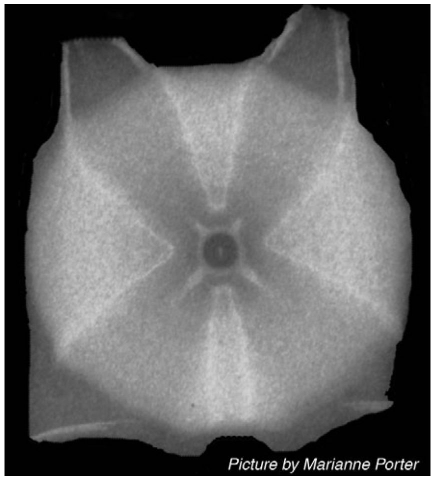
Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

MINERAL PROVIDES MATERIAL BENEFIT



Picture by Marianne Porter

In many vertebrates cartilage supplements a bony skeleton, as its squishy nature absorbs stresses and strains better than bone, which provides the strength and rigidity. Sharks, however, have skeletons made of cartilage. This provides great flexibility but may not be sufficient to resist the forces generated by sudden changes in movement which heavily compress the backbone. Instead, strength and rigidity comes from minerals deposited in distinct ordered patterns around and within the cartilaginous vertebrae. So far, each shark species studied has its own unique pattern: 'You can measure the age of sharks from the rings of mineral as it's laid down – like aging a tree using tree rings,' reveals Marianne Porter. Having previously compared the details of mineral deposits in vertebrae across shark species, Porter focused on cartilage properties in the smooth-hound shark in her latest study (p. 3319).

Working with Adam Summers at the University of California, Irvine, and Thomas Koob of University of South Florida, Porter extracted vertebrae from these small sharks. They soaked a number of vertebrae in the chemical EDTA to demineralize them, so that they could calculate the mineral content. Porter determined that smooth-hound shark vertebrae contain 49.5% mineral on average, which is comparable to other species previously studied. But to her surprise she found that this content varied significantly within smooth-hound sharks, whereas normally there is little variation within a particular shark species. Smooth-hound sharks have a short life span of 10 years and mature quickly, so Porter suggests that the maximal rate of mineral deposition may not have yet been reached in her specimens, resulting in this variation.

To find out how mineral strengthens vertebrae, Porter subjected both mineralized and demineralized vertebrae to compressive forces between two flat plates. From the results the team calculated material properties of stiffness, or resistance to compression, and strength, the maximum stress before breaking. Porter found that, as with other sharks, more mineral correlated with stronger and stiffer vertebrae.

At the same time, Porter took the opportunity to examine how cartilage responds to crushing forces applied at different rates. Cartilage is a viscoelastic material; because it contains fluid it responds differently depending on the rate at which it is crushed. Bone, on the other hand, behaves as an elastic solid, reacting the same way whether it is crushed slowly or quickly. The rate of compression had little effect on smooth-hound vertebrae meaning that at the biologically relevant forces Porter used, shark vertebrae cartilage in fact behaves like a bony elastic solid.

However mineral content is not the only story. 'There seems to be a premium of how much the mineral will do,' explains Porter. By combining her results with those from other species, she showed that a set increase in mineral content didn't account for all the increases seen in stiffness and strength, and that the greater part of this increase had to come from the mineralization pattern. Mineral infiltrates all shark vertebrae like a web, but there are distinct additional mineral deposits around the vertebrae that are unique to each species, possibly because their musculoskeletal systems are arranged differently. While the details remain hidden, these mineral deposits essentially prevent the shark backbone from collapsing under the forces generated by the muscles as the sharks swim.

10.1242/jeb.012310

Porter, M. E., Koob, T. J. and Summers, A. P. (2007). The contribution of mineral to the material properties of vertebral cartilage from the smooth-hound shark *Mustelus californicus*. *J. Exp. Biol.* **210**, 3319-3327.

Sarah Clare

NITRITE FORMS NITRIC OXIDE IN ZEBRAFISH

Ever since nitric oxide (NO) was discovered to relax mammalian blood vessel walls, the list of physiological reactions and organisms that this diminutive molecule is found in has grown and grown. Inspired by the discovery that, in mammals, nitrite acts as a NO donor and that deoxygenated haemoglobin (deoxyHb) catalyses this reaction, Frank Jensen from the University of Southern Denmark took the opportunity to look at the role of nitrite in zebrafish, showing for the first time that NO is formed from nitrite in a lower vertebrate (p. 3387).

Inside the red blood cell, deoxyHb reacts with nitrite to form NO and metHb, a form of haemoglobin that can't carry oxygen. The NO inside the cell can then itself tightly bind to more deoxyHb, forming nitrosylhaemoglobin (HbNO). This form also can't carry oxygen, and is also quite stable, so can be used as a biological marker to measure NO levels inside the fish.

