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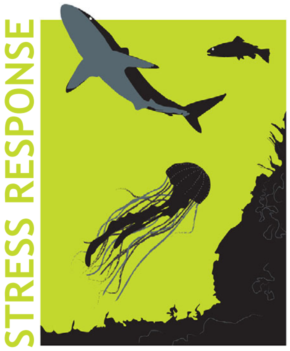
OUTSIDE JEB

An off-the-(nar)whal stress response



The thing about unicorns is, they're hard to find. Legend has it that merely glimpsing the elusive equine requires distant travel to faraway lands, and even then you need luck on your side. Slightly easier would be to locate a narwhal, the toothed whale whose left canine has turned into a unicorn-like tusk protruding from its face. In fact, when Norway's King Frederick III (1609–1670) was regretfully informed that his new throne could not be constructed from unicorn horns as requested, he agreed to narwhal tusks instead. But narwhals are still hard to find, requiring distant travel to the most northern of seas. So, when Terrie Williams set out with her international team of collaborators to investigate the narwhal's physiological stress response, she journeyed more than 6400 km from her University of California, Santa Cruz, USA, base to eastern Greenland's Scoresby Sound.

The team completed the study over two consecutive Augusts, when the weather was agreeable and the indigenous Greenlanders' annual hunt was on. This ensured the researchers found sufficient narwhal to investigate their behavioural, biomechanical, cardiovascular and energetic responses to escape situations. The narwhal's elusiveness and far-flung locale meant that such measurements had never been made before.

Once there, the team suctioned submersible electrocardiograph–accelerometer–depth recorders to the backs of net-entagled – and thus stressed – narwhal. The data loggers then remained in place for up to 3 days of measurements before sliding from the deep-divers' backs, ready for retrieval. So, how cool was the narwhal under stress?

In some ways, very cool; in others, not so much. Escaping narwhal rapidly depressed their heart rate by 92% while simultaneously doubling their swimming stroke frequency and elevating their energy expenditure 6-fold compared with normal swimming conditions. This is unlike other whales' stress responses and is frankly paradoxical. To appreciate the paradox, one must first understand the two types of mammalian stress response: fight or flight, and freezing. A fight or flight response sees the animal's heart, breathing and metabolic rates elevated to ensure about-to-be-used muscles receive sufficient supplies of blood and oxygen. Conversely, a freezing response sees these rates depressed to ensure the animal stays put. These responses are incongruent, and that incongruence travels all the way to the separate regions of the brain that trigger them. Yet, here was the narwhal, simultaneously freezing and fleeing.

The researchers reckon the abnormal stress response is caused by these particular stressful situations, which are novel for the narwhal. Specifically, these situations trigger a complex medley of signals from the narwhal's diving, exercise and neurocognitive fear responses. These signals can oppose one another, and that's not good. For example, diving typically slows heart rate, while exercise elevates it. And in rats, simultaneously inducing these signals under experimental conditions promptly kills them. While the narwhal isn't dying, a response that simultaneously reduces heart rate and elevates energetic demand surely threatens tissue oxygenation and thus homeostasis. And, as rapidly changing Arctic conditions expose the once-isolated species to more predation, hunting by humans, shipping and seismic activity, narwhal will find themselves more frequently in these situations.

In the middle ages, narwhal tusks were seen as entry-level unicorn horns, marketed by Vikings and other northern traders as a practical means of accessing magical powers. One such power was the tusk's ability to purify poisoned water. Whether this also allowed warming water to be magically cooled is unknown, but this power would certainly prove handy for 21st century narwhals. Without it, their adjustment to a changing world – like everyone else's – will hinge on some combination of genetic adaptation, phenotypic plasticity and a bit of luck.

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Williams, T. M., Blackwell, S. B., Richter, B., Sinding, M. H. S. and Heide-Jørgensen, M. P. (2017). Paradoxical escape responses by narwhals (*Monodon monoceros*). *Science* **358**, 1328-1331.

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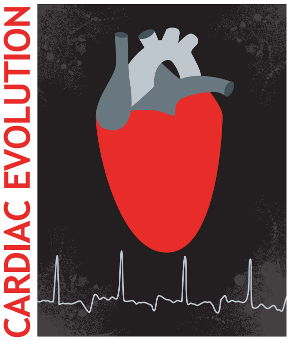
Flying is not for the small-hearted



The evolution of flight in birds required dramatic modifications in anatomy and physiology. Many of the key adaptations, such as the swap of forelimbs for wings, are obvious. For this reason, it is commonly assumed that the earliest birds, which were equipped with wings, were probably capable of flying. But true sustained flight also required other changes deep below the feathers. Notably, powerful flight muscles need to be provisioned with large amounts of oxygen, which is pumped around the body in the blood by the heart. However, reconstructing the cardiovascular systems of extinct, early birds is no mean feat.

