OUTSIDE JEB

Moles power-walk with their thumbs

Moles are busy creatures. When not digging their tunnels, eastern moles (*Scalopus aquaticus*) are living, foraging and traversing through their labyrinthine territories. In a single day, a mole may walk 400 m through pitch-dark, cramped, narrow tunnels; that's a lot of (under) ground to cover. Moles do this in their own particular style – with their palms facing outwards. This unusual forelimb posture is probably an adaptation to a digging lifestyle; equipped with an extra 'false' thumb on their spade-like hands, moles push soil away from their body and against the tunnel walls. But how does an animal specialised for digging deal with the demands of walking such long distances?

Yi-Fen Lin at the University of Massachusetts (UMass) Amherst and colleagues at UMass Lowell and the University of California Merced, USA, set out to record high-precision measurements of how moles walk. The team studied three eastern moles, a species that lives close to Lin's lab. To measure how the moles' skeletons move, the scientists turned to a technique known as X-ray Reconstruction of Moving Morphology, or XROMM for short. XROMM combines X-ray videos of the moving moles with 3D models of their bones and then splices the data together using Hollywood-standard animation software to reconstruct the moles' motion. For the experiment, the moles walked back and forth through a purpose-built tunnel, positioned in front of the X-ray cameras. When the motion was reconstructed from the X-ray videos, the resulting 3D animations were beautiful and precise.

Analysing the movies, Lin found that moles power-walk in a most unusual manner, probably a by-product of their digging tendencies. Whereas all other quadrupeds place their feet on the ground behind their shoulder joint, moles break the rules by holding their feet in front. With each step, they load their weight fleetingly onto their thumbs and false thumbs, way out in front of their body. This instance of weight bearing was so brief that the moles' walking gait almost approached some definitions of running and was more like human power-walking, where people walk as fast as possible without actually breaking into a run. This might seem like an awkward way to get about, but considering that moles walk with shovels permanently attached to their forearms, awkward seems normal.

The XROMM animations also show that moles point their improbably shaped upper-arms to the ceiling, not downwards like other quadrupeds – effectively staging the action high above the shoulder joint instead of below it. As each foot lifts off the ground, the limb moves forward in line with the body, then retracts back. This is a very different motion from that seen in digging, where the moles shovel earth either side by twisting their arms outwards. This walking configuration means that moles fold their limbs into a nice compact package, perfect for squeezing down narrow tunnels.

Lin's moles show us how demanding life can be, sometimes right beneath our feet. Whilst it's incredible that moles have evolved such effective digging modifications at the ends of their limbs, it's even more astonishing that they still perform all of their other daily activities while burdened with these apparently cumbersome appendages. Sometimes a strange lifestyle leads to a unique way of getting around – no other known animal walks quite like a mole.

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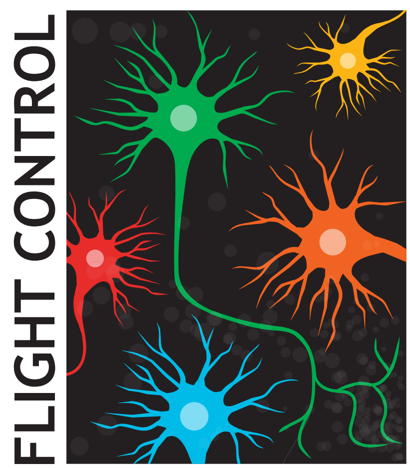
A new spin on flight control



Imagine designing a fly: you would need wings of course, three body segments, six legs, big red eyes and antennae. Unless you are already a fly enthusiast, you might not realize that you also need to include the halteres: small dumbbell-shaped appendages whose evolutionary ancestor is full hindwings. Without these modified hindwings, your fly would crash about clumsily instead of elegantly dipping through the air to land on your food. Halteres beat in the opposite direction to the wings and they are home to dense fields of sensory dome structures that detect tiny mechanical forces when a fly spins or turns, like a gyroscope. Two decades ago, the observation that more nerves carried information from the eyes to the halteres than to the wings led scientists to hypothesize that flies control their flight by first combining information about what the fly sees with a sense of

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rotation from the halteres before controlling the wings.

At the time, technological constraints made this hypothesis untestable. Recently, Bradley Dickerson and colleagues from Caltech, USA, tested the theory using modern genetic tools in tethered flies. They observed haltere muscle activity while the flies were shown spinning patterns, which caused the insects to move their wings as if they were spinning during flight too. The haltere muscles were most active when the patterns turned toward the same side as the muscles, whether the pattern simply rotated horizontally or spun in front of the fly. In other words, the little muscles controlling the halteres respond to what the fly sees, not just how the fly turns.

Next, the team tested the prediction that what the fly sees adjusts how the halteres sense spinning, by imaging nerve signals in the brain that were sent by the sensory domes on the halteres. They saw that nerve signals from the halteres changed depending on whether the spinning patterns rotated horizontally or vertically, or spun in front of the fly. The signals from the haltere indicated the involvement of a greater number of sensory domes, not greater activity from the same domes. This distinction means that muscles at the base of the haltere are likely repositioning the appendage to modulate which groups of sensory domes are active depending on which way the fly is turning.

