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The subtle art of electro-flirting in wild knifefish

What do James Brown and ghost knifefish (*Apteronotus*) have in common? They've both harnessed the power of electric organs in order to deliver declarations of adoration. As close relatives of the electric eel (*Electrophorus electricus*), it's probably not too shocking that ghost knifefish also possess an electric organ that generates an electrical discharge. As well as emitting electricity, ghost knifefish are covered in electro-sensitive receptors that allow them to sense their surroundings, detect the presence of other individuals and communicate with other fish through electric organ discharge. As ghost knifefish are nocturnal and frequently hide amongst rocks and roots, their natural behaviours are rarely ever observed and the characteristics of their electrical discharges have previously been examined almost exclusively through laboratory-based tank studies. Until now, the natural electric conversations of ghost knifefish and their role in wild behaviours have largely remained a mystery.

A recent study reveals the importance of subtle bio-electric communication during the courting and aggression behaviours of wild weakly electric fish. Lead authors of the study, Jörg Henninger and Jan Benda, from the Eberhard Karls University of Tübingen, Germany, set out deep into the rainforests of Panama to record the electro-communications of wild brown ghost knifefish (*Apteronotus rostratus*). Using a grid of electrodes placed in a knifefish-inhabited creek, the team were able to record electric organ discharges with high precision. They found that each fish emitted electric discharges at their own unique frequency, producing 'electrical fingerprints' that they used to identify and track individuals, as well as distinguish between the sexes.

The team discovered that the electric discharges were closely associated with two types of behaviour: male–female courting and male–male aggression. During courting, males would hound females with short electric 'chirps' that lasted less than 20 ms, until the female responded with a long chirp that lasted over 150 ms, signalling her intention to release eggs. These long chirps were responded to with precisely timed response 'doublet' chirps, which indicated the intention of the male to release sperm, resulting in a tightly synchronised fertilisation event. As well as courtship, the team found interesting patterns of electro-communication between males competing for access to females. These events occurred when the electric discharges of a rival male interfered with the signals emitting from a male courting a female and tended to end with the rival male submissively retreating from the area.

The team was also able to demonstrate how these short and long chirps were closely linked to spawning behaviours in another species of brown ghost knifefish (*Apteronotus leptorhynchus*) in a more closely monitored laboratory environment. By simultaneously filming and recording the fish's electric discharges for over 5 months, collecting a staggering 1.3 million individual chirps in the process, the team demonstrated that the same short and long chirps recorded in the wild were closely correlated with critical mating events such as the synchronised release of eggs and sperm.

However, in contrast to previous laboratory-based tank studies, many of the EOD signals detected in the wild were much weaker than expected and barely activated the fish's own electro-receptors. These subtle electric whispers had never previously been recorded in laboratory experiments as the artificial conditions may interfere with natural courtship. This study highlights the importance of investigating physiological phenomena in natural habitats. The team also note that the insights into how weak electrical signals are interpreted by the fish's brains may help us to improve the design of bionic devices, such as retinal and cochlear implants for people with impaired vision and hearing.

10.1242/jeb.170084

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How ticks put the B in blood

Few things wreck a picnic faster than the discovery of a poppy seed tucked into the warm folds behind your knee that turns out to be a tick. If left undisturbed, it will slowly gorge on your blood until it resembles a flattened jelly bean – with legs. For picnickers and parents, it's a revolting transformation fraught with dangers, because ticks are major vectors of a range of debilitating illnesses, including Lyme disease; yet, for biologists, the transformation is nothing short of remarkable.

Ticks can endure weeks of starvation while awaiting the chance arrival of new hosts, but even after tapping into a vein, all they get is a diet of blood, upon which

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they would surely starve. Although blood is rich in proteins and lipids, it lacks other key nutrients required for survival. However, a fascinating new study – by Olivier Duron from the CNRS in Montpellier, France, and his team of colleagues from across the country – shows how ticks overcome this nutritional deficiency.

Ticks are arachnids, but they aren't the only creepy-crawlies that suck. Several insect families are exclusive blood feeders and others live on a diet of plant sap; but their survival doesn't depend on this alone. Typically, these beasts rely on microbial lodgers, known as mutualists, to supply their missing dietary requirements. To test whether a similar symbiosis was at play in ticks, Duron and his colleagues treated ticks with antibiotics to kill off potential bacterial symbionts. The response was dramatic. Male juvenile ticks fed with antibiotics were 10 times less likely to reach adulthood than their untreated counterparts and the ticks that did survive were smaller and deformed. For females, it was even worse. Of 120 treated females, not a single one reached adulthood. So, bacteria matter, but what do they provide for their hosts?

To answer this question, the team dissected ticks and discovered that they were all colonized with a strain from the bacterial genus *Francisella*. These bacteria are usually vertebrate pathogens, but in ticks they showed the hallmarks of long-term mutualists. First, they were extremely numerous and showed a preference for certain tissues. In both males and females, the bacteria accumulated in the Malpighian tubules, a site from which they could alter tick nutrition. More importantly, the bacteria also accumulated in the ovaries of pregnant females, thereby ensuring a stable route of transmission from mothers to offspring. Indeed, all tested tick eggs carried *Francisella.* Second, consistent with other intracellular bacterial symbionts, the *Francisella* genomes were so degraded that these bacteria could never live outside of their hosts. However, despite these losses, the genes for biosynthesis of B vitamins were conspicuously intact.

