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

FEELING THE HEAT

Owl limpets are tenacious little creatures. These territorial marine snails tend a ‘garden’ of algal scum on intertidal rock surfaces, bulldozing off any invaders. Using their foot muscle as a suction cup, limpets hunker down near their garden and resist being prised off by crashing waves or hungry crabs. But there’s a downside to being stuck tightly to your home: when the rock heats up, the limpet’s large foot absorbs the heat. Because limpets have excellent thermal contact with the rock face, they are perfect model organisms for investigating the role of temperature in intertidal ecology, say Mark Denny, Luke Miller and Christopher Harley of the Hopkins Marine Station of Stanford University.

‘These little guys are always in equilibrium with their thermal environment,’ Denny explains. This strong link with their physical surroundings means that limpets may help us understand the biological consequences of global climate change. To create a tool to examine the effects of temperature changes, Denny and Harley set out to construct a heat-budget model that predicts limpet body temperature (p. 2409 Hot limpets…). ‘We translated all the environmental factors that influence rock temperature into one meaningful biological output – limpet body temperature,’ Denny explains. The model’s strength lies in the fact that it relies solely on easily measured physical and meteorological inputs, such as solar radiation, air temperature and the rock’s thermal conductivity.

To test their model, Denny and Harley measured the temperature of artificial limpets and live limpets placed on rocks at the Hopkins Marine Station. They created artificial limpets by filling a limpet shell with wax, casting the wax ‘body’ in silver to ensure good thermal conductivity, and placing it back in the shell. To measure the temperature of live limpets, they sandwiched hair-thin thermocouples between a limpet’s foot and the rock. Denny and Harley found that their heat-budget model predicted the daily maximal body temperatures of artificial and live limpets to within a fraction of a degree, suggesting that their model can be used to explore limpet thermal biology.

To demonstrate how their model might be applied, Denny, Miller and Harley used it to investigate whether temperature sets an upper vertical limit on where limpets can live on intertidal rocks (p. 2420 Thermal stress…). They constructed a thermal history for limpets using an extensive record of ecological data taken at the marine station, including air temperature, solar radiation, wind speed, humidity and wave and tide height measured every ten minutes over a five-year period. Calculating limpet body temperatures at eight shore elevations for nine different rock surface orientations in both exposed and protected conditions, they explored the temperature history of 144 ‘sites’. Denny points out that ‘it would be hard to collect field measurements at such a large number of sites’. The maximum body temperature that the model predicted for any site was 37.5°C, which kills a substantial number of limpets, but not all of them. This suggests that thermal stress does not set an absolute upper limit on limpets’ homes; instead, behaviour or ecological interactions might.

Denny and Harley foresee many practical applications of their model for physiologists. ‘We can now give a physiologist a five-year thermal history of limpets so they can investigate what this means in terms of thermal stress for these animals,’ Denny says. Ecologists can use the model to examine the effects of global warming – it can tell us what a hike in air temperature will do to limpets’ body temperatures. ‘Our model opens up a host of questions for physiologists to explore,’ Denny enthuses.

10.1242/jeb.02356

Denny, M. W. and Harley, C. D. G. Hot limpets: predicting body temperature in a conductance-mediated thermal system. *J. Exp. Biol.* **209**, 2409-2419.
Denny, M. W., Miller, L. P. and Harley, C. D. G. (2006). Thermal stress on intertidal limpets: long-term hindcasts and lethal limits. *J. Exp. Biol.* **209**, 2420-2431.

Yfke Hager

NITRIC OXIDE INHIBITS GUT CONTRACTION

Hatching barely four days after their eggs are fertilized, the humble *Danio rerio* has become the workhorse of many developmental biology labs; their transparent eggs and larvae allow scientists to follow the development of embryonic tissues in the rapidly growing young. Anna Holmberg, Catharina Olsson and Susanne Holmgren from Göteborg University, Sweden, have taken full advantage of this to scrutinise the development of gut motility in tiny zebrafish larvae. Holmberg explains that the team had already discovered that the neurotransmitter acytylcholine appears to stimulate gut contractions. Keen to discover other neurotransmitters that regulate gut activity in the developing fish, the team turned their attention to a molecule that is known to inhibit gut contractions in adults, nitric oxide, to see if the youngsters produce the neurotransmitter and whether their embryonic intestines are able to respond to it (p. 2472).

