### **INSIDE JEB**

### Dolphins are exhalation champs



A dolphin breathing through the pneumotachometer. Photo credit: Dolphin Quest Oahu.

Every air-breathing marine mammal faces a multitude of challenges as it dives beneath the waves: carbon dioxide and nitrogen accumulation in the blood can cause unconsciousness, intoxication and decompression sickness, while low internal pressures in rigid lungs can force blood into the delicate airways. Yet, whales, dolphins and seals rarely seem to suffer these ill effects. Andreas Fahlman from Texas A&M University – Corpus Christi, USA, says, 'It had been assumed that the respiratory systems of all marine mammals are similar, with a stiff upper airway and collapsible lungs'. However, there was little evidence to support the assumption and Fahlman was repeatedly told that the measurements couldn't be made for various reasons, including the incredibly high flow rates generated by exhaling dolphins.

That was until Fahlman met Micah Brodsky, a consultant and wildlife veterinarian with an interest in mechanical engineering. Brodsky decided to take on the challenge of designing a small and portable pneumotachometer (air flow meter) that could be used to measure flows in excess of 200 l s<sup>-1</sup> in less than 100 ms and would survive corrosive salt water. After 9 months of development with the help of Trevor Austin, and knowing that the preliminary measurements couldn't be made in the wild, Fahlman and Brodsky travelled to Dolphin Quest Oahu, to work with bottlenose dolphins. Fahlman recalls that the sympathetic relationship between the dolphins and their trainers, led by Julie Rocho-Levine, was also essential for their success. However, even though the dolphins were trained to respond to their trainers' directions, they only participated voluntarily, 'Which allowed us to test important physiological variables with no stress to the animals', says Fahlman.

Gently placing the pneumotachometer over each dolphin's blow hole as it waited patiently in the water, Fahlman, Brodsky and Greg Levine measured the airway pressures and airflows as the animals breathed normally and heavily. They were impressed to see that the dolphins could inhale at flow rates of up to  $33.4 \, \mathrm{l \, s^{-1}}$ ; however, when they measured the flow rate as the dolphins exhaled, they were astonished to register flows of up to  $137.6 \, \mathrm{l \ s^{-1}}$ . 'The cetaceans are the true champions of respiratory physiology', says Fahlman, putting the dolphins' performance into context by saying, 'The maximal flow rates are at least twice, and probably three times, higher than those in the terrestrial champion, the horse'. And when the team analysed the airflows, they realised that the animals could exchange almost all of the air in their lungs in a single breath: 'Their vital capacity is very high', says Fahlman.

Next, Fahlman and Steven Loring tested how rigid the dolphins' lungs were by calculating their compliance, and they were delighted to confirm Per Scholander's 70 year old hypothesis that the lungs are compressible: rigid human lungs register a compliance of  $0.08 \text{ cmH}_2\text{O}^{-1}$  while the dolphins' compressible lungs had a compliance of  $0.31 \text{ cmH}_2\text{O}^{-1}$ . Fahlman also measured the amount of oxygen that the animals inhaled and exhaled and the carbon dioxide produced to calculate the animals' metabolic rates on a breath-by-breath basis. He explains that measurements like this could directly impact dolphin health. 'Lung function testing may be a novel way to investigate respiratory health of cetaceans under human care and we may be able to provide real-time information about oxygenation state and how animals are doing during a mass-stranding to help save animals', says Fahlman.

So, having overcome many technical challenges and measured the immense

airflows generated by breathing dolphins, Fahlman is keen to study other cetaceans to learn more about their respiratory physiology and the effects of beaching on stranded animals.

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Fahlman, A., Loring, S. H., Levine, G., Rocho-Levine, J., Austin, T. and Brodsky, M. (2015). Lung mechanics and pulmonary function testing in cetaceans. J. Exp. Biol. 218, 2030-2038.

Kathryn Knight

The Company of Biologists

## Chameleons' eyes are not so independent



A chameleon with eyes pointing in different directions. Photo credit: Idan Shapira.