Jensen exposed fish to 3 different nitrite levels in their water – none, 0.6 mmol l^{-1} or 2 mmol l^{-1} – which they took up across their gills. He then measured the levels of the different haemoglobins (deoxyHb, oxygenatedHb, HbNO and metHb) in their blood at 0, 1 and 2 days after exposure, and also after 5 days in fish exposed to 0.6 mmol l^{-1} nitrite. Using a spectrophotometer, Jensen measured the amount of light absorbed by each diluted blood sample. Because each of the four haemoglobin types absorb different wavelengths of light, he was able to use this information in a mathematical model that calculated how much each haemoglobin contributed to producing the recorded absorbance spectra. From this he could then calculate the proportion of each of the haemoglobins in the fishes' blood.

Nitrite exposure clearly led to the production of high levels of NO from nitrite in zebrafish, indicated by a major increase in HbNO production. Jensen also notes that partially deoxygenated haemoglobin would have played a role in this process, because haemoglobin generally only becomes partially desaturated in the blood carried in the veins.

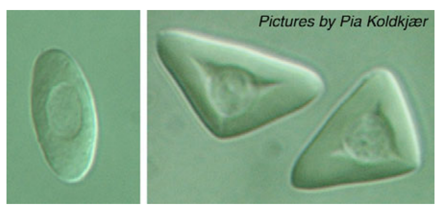
Looking more closely at the effect of the different nitrite concentrations, Jensen found that 2 days of 2 mmol l^{-1} nitrite exposure reduced the total concentration of haemoglobin in the blood samples, suggesting a decline in the number of circulating red blood cells. Of the haemoglobin present, non-oxygen carrying metHb accounted for 59% of the total haemoglobin, and HbNO levels reached 12% of total, reducing the oxygen carrying capacity in these fish to around 30% normal levels. Only some fish in the 0.6 mmol l^{-1} nitrite exposed group had higher metHb and HbNO levels, with HbNO levels stabilising at around 4%. This shows that upon exposure to nitrite, there can be a big decrease in oxygen-carrying haemoglobin.

Because the oxygen carrying capacity is much lower in nitrite exposed fish, Jensen predicted that this would lead to a reduction in their oxygen consumption. But when he measured the oxygen consumption of fish in a respirometer he found that there was no change. He suspects that if the fish are in a relatively resting state, then nitrite levels don't pose too much of a problem, however this could change if the fish are stressed, for example if they need to escape from predators. Then, higher nitrite levels could mean the difference between life or death.

10.1242/jeb.012294

Jensen, F. B. (2007). Nitric oxide formation from nitrite in zebrafish. *J. Exp. Biol.* **210**, 3387-3394.

WHITING SICKLE CELLS



In humans, the sickled red blood cells caused by sickle cell disease condemns sufferers to a whole suite of painful symptoms and a shortened lifespan, all due to one amino acid change in the haemoglobin sequence. This causes haemoglobin molecules to clump together to form long, thin filaments, deforming the cell shape. People don't seem to be the only vertebrates afflicted with sickle cells: researchers had observed sickle cells in other creatures, especially fish. But, many left it at that. This lack of explanation piqued the curiosity of physiologist Michael Berenbrink; 'No one asked why or how this happened', explains his colleague Pia Koldkjær. Therefore the University of Liverpool based scientists decided to investigate how and why red blood cell sickling happens in fish (p. 3451).

Because sickling had been seen in many cod-fish, the team chose to examine one of the cod's relatives, the whiting (*Merlangius merlangus*), which is common in the Mersey estuary near the University. Using a fishing line to catch fish from the pier, and by collaborating with local anglers, the team quickly caught all the whiting they needed. They immediately took blood samples from the some of the fish, before transporting other live fish back to the lab.

Examining the whiting blood samples taken from just-caught fish under the microscope, the team found that around 96% of the red blood cells in samples were sickled. 'You hardly ever see a normal red blood cell,' says Koldkjær. Comparing these samples with those taken from just-caught trout and carp, however, they found that a similar proportion of the red blood cells in these fish were normal shape. The sickle cell count in whiting that had rested for 24 hours in a tank in the lab was 63%, falling to 11% after a week's rest, showing that the fish recovered from sickling. The fish also behaved as normal, as if they were unaffected by sickling.

