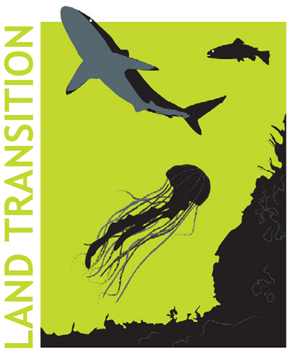
OUTSIDE JEB

Mangrove killifish are stressed for success



While most fish are well suited to living in water, some fish also spend part of their time on land. For these amphibious fish, such as the mangrove killifish (*Kryptolebias marmoratus*), the transition from water to land is taxing. When out of the water, these fish breathe more oxygen and, no longer able to swim, they also get creative with how they move about, sometimes flinging themselves through the air to reach a new location. When the killifish are on land, a stress hormone called cortisol spikes within the first hour but returns to normal after a week out of water. For water-dwelling fishes, long-term increases in this stress hormone help increase how much oxygen a fish breathes in for an extra energy boost during stressful situations. But what if short-term increases in cortisol can help the trying transition from water to land? Sarah Young from the University of Guelph, Canada, and a team of scientists from other Canadian universities turned to the small but mighty mangrove killifish to test this idea.

To understand how cortisol helps their transition from water to air, Young and the team blocked cortisol from being produced in the fish's body, then transitioned them to air for 7 days. They measured how much oxygen the fish breathed in and their cortisol levels immediately after their transition to air, 1 h after the transition, and again at the end of the 7 days. Fish given the cortisol blocker didn't increase their cortisol levels much, but fish that weren't given the cortisol blocker increased their cortisol levels ∼10× after just 1 h in air. This temporary increase in the stress hormone returned to normal after 7 days, but the spike coincided with fish breathing in more oxygen. However, when their cortisol production was blocked, fish didn't breathe in nearly as much oxygen. Together, these findings show that the cortisol blocker worked, and the natural spike in the stress hormone helps the killifish fuel their transition from water to air. Without cortisol and the extra energy boost from breathing in more oxygen, the team thought that fish would probably be less lively on land.

So, the scientists then measured how far a fish could leap, when given a little motivation (a gentle tap on the nose or tail with the tip of a pen). Mangrove killifish have a particularly acrobatic method of jumping, sometimes propelling themselves over 15 body lengths away – the equivalent of a human jumping lengthwise across an entire tennis court. However, when the researchers stopped the fish from producing the stress hormone, the killifish jumped ∼2 body lengths shorter and generally moved around less than fish producing normal amounts of cortisol. This means that the fish aren't as athletic without cortisol, which may be costly when trying to avoid predators.

Young and the team discovered that without cortisol, these amphibious fish can't adjust their bodies and behaviour to land quite as well. More research is needed to discover exactly how cortisol is helping, but it may work together with other stress hormones to tap into the body's extra energy stores, giving killifish a boost when they take on the costly task of transitioning from water to air. With rapid changes to how much oxygen a fish breathes in and how much they jump about within just 1 h in air, the team found just how quickly cortisol can help their life on land. Perhaps unsurprisingly, without cortisol, the mangrove killifish acts like, well, a fish out of water.

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Young, S. J., Rossi, G. S., Bernier, N. J. and Wright, P. A. (2023). Cortisol enhances aerobic metabolism and locomotor performance during the transition to land in an amphibious fish. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **288**. doi:10.1016/j.cbpa.2023.111558

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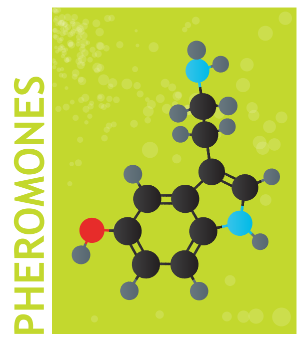
A male tilapia's nose knows fertile females from their feces



Like dogs laying claim to territory, male Mozambique tilapia (*Oreochromis mossambicus*) use urine to assert their dominance. The scents, called pheromones, that dominant males release in their urine keep subordinate males docile and prime females to lay eggs. But male social status and urinary pheromones are just part of the elaborate process of tilapia mating. After males establish their dominance over the subordinate males, the fish dig and defend pits in which the attracted fertile females lay eggs. Females, which choose their mates, also need to communicate they are ready to reproduce so that dominant males know when to strut their stuff. Samyar Ashouri, José Da Silva, Adelino Canário and Peter Hubbard from the University of Algarve, Portugal, suspected that tilapia may release additional scents in their feces to communicate their fertility, so they investigated whether females started defecating any attractive aromas when they were ready to mate.

