## Sea otter mums' metabolic rate rockets

A sea otter mother feeding her pup. © Joe Tomoleoni.

The idyllic sight of a sea otter mum with a pup clasped to her chest, basking in the peaceful waters of Monterey Bay suckling her youngster can conceal the true extent of her devotion. Lactation is one of the most demanding physiological processes that animals can experience, but for sea otter mums, the challenge can be life threatening. 'We had been seeing a disproportionately high amount of adult female southern sea otter mortality at the end of lactation', says Nicole Thometz from the University of California, Santa Cruz, USA, adding, 'This suggested that lactation was a particularly difficult life stage for females, but we had no idea exactly how costly it was'.

Having previously measured the metabolic rates of sea otter pups in a bid to evaluate the cost of parenting, Thometz, Terrie Williams and colleagues had already estimated that sea otter mums might have to double their consumption of food to see the pup through to independence; however, the team needed to measure the resting metabolic rate of a lactating sea otter mother directly to find out exactly how much energy it took to raise a sea otter pup. Yet, with a moratorium on sea otter breeding in captivity, there seemed little chance that they would have the opportunity. That was until a pair of young sea otter females – Mollie and Clara – arrived at the Monterey Bay Aquarium. Thometz recalls that vet Mike Murray gave the animals a physical when they arrived and discovered that Clara was pregnant. 'Mike called me to let me know what an amazing opportunity we had on our hands', recalls Thometz excitedly.

Having relocated the sea otters to Santa Cruz, Williams, Thometz, Traci Kendall and Beau Richter trained Mollie to enter the acrylic dome where they could record her oxygen consumption while resting. However, as the team intended to return Clara's pup to the wild, it was essential that humans altered her behaviour as little as possible, so they gently transferred her into the dome using a net, successfully repeating the manoeuvre when Clara was suckling her pup after the birth. Clara had also caught the team off guard when she gave birth a month early. 'Beau asked one of our other trainers to take a look at Clara and by the time the trainer got over to her, she was pulling the pup out from between her flippers', Thometz remembers.

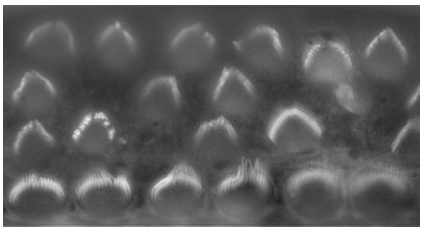
Comparing Mollie and Clara's metabolism over 12 months, the team saw that Clara's resting metabolic rate at the end of the pregnancy was 16.6% lower than her resting metabolic rate after weaning the pup – similar to the metabolic rate drop that has been found in other pregnant marine mammals. They speculate that this reduction could allow sea otters to accumulate fat reserves in preparation for milk production. However, after the birth of her pup and as it grew, Clara's daily energy demands soared – more than doubling to over 21 MJ $day^{-1}$ by the time that the pup was 4 months old – while her resting metabolic rate increased by 51%. 'We found the cost of pup rearing to be significantly higher than previously estimated', says Thometz, adding, 'This represents a substantial energetic burden for a species with already high baseline energy demands and minimal energy reserves and is likely one of the underlying reasons why we are seeing high mortality rates for prime-age females at the end of lactation'.

10.1242/jeb.146225

**Thometz, N. M., Kendall, T. L., Richter, B. P. and Williams, T. M.** (2016). The high cost of reproduction in sea otters necessitates unique physiological adaptations. *J. Exp. Biol.* **219**, 2260-2264.

**Kathryn Knight**

## Sea anemone proteins repair damaged mouse cochlear hair cells



Mouse cochlear hair cells that have been repaired by RP proteins. Photo credit: Pei-Ciao Tang.

