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

## FROG'S COCOONS COULD DO MORE HARM THAN GOOD

Picture by Graham Thompson

Frogs aren't the first species that spring to mind when you think of desert dwellers, but according to Victoria Cartledge, the western Australian deserts are peppered with them. However, the amphibians spend most of their time tucked away beneath the surface, aestivating until the next downpour. How frogs weather years without rain has fascinated scientists for decades, and much has been learnt about their survival strategies from lab studies; some frogs encase themselves in protective cocoons to reduce dehydration rates and store water in their large bladders. But how the frogs apply these strategies in their natural environment remained unclear. It was only when hunting for marsupial moles that Philip Withers and Graham Thompson struck up a collaboration with Aboriginal elders in the Kiwirrkurra Community and realised that the elders could distinguish aestivating frog burrows from other depressions in the sand. Withers and Thompson decided to return to Kiwirrkurra to find out more about the elusive amphibians' water management (p. 3309).

Embarking on a 3200 km round trip from Perth to the remote Aboriginal settlement in the Gibson Desert, Cartledge, Withers, Kellie McMaster and Thompson found no shortage of elders keen to help them locate frog burrows. Having been led to the frogs' resting ground in a sand dune, the team began digging up the dormant animals, as well as collecting samples of the damp sand that had surrounded each frog, to evaluate its hydration state. Cartledge remembers that the dune excavation work was backbreaking, but became much worse when the aborigines led them to a rock-hard clay pan where the frogs were entombed in individual chambers. After their successful first trip in 2003, the aborigines led the scientists to a swale valley (with particle sizes half way between clay and sand) in 2004 to give the team their third frog site.

After days of digging on their first excursion to the sand dune and clay pan, the team were delighted to realise that the aborigines had led them to two different aestivating frog species; *Notaden nichollsi*, which doesn't protect itself with a cocoon, buried in the sand dunes, and *Neobatrachus aquilonius*, which surrounds itself in a protective cocoon to reduce water loss, in the claypan. Returning to the sand dune site on successive years, the team realised that *Notaden* remains fully hydrated in damp years, but suffers in dry years, becoming relatively dehydrated and increasing their blood and urine concentrations while losing water to the environment. *Neobatrachus*, entombed in clay, suffered much more from their incarceration despite their protective cocoon. Half of the animals had exhausted their bladder water supplies, and their plasma and urine concentrations were perilously high, reducing the animal's ability to extract water from the bladder store.

However, the biggest surprise came when the team realised that the cocoon building *Neobatrachus* frogs that they found in the swale depression hadn't constructed a protective cocoon, and were in much better shape than the cocoon-swathed, dehydrated animals in the claypan. It seems that the benefits conferred by the waterproof cocoon in the damper swale could be undone if the cocoon limited the amphibian's access to water from the environment, making it preferable for them to do without and aestivate like the cocoonless *Notaden.*

10.1242/jeb.02476

**Cartledge, V. A., Withers, P. C., McMaster, K. A., Thompson, G. G. and Bradshaw, S. D.** (2006). Water balance of field-excavated aestivating Australian desert frogs, the cocoon-forming *Neobatrachus aquilonius* and the non-cocooning *Notaden nichollsi* (Amphibia: Myobatrachidae). *J. Exp. Biol.* **209**, 3309-3321.

## SLC6 TRANSPORTERS IN *DROSOPHILA*

In the modern age of genomes and phylogenetics, the odds can be stacked against you if you work in a non-model organism. So when Ann Stuart was curious to find out more about one group of transporter proteins (known as SLC6 transporters) in her favourite organism, the barnacle, she knew she would have little luck unless she moved to a better understood creature. Which is why she shifted her gaze to the recently sequenced fruit fly, *Drosophila*. With the benefit of the insect's intact genome, Stuart knew that she and her team would be able to discover which of the SLC6 transporters the insect carried and where they were expressed to get a better understanding of their physiological role (p. 3383).