To test whether *Francisella* provides ticks with the B vitamins essential for their survival, the team fed the antibiotic-treated ticks with a B vitamin supplement and this solved everything! The ticks on drugs and vitamins were as healthy as wild-type ticks.

This and countless other studies make clear the numerous essential roles that bacteria play in animal well-being. In addition, this study highlights the massive evolutionary flexibility of some bacterial groups. Within *Francisella*, there are both mutualists and pathogens that are nasty enough to be used as bioweapons. Interestingly, some other tick species harbor nutritional symbionts from another genus, *Coxiella*, that also contain species used as bioweapons. Remarkably, both were tamed by ticks – which is exciting news for biologists, but probably not enough to calm panicked parents after a day in the woods.

10.1242/jeb.170068

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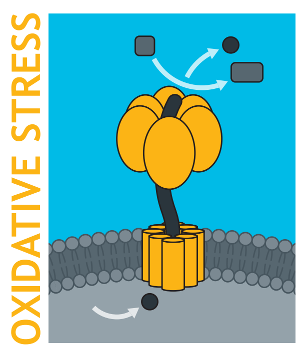
Fasting fish risk oxidative stress



During lean times of food scarcity, animals go all out to get the biggest bang for their energetic buck, including adjusting energy metabolism and downsizing non-essential processes such as growth and reproduction. This strategy has the immediate benefit of stretching limited energy stores, but may have hidden costs not revealed until later in life. One potential trade-off of the metabolic changes made during fasting is increased oxidative stress due to modifications to the mitochondria. These complicated organelles are critical for metabolism, but are also a major source of potentially toxic oxidants in the form of reactive oxygen species (ROS), a by-product of electron movement through the respiratory chain that supports ATP production. The dual role of mitochondria intrigued Karine Salin and her colleagues from the University of Glasgow, UK, and Semmelweis University, Hungary, so they investigated whether the metabolic adjustments made by mitochondria during fasting also caused oxidative stress in brown trout (*Salmo trutta*), a species that naturally experiences periods when food is scarce.

The team either fed trout fry as much as they could eat or deprived them of food in a realistic simulation of the natural food shortages experienced by these animals. After 2 weeks, the team collected the mitochondria from the fish's livers and measured how much oxygen they consumed as they produced energy-rich ATP (known as state 3 respiration) and while maintaining the electric gradient that counteracts proton leak across the mitochondrial inner membrane (state 4 respiration). The team also measured the size of the electric gradient directly using the fluorescent probe safranin.

Comparing the sizes of the livers, the team found that those from the fasted fish were about one-third the size of those from the well-fed fish and had fewer mitochondria, leading to a 50–70% reduction in respiration rate. However, the story was very different at the level of the individual mitochondria: fasted mitochondria increased state 3 respiration and reduced state 4 respiration, meaning they selectively increased their capacity to produce ATP without increasing the amount of energy dissipated through proton leakage. A larger electric gradient across the membranes of the mitochondria from the fasted fish supported these energy-saving modifications to improve the efficiency of energy transduction in the mitochondria further.

So, fasted mitochondria were more efficient, but how did this relate to the risk of oxidative stress caused by the oxygen by-products of metabolism? The team estimated the amount of oxidative stress that the mitochondria were experiencing by injecting a compound into the fish that measured the presence of one of the toxic oxygen by-products: hydrogen peroxide. The fasted mitochondria had nearly twice as much hydrogen peroxide as fed mitochondria, supporting the notion that reduced metabolism can potentially lead to oxidative stress. How this increase in stress occurred is not clear, but it could be due to the increase in the electric gradient (larger gradients are sometimes associated with high oxidative stress) or other aspects of the liver function, such as cutbacks to the system that mops up toxic oxygen by-products as an energy-saving measure.

Regardless of its root cause, increased oxidative stress could lead to long-term life-history challenges for fasted fish, such as DNA damage and ageing. This study also provides mechanistic insight into the trade-off between short-term gains from reducing metabolism during fasting and long-term costs to subsequent life-history traits.

10.1242/jeb.170332

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Waste recycling by gut symbionts

Hibernators, or animals that 'sleep' through the winter to conserve energy, have always fascinated scientists. To date, researchers have studied the adaptations that allow these animals to be inactive for so long and their ability to come back from their slumber at the beginning of spring. However, no hibernator has been more captivating than the wood frog, *Rana sylvatica*. That is because this little critter is able to freeze during the winter and then resurrect as if nothing has happened. We know a great deal about how this animal protects itself from the ice crystals that form in its body as it freezes but very little about the role that the symbiotic bacteria in its gut play during hibernation and emergence from hibernation.