Summer is always the best time of year to get a good blasting at a festival or mega-band concert, but how many of us give a thought to our delicate sense of hearing as our ears are assaulted? Birds are capable of replacing damaged hair cells in the inner ear after exposure to loud sound, but mammals are not, potentially leading to deafness. However, Glen Watson from the University of Louisiana at Lafayette, USA, explains that one remarkably resilient animal has no problem rescuing damaged hair cells. Sea anemones, which detect passing prey with the vibration-sensitive hair cells covering their tentacles, have remarkable regenerative properties that allow them to rebuild the missing halves of their bodies when they tear themselves in two during reproduction. 'It occurred to me that if any animal could recover from damage to its hair bundles, anemones would be the ones', says Watson. Having discovered a cocktail of proteins in the mucus coating sea anemone bodies that allows them to repair injured hair cells in as little as 8 min, Watson and Pei-Ciao Tang decided to find out what effect the restorative proteins might have on damaged mouse cochlear cells.

Recalling that growing the delicate hair cells derived from mouse cochlear cells in the lab was very challenging, Watson explains how he and Tang eventually succeeded in dissecting the minute cochleae and attaching the cultured cells to coverslips thanks to advice from Karen Smith and other colleagues. Watson also describes how hair cells have a bundle of minute hair-like structures on the surface – stereocilia –

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that are tethered at the tips by protein strands in a V-shaped formation and he explains that it is the tethers that break when hair cells are damaged, causing the stereocilia to collapse. Knowing that calcium is an essential component of the tether structures, Watson and Tang transferred the hair cells to an environment lacking calcium for 15 min in an attempt to reproduce the destruction produced by sound in mammalian cochleae. Describing the effects, Watson says, 'The stereocilia splayed rather than occurring in well-organised bundles'. In addition, the hair cells were unable to take up a dye that undamaged hair cells absorb readily; they were severely damaged, in much the same way that the cells in our cochleae are damaged by loud sound.

Having confirmed that the low calcium environment was destructive, Watson and Tang collected the mucus from starlet sea anemones that had damaged tentacle hair cells, isolated the repair proteins and added the protein cocktail to the damaged mouse hair cells for 1 h.

The hair cells recovered significantly – the stereocilia were no longer splayed and the cells improved sufficiently to absorb the dye; the sea anemone proteins had repaired the damaged mouse cells. Watson and Tang then searched the mouse genome for examples of the crucial repair proteins and found evidence that mice produce many proteins that are closely related to the sea anemone repair proteins, suggesting that it may be possible to mobilise the same repair mechanisms in mammals with damaged hearing. Watson hopes that this ground-breaking discovery will eventually lead to a treatment for patients with hearing loss. However, he acknowledges that this research is in its infancy and is keen to discover the mechanism that could eventually allow sea anemones to restore our hearing.

10.1242/jeb.146217

**Tang, P.-C., Smith, K. M. and Watson, G. M.** (2016). Repair of traumatized mammalian hair cells via sea anemone repair proteins. *J. Exp. Biol.* **219**, 2265-2270.

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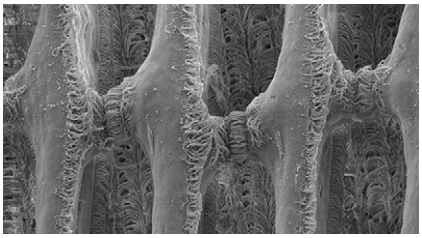
## Mussels get a cheap deal on waste disposal



Mussel gills and their cilia. Photo credit: Nicholas Holland.

Once the tide comes in, all a mussel has to do is open its shell and beat the minute hairs that line some of its body surfaces to waft tiny particles of food across the gills for nourishment and to deliver oxygen to the animal. However, Jörn Thomsen, Martin Tresguerres and colleagues from the Scripps Institution of Oceanography, USA, and two German institutions – Christian Albrechts University and the Alfred Wegener Institute – explain that it wasn't clear whether the mollusc also takes advantage of these micro-currents to dispose of the bivalve's ammonia waste at negligible cost. Many aquatic invertebrates excrete their toxic ammonia waste through expensive ion pumps that consume energy in the form of ATP, while some waste is carried out of cells by passive diffusion through a group of gas-permeable channels known as Rhesus-like proteins, ready to be washed away by the water. Little was known about how mussels excrete nitrogenous waste and through which organs; were the animals using the kidney, the gills or even the plicate organ, which is unique to this family of mussels and was believed to contribute to gas exchange for respiration? Thomsen and his colleagues decided to investigate.

