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

ALL WORK AND NO PLAY FOR ACID-LOVING ALGAE


Picture by Linda Amaral-Zettler

Meandering through southern Spain, the Rio Tinto's blood red colour warns that this river is not a pleasant home; it's extremely acidic and brimming with heavy metals. Yet single-celled *Chlamydomonas* algae thrive in this toxic brew, leading Mark Messerli and his colleagues at the Marine Biological Laboratory in Woods Hole to wonder how these microalgae prosper in acid (p. 2569).

Messerli assumed that the acid-loving algal cells are just like other organisms' cells, on the inside at least. To make sure that their internal biochemistry works as it does in other organisms, the algae must maintain a relatively neutral pH in their cells. But this is not a trivial matter if you live in an acidic river teeming with protons that are constantly storming your cell membrane.

Messerli and his Woods Hole colleagues decided to see if the microalgae maintain a neutral pH inside their cells. When they loaded a fluorescent pH-sensitive dye into the algal cells and monitored the cells' fluorescent intensity, they found that the pH inside the algal cells is indeed close to neutral. And when they moved the algal cells to different pH levels, their internal pH didn't change; the cells clearly control their neutral internal pH. 'So there is a huge proton gradient between the neutral algal cell and the acidic river,' Messerli says.

To find out how the microalgae deal with the onslaught of protons from the Rio Tinto, the team measured the microalgae's transmembrane electric potential, which is the electrical difference between the inside of the cell and the river. When they impaled the cells with an intracellular electrode, they were astonished to find that the membrane potential was practically zero. Nearly all plant and animal cells maintain a negative membrane potential, which drives positive ions into the cell. Maintaining zero membrane potential is a smart move in an acidic river, because this eliminates the electrical gradient that would otherwise drive protons from the river into the cell. 'But a membrane potential of zero usually means the cell is dead!' Messerli says. So were they really recording inside living algal cells? To prove that they were inside the cells, the team probed the ion channels in the algal cell membranes using a voltage clamp. Sure enough, the channels still worked while the algae were impaled, so they were recording inside the cells. And they knew that the algal cells were alive because 'their flagella were still wriggling after the cells were impaled,' Messerli says.

But do the microalgae actively maintain their neutral internal pH? If they do, the team expected the cells to consume more ATP at acidic than neutral pH. To test this, they monitored cellular ATP levels in microalgae kept at pH 2 and 7 using firefly luciferin/luciferase, which emits light when ATP is present. They found that the algae burn 7% more ATP at pH 2 than the same cells burn at pH 7. In other words, living in an acidic river is energetically costly.

So why do the microalgae flourish in the Rio Tinto? Messerli suspects that the answer lies in the fact that no multicellular predators can survive the river's acidity. If you're a microalgal cell, it seems that pumping out protons is a small price to pay to ensure that you remain unmolested.

10.1242/jeb.01718

Messerli, M. A., Amaral-Zettler, L. A., Zettler, E., Jung, S.-K., Smith, P. J. S. and Sogin, M. L. (2005). Life at acidic pH imposes an increased energetic cost for a Eukaryotic acidophile. *J. Exp. Biol.* **208**, 2569-2579.

OOZING OXYGEN: IT'S ALL IN THE GEL


Picture by Trisha Towanda

Gently bobbing along on ocean currents, jellyfish have a penchant for turning up on eutrophied shores, where they feast on flourishing zooplankton populations. Most other predators sensibly avoid these oxygen-depleted waters. Since jellyfish don't have circulatory systems to deliver oxygen to their tissues, Erik Thuesen

wondered how they cope where most other animals can't (p. 2475).

Jellyfish bodies largely consist of a gel called mesoglea, which provides hydrostatic support. Since the creatures' metabolically active tissues are embedded in this gel, Thuesen figured that it might also have a physiological function. He decided to examine whether the gel stores oxygen to supply the animal's active tissues when external oxygen levels plummet. If it does, he reasoned that jellyfish should be able to cope with a sharp drop in oxygen levels without changing their oxygen uptake.

To test this, Thuesen collected *Aurelia labiata*, 'the lab rat of jellyfish research,' from Puget Sound in Washington. Back in the lab, he and Ladd Rutherford placed them in closed tanks at different oxygen levels. To monitor their oxygen consumption, Thuesen and Rutherford stuck a fibre optic oxygen sensor into each tank. They saw that jellyfish happily swam around and didn't change their oxygen uptake, no matter how little oxygen the water contained. When Thuesen dropped the tanks' oxygen levels to 10%, jellyfish still took up the same amount of oxygen, despite the fact that most other animals faced with this situation would decrease their oxygen uptake. Thuesen concluded that jellyfish use internal oxygen stores to regulate their oxygen uptake, so they're not affected when oxygen levels fall.

To see if jellyfish use their gel as an oxygen reservoir, Thuesen and Rutherford teamed up with Patricia Brommer to measure oxygen levels inside the animals' bodies. The team crafted fine mesh harnesses to support the fragile creatures and used a micromanipulator to carefully push an oxygen sensor through each animal's gel. They saw that oxygen levels in the gel dropped as the sensor got closer to the layer of metabolically active tissue; the tissue was extracting oxygen from the surrounding gel.