Famed for their ability to change colour, chameleons have yet another mindboggling talent: their eyes appear to swivel completely independently. This means that they can simultaneously track two completely different views of the world, which is quite impressive from our primate perspective. However, Hadas Ketter Katz and Gadi Katzir from the University of Haifa, Israel, explain that this phenomenon is more common than we may realise: many fish and birds also have independently wandering eyes, with each eye connecting to the opposite half of the brain – meaning that the left half of the brain should only know what the right eye is seeing and vice versa. However, the duo and their colleagues were less sure that the chameleon's extraordinary eye movements were as independent as they first appeared. Could the left eye know what the right eye is doing when tracking completely different objects? Katzir and his team decided to try

foxing chameleons with an illusion to find out.

Fortunately, the chameleons that Katzir and his team work with are more than happy to play computer games. So, when Ketter Katz and Ehud Rivlin from the Technion, Israel, showed a Minecraftstyle insect scuttling across a screen to the chameleons, the enthusiastic gamers focused on it with one eye and precisely estimated the distance to the pixilated treat. Then they locked on to the target with both eyes and stuck out their tongue ready to fire at the screen. 'If you have a very precise behavioural pattern like this, you can tell that both eyes are looking at a target', says Katzir. But then Ketter Katz, working with Avichai Lustig and Tidhar Lev-Ari, played a cunning trick on the chameleons: they split the cyber fly in two. So, having comfortably focused both eyes on the tempting cybertreat, the chameleon was thrown onto the back foot. Pulling its tongue back, the reptile suddenly had to contend with each eye tracking its simulated fly independently as the images scampered in opposite directions towards the edges of the screen. How would the chameleons react?

'There are a few seconds of indecision. It knows that it has targets to shoot at but it cannot decide which target is the one to be shot', chuckles Katzir. However, once the chameleon has made its decision and swivelled the second eve around to focus on the same simulated fly as the first eye, then the chameleon prepares to fire its tongue within a matter of milliseconds. 'If the eyes were independent you would not have expected one to stay put and the other to converge', says Katzir. The fact that the chameleons are able to track objects moving in opposite directions before deciding which object to target and are then able to direct the second eye to where the first eye is gazing suggests that the second eye has some knowledge of where the first eye is directed. They are not independently controlled; there must be cross-talk between the eyes, similar to the cross-talk that gives us binocular vision.

'The capacity of animals to control their sensory systems is greater than we sometimes anticipate', says Katzir, who is now interested in seeing how well the chameleon's eyes coordinate in response to moving threats. 'I think there should be more attention to what animals are actually acquiring as visual information and how they are processing it. Their visual world might be different from ours and we should look into their solutions', says Katzir.

10.1242/jeb.126953

Ketter Katz, H., Lustig, A., Lev-Ari, T., Nov, Y., Rivlin, E. and Katzir, G. (2015). Eye movements in chameleons are not truly independent – evidence from simultaneous monocular tracking of two targets. J. Exp. Biol. 218, 2097-2105.

Kathryn Knight

# Melanin waterproofs mountain-dwelling weta



Alpine tree weta (*Hemideina maori*). Photo credit: Keith King.

It can be hard to get your head around just how big some weta are if you're used to working on the Drosophila scale, but giant weta routinely tip the scales at up to 70 g and even the more diminutive tree weta tower over other more familiar insects. While some offshoots of the family make their homes in low-lying areas, others have pushed the boundaries into the mountains, where they are exposed to high winds, low oxygen, the searing sun and dry air. 'Together, these environmental challenges...mean montane insects are at a significantly higher risk of desiccation', say Keith King from the University of Otago, New Zealand, and Brent Sinclair from the University of Western Ontario, Canada. So how do these high-altitude insects cope with these conditions? Suspecting that they might reduce their water-loss rates, King and Sinclair set about measuring water-loss across the insect's cuticle in weta ranging from sea-level species (the Wellington tree weta, the tokoriro and the Canterbury

tree weta) to the mountain dwellers (the alpine tree weta and the Banks Peninsula tree weta).