Searching for the presence of Rhesus-like proteins in the mollusc's tissues (only one had previously been found in bivalves or crustaceans), Thomsen and Tresguerres located the channels in the plicate organ and to a lesser extent in the gill. However, there was no evidence of the channels in the kidney, which was surprising, as the team had suspected that the kidney would contribute to ammonia excretion. And when Thomsen and Franz-Josef Sartoris cautiously inserted a fine capillary into the mollusc's kidney to extract urine to find out whether the ammonia concentration in the urine was higher than that of the haemolymph, they discovered it was not.

Having ruled out the kidney as a site of ammonia excretion, the team turned their attention to the gills and the plicate organ. Testing for evidence of the V-type $H^+$-ATPase protein pump – which is involved in ammonia excretion in other aquatic organisms – Thomsen, Nick Holland, Nina Himmerkus and Markus Bleich found that the protein is produced in the plicate organ. However, when they inactivated the pump, the ammonia production rate did not drop; the pump was not contributing to ammonia excretion, suggesting that ammonia was being excreted passively through the Rhesus-like channels. Knowing that passive diffusion through protein channels only works if the external concentration of ammonia is lower than the internal concentration, and that the external concentration of the waste can only be reduced if it is carried away by water currents, Thomsen stilled the cilia on the gill and plicate organ using dopamine and this time the ammonia excretion rate fell.

'Water pumping by ciliary beating therefore appears to be an energetically inexpensive mechanism to remove ammonia from the body because mussels constantly beat their cilia to filter water for feeding', says Thomsen, who is now keen to know whether another protein, the ammonia transporter, recently identified in mosquitoes, might also contribute to mussel ammonia excretion. Meanwhile, it looks as though mussels are getting a cheap deal on their waste disposal in addition to the low cost of meal deliveries, thanks to water currents generated by their ever-beating cilia.

10.1242/jeb.146241

**Thomsen, J., Himmerkus, N., Holland, N., Sartoris, F.-J., Bleich, M. and Tresguerres, M.** (2016). Ammonia excretion in mytilid mussels is facilitated by ciliary beating. *J. Exp. Biol.* **219**, 2300-2310.