One strategy that wood frogs use to preserve water and salts during hibernation is to produce urea when they break down nitrogen-rich proteins. Urea is less toxic than other forms of nitrogen waste, such as ammonia, which has to be diluted and leads to the loss of water and salts. However, the amphibians lack the ability to recuperate the valuable nitrogen from urea. Might the frog enlist some 'helpers' – such as their intestinal flora – to retain the nitrogen? A team of researchers from Miami and Pittsburgh Universities, led by James Wiebler, examined the frog's gut to look for bacteria that can produce the enzyme urease, which breaks down urea to produce nitrogen.

First, the researchers isolated the gut from hibernating adult male frogs and separated out three segments: the foregut, midgut and hindgut. In each of these segments, the group measured the size (length and mass) of the segment, the bacterial load and type, and urease activity. They found that, even though the hindgut was the smallest segment, it contained the largest number of bacteria and also had the largest urease activity out of the three. Next, the authors injected active frogs with urea and allowed them to recover for 10 days, to determine whether high levels of urea in the body – which are typical in frogs nearing hibernation to maintain water and ion balance during their slumber – increases urease activity in the gut bacteria. The group determined that high urea levels in the blood did not influence the number of bacteria present in the hindgut but did increase the activity of the urease enzyme by 2.7 times.

The researchers then collected guts from active frogs during the summer for comparison with guts from the hibernating animals. Even though the digestive tracts of the hibernators weighed 61% less and were 25% shorter than those of the active frogs, there was no difference between the size of the hindgut portion in the two groups. In fact, Wiebler and his colleagues reported that the hindguts of the hibernators had 33% fewer bacteria than those of the active frogs, but the amount of the urease enzyme and activity was 2–3 times higher in the hibernators. In addition, the authors reported that the types of bacteria present in the hibernators were different from those in the active frogs, suggesting that gut bacteria composition changes in these animals as they begin their hibernation.

Frogs have a relatively low-energy lifestyle, which allows them to tolerate low-nutrient environments, so retaining as many nutrients as possible is key to survival, particularly during periods of fasting, such as hibernation. Through this work, Wiebler and his group have provided the first evidence of nitrogen recycling from urea in wood frogs and have highlighted the important role that friendly gut bacteria may play in all amphibians. Talk about waste recycling at its best!

10.1242/jeb.170142

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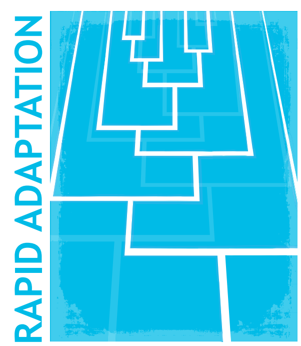
A fish out of saltwater



Common knowledge says that evolution takes a long time, right? It might take thousands of years for us to detect the effect of a selective pressure. But for steelhead trout, evolution has happened much faster. Game fisherman stocked the freshwaters of Lake Michigan with steelhead trout from the saltwater coast of California in the late nineteenth century. Since then, the steelhead have settled into their new Great Lakes home and adapted to freshwater life in just over 100 years.

Janna Willoughby and her colleagues from Purdue University, Michigan State University, and Oregon State University, USA, were interested in how a saltwater fish could make the move to freshwater. How had the genetic code of these fish changed and evolved to allow them to

Journal of Experimental Biology (2018) 221, jeb170084. doi:10.1242/jeb.170084

grow, reproduce and live in a completely new environment? To uncover the answer, Willoughby and her colleagues sequenced the entire genome for over 250 steelhead trout, some from the modern California population and others from the Lake Michigan population a century on from their original trans-continental road trip.

The team then scrutinized the two genomes, looking for sequences that varied between the two populations and found three regions that differed dramatically. Two of the three genomic regions play a role in osmoregulation, which is the ability of fish to maintain their internal salt balance; the freshwater fish have to work hard to keep salt ions in their bodies, while the saltwater fish have to work hard to pump excess salt ions out of their bodies. This means that in just 100 years, the transplanted saltwater trout population had experienced dramatic changes in its genetic code, which allowed it to cope better with freshwater living.

The third gene region identified by the team plays a key role in wound healing, indicating that freshwater steelhead likely heal more effectively than their saltwater counterparts. This change might seem strange at first glance, but the ability to heal rapidly should prevent the loss of internal salts following an injury or attack. Additionally, the introduced fish are vulnerable to attacks by parasitic lampreys – residents of the Great Lakes that latch onto fish with razor-sharp teeth – which they would never encounter in their native California coast. The ability to heal quickly from a lamprey attack would be another bonus for freshwater steelhead.

Fascinatingly, the rapid evolution that Willoughby and colleagues have revealed in the steelhead genome occurred despite the limited genetic diversity in the small population of transplanted fish. Only a tiny portion of the California population was moved to Lake Michigan in the late 1800s, creating a population bottleneck that could reduce the population's genetic diversity. Less diversity could potentially restrict their opportunity to evolve in their new home. Yet, this new study excitingly highlights how animals can evolve and adapt rapidly to changing environments and, more promisingly, shows that certain animals, such as the steelhead trout, can succeed even if they started with fewer cards in their genetic deck.

10.1242/jeb.170134

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