INSIDE JEB

Elephant seals' CO as high as heavy human smokers'

Two adult male elephant seals. Photo credit: Michael Tift.

Carbon monoxide (CO) is a silent killer. Produced by incomplete combustion, the colourless, odourless gas binds to the oxygen carrying protein – haemoglobin – in the blood of smokers and fire victims, insidiously clogging the protein to prevent it from transporting oxygen, resulting in suffocation. However, in recent years, CO has undergone something of a rehabilitation. Michael Tift, from the Scripps Institution of Oceanography, USA, explains that we all produce minute quantities of CO naturally and at very low concentrations, the gas has beneficial effects. He says, 'CO reduces inflammatory responses and the amount of apoptosis – cell death – due to heart attack and stroke.' Knowing that only 1% of human non-smokers' haemoglobin is incapacitated by CO – in the form of carboxyhaemoglobin – and that CO is produced naturally by haemoglobin breakdown, Tift's colleagues, Dan Crocker and Paul Ponganis, wondered how much CO an elephant seal might carry in its blood, as they have the largest blood volumes of any animal of their size (p. 1752).

At the time, Tift was working closely with colleagues at the University of California, Santa Cruz, USA – which has been studying elephant seals at the Año Nuevo State Park for 25 years – so he could call on their experience of working with the seals. Mildly sedating animals ranging from young pups to adults, Tift collected blood samples from the elephant seals to measure the proportion of carboxyhaemoglobin in their blood. The team was also curious to find out how the elephant seals' carboxyhaemoglobin levels altered with age, as the oldest animals have the largest blood volumes and highest levels of haemoglobin.

Measuring the relative proportions of the haemoglobin bound with the oxygen, carbon dioxide and CO in the seals' blood using a blood gas analyser, Tift was amazed to discover that the animals' carboxyhaemoglobin levels were stratospherically high at 10.4%; 'They have values similar to humans that smoke up to 40 cigarettes a day', says Tift. Next the team compared the carboxyhaemoglobin levels of the youngsters with those of the mature animals and found that the proportion of carboxyhaemoglobin in the blood increased as the animals aged, probably because of the veterans' higher haemoglobin levels. And when Tift monitored the carboxyhaemoglobin levels of a pair of juveniles over a day, it was clear that the amount of carboxyhaemoglobin in the animals' blood was incredibly stable.

So why are the seals' carboxyhaemoglobin levels so high? Tift explains that there is only one way for an animal to clear CO from its body, and that is to exhale it. He suspects that elephant seals accumulate an immense reserve of carboxyhaemoglobin because they hold their breath for about 75% of their lives. 'If they are at sea, they are constantly diving; if they are on land, they are going into sleep apneas [breath holds]. Since they are producing this stuff, they may be producing it at a similar rate to humans, but they may not be getting rid of it at the same rate,' says Tift. Alternatively, he suggests that the seals may produce more CO than other species either because they break haem proteins down faster or turn over more of the molecules.

Tift also suspects that the animals' high CO concentrations could protect them from oxidative damage incurred when blood rushes back into peripheral tissues after they hold their breath as part of the dive response. Explaining that CO can protect tissues from damaging inflammation when the oxygen returns, Tift says, 'High CO values could significantly benefit these animals in reducing the amount of injuries from the natural dive response.'

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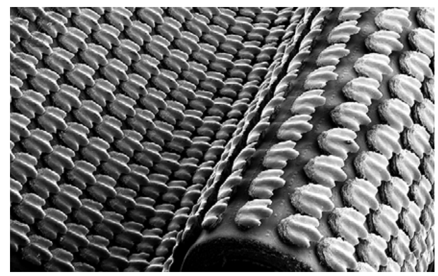
Tift, M. S., Ponganis, P. J. and Crocker, D. E. (2014). Elevated carboxyhemoglobin in a marine mammal, the northern elephant seal. *J. Exp. Biol.* **217**, 1752-1757.

Kathryn Knight

Simulated shark skin boosts swimming



Artificial shark skin with rigid denticles attached to a flexible membrane. Photo credit: James Weaver.

It's only when you get up close to a shark that you realise how rough the sleek-looking skin really is: it is peppered with millions of microscopic overlapping tooth-like scales. These so-called 'denticles' disrupt the smooth flow of water over the animal's surface, reducing the drag that holds them back. Engineers and scientists have been mesmerised by the advantage that the razor-sharp surface gives sharks, but it is impossible to get to the nub of how denticles give sharks a boost without testing how alterations to the skin affect the fish. 'You can't modify real shark skin', explains George Lauder from Harvard University, USA. So Lauder and his colleagues Li Wen and James Weaver decided to try to create artificial shark skin instead (p. 1656).