But does this stored oxygen allow the animals to keep on swimming when surrounding oxygen levels crash? To find out, Kurt Garrison, Magdalena Gutowska and Trisha Towanda watched jellyfish swim in tanks with normal, low and no oxygen. To their surprise, the creatures behaved normally at low oxygen levels. They only noticed a change in behaviour in zero oxygen; jellyfish pulsated more slowly and swam less far. And when they measured gel oxygen levels afterwards, they found that the animals hadn't even depleted all their oxygen stores. 'The oxygen stored in the gel keeps the animals going, even when there's no oxygen around,' Thuesen concludes.

When the first jellyfish roamed the planet's early seas, oxygen levels were drastically lower than they are today. Thuesen views oxygen diffusion pathways in jellyfish gel as a prehistoric step in the evolution of more sophisticated circulatory systems. It may be primitive, but their gel has certainly helped jellyfish stick around for a very long time.

10.1242/jeb.01716

Thuesen, E. V., Rutherford, L. D. Jr, Brommer, P. L., Garrison, K., Gutowska, M. A. and Towanda, T. (2005). Intragel oxygen promotes hypoxia tolerance of scyphomedusae. *J. Exp. Biol.* **208**, 2475-2482.

BLUE BLOODS: HOW A CRAB MAKES ITS SHELL


Picture by Margaret Ryan

While our blood cells bustle around carrying red iron-containing hemoglobin to deliver oxygen to our tissues, crabs and lobsters use a blue copper-containing protein called hemocyanin to transport their oxygen. Cryptocyanin, another protein found lurking in crabs' bloodstreams, is closely related to hemocyanin but can't transport oxygen. So why do crabs bother to produce it, Nora Terwilliger wondered (p. 2467).

Terwilliger's first clue surfaced when the late crustacean biologist Dorothy Skinner suggested that differences in hemocyanins between juvenile and adult crabs might be related to crabs' molt cycles; crabs regularly have to shed, or molt, their hard outer shell because it limits their growth. To find out if Skinner was right, Terwilliger tracked crabs' blood protein levels during the molt cycle. She caught some crab larvae in an Oregon bay, took them back to the lab and sampled their blood as they grew. But to her surprise cryptocyanin, not hemocyanin, changed throughout the cycle; cryptocyanin peaked during premolt and plummeted just before crabs crawled out of their old shells.

Intrigued by this finding, Terwilliger reasoned that hemocyanin and cryptocyanin are regulated by different mechanisms. To understand how the proteins are regulated, Terwilliger and Margaret Ryan teamed up with David Towle to examine the mRNA expression of both proteins in different crab tissues during the molt cycle. They found that both proteins are expressed in the hepatopancreas, a digestive organ. They saw that hemocyanin mRNA levels are relatively constant throughout the molt cycle, but cryptocyanin mRNA levels follow the same pattern as the presence of cryptocyanin in the bloodstream during the molt cycle. 'The fact that hemocyanin and cryptocyanin are present in different amounts and at different times during the molt cycle suggests that the two proteins have different control mechanisms,' Terwilliger says.

Suspecting that cryptocyanin might be under hormonal control, Terwilliger and Ryan snipped off crabs' eyestalks, which contain a molt-inhibiting hormone. Monitoring the crabs' bloodstreams during subsequent molt cycles, they saw that hemocyanin levels were unaffected, but cryptocyanin disappeared. 'Cryptocyanin is regulated by molting hormones,' Terwilliger concludes.