SLC6 transporters are a large group of proteins that transport a whole suite of physiologically significant molecules across cell membranes. Powered by sodium and chloride gradients, many of these transporters are involved in neurotransmitter transport and are found in the central nervous system, where the ionic environment is dominated by sodium. Matthew Thimgan and bioinformaticist Jonathan Berg began searching the insect's genome with the sequences of serotonin, dopamine and norepinephrine SLC6 transporters.

Thimgan remembers that he and Berg swiftly drew up a list of 21 *Drosophila* SLC6 genes, but refining the phylogenetic tree showing the relationships between various members of the diverse family took much longer. Out of the 21 genes, Thimgan and Berg found members of six of the SLC6 subfamilies, including the monoamine transporters, GABA transporters and amino acid transporters.

Curious to know which tissues express the *Drosophila* SLC6 genes, Thimgan began exploring microscopically thin insect body sections with RNA probes, designed to recognise mRNA from all 21 SLC6 genes, to find out where each gene is expressed. Thimgan remembers that planing the frozen insects' tiny bodies was complicated by their exoskeletons, but after a few weeks, he'd mastered the technique.

Knowing that many of the genes were involved in neurotransmitter transport, Thimgan concentrated on searching for SLC6 genes in the insect's head, and sure enough, the two main neurotransmitter (seratonin and dopamine) transporters were expressed in the head and thorax. Meanwhile, the *Drosophila* GABA transporter turned up in the glia cells surrounding neurones in the central nervous system; glia cells are often responsible for GABA reuptake from synapses. Surprisingly, Thimgan only found one amino acid transporter in the insect's central nervous system. He explains that some amino acids are key neurotransmitters with dedicated transporters in mammals, but he suspects that the sole insect amino acid transporter is not specific and probably transports a wide range of amino acids.

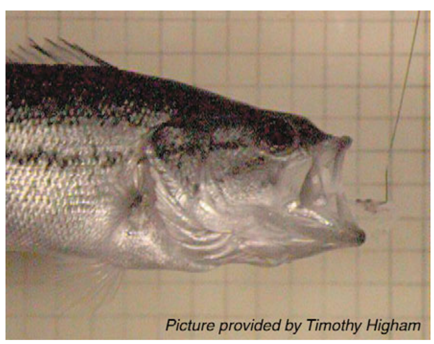
The main surprise came when Thimgan began looking for the recently identified group of insect amino acid transporters (IAAT) that are driven by either sodium or potassium gradients, allowing them to function in tissues other than the central nervous system. *Drosophila* has six IAATs, which cropup throughout the insect's body including in the digestive tract, Malpighian tubules and the female's reproductive system. While the IAATs probably transport neurotransmitter amino acids in the central nervous system, Thimgan suspects that they transport digested amino acids in the digestive tract and could be involved in loading nutrients into females' gametes ready to fuel embryos through gestation.

Thimgan hopes that this thorough analysis of SLC6 transporters in *Drosophila* could be 'a jumping off point in *Drosophila* physiology… improving its function as a physiological model organism' he adds.

10.1242/jeb.02474

**Thimgan, M. S., Berg, J. S. and Stuart, A. E.** (2006). Comparative sequence analysis and tissue localization of members of the SLC6 family of transporters in adult *Drosophila melanogaster*. *J. Exp. Biol.* **209,** 3383-3404.

## WHAT HAPPENS WHEN FISH SUCK?



*Picture provided by Timothy Higham*

While slurping one's food is frowned upon in some societies, fish do it all the time; they simply throw their jaws wide, decrease the pressure within, and suck. In 1982, Mees Muller and his colleagues derived a sophisticated mathematical model that allowed scientists to calculate both the pressure in the mouth and speed of fluid drawn in by simply measuring the mouth's dimensions and its rate of expansion. However, no one had ever directly measured the fluid flows generated by a gulping fish while simultaneously recording the mouth pressure until Timothy Higham, Steven Day and Peter Wainwright put bluegill sunfish and largemouth bass, two members of the centrarchid family, into a DPIV tank (p. 3281).

