OUTSIDE JEB

In acidic water, crabs stink at smelling

Human activities that burn fuel for energy are driving more carbon dioxide (CO_2) into the atmosphere than the earth can handle. Consequently, the CO_2 is being absorbed by the ocean's waters, making them more acidic and interfering with the behaviour and senses of many marine animals. Crabs have poor eyesight and rely primarily on their sense of smell to find food and avoid predators, and unfortunately acidic waters are wreaking havoc with this. Scientists are unsure exactly how higher acid levels in the ocean are changing crabs' smelling skills, so Andrea Durant, Elissa Khodikian and Cosima Porteus of the University of Toronto Scarborough, Canada, set off to investigate the mechanics of a bad sniffer.

Crabs sniff by flicking their antennae and flicking them faster when there's a good (or bad) smell around. To test how this sniffing behaviour changes, the scientists first put Dungeness crabs (*Metacarcinus magister*) into slightly acidic seawater for 10 days and then measured their response to either a 'good' or 'bad' smell at different strengths. Because some aquatic animals release ammonia-laden urine when they're startled, ammonia was chosen as the bad smell. But this 'alarm cue' didn't bother the crabs at all, whether they were in normal seawater or acidic seawater. Instead, they 'sniffed' the bad smell just as they would any other scent. For the good smell, the scientists used a scent called cadaverine, which is released when animals are decomposing and apparently smells delicious to hungry crabs. Normally, crabs would flick their antennae enthusiastically when they sniff this alluring aroma. Unfortunately, the crabs from acidic seawater had a hard time detecting this enticing smell. In fact, they needed a cue that was 10 times stronger than normal to set their antennae flicking, suggesting it was harder for the crabs to 'sniff out' their food.

To understand why crabs in acidic waters have a poorer sense of smell, the scientists turned to the olfactory (smell-sensing) nerves. The team tested how sensitive these smell-sensing nerves of the crabs' antennae were to cadaverine. As expected, the nerves of the crabs in acidic seawater reacted poorly to the feeding cue, barely responding to the delicious scent no matter how strong the dose of cadaverine was. The scientists then examined the structure of the nerves and assessed which proteins were present because the smell-sensing nerves in crabs rely on particular proteins to sense and process smells. Interestingly, the crabs in acidic seawater had smaller nerve cells and fewer receptors for scents. Unexpectedly, they also had more ammonia-transporting proteins, even though ammonia didn't affect their sniffing abilities or nerve function. What these ammonia transporters are doing in these nerves remains a mystery.

This work by Durant and colleagues adds to the growing body of evidence that crabs are incredibly vulnerable to the increased acidity of the ocean resulting from climate change. Dungeness crabs in particular are one of the most economically important species in Northwest America and Canada and already experience periods of more acidic seawater, which will only intensify in the future. If only 10 days in acidic seawater makes it harder for crabs to smell food, well, that stinks.

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Durant, A., Khodikian, E. and Porteus, C. S. (2023). Ocean acidification alters foraging behavior in Dungeness crab through impairment of the olfactory pathway. *Glob. Chang. Biol.* **29**, 4126-4139. doi:10.1111/gcb.16738

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Learning how Rice's whales eat might save them

Oceans are vast and home to many magnificent creatures, including colossal whales, which few of us have ever seen in real life but encounter frequently on our screens. Rorquals are a family of large whales that have a unique way of dining: they lunge forward, filling their mouths with water and food before filtering out the water and swallowing what remains. One species, Rice's whale (*Balaenoptera ricei*), is found only in the Gulf of Mexico, with a population of less than 100. Little is known about their eating habits and how they use energy when moving and searching for food, but this knowledge is essential for us to identify possible threats, such as entanglement in fishing gear, which could be modified to preserve the endangered species. This motivated a collaborative team of scientists from the Scripps Institution of Oceanography, USA, the University of California San Diego, USA, and the National Marine Fisheries Service, USA, to collect information about the lifestyle of two Rice's whales in their natural habitat.

Annebelle Kok (Scripps Institution of Oceanography) with six colleagues named the two animals Milky Way and Edna and collected information about their maneuvers using suction-cup tags,

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which recorded the whales' movements, water pressure and sound of the water rushing past as they moved over several years. The team then used the pressure information to determine how deep the whales were diving and the movement information indicated how the animals changed direction, either by moving their head upwards or rolling their bodies. Then, the team used the sound recordings to determine how often the whales breathed by identifying the sound of exhalation when the whales reached the surface. Finally, Kok and colleagues calculated how much energy the whales consumed when foraging by estimating each animal's mass, their acceleration, velocity and the time they spent diving, breaking down the time underwater into four major events: time descending, at the bottom of the dive, while lunge-feeding and returning to the surface.