The team then tested the prediction that changing the firing pattern of the nerve signals from the halteres would change how the wings beat. They genetically engineered special flies that produced light-sensitive proteins that cause the haltere motor neurons to fire when light is shone on them. At the same time, the team recorded electrical activity in two motor neurons controlling the wing: one that fires consistently when the wing is at the top of the wingbeat and one that fires sporadically. They found that when they artificially activated haltere muscles, the consistent motor neuron fired earlier than usual, and the sporadic motor neuron fired more often. This observation supports the prediction from 20 years ago that muscles controlling the wings are dependent on haltere muscles during flight.

Dickerson and colleagues have tested the predictions borne out of earlier experiments in larger flies and found that halteres are not just gyroscopic sensors; instead, they are actively controlled based on what the fly sees and they can, in turn, modify how muscles control the wings. From these observations, the team put forth a new evolutionary hypothesis: that halteres are better equipped to sense both rotation and motion because they do not produce aerodynamic force, like the wings do. In this way, flies can use their halteres to orchestrate wing muscles, thereby exerting greater control over their route to your food.

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Rising CO_2 saves lives

Anthropogenic climate change is a multi-faceted problem. Rising CO_2 levels in the atmosphere cause a warming of global temperatures, acidify the oceans and may ultimately deplete aquatic oxygen, leading to hypoxia. By itself, any one of these conditions can pose a real challenge to an animal, but how the stressors interact is still largely unexplored. Aquatic hypoxia may be especially intricate and, unlike the hypoxic conditions that one might experience when climbing a mountain, is almost always caused by organismal respiration in the water and, thus, is associated with an additional, simultaneous increase in CO_2. The linkage between oxygen and CO_2 levels in water has long been known, but how fish are affected by the combined challenge is poorly studied.

To address the issue, Daniel Montgomery and his colleagues at the University of Exeter, UK, set out to test the effect of rising CO_2 on the hypoxia tolerance of European seabass. They measured the oxygen consumption of the fish while lowering the available oxygen in the water; the point at which the animal can no longer extract oxygen from the depleted water and their oxygen consumption starts to decline is a popular indicator of hypoxia tolerance in fish. Knowing that higher levels of CO_2 tend to acidify the blood of fish – compromising the ability of haemoglobin to bind oxygen and hindering the fish's ability to extract oxygen from the water – Montgomery then supplemented one of the treatments with a simultaneous increase in CO_2, expecting that the combined challenge would render the fish more susceptible to hypoxia.

However, contrary to these predictions, CO_2-treated fish excelled at extracting oxygen from the water and outperformed the low-CO_2 group in terms of hypoxia tolerance. When Montgomery took a closer look at the fish's blood, he found no difference in blood pH between the treatments, indicating a remarkable capacity of the seabass to counteract the acidifying effect of elevated CO_2; surely this is good news for fish that must cope with global rises in CO_2. In addition, fish in the rising CO_2 treatment had a higher haemoglobin–oxygen affinity compared with the low-CO_2 group – a surprising finding, given the common blood pH in the two groups – and this may explain their improved ability to tolerate hypoxia. Haemoglobin is housed within red blood cells, which may actively alter their internal environment to modulate oxygen transport. Whether this is part of the mechanism by which CO_2-exposed seabass increased their haemoglobin–oxygen affinity remains to be seen.

Studying the effects of climate change is complicated as numerous factors may interact to challenge an organism. Understandably, researchers often choose to study single factors in isolation, as this simplifies the interpretation of the data. However, Montgomery and colleagues show that overlooking interaction effects may limit our ability to predict the response of animals to climate change in the wild. In European seabass it appears that the combined condition of a decreasing oxygen level and an increasing CO_2 level in the water, as is found in nature, improves hypoxia tolerance. To account for such effects in future work, current methods to assess hypoxia tolerance in fish should be revised to control for, or at least measure and report, CO_2 levels in the water. Rising CO_2 levels receive a lot of bad press and, in the context of climate change, rightfully so. However, at least for seabass that find themselves gasping for oxygen, the natural rise in CO_2 that comes with aquatic hypoxia may actually save lives.

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Electric fish turn down the power

Animals have all sorts of neat tricks to find their way in the dark. Bats have sonar, ants follow their noses, and some fishes surround themselves with electric fields that can detect nearby objects. Electrical navigation is wonderful for fishes that live in murky water, but it comes at a cost – electricity is expensive to generate and can represent almost a third of the overall energy budget in these fishes. When conditions are good, paying this cost doesn't seem to be a problem. But electrical sensing is most useful in murky and stagnant habitats that also tend to be low in oxygen. Oxygen is critical for fuelling metabolic energy production and survival in oxygen-limited environments often, therefore, depends on the ability of animals to reduce their metabolic rates. How do electric fishes balance this energetic budget and deal with the expense of electricity generation in the face of severe oxygen austerity?

