event may enhance the fly's attention and enable the recollection of memories. This study highlights the unexpected role of carbon dioxide in insect intelligence. I wish carbon dioxide could improve my memory!

doi:10.1242/jeb.236893

Muria, A., Musso, P.-Y., Durrieu, M., Portugal, F. R., Ronsin, B., Gordon, M. D., Jeanson, R. and Isabel, G. (2021). Social facilitation of long-lasting memory is mediated by CO2 in Drosophila. *Current Biology* **31**, 2065-2074. doi:10.1016/j.cub.2021.02.044

Ravindra Palavalli-Nettimi (0000-0002-4523-8245)
Florida International University
rvndrpn@gmail.com

Long-legged lizards get blown away

Hurricanes do not affect all lizards equally. Research has shown that survivors of hurricanes are generally those individuals with both larger toepads and shorter femurs than the average population before the hurricane. The larger toepad phenomenon can be explained by grip force; those with larger toepads were able to hang on to their perch in the strong winds, and those with smaller toepads likely got blown away. However, until now, the phenomenon of shorter-legged survivors was unexplained. A group of researchers lead by Shamil Debaere at the University of Antwerp, Belgium, investigated the reason behind the shorter femurs in surviving *Anolis* lizards and why longer legs may be disadvantageous in high winds during tropical hurricanes.

During windy storms in the wild, anoles generally hold on to vertical branches, with their head facing up, arms close to the body and legs either stretched out down the branch, or bent in a crouched position. In this way, the branch acts as a windshield that the lizards can effectively hide behind to withstand stronger winds. Debaere's team suspected that longer legs may stick out from that windshield, allowing them to be torn free of their hold by fierce winds.

To test whether longer legs might affect the amount of drag experienced by the reptiles in different positions, the researchers calculated the aerodynamic forces exerted on the lizard body, in much the same way that engineers calculate the aerodynamic forces on a sports car. Using this method, they were able to see which parts of the lizard were causing the most drag. They used previously recorded videos of *Anolis* lizards gripping on to vertical perches while being blown by a leaf blower mimicking a hurricane, to make accurate 3D models of the buffeted animals. The researchers then modeled the forces exerted on the 3D models across five different postures, two of which were given 10% longer legs – one with more flexed knees and one with the pelvis protruding further from the virtual perch.

They found that the postures with 10% longer limbs had the highest drag of all five postures. The longer femurs force the lizard's pelvis to extend further from the branch, outside of the wind-shielding effect of the branch. Imagine hurricane-force winds attempting to remove you from your perch; any object sticking out of the aerodynamic shadow from the branch will cause drag, much like sticking your hand out of a moving car.

Tucked in, hiding from the wind behind the branch, the lizards are better able to hang on during hurricanes, resulting in a surviving population of lizards with significantly shorter hindlegs. Because shorter legs are less likely to get caught in the wind, those lizards can hang on better than their longer-legged counterparts and are more likely to be able to hold on tight to their perch.

doi:10.1242/jeb.236901

Debaere, S. F., Donihue, C. M., Herrel, A. and Van Wassenbergh, S. (2021). An aerodynamic perspective on hurricane-induced selection on *Anolis* lizards. *Funct. Ecol.* doi:10.1111/1365-2435.13848

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

Pregnant salmon: the real Olympians

Once in their lifetime, coho salmon undergo an arduous upstream migration to reach the same spawning ground they left as larvae years earlier. Transitioning from sea to freshwater, swimming against the current for hundreds of kilometres, navigating rapids and avoiding predators, salmon migrations are perilous journeys that require elite athletic performances to complete. Recent increases in river temperatures are further challenging the stamina of salmon, meaning that fewer fish may survive the migration and reproduce, which worries conservationists, fishers and indigenous peoples. The mortality is often higher in female salmon, which is perhaps unsurprising, as they must complete the migration while hauling a large mass of eggs up the river – the fish equivalent to running an up-hill marathon, in the summer heat, while pregnant. Just like you don't see many pregnant Olympians, female salmon may also be at a disadvantage when it comes to exercise, but whether pregnancy is to blame entirely is still unclear.

Krista Kraskura at the University of California Santa Barbara, USA, and her international team of collaborators set out to test whether mature female salmon struggle with exercise and whether higher water temperatures affect them more than the males. In a tremendous sampling effort, the team intercepted migrating salmon in the rivers of British Columbia, Canada, and transported them to the lab. There, they measured the athletic performance of females and males in a swim tunnel, a fish-treadmill of sorts, at three different temperatures: present low (9°C) and high (14°C) river temperatures, and a climate change scenario (18°C) that the fish do not yet see in the wild. The results were a surprise.

Across all of the temperatures, female salmon, despite carrying their heavy egg loads, matched the performance of the leaner males in all tests: swimming to exhaustion, escaping a simulated predator and recovering from bursts of activity. During exercise, female and male salmon needed similar amounts of oxygen, indicating that the larger female gonads did not impair performance as had been predicted. On the contrary, at rest and during recovery from exercise, female salmon actually required less oxygen than males, indicating that females may be more conservative with their energy stores, which is critical as salmon stop feeding during their journey. But once the temperatures were raised, both female and male salmon struggled in some way.

In warm waters that mimic predicted river temperatures due to climate change, salmon of both sexes were able to maintain their swimming performance surprisingly well. However, at the higher temperatures, all salmon needed longer to recover from the effort. This is a critical finding, as migrating salmon must repeatedly overcome rapids and other obstacles that require exhaustive bouts of exercise. A longer recovery period would delay their upriver journey and offset their timing, which is key when the next generation depends on everyone meeting at the same place, at the same time. Combined, these findings stoke the fears of conservationists that fewer salmon will be able to reproduce in a warmer future, putting populations at risk.

However, the main question of the study still remains a mystery: why do female salmon experience higher migration mortality rates? Kraskura and the team showed that elevated river temperatures affect both sexes and, if anything, female salmon are more efficient at metering out their limited energy. All things considered, female salmon are the real Olympians, which not only match the athleticism of males but also raise the stakes by carrying the future salmon brood up the rivers.

doi:10.1242/jeb.236927

Kraskura, K., Hardison, E. A., Little, A. G., Dressler, T., Prystay, T. S., Hendriks, B., Farrell, A. P., Cooke, S. J., Patterson, D. A., Hinch, S. G. and Eliason E. J. (2021). Sex-specific differences in swimming, aerobic metabolism and recovery from exercise in adult coho salmon (*Oncorhynchus kisutch*) across ecologically relevant temperatures. *Conserv. Physiol.* **9**. doi:10.1093/conphys/coab016

Till Harter (0000-0003-1712-1370)
Scripps Institution of Oceanography
tharter@ucsd.edu