OUTSIDE JEB

Carbonic anhydrase: an evolving story in bony fishes

In the early 1900s, August Krogh shaped the way we think of oxygen uptake in mammalian lungs and oxygen release at the level of tissues as two processes that occur passively, from an area of high oxygen partial pressure to one of lower partial pressure until the two sides are in equilibrium. Using this mammalian model, we have assumed that these processes are identical across all vertebrate species. However, recent studies in teleost fish have challenged the assumption that the diffusion proceeds unaided, and have provided evidence that fishes, particularly those that withstand long migrations, have evolved a mechanism that increases oxygen unloading efficiency at the level of tissues: a protein called carbonic anhydrase that is located in the blood plasma.

Till Harter, from University of British Columbia, Canada, along with a group of researchers from Memorial University of Newfoundland, Canada, decided to challenge the dogma on the fate of oxygen in the fish body by demonstrating that the evolution of the plasma carbonic anhydrase allows for efficient oxygen unloading at the tissues following a swim challenge. To this end, the researchers acclimated Atlantic salmon (*Salmo salar*) – which perform epic migrations from their spawning grounds out to sea and back – to water containing either 95–100% oxygen air saturation (normal oxygen levels) or 40% oxygen air saturation (low oxygen) for 6–10 weeks.

The team then set both groups of fish swimming for 1.5 h in well-oxygenated water before injecting them with a chemical that blocks the activity of the blood plasma carbonic anhydrase, C18, which should reduce the ability of the fish to supply oxygen to their tissues, making the heart work harder if they were dependent on the enzyme to boost the oxygen supply to their tissues. The scientists found that the volume of blood pumped by the heart per minute (known as the cardiac output) increased immediately after the injection and was ~27% higher, regardless of whether they were adapted to swimming in normal water or low oxygen conditions. So, having knocked out the plasma carbonic anhydrase, the fish hearts were compensating for the loss by working harder to increase oxygen delivery to the tissues. However, during the fish's recovery, the role of blood plasma carbonic anhydrase in oxygen delivery became more apparent. The hearts of the fish that had been that been living in poorly oxygenated water continued pumping harder than the hearts of the fish that been kept in fully oxygenated water as they got over the exertion, suggesting that prior acclimation to low oxygen had conditioned the fish to rely more on plasma carbonic anhydrase to increase tissue oxygen unloading efficiency.

This study is one of the first to show the significant role that plasma carbonic anhydrase plays in the tissues of migratory bony fishes. The authors have shown that, by increasing the oxygen unloading capacity at the tissues through the action of the plasma carbonic anhydrase, migrating fishes are not only able to decrease the load on their hearts by 30%, but can also employ this mechanism while exercising and during recovery. Having an effective way of getting oxygen to the tissues is an incredible adaptation, one that Atlantic salmon take full advantage of, especially during their 300 km migrations to their spawning grounds!

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Fatty fish frolic in freshwater

Why are some species so much more successful in colonizing new ecosystems than others? Are they serendipitously in the right place at the right time? Are some habitats friendlier to newcomers than others? The three-spined stickleback (*Gasterosteus aculeatus*) exemplifies a successful colonizer with freshwater and marine populations on three different continents. However, its close relative, the Japan Sea stickleback (*G. nipponicus*) does not – they live only in marine environments, except for occasional forays inland to spawn. Why have some sticklebacks moved permanently inland while others have remained out at sea?

Work led by Asano Ishikawa, from the National Institute of Genetics and the Graduate University for Advanced Studies in Japan, argues that it depends on what animals can (or cannot) eat. Food is surprisingly different between freshwater and marine environments: freshwater ecosystems typically lack nutrients, especially long-chain polyunsaturated fatty acids such as DHA (docosahexaenoic acid). Hypothesizing that the scarcity of fatty acids limited the colonization of freshwater habitats,

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Ishikawa and colleagues integrated rearing experiments, genomics and genetically modified fish to compare how globetrotting three-spined sticklebacks and homebody Japan Sea sticklebacks cope with fat-free diets.