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.

The researchers created groups of fish with two males and four females in each group, then placed each group into its own tank. They tracked the behaviors of the males for a week and decided which one was dominant based on its tendency to bite, dig and mate. They also tracked the reproductive cycles of the females for 3 months to determine when the females were about to ovulate and most likely to draw the attention of males. After establishing which male was dominant and each female's fertility, the researchers collected feces from the fish to analyze their contents; they found that dominant males and fertile females had higher quantities of several amino acids and two bile acids – chemicals which help digest fats and other nutrients – in their feces. The team suggested that these differences are due to reproductive hormones, rather than dietary changes associated with motherhood, because they occurred in both sexes.

Still, the amino acids and bile acids can only signal that the females are ready to reproduce if other fish can smell them. To check whether the males could smell the scents from the female's feces, the researchers put male fish in small tanks, pumped different substances into the water and measured whether the nerves in their noses responded to the scents. Males picked up the scent of the amino acids and bile acids in the feces of the ready to reproduce female fish, and when the scientists pumped the two bile acids simultaneously over the fish's nose, the resulting electrical activity in the nerves suggested that males even have the capacity to recognize each bile acid individually.

Confident that the male fish could smell the chemicals, the researchers checked to see whether the scents caused any behavioral changes in the males. First, they placed a male tilapia in front of a mirror to fool the fish into thinking he had a competitor to fight, and then pumped in the chemicals from the female's feces to see whether the males mellowed. Because none of the amino acids or bile acids reduced the male's aggressiveness, the researchers concluded that the fecal odors don't act like male urinary scents. However, when the scientists placed males in tanks with three distinct chambers that the fish could swim into, they observed that pumping feces from fertile females into one of the chambers attracted the males and prompted them to start digging more frequently in preparation for mating. When the team pumped the bile acids alone into the tank, the males also swam into the scented section and dug more, but not if the researchers only pumped in the amino acids. This suggests that there is a connection between a female tilapia's fertility and the bile acids she produces, and a male's nose knows it.

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Ashouri, S., Da Silva, J., Canário, A. and Hubbard, P. (2023). Bile acids as putative social signals in Mozambique tilapia (*Oreochromis mossambicus*). *Physiol. Behav.* **272**, 114378. doi:10.1016/j.physbeh.2023.114378

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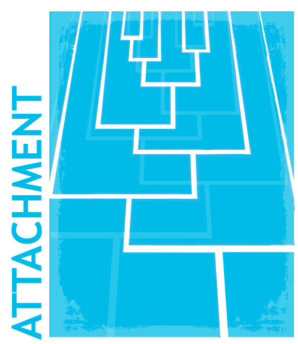
Windswept weevils hold on with their feet



Island environments can be a tough world for the species that call them home. Even though some islands are exposed to extreme winds and rain, especially in the event of climatic events such as tropical cyclones, animals and plants persist. One adaptation to these environmental pressures is that the tiniest island dwelling creatures, insects, are more often flightless, to protect themselves from being carried away by unexpected winds. But how do they cling on to stay put when the weather turns extreme? Knowing that flightless weevils live high up in the windy forest canopy of Orchid Island, Taiwan, Lu-Yi Wang from the University of Melbourne, Australia, and an international team of colleagues decided to investigate how sticky the wind-beaten weevils' feet are, to understand how they manage to stay put with the constant threat to being blown away.