A new study, led by Shelby Clarke at McGill University, Canada, has unravelled the details of this trade-off by studying the electric fish *Petrocephalus degeni*. The authors captured wild fish from a low-oxygen Ugandan swamp and brought them into a lakeside laboratory, where they measured metabolic rate and electricity production first under conditions of abundant oxygen and then after the fish were challenged with low-oxygen conditions.

As oxygen levels decreased in the experimental chamber, electricity production initially remained steady. However, under more severe conditions – when about 80% of the oxygen was gone – electrical activity began to decrease. The energy saved from minimizing electrical output could then be allocated to other vital processes, allowing the fish to continue to obtain enough oxygen to maintain normal metabolism until almost 90% of the oxygen was gone from the water. Amazingly, even below this critical point where the fish could not breathe as much oxygen as they required, electrical production did not cease despite its high energetic cost. Low levels of electricity persisted, perhaps representing a desperate attempt to find an escape route.

If electrical activity is constrained by oxygen supply, the authors reasoned the electric fish should get even more electric if oxygen is abundant. To test this idea, Clarke moved electric fish from their typical low-oxygen swampy habitat to a life of luxury in well-aerated aquariums. After several weeks in this housing arrangement, electrical production was indeed higher than in fish from the harsh wild conditions. However, the ability of these pampered fish to tolerate low-oxygen conditions was diminished. The authors conclude that when these electric fish have easy access to oxygen, they spend less energy on the organs used to acquire more of the gas, such as the heart, gills or blood. Instead, the energy is allocated to increased electrical capacity that presumably improves their ability to perceive their physical environment.

Like any utility company, electric fish must continually evaluate the budgetary landscape when deciding how much to invest in electricity production. And, while the mechanistic details of electrical output regulation remain to be discovered, it is clear that these fish have an impressive ability to re-organize their power system over both the short and long term, allowing them to cope with whatever conditions nature throws their way.

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The big problem with microplastic pollution

Plastic pollution is one of the most pervasive and sinister ecological threats worldwide. Most of us are aware of animals ingesting and dying from plastic waste. However, much less is known of the more insidious problem caused by much smaller microplastics, which are formed after larger pieces of plastic are degraded, by sunlight or wave action, into very tiny particles. Owing to their size, microplastics are extremely difficult to remove from the environment and are easily consumed by animals, yet their biological effects are unclear. For these reasons, Giacomo Limonta and scientists from the Universities of Siena and Ferrara, Italy, wanted to know the various ways in which microplastics impact animals, from their miniscule DNA molecules to their ability to move.

The team tested the effect of two plastics – polyethylene (the principal source of microplastic globally) and toxic polystyrene – on a standard laboratory animal, the zebrafish. They exposed the fish to microplastics that were smaller than one-tenth of a millimetre, at concentrations similar to those found in highly polluted waters, for 20 days, before looking at how the plastics affected the fish's gills and intestines, their movement and the gene-expression patterns of their liver. The researchers focused on the liver because it detoxifies blood and contributes to immune function, while the intestine absorbs nutrients into the body and the gills are necessary for breathing. To identify how microplastics changed the structure of the gills and intestine, the researchers stained the tissues and scrutinised them under a microscope. Lastly, they used infrared sensors to observe how the pollution affected the fish's swimming behaviour.

Overall, the microplastics severely compromised the zebrafish's immunity. For instance, microplastics elevated the activity of genes that help certain immune cells (lymphocytes) recognise liver tissue and trigger immune responses. This worrying finding suggests that the fish's own immune system could mount overzealous attacks on the liver, impairing its function. In addition, the team realised that germs might be able to infiltrate liver cells more easily, because genes linked to antimicrobial resistance and cell-to-cell attachment were less active. Meanwhile, genes for metabolic pathways that manage fat in the fish's bodies – including those for cholesterol and fatty-acid synthesis – were turned down. Given the importance of these molecules for immunity, the authors suggest that microplastics could seriously affect the fish's ability to fight off infections.

The microscopic analysis also showed the layer of cells (mucosa) covering the intestine and gills was profoundly weakened by microplastics. Indeed, the intestine showed evidence of mucosal degradation (a sign of tissue damage) and it had fewer mucus-producing cells (goblet cells) essential for intestinal function. In the gills, the microplastics thickened the secondary lamellae (the flap structures where gases pass into and out of the body) and caused excessive mucus secretion, which together increases the distance the gasses travel to and from the bloodstream. Additionally, the microplastics elevated the number of neutrophils (immune cells) that respond to inflammation and kill bacteria in the gills and intestine, pointing to severe microbial infection. And when the team monitored the zebrafish's activity levels, the microplastics made them unusually active at night, indicating the damaging effect that these particles can have on an animal's internal clock.

This study demonstrates that microplastics perversely impact all aspects of an animal's biology, from gene expression to first and secondary lines of defence against infection and their body clock. Considering the critical roles these play in everyday survival, microplastics have the potential to imperil whole populations of animals and wreak havoc on ecosystems. The alarming discoveries presented by the authors are yet another warning of the dangers of pollution and the need to reduce plastic use.

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