How do you feel after a big steak? Stuffed and sleepy? Or perhaps more alkaline? After guzzling a protein-rich meal, many mammals experience a phenomenon known as the alkaline tide; when stomach cells secrete acid to aid digestion, they pump their surplus bicarbonate ions into the bloodstream, leading to a rise in blood alkalinity. But how does feeding affect blood pH in other types of animal? Chris Wood, a keen fisherman and physiologist at McMaster University, has now discovered alkaline tides in an elasmobranch fish for the first time (p. 2693).

To find out whether fish have alkaline tides, Wood and his colleagues Makiko Kajimura, Tom Mommsen, and Pat Walsh at Bamfield Marine Station needed to study a protein-munching species. They chose the Pacific spiny dogfish, a shark that feasts on bony fishes and invertebrates. But working with sharks wasn’t easy, as Wood explains: ‘they’re pretty vicious and I’ve been bitten once, but what do you expect from a top predator? They are also uncooperative animals and don’t feed well in captivity.’ Wood soon found a solution to that; to ensure that the dogfish ate their dinners, he fitted the sharks with feeding tubes. He pumped a flatfish paste into their stomachs and took blood samples from the fish to search for evidence of an alkaline tide. He saw a marked increase in blood pH after three hours, peaking around 6 hours and returning to pre-feed levels by 17 hours. Levels of bicarbonate ions in the blood rose and ebbed in a similar manner – the hallmark of a classic alkaline tide.

We know very little about nitrogen metabolism in elasmobranchs, so Wood also examined nitrogen excretion after he fed the dogfish. He explains that elasmobranchs exhibit a peculiar form of osmoregulation. ‘Unlike bony fish that constantly need to excrete salt in order to maintain their bodies at a lower salt concentration than the sea, dogfish keep their blood and tissues at an osmotic concentration close to seawater,’ he says. One way to achieve this is to hang on to organic molecules like urea, the nitrogenous waste from a protein-rich diet. But dogfish are sporadic feeders, gorging themselves about twice a week, so their nitrogen reserves are scarce. Wood was curious to know if dogfish conserve nitrogen from their diet to make urea or excrete it in ammonia, which is much cheaper to produce. By measuring the ammonia and urea excretion of the tube-fed dogfish, Wood found that the sharks retained virtually all the nitrogen from their food. This nitrogen conservation suggests that dogfish can produce enough urea to maintain the correct osmotic concentration, despite their irregular feeding habits.

Dogfish may also benefit from their alkaline tide. Mammals counteract the alkaline tide by hoarding acid-generating carbon dioxide in their blood. But Wood didn’t see carbon dioxide levels escalate in fed dogfish, which suggests that their blood pH rises unchecked. He suspects that the alkaline tide has knock-on effects for the sharks’ metabolism, and may even kick-start urea production. Wood now has plenty to get his teeth into, as he tries to piece together what happens when sharks feed.

10.1242/jeb.01721


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TROUT TAKE ON THERMAL CHALLENGE

Lake-dwelling fish are restricted to their watery homes, so they can’t escape from seasonal temperature swings. Since fish are cold-blooded, they are sensitive to changes in temperature. So it’s likely that their muscle performance – and therefore their swimming ability – is affected by hot and cold spells. But when Norman Day and Patrick Butler studied brown trout, they were surprised to find that the fish swam at the same top speed in both winter and summer. They decided to investigate how trout achieve this (p. 2683).

When temperatures soar in summer or drop to frosty levels in winter, cold-blooded animals resort to thermal compensation; they make physiological and biochemical adjustments to keep their bodies going about their daily business. But does thermal compensation kick in if fish find themselves in warm water in winter, or are...
forced to cope with chilly water in summer?

To find out, Day and Butler collected brown trout from a fish farm, but soon discovered that they are not very cooperative. 'Adult brown trout are very aggressive and initially were a real challenge to keep in the lab,’ Day says. Once Day and Butler had worked out how to stop the fish from attacking each other, they were ready to see how trout cope with a thermal challenge. They acclimated some brown trout to normal seasonal temperatures (keeping fish at 5°C in winter and fish at 15°C in summer) and acclimated others to reversed-seasonal temperatures (keeping fish at 15°C in winter and fish at 5°C in summer). To see how this affects swimming ability, they measured each fish’s speed as it swam against an increasing current. They were surprised to find that seasonally-acclimated fish swam faster than trout acclimated to reversed-seasonal temperatures. Clearly, exposing trout to reversed-seasonal temperatures wreaks havoc on their swimming prowess.

Eager to explain this, Day and Butler examined trout tissue samples for morphological and biochemical clues that might reveal why brown trout don’t adjust to reversed-seasonal temperatures. From previous studies on trout, they knew that ammonia build-up in white muscle reduces swimming ability. But when they measured ammonia and another waste product (lactate) in the trout’s white muscle, they found that fish swimming at reversed-seasonal temperatures actually had lower levels of both waste products than fish swimming at seasonal temperatures. Resting fish also had lower ammonia levels in their white muscle at reversed-seasonal temperatures than at seasonal temperatures, in winter at least. So a waste build-up can’t be the reason for their lethargic swimming. But the lower level of waste products does suggest that trout may swim poorly at reversed-seasonal temperatures because they don’t use their white muscle very much.