To test the 'stickiness', or adhesion, of these insects' feet, the team collected live weevils from the island and put them on a disc that was spun fast, to force them to cling on. By increasing the spinning speed and recording when the bugs fell off, the team could calculate how well each bug stuck to the surface with the sticky pads on their feet. In addition, the team changed the roughness of the disc surface to see how the insects' ability to cling on changed as the surface became rougher. Lastly, knowing that the weevils' feet come equipped with a claw for hanging on, the team also tested how this grappling hook contributed to the insects' ability to cling on by repeating the disc-spinning experiment after snipping off their claws. The team then compared how tightly the Orchid Island weevils hung on with and without their claws, as well as the sticking power of these island weevils and those that live in less windy landlocked locations.

Wang and colleagues found that the Orchid Island weevils show much stronger attachment forces than previously measured for non-island-dwelling insects. On rough surfaces, the island weevils' attachment forces were 12% higher than those recorded for other insects. The weevils also had a 7% higher safety margin, meaning that their maximum attachment force exceeds a higher multiple of their body weight compared with other insects. On slick glass surfaces, the weevils held on 14 times more strongly than bugs that came from less windy locations. When the team compared how well the island dwellers hung on with and without their claws, the clawless weevils that only had their sticky pads to cling on with held on as tightly as the clawed weevils, so the insects' claws did not contribute to their ability to hang on tight in the wind. And when the team took a detailed look at the sticky pads on the insects' feet using an electron microscope, they found that the weevils have long but soft hairs on their footpads, which can mould snuggly to any surface, creating a large contact area to hold the insect in place through molecular forces.

This study shows that these endangered and protected Orchid Island weevils show impressive attachment ability, which is an important adaptation to island life. Stronger adhesion not only prevents them from being carried away by strong winds but also avoids them falling prey to other small bugs such as ants. The ability of these creatures to attach strongly to a surface is caused by the structure of the sticky pads on their feet, as well as the softness of the base and tips of the adhesive hairs that cover the sticky pad surface, allowing the weevils' toes to make intimate contact with any surface, from the roughest to the smoothest. These insights might help researchers understand how species adapt to extreme weather, which might occur more often as a result of climate change.

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Wang, L., Lin, C., Gorb, S. N. and Rajabi, H. (2023). Strong attachment as an adaptation of flightless weevils on windy oceanic islands. *J. R. Soc. Interface* **20**, 20230447. doi:10.1098/rsif.2023.0447

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Salmon stress inherited from parents

Parents shape the lives of their children in countless ways. Even before we are born, the genetics and environment of our parents build the foundation of our future. That said, additional stress that parents experience during reproduction may influence the next generation of offspring. One stress event that uniquely impacts fish is catch-and-release angling – a conservation technique that encourages recreational fishing while maintaining productive fish populations. The idea is that many fish species can recover from the exercise, air exposure and potential injury; however, it remains unclear how catch-and-release angling during the spawning period, when fish reproduce, might affect developing eggs and subsequent offspring. Magdalene Papatheodoulou and colleagues at the University of Glasgow, UK, were particularly interested in how catch-and-release angling close to the spawning period of wild Atlantic salmon (*Salmo salar*) might impact the development of their offspring. Papatheodoulou and her colleagues predicted that salmon parents that experienced catch-and-release angling would produce offspring that were less likely to survive.

To study this, the researchers captured 56 male and female salmon from the river Blackwater in Scotland before the spawning period. The team then transported the fish to holding tanks, where they chased some individuals around for 3.5 min before catching them and holding them in the air for 1–2 min, to mimic the stress of being caught by an angler. They also chased other fish around, to simulate the less stressful experience of being caught but escaping before they were reeled in, and another group of fish were just allowed to swim undisturbed. The fish then rested in the holding tanks for 8–18 days until they spawned, at which point the researchers mixed eggs and sperm from fish that were part of the study with gametes from fish that did not participate in any part of the study. This way, the researchers were sure that only one parent of the fertilized eggs was stressed. After monitoring the eggs through development at a nearby hatchery, the team transferred the fry back to the University of Glasgow where they could watch how the offspring grew and developed.