After finding a mako shark in a local fish market, Lauder took a small sample of the skin for scanning to get a high-resolution view of the surface. Next, he and Wen zoomed in on one representative denticle to build a detailed model of the structure before reproducing it thousands

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of times in a computer model of the skin. Then the team had to find a way to construct the model. 'After considering a number of approaches, we decided that the only way to embed hard denticles in a flexible substrate was the 3D printer', recalls Lauder, but this proved to be easier said than done. 'We had to figure out how to print them with multiple materials… The denticles are embedded into the membrane and overlap, which posed a key challenge', recalls Lauder. However, after a year of testing different materials, printing protocols and enlarging the denticles and their spacing, Weaver finally produced a convincing sample with the denticles secured in a flexible support. 'Seeing the [scanning electron micrograph] SEM of the curved membrane with the denticles was a great moment for us', smiles Lauder, who admits that the image of the SEM in the research paper is one of his favourite research images of recent years. But how well would the artificial skin function in practice?

Wen and Lauder attached samples of the manufactured skin to both sides of a flexible foil that could be held stationary in flowing water or flapped to move like a swimming fish, and then measured the forces exerted on the swimming and stationary foil. After repeating the experiments with samples of the flexible membrane alone, so that they could dissect out the effect of the denticles on the swimming performance, they were impressed to see that the static shark skin mimic reduced the drag on the foil by 8.7% at the lowest flow speeds, although at the highest flow speed – almost $0.6\ \mathrm{m\,s^{-1}}$ – the shark skin produced 15% more drag than the smooth membrane. However, when the team began flapping the foil like a fish, displacing its body by 1 cm as it wriggled at 1.5 Hz, the shark skin's performance improved significantly, increasing the foil's swimming speed by 6.6% and reducing the energy expended by 5.9%. 'This is the first time that anyone has measured the energetic cost of shark skin and the reduction in swimming cost relative to a smooth surface', says Lauder.

Reflecting on their success, Lauder attributes the breakthrough to the design, 'Artificial shark skin needs to have rigid denticles/scales on a flexible substrate so that the biomimetic skin can flex and bend like real shark skin', he says. And he is now keen to test how altering the arrangement of the denticles will affect performance. However, he doesn't think that we'll be wearing swimming costumes made from artificial shark skin soon: 'The manufacturing challenges are tremendous', he chuckles.

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Wen, L., Weaver, J. C. and Lauder, G. V. (2014). Biomimetic shark skin: design, fabrication and hydrodynamic function. *J. Exp. Biol.* **217**, 1656-1666.

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Varroa mites do not suppress honey bee immunity

A warm slice of toast dripping with honey is one of life's guilty pleasures, but the fate of the humble workers that produce this golden treat hangs in the balance. Ryan Kuster, Humberto Bonicristiani and Olav Rueppell from the University of North Carolina at Greensboro, USA, explain that European honey bee populations are in rapid and dramatic decline and that more than just the honey harvest is at stake: 'Honey bees (*Apis mellifera* L.) are the most important commercial pollinators worldwide', they explain. One of the most pernicious threats to honey bee health is the parasitic *Varroa destructor* mite. The mites not only harm their victims directly, but they also transmit other life-threatening diseases to the unwitting hosts. The team says, 'Vector-borne diseases and their vectors often share the common interest to attenuate the host's immune response to facilitate feeding and reproduction.' However, whether the *V. destructor* mite supresses the honey bee's immune system was unclear. Rueppell and his team embarked on a comprehensive survey of the parasite's impact on honey bees to lay the question to rest (p. 1710).

After deliberately infecting some honey bee larvae with one, two, or three or more *V. destructor* mites, or simulating the wound inflicted by a feeding mite on other larvae, the team then resealed the youngsters back into their cells to develop. Then, over a period of 10 days, they monitored the insect's immune response by measuring the expression of various genes related to the larvae's immune response. They also analysed the infection levels in the larvae of a virus (deformed wing virus) that is transmitted by the mites and is harmful to the developing insects.

However, when the team examined the larvae's immune response to the mite infection, they were surprised to see that it was unsuppressed. Only two of the immune response proteins that they monitored showed a drop in expression over the course of the infection: a pattern recognition receptor (*PGRP-S2*) 10 days after the mites were introduced and the encapsulation response effector (*PPOAct*) after 5 days. And the team was surprised to see that three immune effector genes (*defensin*, *hymenoptaecin* and *abaecin*) increased their expression levels, as did the intermediary protein, relish, in response to the *V. destructor* mites. The scientists add that the degree of *V. destructor* infection (one or more than three mites) did not alter the larvae's immune response, although they say, 'Our findings do not exclude the possibility that *V. destructor* parasitism has a delayed effect on adult honey bees'.

Next the team investigated how the presence of the mite affected the degree to which the larvae were infected by deformed wing virus, and this time there was a clear correlation: the greater the number of introduced mites in a larva's cell, the higher the level of deformed wing virus. 'This pattern seems to be established at 72 h post capping [of the cell]', the team says. In addition, the larvae that had been wounded – to simulate a mite feeding without introducing infection – were more vulnerable to the harmful virus, which they carry naturally and is usually asymptomatic.

Summing the data up, the team says, 'These results suggest that mite feeding activity itself and not immunosuppression may contribute to the synergy between viruses and mites.' They conclude by offering a possible strategy for combating the mites, saying, 'Increased expression of honey bee immune genes decreases mite reproductive success, which may be explored to enhance mite control strategies.'