Digital particle image velocimetry (DPIV) is mainly used to calculate the hydrodynamic forces acting on an animal as it scythes through the water; a fish swims through a suspension of microscopic reflective beads illuminated by a thin plane of laser light, allowing scientists to track the eddies generated by the fish. By swimming the fish in a flow tank that matches the water's flow to the speed of the swimming fish, it's possible to hold the fish in one position within the laser plane and record the glittering flow patters. However, this isn't an option when you're measuring fluid flows near to a lunging fish's mouth. Fortunately Higham realised that he could entice the fish to lunge reproducibly in the laser plane by tempting them with a tasty morsel suspended in the laser light; they always open their mouths at the same point when approaching a meal. But even then, the majority of the fish weren't correctly positioned in the plane of light and Higham had to discard the data.

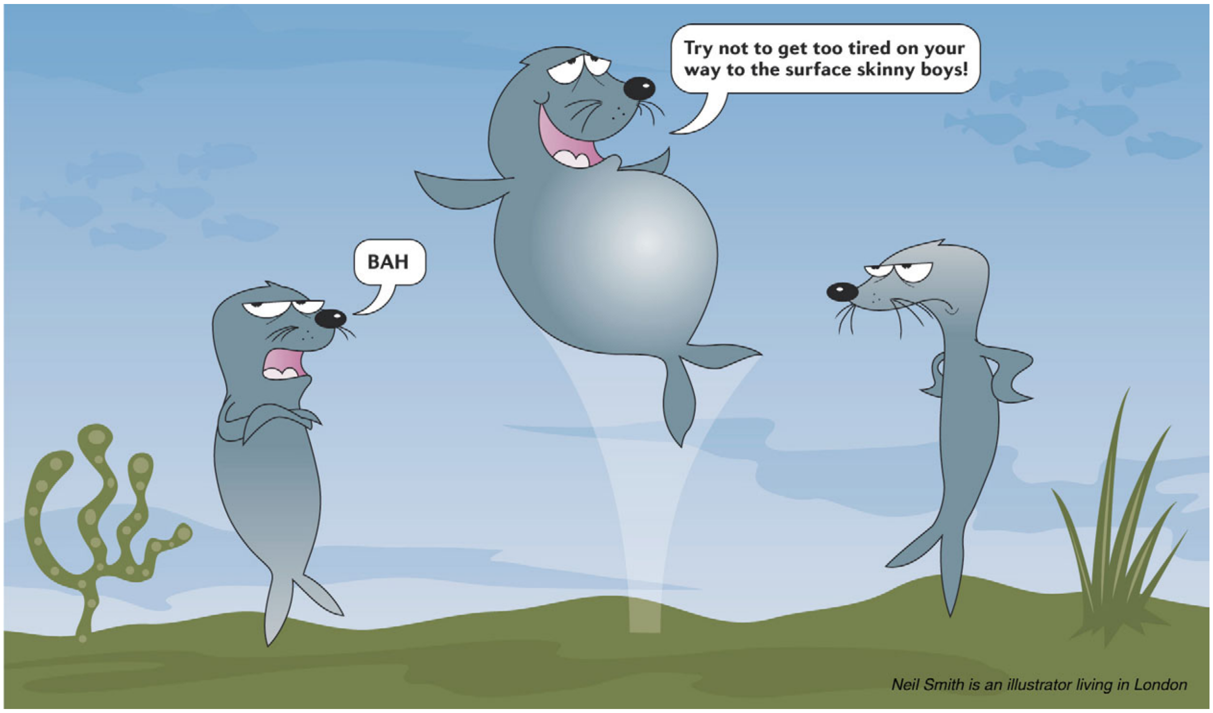
After months of patiently filming fish feeding while simultaneously recording the pressures in their mouths, Higham was able to calculate the fluid speed as each fish sucked. Comparing the sunfish with the bass, Higham could see that the fluid speeds were strongly correlated with the time it took the sunfish to open its mouth fully, but the relationship was weaker for the bass. The sunfish's powerful suck was generated simply by opening its mouth, but Higham explains that the weaker relationship between the time the bass took to open its mouth fully and fluid flow suggests that the animal modulates its slurp with other mouth structures.

