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.

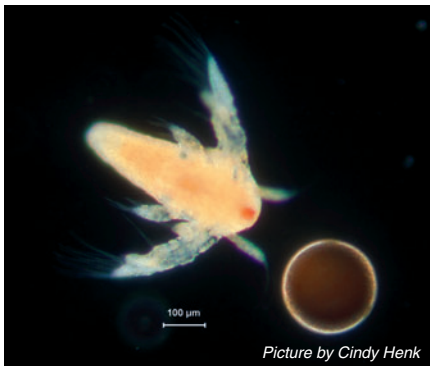
Eager to explain this, Day and Butler examined trout tissue samples for morphological and biochemical clues that might reveal why brown trout don't adjust to reversed-seasonal temperatures. From previous studies on trout, they knew that ammonia build-up in white muscle reduces swimming ability. But when 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.

10.1242/jeb.01720

Day, N. and Butler, P. J. (2005). The effects of acclimation to reversed seasonal temperatures on the swimming performance of adult brown trout, *Salmo trutta*. *J. Exp. Biol.* **208**, 2683-2692.

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 ^{31}P-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.

10.1242/jeb.01722

Covi, J. A. and Hand, S. C. (2005). V-ATPase expression during development of *Artemia franciscana* embryos: potential role for proton gradients in anoxia signaling. *J. Exp. Biol.* **208**, 2783-2798.
Covi, J. A., Treleaven, W. D. and Hand, S. C. (2005). V-ATPase inhibition prevents recovery from anoxia in *Artemia franciscana* embryos: quiescence signaling through dissipation of proton gradients. *J. Exp. Biol.* **208**, 2799-2808.