Day and Butler were even more intrigued to find that there were clear differences in muscle morphology and biochemistry between fish acclimated to 5°C in summer and those acclimated to 5°C in winter. Since both groups were acclimated to the same temperature, these differences cannot be due to temperature alone. There must be other ‘seasonal’ factors at work, and Day and Butler list photoperiod, geomagnetism and the internal biological clock as possible suspects. It’s clear that seasonality has complex physiological ramifications, so Day and Butler have their work cut out for them.


PROTON PUMP IS KEY TO SURVIVING ANOXIA

Safely ensconced in their protective cyst shells, brine shrimp embryos are some of the toughest creatures on the planet, happily surviving for years where other animals would suffocate. Researchers have painstakingly worked out that a key factor for the hardy little creatures’ survival in oxygenless water is acidification of their cells. This triggers an almost complete metabolic shutdown and stops an embryo’s development in its tracks, conserving precious energy until it’s safe to reverse the acidification and kick-start development again. But the tough shell that provides such effective refuge makes it almost impossible to study an embryo’s insides, so the mechanism that causes this acidification has proved to be frustratingly elusive, baffling researchers for 20 years. Joseph Covi and Steven Hand set out to explain the reversible acidification that’s crucial for anoxia tolerance (p. 2783 and p. 2799).

Covi explains that brine shrimp embryo cells don’t yet have fully formed cell membranes, so an embryo is essentially one big cell. Suspended inside it are organelles like lysosomes and yolk platelets. Covi and Hand suspected that when embryos are afloat in comfortable oxygen levels, a common proton pump called V-ATPase pumps protons into these organelles, turning them into proton storage units. But when oxygen levels suddenly plummet, ‘the embryo’s ATP levels crash, the ATP-dependent V-ATPase stops working, and the organelles leak their protons into the embryo’s cytoplasm,’ Covi says. Could this explain the intracellular acidification seen in anoxic embryos?

To show that this explanation is plausible, Covi and Hand first had to establish that brine shrimp embryos have V-ATPase. Scooping floating cysts out of Utah’s Great Salt Lake, they took the embryos back to the lab. They compared brine shrimp embryos’ cDNA with known sequences of V-ATPases from other animals. Sure enough, they found that the embryos possess V-ATPase. They also noticed that it’s expressed differentially as embryos develop, suggesting that it plays a role during development. To show that V-ATPase is positioned to sequester protons in the organelles, Covi and Hand used an antibody to locate the proton pump in isolated cell fractions. They discovered that V-ATPase is distributed in various membranes, including those of organelles.

But does V-ATPase set up a proton gradient between an embryo’s organelles and its cytoplasm? To find out, Covi and Hand tried to stop the proton pump by incubating dechorionated embryos with bafilomycin, a V-ATPase inhibitor. It was a long shot; nobody had breached the cysts’ chitin layer before. To their amazement, the embryos stopped developing. ‘I dropped everything else I was working on,’ Covi recalls. He called in Dale Treleaven, an expert in 31P-NMR, a non-invasive technique to measure intracellular pH. Monitoring embryos’ pH as they recovered from anoxia, Covi saw that the cytoplasm of bafilomycin-treated embryos remained acidic. So the pumping of protons from the cytoplasm into organelles by V-ATPase is crucial to the reversal of acidification, and is therefore a key factor in an embryo’s recovery from anoxia. Finally, to confirm that the release of protons stored in the organelles causes intracellular acidification, Covi incubated embryos in oxygenated seawater with CCCP, a chemical that makes membranes leaky to protons. Sure enough, the cytoplasm acidified, just as it does in anoxic embryos. ‘The ability to dissipate internal proton gradients under anoxia appears to set brine shrimp embryos apart from other animals,’ Covi concludes.


A bluegill sunfish uses a cunning combination of ram and suction to close the gap between itself and its dinner; it propels itself forwards while rapidly expanding its jaws. But is a sunfish more likely to catch a juicy treat if it lunges faster? Timothy Higham, Steven Day and Peter Wainwright have now worked out how a sunfish’s attack speed affects the speed, area and shape of the fluid that it swallows (p. 2653).

To visualise sunfish sweeping food into their mouths, the team scattered microscopic silver beads in a tank and shone a laser light sheet onto them to see water flow. They dangled a tasty morsel 0, 30 and 50 cm from a fish’s mouth to entice it to lunge forwards at different speeds, and recorded the prey-engulfing process on high-speed video. The team measured ram speeds of up to 25 cm s⁻¹. They saw that the speed of water rushing into the fish’s mouth wasn’t affected by the animal’s swim speed. But faster sunfish did suck up water from a narrower region right in front of their mouths, which is where the prey is likely to be. Faster fish also gulped down water from further away, which could limit the escape chances of prey struggling against the current. But there’s a potential drawback when sunfish boost their attack sprint; sucking up a narrower volume of fluid means that the fish need to attack pretty accurately. The team concludes that a moderate ram speed might improve a sunfish’s chances of seizing a hapless victim.

10.1242/jeb.01723


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