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OUTSIDE JEB

Rockfish are anxious about climate change too!

These days, it's hard to exhale without thinking about carbon emissions. One major consequence of climate change is ocean acidification, which is an increase in hydrogen ions as water chemistry balances the atmospheric carbon dioxide load. Aquatic animals are affected by ocean acidification because pH (a measure of hydrogen ions) is an important factor in all biological processes. Fish, for example, compensate for a change in environmental pH by adjusting certain ions in the blood and tissues. This shifting of ions is important for the fish to maintain optimal pH inside its body, but there are consequences for cells like neurons that rely on ions for communication. For example, the neurotransmitter gamma-aminobutyric acid (GABA) binds to its cell membrane receptor to allow the passage of chloride ions into the neuron as they flow down their concentration gradient, ultimately stopping that neuron from talking to its neighbors (inhibitory). A change in chloride ion concentration on either side of cell membranes would reverse the direction of ion flow through the GABA receptor and ultimately encourage that neuron to chat (excitatory), and this could result in anxious behavior. Trevor Hamilton and Adam Holcombe at MacEwan University in Alberta, Canada, teamed up with Martin Tresguerres at the Scripps Institution for Oceanography in California, USA, to determine whether ocean acidification influences the behavior of rockfish by shifting the GABA receptor from inhibitory to excitatory.

The international research team collected rockfish off the coast of California, where ocean currents already create intermittent periods of pronounced acidification. Back at the lab, half of the rockfish happily swam in normal seawater while the other half experienced simulated ocean acidification for 1 week. To see how water pH affected the fish's anxiety-like behavior, the team then placed individual fish in aquaria that had either a combination of black and white walls or a novel object in the center, and then they recorded the fish's position in the aquarium for 15 min with a video camera. The researchers then repeated the behavioral testing on the fish from the acidified and normal seawater, but this time they gave the fish one of two drugs – one drug closed the GABA receptor (antagonist) and the other drug opened it (agonist). Hamilton and colleagues predicted that if the function of the GABA receptor is reversed in response to ocean acidification as ions re-shuffle across membranes, then fish from normal seawater will be more anxious with a closed receptor, while fish from acidified seawater will be extra anxious with an open GABA receptor.

Compared with the fish from normal seawater, rockfish from the acidified seawater spent more time near the black walls, meaning they were anxious. Rockfish from normal seawater that were exposed to the GABA receptor antagonist also preferred black walls; so closing the GABA receptor in control seawater prevented its inhibitory function and resulted in anxious behavior. Next, the researchers compared how much time the fish spent near a novel object in the testing aquarium if they were given a GABA receptor agonist prior to the behavioral test. Typical of increased anxiety, rockfish from acidified seawater spent more time near a novel object in the aquarium compared with fish from normal seawater that were also given the agonist.

So, if a rockfish changes its ion distribution across neuron membranes to accommodate ocean acidification, it is more anxious because opening the GABA receptor excites the neuron that should be inhibited, as chloride ions flow out instead of in. Hmmm, this GABA receptor switcheroo sounds a bit like the hokey-pokey… 'You let your chloride in, you let your chloride out…'

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Hamilton, T. J., Holcombe, A. and Tresguerres, M. (2014). CO_2-induced ocean acidification increases anxiety in rockfish via alteration of $GABA_A$ receptor functioning. *Proc R. Soc. B* **281**, 20132509.

Sarah Alderman
University of British Columbia
salderman80@gmail.com

Frog's little helpers

Amphibians across the globe are facing calamitous declines. Around a third of species are critically threatened, while extinction rates for amphibians are 200 times higher than for other vertebrates. Several factors underlie these changes: widespread habitat loss, climate change and toxic chemicals in environmental run-off, among others. However, the most prominent cause of epidemic mortality is the fungal pathogen *Batrachochytrium dendrobatidis*, known more simply as Bd. Where it is common, Bd decimates populations. Puzzlingly, however, while Bd infection rates in some ponds are extremely high, other ponds are hardly affected. New research published in *Current Biology* offers a compelling explanation for this variation. Simultaneously, the results offer a novel route to mitigate Bd-induced amphibian loss.