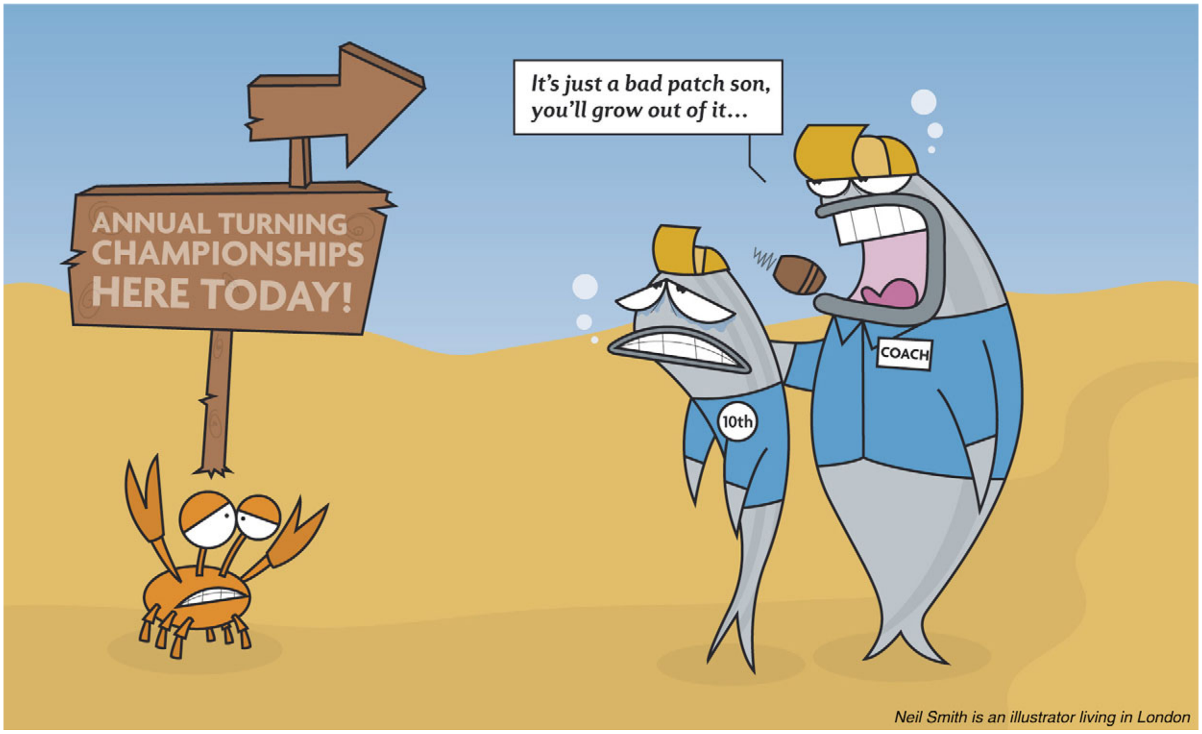
The team also knew that extreme stress reduces the pH of a fish's blood, so reasoned that the stress of being caught on a fishing line could be causing the sickling they saw. When they reduced the pH of the solutions containing the cells, this was exactly what they found: lowering the pH caused the red blood cells to sickle, probably caused by the increased number of protons inside the cells. Dissolving more oxygen in the solution lowered the pH needed to sickle the cells, suggesting that high oxygen levels partially protect against sickling. Since oxygen pressures are higher in some parts of a fishes' circulation than others, such as the retina, this could have a protective effect in areas where very low pH values have been previously measured.

Not only does stress lower pH, but also raises adrenaline and noradrenaline levels. These catecholamines stimulate a receptor on the surface of the red blood cell, which in turn activates a sodium/proton pump via an enzyme cascade. The action of this pump has the net effect of increasing pH inside the cell, which protects against sickling. Adding the adrenaline-like molecule isoprotenerol, which reliably stimulates the receptor, enabled the cells to partially reverse the sickling process, even at very low blood pH values only encountered during severe exercise. Although, a big mystery still remains: why sickling happens. 'There could be an advantage, such as protection from parasites', Koldkjær says, 'but we just don't know yet'.

10.1242/jeb.012302

Koldkjær, P. and Berenbrink, M. (2007). *In vivo* red blood cell sickling and mechanism of recovery in whiting, *Merlangius merlangus*. *J. Exp. Biol.* **210**, 3451-3460.

TURNING PERFORMANCE IN GROWING ZEBRAFISH



There are few things as important to a growing zebrafish (*Danio rerio*) as finding food, and as part of their normal foraging behaviour they show spontaneous turns that help them find nourishment in the water column. Given that turning is a vital zebrafish behaviour, Nicole Danos and George Lauder from Harvard University wondered how it changes as the fish grow (p. 3374). To find out, the team analysed the kinematics of turning fish, measuring 0.38–1.97 cm from snout tip to the centre of their tail forks, by filming them at 1000 frames per second. They used a technique called digital particle image velocimetry, a tool normally used for quantifying fluid motions, to track and measure the motions of the body, such as how fast the fishes moved their bodies and fins during turning.

The team found that smaller fish turned more frequently and beat their tails many times out of a turn, while larger ones tended to have one strong tail beat out of their less frequent turns before coasting to a halt. They also found that the different components involved in turning changed in different ways as fish grow. In fish up to 1 cm long, the angle of the turn and the angular velocity got smaller, however when the fish were more than 1 cm long, these measurements became bigger with body size. This transition coincides with major morphological changes to the muscles and fins. It implies that in order for the fish to develop properly, ensuring that they can maintain turning performance as adults, some aspects of turning get worse before they get better as the fish develop.

The velocity of the pectoral and caudal fins and the time taken to turn were all larger in bigger fish, probably because the muscles in larger fish are better developed. However, how much the body curved during turning got smaller as fish increased in size, probably because the skeleton is slightly stiffer in bigger fish. The team conclude that it is the development of a zebrafish's fins and skeleton that are important in maintaining its turning performance as it grows up.

10.1242/jeb.012286

Danos, N. and Lauder, G. V. (2007). The ontogeny of fin function during routine turns in zebrafish *Danio rerio*. *J. Exp. Biol.* **210**, 3374-3386.

Laura Blackburn
laura@biologists.com