Next, Higham compared his measurements with fluid speed predictions from Muller's model and found that the model consistently overestimated the fluid speed over a range of distances in front of the fish's mouth. Ever since the model's development, fish kinematicists have used mouth pressure measurements to predict fluid flow speeds, but Higham's simultaneous recordings suggest that simply knowing the pressure in a fish's mouth isn't enough to accurately estimate the flow from Muller's model. However, he suspects that it is now feasible to increase the model's complexity to better reflect the true movements in a gaping fish's mouth, given the massive increase in computing power since the early 1980s.

10.1242/jeb.02475

**Higham, T. E., Day, S. W. and Wainwright, P. C.** (2006). The pressures of suction feeding: the relation between buccal pressure and induced fluid speed in centrarchid fishes. *J. Exp. Biol.* **209**, 3281-3287.

# DIVERS ADAPT AS FATNESS VARIES




Most of us remember our first sortie into a swimming pool supported by buoyancy aids. But while children's flotation devices are designed to give them support, buoyancy can be a major hurdle for some diving species; they have to swim hard to overcome its effects. And some animal's buoyancy isn't a constant impediment, it can vary significantly between individuals and from season to season as an animal gains and loses fat. How diving animals adapt as their buoyancy changes wasn't clear until Yuuki Watanabe and his colleagues from Japan and Russia attached data loggers to freely swimming Baikal seals, ranging in weight from 54 kg to 83 kg, to see whether each seal had a unique dive signature that reflected their different densities (p. 3269).

Securing detachable data loggers to three animals in Lake Baikal, the team let the animals swim free. After a nail-biting wait, the data loggers bobbed back to the surface a day later and the team retrieved them with the help of a VHF signal before downloading the animals' stroke pattern data. Sure enough, the seals had very different dive signatures. Two of the seals descended with little effort, yet swam hard to return to the surface. The third seal returned to the surface with ease, while swimming with fast strokes while diving. The animals were all using different swimming stroke rates during descent and ascent, probably in response to their different body densities.

But how would the animals adapt to a seasonal change in buoyancy? The team secured a 1.5 kg lead weight, designed to fall off after 24 hours, to a seal and monitored its diving behaviour for 72 hours. But the experiment seemed doomed when the VHF signal failed and it couldn't be retrieved. Fortunately, a passing tourist retrieved the valuable data logger and returned it to a relieved Watanabe two weeks later.

Analysing the precious data, the team saw that the animal's stroke pattern changed significantly after the weight detached and the animal's 'fatness' suddenly increased. While carrying the weight, the seal's density was high, as if it carried little fat, so the animal glided during its descent while swimming continually to return to the surface. However, after the weight fell off the seal had to adapt to its apparent fat increase by raising its stroke rate while diving, to overcome its increased buoyancy, and alternating swimming and gliding stroke patterns to return to the surface. The seal had drastically changed its swimming style in response to its altered buoyancy.

Curious to know how the 1.5 kg lead weight compared with a real change in the animal's fat deposits, the team calculated that carrying the lead weight was equivalent to the seal losing 14% of its fat, a realistic loss for animals marooned on shore when moulting and raising young. So, the diver's drastic swimming style change is probably a realistic reflection of the adjustments the seals make on a seasonal basis.

10.1242/jeb.02477

**Watanabe, Y., Baranov, E. A., Sato, K., Naito, Y. and Miyazaki, N.** (2006). Body density affects stroke patterns in Baikal seals. *J. Exp. Biol.* **209**, 3269-3280.

**Kathryn Phillips**
**kathryn@biologists.com**