Fortunately, there is an ancient division within modern birds that helps shed light on the earliest avian origins. ‘Neognaths’ are represented by the majority of modern flying birds, whilst ‘paleognaths’ include ratites (flightless birds such as ostriches, emus and kiwis) and tinamous. Tinamous are peculiar chicken-sized residents of Latin America that are capable of short bursts of flight but not sustainable, long-distance flapping flight. They are also characterised by having much smaller hearts than other birds. This prompted Jordi Altimiras from Linköping University, Sweden, and his colleagues to detail the cardiovascular physiology of tinamous in Bolivia and Chile in order to predict how the hearts of early birds may have worked.

By weighing the hearts of tinamous of different ages, Altimiras and colleagues first confirmed that their hearts are tiny: chicken hearts are about twice as big. Indeed, tinamou hearts are more comparable in mass to those of similarly sized alligators, which represent the non-flying reptilian ancestors that birds evolved from. The team also measured cardiovascular function in anaesthetised birds and revealed that the smaller hearts of tinamous generate cardiac output that is 2.5–3 times lower than that of chickens, although they have similar blood pressure.

The researchers also made metabolic measurements in tinamous and chickens after they chased the birds into flying for 3 min. The tinamous were physically exhausted by this and took far longer than chickens to return to resting levels of oxygen consumption. Lactate, a marker for anaerobic metabolism, also reached about twice the concentration in the tinamou blood after exercise, strengthening the view that their small hearts could not provide enough oxygen to adequately sustain flight muscles.

Based on this evidence, Altimiras and his team suggest that the evolution of flight must have coincided with the evolution of an enlarged heart, which they term the ‘heart to fly hypothesis’. They further suggest that this most likely evolved after the split between paleognaths and neognaths. This is, however, controversial. Whilst tinamous have small hearts, most ratites, despite being flightless, have much larger hearts that are comparable to those of flying birds. If Altimiras and colleagues are correct, ostriches and their kin must have evolved their large hearts independently from other birds. This is possible – it may be an adaptation for their famous running abilities – but is difficult to prove. Nonetheless, the data provide a unique insight into the cardiovascular changes that were required for birds to conquer the skies and the hypothesis deserves further study.

10.1242/jeb.169912

Altimiras, J., Lindgren, I., Giraldo-Deck, L. M., Matthei, A. and Garitano-Zavala, Á. (2017). Aerobic performance in tinamous is limited by their small heart. A novel hypothesis in the evolution of avian flight. *Sci. Rep.* **7**, 15964.

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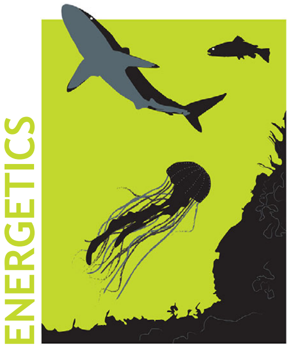
Slow swimming exhausts fish



Fish must spend energy to swim when they forage, reproduce and avoid danger. While it’s well known that fish metabolize energy quicker at fast speeds to propel themselves, slow swimming should, theoretically, also demand high energy costs as the fish works to maintain its posture in the water. But this predicted U-shaped relationship between energy and swimming speed has never been found in fish. Intrigued by this paradox, Valentina Di Santo and her colleagues from Harvard University and Boston College, USA, wondered whether the fabled U-shaped curve had evaded other researchers because they only analysed a narrow range of swimming speeds, so they set about measuring how a fish’s energy demand changes over a much larger range of speeds.

To test their hypotheses, the authors had clearnose skate swim upstream in a controlled water flow so that the fish maintained a stationary position in the tunnel by swimming against the water at the same speed. By measuring the amount of oxygen in the water as the 9 cm-long fish swam over a wide range of speeds, ranging from about 7 to 20 cm s^{-1}, the authors inferred the energy that the skate used at each speed. However, after each swimming bout, the team allowed the fish to rest to avoid fatigue, while they continued measuring the skates’ oxygen consumption, in order to determine whether the fish were supplementing the oxygen consumed during their swim with anaerobic respiration: elevated oxygen consumption during the rest period would indicate that anaerobic metabolism had contributed to the skates’ energy demand while they were swimming. In addition, the team filmed the fish swimming in the tunnel, to find out how they adapted their movements at different speeds.

When the team plotted the oxygen used by the fish during the swimming bouts, they confirmed that the fish’s energy demands were high at slow swimming speeds, low at intermediate speeds and high again at the highest swimming speeds, to produce the U-shaped graph that was predicted by theory. After analysing the fish’s movements, the team also realised that their high energy demand at slow speeds was explained by the need to create upward lift to prevent themselves from falling to the bottom. To increase lift, the skates beat their fins faster and tilted their head higher than their tail, much like an airplane would at take-off. Following each swimming bout, the team also found that the skates’ oxygen levels remained high. This suggests that in addition to using aerobic respiration while swimming, the fish were also using anaerobic metabolism. And, when the anaerobic contribution was included in the total energy demand, the team found that the rate of energy use increased by ∼50%.