Regardless of salinity, three-spined sticklebacks survived better than Japan Sea sticklebacks on a DHA-free diet. Interestingly, survival only really started to drop off for the latter about 40 days after fertilization, the same age at which young Japan Sea sticklebacks migrate seaward in the wild. The poor survival matched lower brain and eye DHA levels, suggesting that Japan Sea sticklebacks cannot make and/or store DHA as effectively as their cosmopolitan cousins. Feeding Japan Sea sticklebacks fat-enriched meals solved the problems brought on by freshwater, linking freshwater morality and lack of DHA.

Why did diet matter for Japan Sea stickleback survival in freshwater? Perusing the sticklebacks' genomes, the team found extra copies and higher activity of the *fatty acid desaturases 2* (*Fads2*) gene in three-spined sticklebacks compared with their homebody relatives. *Fads2* encodes an enzyme that metabolizes fatty acids such as DHA.

Encouraged by their findings, the team delved deeper into how *Fads2* related to freshwater survival. They produced Japan Sea sticklebacks with a more active *Fads2* gene to examine how this gene played into freshwater tolerance. Japan Sea sticklebacks with boosted *Fads2* survived better in freshwater and had more DHA, mimicking the three-spined sticklebacks. Survival in freshwater depends on getting enough fats, and the *Fads2* enzyme made all the difference for sticklebacks. It also turned out that three-spined sticklebacks naturally have an extra copy of the *Fads2* gene. How did they manage to turn one copy of the *Fads2* gene into two?

Looking closely at the structure of the three-spined stickleback genome around the extra *Fads2* gene, the team found that it is messy and cluttered with short, repetitive sequences called transposons. These elements copy and paste themselves – and anything in their vicinity – randomly throughout the genome. Nearly a million years ago, some transposons copied *Fads2* and took it on an adventure to a new location in another chromosome, opening a world of possibilities for the three-spined but not the Japan Sea stickleback.

A messy copy-and-paste job changed the course of three-spined stickleback history. Unlike their close relatives, these fish managed to eke out a living in a new, but nutrient-thin, environment. Understanding why only some species seize the opportunities of new habitats is a complicated question, but for fishes, more *Fads2* makes freshwater colonization a piece of cake.

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How many years does a big brain cost?

Make sure you have access to food and avoid being snatched by a predator – these are two ingredients for living a long life in the animal kingdom. Having a big brain might also help, because big-brained animals tend to be smarter and are able to solve life-threatening problems that might otherwise cause an early death. It is therefore logical to assume that, over generations of being able to outsmart external causes of mortality such as predation, animals with bigger brains will evolve to age more slowly.

However, there is a contradictory theory lurking behind this seemingly logical relationship. Brain tissue is the most energetically expensive tissue in the body. Growing a big brain means that animals have to divert energy and resources away from other bodily processes such as growth, reproduction and general tissue repair and maintenance. So, according to this theory, it would be logical to assume that animals with bigger brains should actually age faster, as they are unable to maintain and repair their bodies over time.

To disentangle these competing ideas, Alexander Kotrschal, Alberto Corral-Lopez and Niclas Kolm, from Stockholm University, Sweden, studied ageing in a unique line of artificially selected Trinidadian guppies. These fish were selected over four generations to be either large- or small-brained, resulting in a whopping 12% difference in relative brain size by the fourth generation. The researchers raised the guppies from birth in a safe environment where they always had access to food and there was no threat from predation. This gentle rearing environment therefore removed any external causes of mortality. The guppies were even kept in individual tanks to avoid any competition or aggression among the fish, but they could still see each other so they would not get lonely.

The team monitored the guppies over their entire lifespans to reveal that the large-brained fish had a 22% shorter lifespan than their small-brained counterparts. Large-brained fish lived for an average of 2.9 years, while small-brained fish lived for 3.5 years. This study certainly took up some time for the researchers, because the longest-lived small-brained fish was over 5 years old when it died. Their findings support the idea that larger brains are costly and there is a trade-off with ageing. The big-brained guppies in this study were likely re-allocating their bodily resources to brain growth. There is already some support for this notion, because the team had previously found that these large-brained guppies also have smaller digestive tracts and poorer immune systems.