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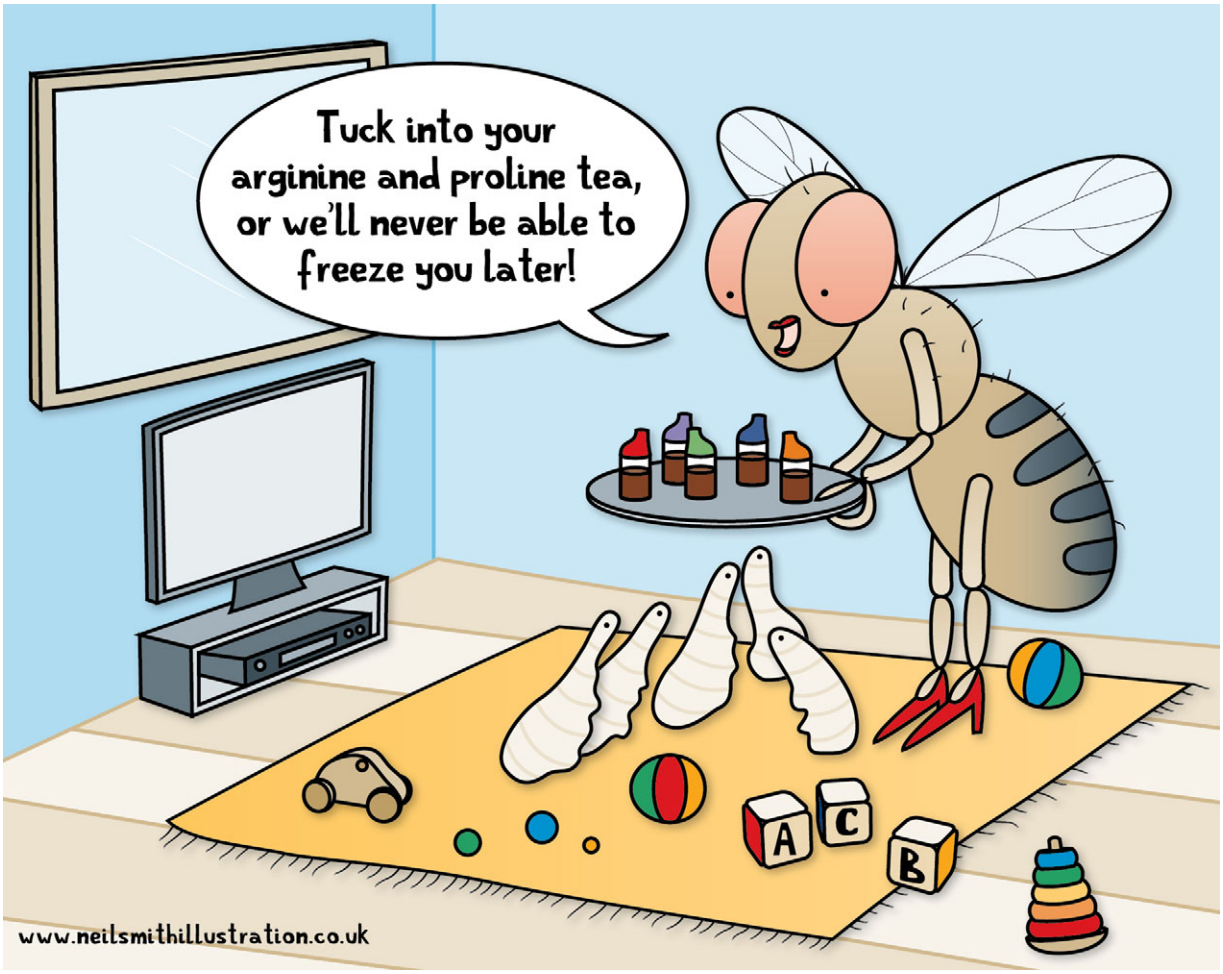
# Amino acid diet improves frozen fly survival




For many people, the humble fruit fly is a pest that competes with us for the ripest fruit, but to many biologists, the pesky insect has become a fount of knowledge since the development of powerful molecular tools that allow scientists to answer fundamental biological questions. However, Vladimír Koštál from the Czech Republic's Institute of Entomology explains that presently it is not possible to store the genetically modified insects by freezing in the way that scientists routinely store cells and bacteria. 'Current practice relies on continuous rearing of *Drosophila* flies. This is very tedious, expensive and risky', Koštál explains. So, in a bid to develop a technique to preserve the insects at an earlier stage of development, Koštál and his colleagues designed a protocol to freeze larvae in which they washed the insects, wrapped them in a ball of moist cellulose and then added an ice crystal to trigger ice formation in the larvae's bodies down to –5°C. Then, having thawed the larvae, the team monitored the insects as they developed into adults and found that they could improve survival from 0.7% to an impressive 12.6% by cooling the larvae slowly. But could they find a dietary additive that would improve the insects' survival still further?

Systematically feeding the larvae diets fortified with individual amino acids and other amine compounds before freezing and testing their survival, the team was impressed to find that the survival of the adult flies improved dramatically to 50.6% and 42.1% when the larvae had been fed a diet including either 25 mg $g^{-1}$ arginine or 50 mg $g^{-1}$ proline. Discussing possible mechanisms that may allow the two amino acids to protect structures and prevent damaged proteins and lipids from forming destructive aggregations in cells, the team is optimistic that their evaluation of the impact of different amino acids on freeze tolerance will eventually contribute to the development of a successful protocol that will allow researchers to store valuable insect strains for extended periods at low temperatures.

10.1242/jeb.146233

**Koštál, V., Korbelová, J., Poupardin, R., Moos, M. and Šimek, P.** (2016). Arginine and proline applied as food additives stimulate high freeze tolerance in larvae of *Drosophila melanogaster*. *J. Exp. Biol.* **219**, 2358-2367.

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