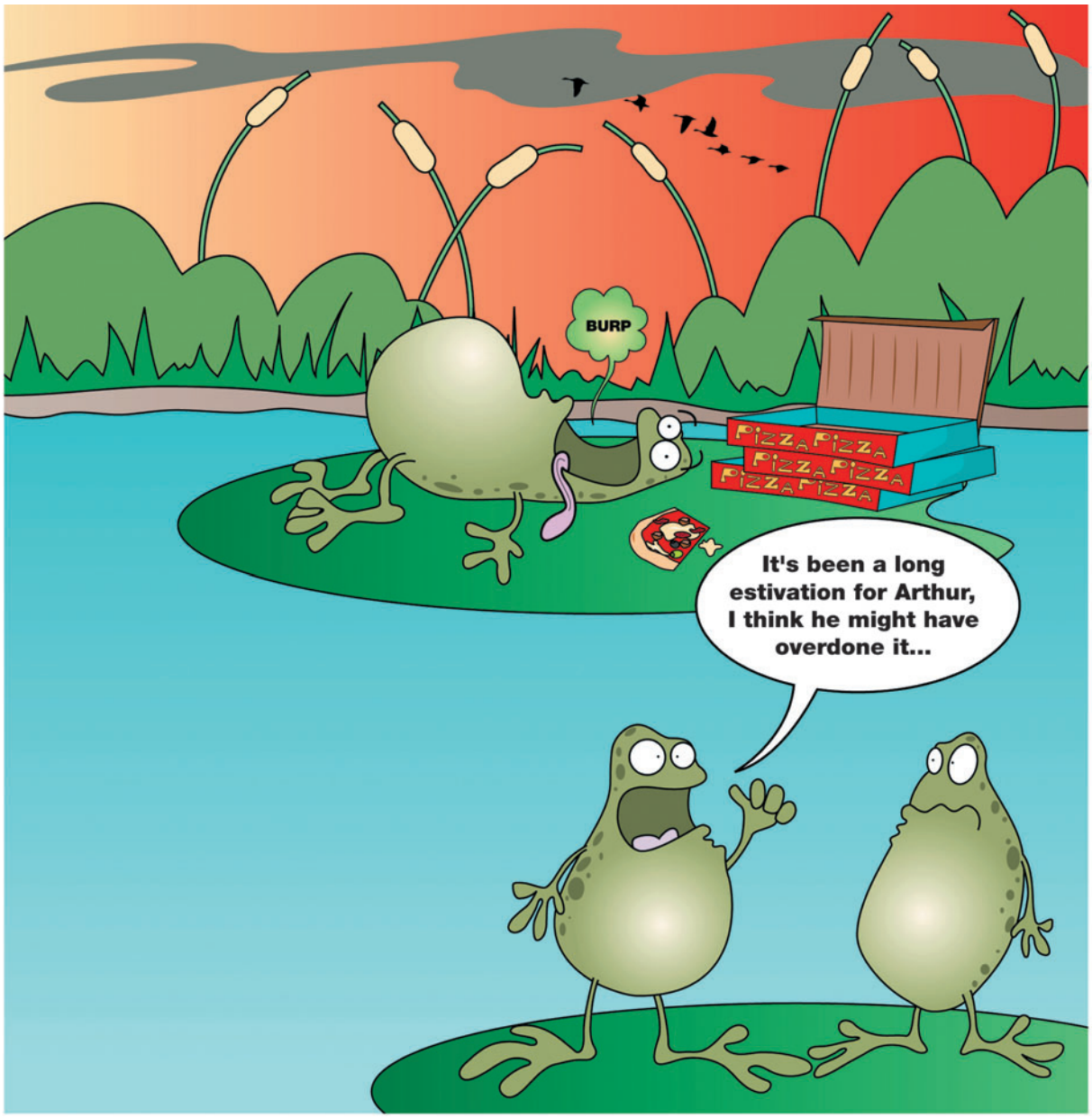
Since they had found such different mRNA expression patterns and control mechanisms for the two proteins, Terwilliger and Ryan figured that the proteins must be synthesized in different cells. To discover which types of hepatopancreas cell synthesize the proteins, they made labelled probes to locate the mRNA in the cells. They were astonished to find that hemocyanin and cryptocyanin are in fact synthesized in the same cell type.

Despite being produced by the same cells, the two proteins have very different jobs. Terwilliger and Ryan uncovered a final clue concerning cryptocyanin's function when they discovered that the protein is present in crabs' newly secreted exoskeletons. They conclude that when cryptocyanin lost its oxygen-transport abilities, it was assigned a new job by evolution: to help growing crabs build roomier shells.

10.1242/jeb.01719

Terwilliger, N. B., Ryan, M. C. and Towle, D. (2005). Evolution of novel functions: cryptocyanin helps build new exoskeleton in *Cancer magister*. *J. Exp. Biol.* **208**, 2467-2474.

FASTING FROGS



Neil Smith is an illustrator living in London

While squirrels snuggle up to hibernate through frosty winters, some frogs and toads estivate to escape unbearably hot and dry summers. Cocooned underground during the heatwave, they fast for months and emerge to feast when the monsoons start. Curious about the digestive consequences of estivation, Stephen Secor examined whether three estivating anuran species show different responses to fasting and feeding than their non-estivating relatives (p. 2595).

Estivating frogs and toads face the same metabolic challenges as sit-and-wait predatory snakes, which often go without a meal for a long time. These snakes have a simple solution to save energy and survive longer on their limited energy stores; they simply shrivel up their guts while they're fasting and then pump them up again when they get lucky and snare a hapless victim. Do estivating anurans do the same thing?

To find out, Secor compared the digestive action of estivating and non-estivating frogs and toads after they wolfed down a meal following a two-week fast. When the three estivating species devoured newborn rats, he noticed a spectacular doubling of intestine mass and a 6- to 10-fold surge in their guts' nutrient uptake. But the non-estivating species' response was less impressive; Secor saw a modest 50% increase in intestine mass and a 69% increase in nutrient uptake. When he triggered estivation by dehydrating two of the estivating species, he saw that after one month of estivation their gut mass had dropped by 44% and their nutrient uptake by 60%. Secor concludes that estivating anurans have adopted the same strategy as sit-and-wait snakes to save precious energy while they wait for conditions to improve.

10.1242/jeb.01717

Secor, S. M. (2005). Physiological responses to feeding, fasting and estivation for anurans. *J. Exp. Biol.* **208**, 2595-2608.

Yfke van Bergen
yfke@biologists.com