Gently immobilising the embryos and larvae in soft agarose, the team were able to monitor the microscopic animals' gut contractions as they exposed the developing fish to compounds that stimulated and suppressed nitric oxide synthesis. Holmberg explains that the developing youngsters intestines only begin pulsating about 3-4 days after fertilization, so it wasn't clear whether or not the embryos' intestines responded to nitric oxide at 3 days. However, by 4 days development, Holmberg could clearly see that the embryonic fishes' guts were affected by the presence and absence of nitric oxide; nitric oxide appeared to inhibit gut contraction even before the youngsters begin feeding. And the neurotransmitter's effects became stronger as the youngsters developed.

Curious to know whether the 3-day-old youngsters were unable to synthesise nitric oxide or simply lacked the equipment to respond to it, the team probed the intestines of the developing fish, ranging from 2-day-old embryos to 7-day-old larvae, for nitric oxide synthase, the enzyme that synthesizes nitric oxide, to see if the tiny fish were capable of producing the neurotransmitter. Surprisingly, even the 2-day-old embryos expressed nitric oxide synthase. The team suspect that even though the developing fish can produce nitric oxide, their intestines are too immature to propagate the gut's rippling contractions.

According to Holmberg, the guts of zebrafish larvae begin responding to the inhibitory effects of nitric oxide at about the same time that they become responsive to acetylcholine, and she suspects that 'there is probably a co-functionality between these two pathways to balance sweeping gut contractions at a desired frequency'.

10.1242/jeb.02355

Holmberg, A., Olsson, C. and Holmgren, S. (2006). The effects of endogenous and exogenous nitric oxide on gut motility in zebrafish *Danio rerio* embryos and larvae. *J. Exp. Biol.* **209**, 2472-2479.

HOW COD COPE IN THE COLD

When your body temperature is determined by your surroundings, and your metabolism is tightly tied to your temperature, life becomes an energy-balancing act when you have to counteract the cold. But how do cold-blooded creatures regulate their physiology to survive at low temperatures? Knowing that the tissues of cold adapted eurythermal marine species respond to the cold by increasing their mitochondrial content, Hans-Otto Pörtner and his colleagues from the Alfred-Wegener Institute for Polar and Marine Research decided to find out how cod populations from icy Arctic waters and the more temperate North Sea regulate their mitochondrial physiology to cope in cold conditions. Focusing on mitochondrial citrate synthase and cytochrome oxidase, two key metabolic mitochondrial enzymes, Nils Koschnick, Lars Eckerle, Magnus Lucassen and Pörtner monitored the proteins' transcription levels in fish from both populations and found that citrate synthase transcription and enzyme activity in white muscle are tightly correlated with the fishes' environmental temperature (p. 2462).

But first Koschnick and Lucassen had to clone and sequence both the cod citrate synthase and cytochrome oxidase genes before they could begin tracking the transcriptional responses under different environmental conditions. Having successfully sequenced regions of both genes, the team acclimated fish from the Arctic and North Sea populations to temperatures of 4 and 10°C, before collecting samples of two major metabolic organs, white muscle and liver, from each group and analysing the mRNA levels and enzyme activities.

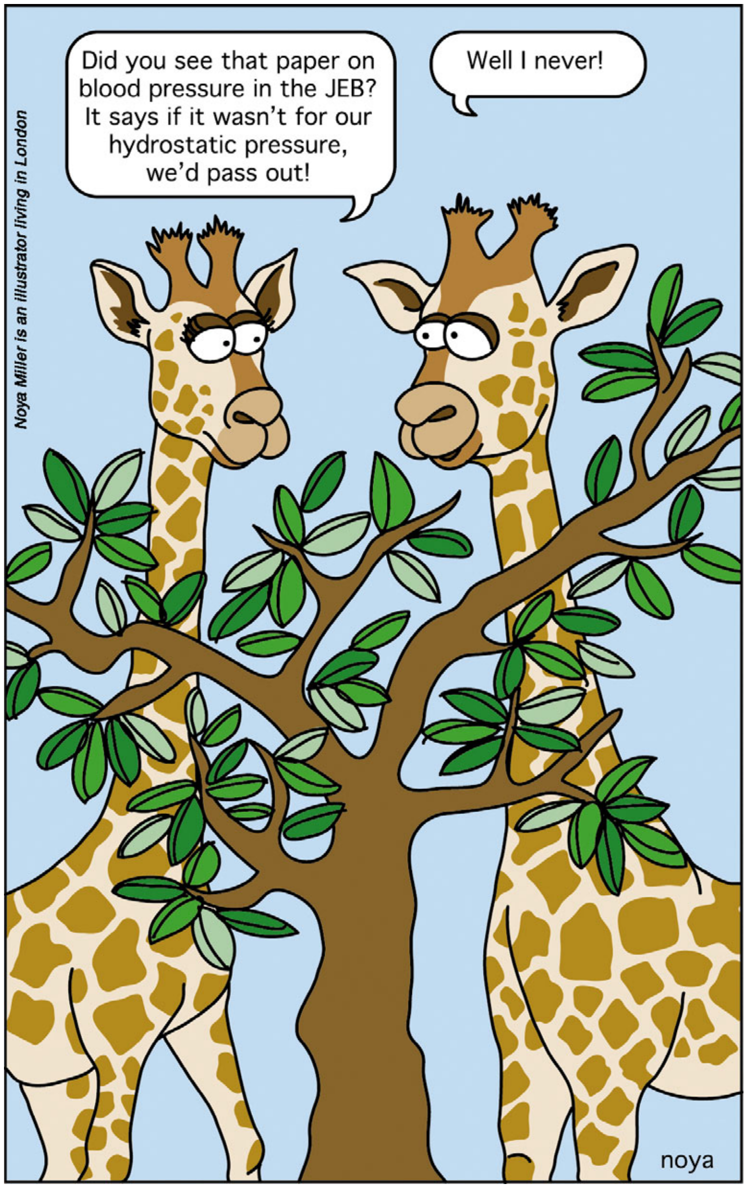
While there were no readily noticeable trends in either enzymes' expression pattern in the livers of warm and cold acclimated Arctic and North Sea fish, the team found a strong correlation between the citrate synthase transcription levels and enzyme activities in the white muscle of both populations. Both populations of cold acclimated fish increased their citrate synthase mRNA levels massively when acclimated to 4°C, but the Arctic population seemed to have a much greater adaptive capacity to the cold, increasing their citrate synthase mRNA levels twice as much as the North Sea cod. The cod's citrate synthase levels were under tight temperature regulated transcriptional control and the Arctic fish had a greater expression capacity than their temperate cousins.