Kortrschal's experiment is one of the first to experimentally show how ageing is affected by brain size. While big brains appear to be quite bad for longevity (a cost of 0.6 years – almost a quarter of their lifespan – for large-brained guppies!), the team also highlights that their findings are restricted to a benign and protected environment. More work is now needed to understand how brain size and ageing interact with external causes of mortality, such as predation or starvation, which can also affect animal survival in the wild.

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Kotrschal, A., Corral-Lopez, A. and Kolm, N. (2019). Large brains, short life: selection on brain size impacts intrinsic lifespan. *Biol. Lett.* **15**, e20190137. doi:10.1098/rsbl.2019.0137

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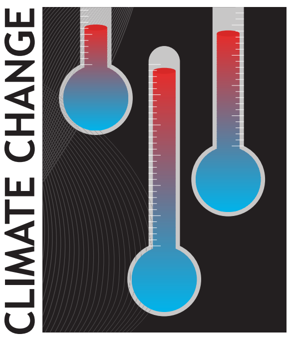
Environmental warming leaves fish all at sea



The impacts of climate change on animals are not uniform, nor are they simple. The magnitude of heatwaves, for example, varies among the world's climate zones. Similarly, the susceptibility of animals and plants to global warming differs among species and among regions. Because our predictions of Earth's future are often clouded by uncertainty, how can we best figure out who will carry on and who will go the way of the dinosaurs? An answer could lie in using broad ecological patterns, identified by combining detailed information on the biology of as many species as possible, to improve conservation practice and policy.

Malin Pinsky, from Rutgers University, USA, and a team of researchers from the USA, Norway and Canada assessed the vulnerability to climate change of animals living in two very different environments: on land and in the ocean. Specifically, they studied ectotherms – animals whose body temperature is directly influenced by environmental temperature ('ecto'=outside, 'therm'=heat), such as insects, reptiles, fish and molluscs. Extreme temperature not only negatively impacts individuals, but also whole populations, so the researchers wanted to find out how often these animals live under physiologically stressful conditions. They calculated the 'thermal safety margin' – the difference between the highest temperature a critter can withstand before it dies and the highest body temperature it will reach in its habitat – for more than 400 species, under current and predicted future warming conditions. They reasoned that the narrower the thermal safety margin, the more likely an animal was to be adversely affected by climate change.

The team found that the thermal safety margins of land-dwelling ectotherms are narrowest in regions midway between the equator and the poles, whereas marine species are most vulnerable at the equator. They also discovered that marine species have narrower thermal safety margins in general, leaving them more open to heatwave hardship. Additionally, the size of the thermal safety margins under a high greenhouse gas emissions scenario tighten to half the size of those under low emissions for both animal groups. On land, if animals lose the shade needed to avoid extreme temperatures, their chances of survival will all but vanish.

The authors suggest that the narrow thermal safety margins of marine ectotherms may have already caused local extinctions (the die-out of a species in a specific area). In fact, they estimate that twice as many local extinctions have occurred on the warmer edge of marine species' ranges than for terrestrial species. It is possible that available habitat is key in mitigating the effects of climate change on land and underwater. If marine species are able to successfully move out of uninhabitable areas and colonise new locations, the team expects higher levels of local population extinction, but lower levels of global extinction, in marine versus terrestrial ectotherms. Land animals are not able to move as far to avoid warming temperatures, so access to protective thermal refuges – hideaways from the heat – will be critical.

The authors' take-home message is that fish, lobsters, abalone and other ectotherms living in the ocean are highly vulnerable to global warming and it will not take much more heat to send populations crashing into the abyss. They emphasise that the collapse of marine ecosystems will have dire economic and nutritional consequences for humans, and the best way to avoid this catastrophe is to reduce our greenhouse gas emissions. The human race is shooting itself in the foot because climate change begins, and ends, with us.

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