Di Santo and colleagues then decided to test how different swimming protocols affect the maximum sustainable speed that a fish can achieve. Comparing the ramp-up protocol – where the speed is gradually increased, without breaks, until the fish fatigues – against their new protocol with

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rest periods between swimming bouts, the team found that the fish fatigued at a slower speed when they were using the ramp-up protocol than when they were allowed to rest between periods. This suggests that the widely used ramp-up protocol underestimates the maximal sustainable speed, possibly because of fatigue caused by anaerobic metabolism when swimming at slower speeds.

Di Santo and colleagues have confirmed that fish that swim slowly spend much energy to sustain their posture and show that anaerobic metabolism contributes to the energy demand even at slow speeds. These results give a more complete description of fish energetics from which we can begin to appreciate why fish exhaust themselves when swimming slowly.

10.1242/jeb.169854

Di Santo, V., Kenaley, C. P. and Lauder, G. V. (2017). High postural costs and aerobic metabolism during swimming support the hypothesis of a U-shaped metabolism-speed curve in fishes. *Proc. Natl. Acad. Sci. USA* **114**, 13048-13053.

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Salmon nourish their children even after death

Parents influence their offspring in one form or another, whether it is the initial input of genes or ongoing involvement throughout development. Importantly, parental contribution can impact the evolution of a species. But what if parents are prevented from contributing to their offspring's future? How would that affect the evolution of a population? Sonya Auer, from the University of Glasgow, UK, and colleagues explain that Scottish Atlantic salmon (*Salmo salar*) are no longer able to reach many of their spawning grounds thanks to dams that have been installed by humans. So, to ensure that salmon populations are not lost, people now capture adult fish as they return from the sea ready to spawn, collect the eggs and sperm and place the fertilised eggs upstream in the spawning areas where they need to hatch. However, during this process, an essential ecological component is lost: adult salmon usually die after spawning, leaving their carcasses as nutrients for the ecosystem. Auer and colleagues wondered whether this loss of nutrients can alter the survival, growth and metabolic rates of their young and, therefore, the evolutionary trajectory of the salmon in Scotland's River Conon catchment.

Auer and her team took advantage of this human-modified ecosystem by adding nutrients in the form of dried hatchery salmon pellets – which are essentially salmon carcasses – to five streams that form part of the river catchment during the breeding season. Simultaneously, they transferred 3000 eggs from 30 different salmon families into each of the pellet-enriched streams and five other streams that remained untreated. The team also took fertilised eggs from each of the salmon families to grow and develop in the lab so that they could measure the metabolic rates of these lab-reared siblings at the age of 2 months. In addition, Auer and colleagues collected insects, the preferred meal of juvenile salmon, from each of the 10 study streams a couple of months after the young salmon should have hatched to assess how the added nutrients influenced the food available to the developing juveniles. Unsurprisingly, the increased amount of nutrients provided by the makeshift corpses boosted the insect population 2.5-fold. But could this insect bounty influence the development of salmon young?

First, the team explored this by capturing young salmon from the streams to measure their size and obtain fin clips for DNA analysis to determine which families the juveniles belonged to. The insect glut indeed led to heftier juveniles. Intriguingly, the surviving salmon in the pellet-supplemented streams included individuals from most of the original families, whereas several of the families in the unsupplemented streams died out. And when the team compared the maximum metabolic rates of the lab-reared siblings, it was clear that the siblings from families that survived in the unsupplemented streams had higher maximum metabolic rates than those of the families that perished. These families with higher maximum metabolic rates are likely to be more dominant and can obtain and protect better territories, which should be beneficial in a habitat where food is scarce and competition fierce. Conversely, as the family death rate in the supplemented river was low and there was significant diversity in the maximum metabolic rates measured in the laboratory siblings, the team suspects that the variation of maximum metabolic rates of the fish in these streams was greater, possibly as a result of more food and less intense competition.

It seems that nutrients provided by the rotting corpses of salmon parents can ease the squabbling over food among young, resulting in vaster genetic diversity. Yet, the circumstances created by the damming of the River Conon catchment have influenced the evolution of salmon by favouring individuals that are better able to defend prime territories.

10.1242/jeb.169896

Auer, S. K., Anderson, G. J., McKelvey, S., Bassar, R. D., McLennan, D., Armstrong, J. D., Nislow, K. H., Downie, H. K., McKelvey, L., Morgan, T. A. J. et al. (2017). Nutrients from salmon parents alter selection pressures on their offspring. *Ecol. Lett.* doi:10.1111/ele.12894.

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