As Papatheodoulou and her colleagues expected, the fertilized eggs of the stressed parents, which had been chased and held aloft, died at a greater rate than those of salmon that had been less stressed or not stressed at all. However, the fertilized eggs of parents that had been stressed 8 days before spawning survived much better than those of parents that had been chased and taken out of the water 18 days before they spawned, which suggests that catch-and-release angling may have less of an effect on egg development when the parents are caught close to spawning. And when the eggs hatched, the larvae produced by stressed parents had a higher death rate than those produced by unstressed parents, particularly following the outbreak of a fungal infection. Papatheodoulou thought this was especially interesting because stressing salmon parents seem to change the ability of the larva's immune system to fight fungal infection. In addition, the yolk sacs of the larvae produced by stressed mothers were smaller, resulting in fry that were smaller when they first began to feed. However, the offspring produced by stressed fathers were a normal size when they started feeding, which makes sense as mothers supply most, if not all, of the nutrients provided by egg yolk sacs.

Overall, Papatheodoulou and her colleagues have shown that catching and releasing Atlantic salmon as they rush upriver to spawn impacts the next generation. It is essential to protect both sexes during the critical spawning window, which is important knowledge for fisheries managers to better safeguard salmon while spawning, possibly by closing the recreational fishing season before and during this crucial period.

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Papatheodoulou, M., Metcalfe, N. B. and Killen, S. S. (2023). Effects of simulated catch-and-release angling of Atlantic salmon shortly before spawning on the viability and development of their offspring. *Can. J. Fish. Aquat. Sci.* doi:10.1139/cjfas-2022-0306

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Carrying an eggsac is not an issue for wolf spiders

People are often afraid of spiders. Perhaps one of the most fearful moments is when they move fast unexpectedly, even though they might be trying to avoid humans. And the spider's ability to move fast or slow depends on the temperature of the environment, because they are ectothermic creatures. It is also known that changes in their body mass and their build can influence their moving speed. However, there is not much information on how the combination of environmental temperature and changes in body composition together influences how fast spiders move. This prompted scientists from various countries in Latin America and the USA to investigate how temperature and carrying an eggsac affects the ability of female *Pardosa* wolf spiders (Lycosidae) to move.

Damián Villaseñor-Amador from the Universidad Autónoma de Mexico, and researchers from Brazil, Chile, Argentina and the USA collected 86 female wolf spiders from the sub-Alpine zone of the Buena Vista peak in the Talamanca Mountain range, Costa Rica, where temperatures can be extremely high or low, and conditions can change suddenly. Thirty-six of the spiders were carrying eggsacs, which can weigh as much as 77% of their body mass, while the remaining females were eggsac-free. After transporting the spiders in individual tubes to a warehouse 22 km away, Villaseñor-Amador and colleagues placed half of the eggsac-carrying females in a cooler for 5 min, to chill the spider. Then, they monitored each spider as it ran around an arena with a leaf floor covered in clear plastic, marking the spider's locations on the plastic surface as the animal moved, before using a cotton string to track the markings and measuring the string length to calculate the distance travelled by the spider. Next, they placed the remaining egg-carrying females in a 30°C heater for 5 min to raise their temperature before recording the warmed spiders' movements. The team then tracked the movements of the females that had no eggsacs at 7°C, 30°C and also at room temperature (13°C), in addition to recording how long all of the spiders (with and without eggsacs) kept moving until they paused for at least 5 s.

Not surprisingly, the wolf spiders moved faster when the environment was hotter; however, the largest spiders moved the slowest in hot temperatures, while the opposite occurred in cold temperatures. This may be because it is easier for the smallest wolf spiders to heat up to move faster in hot conditions. In contrast, at cold temperatures, the largest spiders may be more efficient at using their energy.

However, in contrast to expectation, the heavy burden carried by females with eggsacs did not affect their speed. Villaseñor-Amador and colleagues suggest that *Pardosa* wolf spiders may modify their body posture to compensate for the extra weight and drag that the eggsac generates. Alternatively, egg-carrying females may simply invest more energy in movement, allowing them to run as fast as unburdened females.

So, the extreme temperatures experienced by *Pardosa* wolf spiders in the Talamanca Mountains affect how fast the animals can move, yet their speed is unaltered when carrying an extremely heavy load, allowing them to escape predators as successfully as egg-free females, to ensure the future of the next generation.

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