The duo was impressed to see that the low-level dwellers' cuticular water loss rate was  $0.444~\mu mol^{-1}~g^{-1}~h^{-1}$  while the mountain species' cuticular water loss rate was 45% lower at  $0.245~\mu mol^{-1}~g^{-1}~h^{-1}$ . They also found that the mountain dwellers had cut their respiratory water loss by 55%. And when they compared how much water the species lost relative to their metabolic rate, the mountain species were much more conservative than their low-altitude cousins, losing 64% less water.

King and Sinclair then explain that the amount of melanin pigment in the cuticle of *Drosophila* is known to affect insect desiccation rates, with lighter flies losing water across the cuticle faster than darker species. Knowing that there are two forms of the alpine tree weta – one with a melanin-tinted cuticle and a lighter version – the duo decided to test whether the degree of melanisation affected the insects' cuticular water loss rates. Through a direct comparison of cuticular water loss rates King and Sinclair could see that the darker form lost 46% less water than the lighter form. And when they stripped the waterproofing hydrocarbons from the surface of the cuticles of both insects and measured their cuticular water loss rates, the duo found that although the insects' water loss rates rocketed, the darker weta still lost water 57% more slowly than the lighter insects. The high melanin content of the cuticle in the darker weta offers more protection from desiccation than the cuticle of the lighter weta.

So, mountain-dwelling weta have developed various strategies to protect themselves from desiccation in their harsh mountain homes and some have probably benefited from the waterproofing effects of melanin as an unintended consequence when they became darker for camouflage.

10.1242/jeb.126961

King, K. J. and Sinclair, B. J. (2015). Water loss in tree weta (Hemideina): adaptation to the montane environment and a test of the melanisation desiccation resistance hypothesis. *J. Exp. Biol.* 218, 1995-2004.

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# I'm glad I protected my bones by suppressing resorption, I haven't moved for 6 months!

### Hibernating bears protect bones by reducing resorption

Even a short period of inactivity can be extremely bad for our bones, and for astronauts facing months in zero gravity, the risks are serious. But there is an animal that has already solved all of the problems faced by immobile humans. Black bears routinely hibernate for 6 months without stirring, and although it can take several weeks for them to regain their full metabolic vigour, their bones seem largely unaffected by the lengthy period of inactivity. Yet, how these impressive beasts protect their bones was a mystery, with previous studies yielding contradictory results. Intrigued by bone regeneration, Seth Donahue from Colorado State University, USA, and an international team of collaborators decided to monitor the blood levels of specific enzymes and hormones that are known to contribute to bone formation and resorption to find out how bears protect their bones during hibernation.

The team captured 13 female bears over four hibernation seasons, and collected

blood and bone samples from the animals before releasing them back into the wild the following spring. Carefully analysing the blood samples for bonespecific alkaline phosphatase (BSALP) and tartrate-resistant acid phosphatase (TRACP) - which indicate bone rebuilding – the team found that the blood enzyme levels fell in the hibernating bears, suggesting that the hibernating animals were suppressing bone remodelling. And when the team analysed the levels of boneregenerating osteoblast cells at the surface of the bone, they plummeted from 2% before hibernation to 0.15% during hibernation. However, the levels of cocaine and amphetamine regulated transcript (CART) - which is known to reduce bone resorption – increased 15fold during hibernation. So, instead of constantly rebuilding, the bears were protecting their bones by suppressing resorption of the tissue. The team also found that the bears' blood calcium

levels varied little from season to season, suggesting that the animals balance bone resorption and formation during hibernation to maintain stable blood calcium levels, which are also essential for healthy organ function and fat and energy metabolism.

'Hibernating bears are metabolic marvels', say Donahue and his colleagues, adding that these animals appear to be immune to two major epidemics sweeping the world – obesity and inactivity – suggesting that we could learn a thing or two from these fantastic creatures.

10.1242/jeb.126946

McGee-Lawrence, M., Buckendahl, P., Carpenter, C., Henriksen, K., Vaughan, M. and Donahue, S. (2015). Suppressed bone remodeling in black bears conserves energy and bone mass during hibernation. *J. Exp. Biol.* **218**, 2067-2074.

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