The team is now keen to find out what regulates the differences in both populations' citrate synthase responses. Pörtner explains that there are few other examples where two populations of the same species have different expression capacities for the same gene, and says that the cod's mitochondrial response could possibly explain the Arctic fish's low growth and high oxygen consumption rates relative to more temperate cod populations. Pörtner adds that the Arctic cod is not the only fish to experience ice cold waters; stenotherms trapped behind the Polar Front in the Antarctic Ocean are subject to a slightly colder, but highly stable, thermal environment. According to Pörtner, cold acclimation is energetically more costly for eurythermal fish adapted to a wide range of temperatures than for stenothermal fish, giving us another perspective on the alternative approaches adopted by cold-blooded creatures to survive at low temperatures.

10.1242/jeb.02353

Lucassen, M., Koschnick, N., Eckerle, L. G. and Pörtner, H.-O. (2006). Mitochondrial mechanisms of cold adaptation in cod (*Gadus morhua* L.) populations from different climatic zones. *J. Exp. Biol.* **209**, 2462-2471.

HOW GIRAFFES KEEP THE PRESSURE UP



You'd have thought that maintaining a decent blood supply to your brain when your head is almost 2 m above your heart could be a serious headache, but giraffe's have evidently overcome any difficulties they might encounter. However, the mechanism that maintains sufficient blood flow to the animal's head has remained a topic of hot debate for several decades. One suggestion had been that the major blood vessels in the mammal's graceful neck form a siphon that draws blood up to the brain. In this issue of *The Journal of Experimental Biology*, Graham Mitchell, Shane Maloney, Duncan Mitchell and James Keegan add another contribution to the argument, suggesting that hydrostatic pressure generated by the column of blood in the carotid artery, and not a siphon, could account for the astounding blood pressures need to keep the giraffe's brain supplied (p. 2515).

The team built a mechanical model of a giraffe's neck and head, consisting of a 1660 mm long 'carotid artery' tube, a 1638 mm long collapsible 'jugular' tube, a linking tube to simulate blood flow through the brain, and a pump that simulated the heart. Adjusting the relative positions of the bottoms of the carotid and jugular tubes to create a siphon, the team tested several permutations of rigid and flexible blood vessels, and found that the siphon failed to deliver sufficient pressure. However, when the team activated the pump to simulate the heart's intrinsic hydrostatic pressure, they successfully generated pressures similar to those measured in the giraffe's carotid artery. And when the team constricted the lowest portion of the jugular tube, they found that the blood pressure rose dramatically. They suggest that by constricting the jugular at this point, giraffes could maintain sufficient blood pressure, when they raise their heads after drinking, to prevent themselves from passing out.

10.1242/jeb.02354

Mitchell, G., Maloney, S. K., Mitchell, D. and Keegan, D. J. (2006). The origin of mean arterial and jugular venous blood pressures in giraffes. *J. Exp. Biol.* **209**, 2515-2524.

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