From this, the team were able to identify how often Milky Way and Edna performed deep dives to feed, which told them what kinds of food the whales were dining on; fish tend to reside at depth and are more calorific than small animals, such as krill and zooplankton, which live nearer to the surface. The scientists determined that prior to feeding, the whales circled around their prey before speeding up to engulf a colossal mouthful of water containing a school of fish. Sometimes the whales almost reached the bottom of the sea when they embarked on their deepest dives, which they mainly undertook during the day, completing no more than two dives at night, when they mainly stayed close to the surface feeding off krill and zooplankton. According to Kok and the team, the two whales spent most of their energy when circling their prey and lunging to engulf as many fish as possible. Interestingly, although both mammals behaved similarly during the day and night, Edna dived more frequently and consumed more high-energy food than Milky Way did. Kok and colleagues think that these differences may arise because Milky Way was caring for a calf, thus requiring lower energy consumption, as previously reported, whereas Edna might be leaner with higher energy needs.

The ability to collect information about animals in the wild and learning in detail about their foraging patterns around the clock is important if we want to protect them from extinction. Kok and colleagues also argue that Rice's whales are in danger of injury by boats at night, in addition to being at risk of becoming entangled in bottom longline fishing gear, since fishers are also trying to collect the fish that these mammals are looking for.

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Kok, A. C. M., Hildebrand, M. J., MacArdle, M., Martinez, A., Garrison, L. P., Soldevilla, M. and Hildebrand, J. A. (2023). Kinematics and energetics of foraging behavior in Rice's whales of the Gulf of Mexico. *Sci. Rep.* **13**, 8996. doi:10.1038/s41598-023-35049-z

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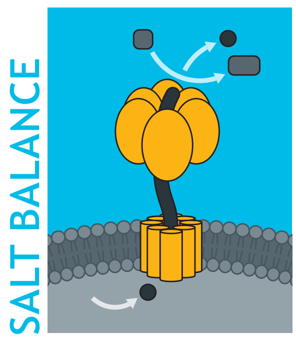
Freshwater sculpins struggle with their marine roots



The ancestors of today's freshwater fishes came from the ocean. Although many fish stayed in the salty waters of the seas, those that moved into freshwater rivers and lakes faced some major challenges such as controlling their salt and water balance, a process termed osmoregulation. Over time, the formerly marine fish adapted to the strange fresh waters of their new home by doing the opposite of what their saltwater ancestors did: taking salt in and keeping water out. But what happens when the newly freshwater fish rediscover their oceanic roots? The prickly sculpin (*Cottus asper*), an ancestrally marine species with both freshwater and seawater populations, can be found in three types of habitats: inland lakes, coastal lakes and coastal rivers that flow into salty estuaries. Both lake habitats are freshwater, but the inland lakes have been isolated from the ocean longer than the coastal lakes and the sculpins that live in coastal rivers are barely removed from their salty origins. In a recently published study in Physiological Biochemical Zoology, Shaung Liu and colleagues from the University of British Columbia, Canada, investigated how moving into freshwater has changed the prickly sculpin's ability to control its salt balance.

Liu and colleagues suspected that lake sculpins would struggle with the high salt environment of seawater because of their freshwater specialization and that this would be most apparent in populations from inland lakes. After scouring southern British Columbia to collect sculpins from all three of the habitats, they transferred the fish to saltwater for several weeks and compared how well the different populations kept their salt and water levels in check. The freshwater sculpins struggled in seawater because the salt-managing machinery in their gills and intestine wasn't as good as that in their marine counterparts at getting rid of the extra salt.

The best thing that a marine fish can do for salt balance is take in as little salt as possible. When held in seawater, the coastal river sculpins kept sodium and chloride levels in their blood lower than in the sculpins from both lakes, indicating that they did a better job of limiting their salt intake compared with their freshwater relatives. The researchers turned their attention to two protein pumps that are essential for keeping salt balanced in the body, Na^+/K^+-ATPase and H^+-ATPase. They discovered that these proteins from the lake sculpins weren't as good as the river sculpin proteins at keeping salt out of their cells.