Bd in nature can be divided into two stages: a host-associated stage and a free-living motile stage called a zoospore. Zoospores are the agents of new infections, and where there are lots of zoospores there tends to be lots of disease. But why are some ponds zoospore hotbeds while others are not?

Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

The simple answer, it turns out, is in the water.

Dirk Schmeller from Leipzig in Germany and an international team of collaborators compared zoospore survival in water taken from ponds with a high incidence of infection with survival in water from ponds with little infection. They observed striking differences: zoospores incubated in water from sites with little infection died much more rapidly. But this difference was not caused by pH, temperature or, indeed, anything chemical in the water. Instead, the team found that the zoospores were being eaten by tiny rotifer, paramecium and ciliate predators that were abundant in ponds with low rates of infection but largely absent from ponds with lots of infection. Moreover, if they removed the predatory microfauna using filters, the survival of the Bd zoospores dramatically increased. In short, the parasites had become prey.

But how does zoospore predation influence amphibians? To address this, Schmeller and his collaborators exposed susceptible tadpoles to a mixture of zoospores and different microfaunal predators. Mimicking the results from natural ponds, they found that predators significantly reduced the incidence and intensity of Bd infection. Indeed, in the presence of one particular *Notommatid* rotifer predator, the prevalence of infection declined to zero. These experiments thus established a crucial causal link between zoospore consumption of Bd microbial predators and amphibian disease.

Two clear messages for conservation emerge from this study. First, by seeding microbial predators into ponds it might be possible to directly and rapidly reduce amphibian Bd infection rates by removing infectious Bd zoospores. Solutions to amphibian decline are critically needed and this novel approach certainly warrants further consideration. Second, even with successful intervention, this study highlights the fact that amphibian conservation requires habitat preservation, not only to support the direct physical requirements of amphibians but also to protect the species that are not themselves threatened but whose presence can dramatically influence those that are. Saving rotifers may not be high on the agenda of Greenpeace, but doing so might be the difference between a twilight chorus of frogs and a silent spring.

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Schmeller, D. S., Blooi, M., Martel, A., Garner, T. W. J., Fisher, M. C., Azemar, F., Clare, F. C., Leclerc, C., Jager, L., Guevara-Nieto, M. et al. (2014). Microscopic aquatic predators strongly affect infection dynamics of a globally emerged pathogen. *Curr. Biol.* **24**, 1-5.

Daniel E. Rozen
University of Leiden
d.e.rozen@biology.leidenuniv.nl

Treat a bird as if he were what he ought to be

For social animals, status is extremely important. The highest-ranking individuals in a social group will enjoy a myriad of benefits, such as better mating success, more food resources, and better access to shelters. However, if group members are constantly bickering over social status, it wastes time and energy for everyone in the social group and risks unnecessary injury. In established groups where individuals interact with one another frequently, it is more efficient for animals to communicate their social status through a signal, rather than constantly fighting to establish who is the strongest.

Animals often communicate their rank through physical traits called badges of status. These status symbols can be brightly coloured feathers on a bird, or big and powerful claws on a crab. Badges of status are often costly to produce and maintain and so these signals must be honest. For example, only the strongest deer can grow large and elaborate antlers, and so antlers are an honest signal of quality. However, other badges of status are not necessarily linked to some costly physiological process. In these cases, what keeps animals honest? How do animals know what status signal to produce?

Cody Dey and his colleagues from McMaster University, Canada, and Massey University, New Zealand, decided to investigate social status signaling in the pukeko (*Porphyrio porphyrio melanotus*), a bird with a complex social system. Pukeko are cooperative breeders, and multiple males and females live together as a family unit. These birds have strict social hierarchies. While all of the birds in the group defend the territory and take care of the offspring, only the top-ranking birds reproduce.