Oceangoing fish remove calcium and magnesium salts from the seawater that they drink by producing carbonate pebbles in their intestines as part of the process that reduces the salt levels in their bodies. Knowing this, the team wondered if the sculpins from the saltiest environment – the coastal river – might carry more the pebbles in their intestines than the fish from the freshwater lakes. The researchers counted the number of pebbles in the intestines of the fish from the different habitats. They found that the sculpins from the coastal river had the most pebbles in their intestines and, therefore, were the best at getting rid of salt from the water they had drunk, ensuring that their bodies are well hydrated. In contrast, the sculpins from the inland lakes had very few pebbles in their intestines and have likely lost most of the ability to get rid of salt ions from the water they drink. The researchers also discovered that the number of carbonate pebbles in the sculpin intestines increased with the quantity of Na^+/K^+-ATPase pumps in the tissue, once again highlighting the importance of this protein for keeping the fish's salt levels balanced, enabling them to survive in freshwater.

Separated from the sea for over 10,000 years, freshwater prickly sculpins have lost some of their salt-regulating skills and struggle when returned to their ancestral habitat. This study emphasizes an underappreciated cost of adapting to a new environment: sometimes it requires losing something that gave you an advantage in the old one.

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Liu, S., Taylor, E. B. and Richards, J. G. (2023). Osmoregulatory performance among prickly sculpin (*Cottus asper*) living in contrasting osmotic habitats. *Physiol. Biochem. Zool.* **96**, 233-246. doi:10.1086/725208

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Aphids host, parasitic wasps mix venom cocktails

There is nothing friendly about parasites. One, a parasitic wasp (*Aphidius ervi*), uses pea aphids (*Acythrosiphon pisum*) as growth chambers for its young, which consume the aphids from the inside before emerging from their hosts' mummified husks. To ensure the aphid's body feeds her offspring well, a mother wasp injects venom along with her egg. The mother's venom is a cocktail of molecules that cause the aphid to stop reproducing and instead produce more nutrients for the developing wasp. The most abundant molecule in the venom is the protein Ae-γ-GT, which has been shown to cause the degradation of the reproductive organs of aphids. Scientists have determined the protein's role by isolating it and injecting large quantities into aphids, but previously no one had checked the effects of Ae-γ-GT on an aphid infected by the other components of the venom and the developing wasp. Elia Russo, Ilaria Di Lelio, Andrea Becchimanzi and Francesco Pennacchio of the University of Naples, Italy, with Min Shi of Jiaxing Nanhu University, China, decided to change that.

The team identified young wasps that were pupating in mummified aphids and injected the insects with bits of RNA designed to target and deactivate the genes encoding Ae-γ-GT. When the treated wasps emerged as adults, they could then produce all the components of their venom except for the Ae-γ-GT protein. The researchers provided treated and untreated wasps with aphids to infect and tracked the development of the wasps' offspring and the destruction of their hosts. They then compared the two groups to learn more about the role of Ae-γ-GT.

As the scientists expected, aphids infected by the treated wasps did not lose their reproductive capabilities, but the scientists were surprised to observe other differences as well. The aphids that had not been exposed to Ae-γ-GT grew much larger than their counterparts, fueled by an explosion in the number of beneficial bacteria in their bodies. Aphids, which subsist on sap, carry a special type of bacteria (*Buchnera aphidicola*) that produce essential amino acids that would otherwise be absent from their sugar charged diet. Those amino acids are also important for developing wasps, and while previous studies involving untreated wasps had found that aphids had more bacteria after infection, removing Ae-γ-GT from the equation supercharged the bacterial proliferation.

Within these bacteria laden aphids, the developing wasps grew larger as well and ultimately emerged as bigger adults. At first glance, bigger children might seem like a win for the treated wasps, but the researchers found that those big offspring also died younger and produced fewer offspring of their own in comparison to the children of the untreated wasps. In the long run, wasps that fail to produce Ae-γ-GT would be left with fewer descendants. The molecules that a mother wasp injects along with her egg interact with each other and it appears that Ae-γ-GT limits the bacterial growth caused by some other molecule in the venom. This function may be even more important to the health of the developing wasp than shutting off the aphid's reproductive system. Isolating Ae-γ-GT allowed scientists to begin to study it, but the protein is a part of a mixture, and a mixture cannot be fully understood by studying its parts in isolation.

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Russo, E., Di Lelio, I., Shi, M., Becchimanzi, A. and Pennacchio, F. (2023). *Aphidius ervi* venom regulates *Buchnera* contribution to host nutritional suitability. *J. Insect Physiol.* **147**, 104506. doi:10.1016/j.jinsphys.2023.104506

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