These blue and black birds also have distinctive red bills and red facial shields. The researchers hypothesized that this bright red facial shield might be a badge of status. First, the scientists measured shield size in a large number of wild pukeko, and watched the birds to determine their position within their social hierarchies. They found that shield size was highly correlated with rank, even when controlling for body size and sex.

The scientists then took the study a step further. If the red facial shields are badges of status, how is honesty maintained? How do pukeko know what size their red shield ought to be? To answer these more complex questions, the researchers cosmetically altered the size of the red frontal shields in some of the pukeko. They watched the birds within their social groups, and after a week, they recaptured the same birds and re-measured their true shields.

The scientists found that birds with cosmetically reduced shields were treated as more subordinate by other group members, and were aggressively challenged more often. And after a week of such treatment by their social group, the birds with cosmetically reduced shields had actually reduced the size of their true shields.

This study lends insight into the dynamic nature of status signaling in animal societies. Goethe may have been more right than he thought when he said, ‘If you treat an individual as he is, he will stay as he is; but if you treat him as if he were what he ought to be, he will become what he ought to be.’ This study suggests that a status signal not only reflects an animal's intrinsic strength or health but is also influenced by how the animal is treated by other members of the social group.

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Dey, C. J., Dale, J. and Quinn, J. S. (2014). Manipulating the appearance of a badge of status causes changes in true badge expression. *Proc. R. Soc. B* **281**, 20132680.

Constance M. O'Connor
McMaster University
coconn@mcmaster.ca

Stinky secretions for keeping clean

Earwigs are not nature's most cuddly animals. If attacked, they defend themselves with a large set of pincers (called cerci) on the ends of their abdomen. If they're disturbed enough, they'll ooze a smelly liquid, which is occasionally bright yellow, from their abdomen. Despite this, they'll often spend the winter snuggled up together.

Living in large aggregations can facilitate the spread of disease, so PhD student Tina Gasch and her colleagues at Justus Liebig University Giessen in Germany set out to see whether the earwigs' chemical secretions could be used to combat the microorganisms found in their environments. They collected three different earwig species – *Apterygida media*, *Chelidurella guentheri* and *Forficula auricularia* – to examine the chemical composition of their defensive ooze and to investigate whether it might kill pathogens.

First, the researchers dissected out the sacs that held the defence liquid and then used gas chromatography coupled to mass spectroscopy to identify the chemical components. They found that the secretions of all three earwig species were chemically quite simple, with no more than four unique compounds found in any species' gunge. One compound, 2-ethyl-3-methyl-1,4-benzoquinone, had never been found in insects before, but it is used as a defensive compound by harvestmen arachnids.

Next, the researchers set out to challenge the ooze with a shooting gallery's worth of potential pathogens to see whether the earwigs were mounting a chemical defence when they secreted their defensive compounds. Gram-positive and gram-negative bacteria, two species of fungi and the nematode *Caenorhabditis elegans* were all doused with the defensive fluid from *F. auricularia*, which turned out to be an effective pathogen slayer.

Finally, the researchers wanted to know whether the smell from natural aggregations of *F. auricularia* contained the chemicals found in the defensive emissions. They placed bamboo traps containing swabs that absorbed volatiles in areas where the earwigs liked to aggregate. Analysing the swabs, the team found that the characteristic odour of huddled *F. auricularia* groups contains the defensive chemicals that they found in the ooze.

While we might not like it much, the stink of the defensive ooze might be the 'sweet, sweet smell of home' for earwigs, which provides them with a sterile space free of pathogens.

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Gasch, T., Schott, M., Wehrenfennig, C., Düring, R.-A. and Vilcinskas, A. (2013). Multifunctional weaponry: the chemical defenses of earwigs. *J. Insect Physiol.* **59**, 1186-1193.

Katie Marshall
University of British Columbia
kmarshall@zoology.ubc.ca