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Kuster, R. D., Boncristiani, H. F. and Rueppell, O. (2014). Immunogene and viral transcript dynamics during parasitic *Varroa destructor* mite infection of developing honey bee (*Apis mellifera*) pupae. *J. Exp. Biol.* **217**, 1710-1718.

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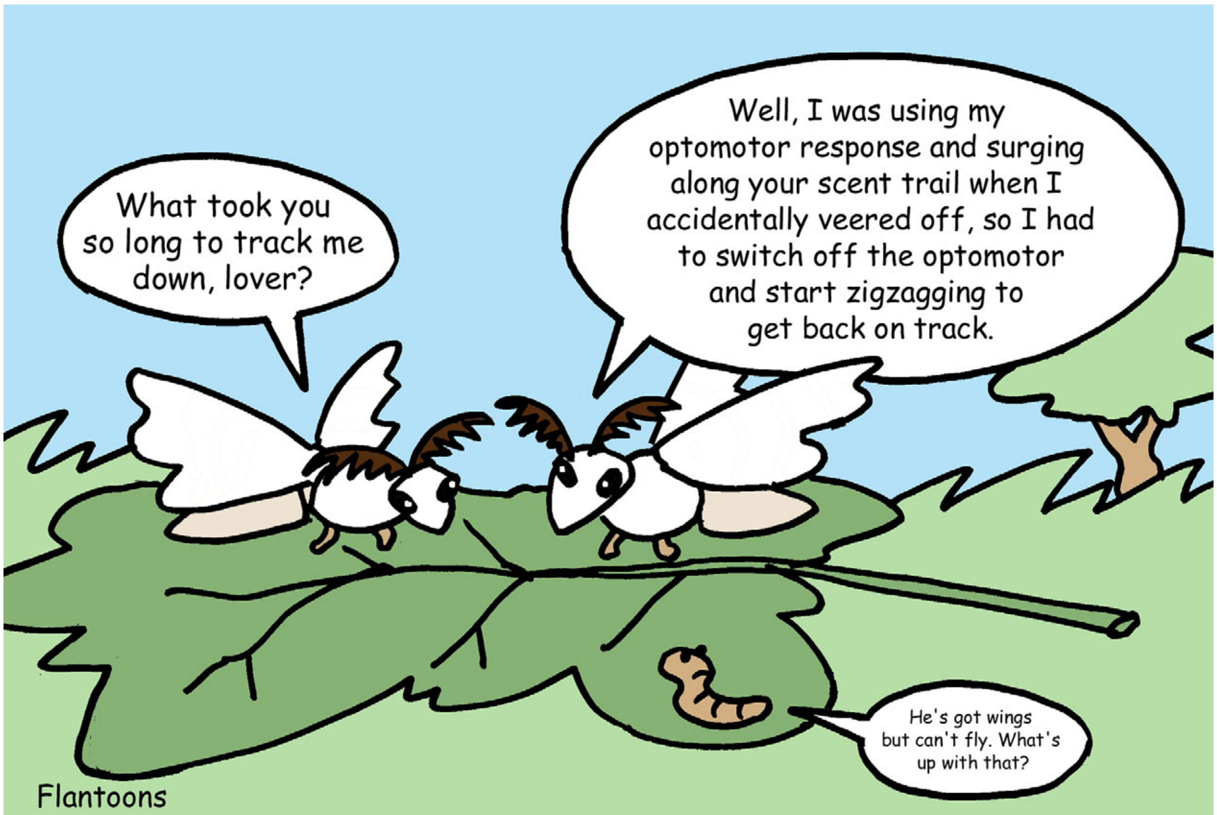
Silkmoths alternate optomotor response



Once a male silkmoth picks up the whiff of a female, very little will deter him from pursuit. Focusing his full attention on the task, the flightless male doggedly walks (surges) along her tantalising pheromone trail, switching to various zigzagging motions when he slips out of the odour plume in a bid to regain entry to track her down. Poonsup Pansopha, Noriyasu Ando and Ryohei Kanzaki from the University of Tokyo, Japan, explain that in addition to using their olfactory senses to track pheromones, insects also use visual cues to help them stay on track, but it wasn't clear how male silkmoths integrate their visual inputs with their olfactory senses to control the surge and zigzag behaviours. According to Kanzaki and his team, insects visually control progress along an odour plume by steering in the direction in which their surroundings appear to move. They do this by moving so that the image of their surroundings – the optic flow – remains static on the eye, and this response is known as the 'optomotor response'. However, they suspected that the optomotor visual guidance strategy could override the moths' zigzagging behaviour when they slip out of the plume, raising the question of how these males integrate the visual and olfactory senses during a pursuit (p. 1811).

Pulsing puffs of odour to simulate the males' olfactory experience of an odour plume while surging and zigzagging, the team also simulated the optic flow to find out how the insects use this visual information during the different stages of a pursuit. As they suspected, the moths did not use optic flow while zigzagging, but they did use it while surging along the pheromone plume. Kanzaki and colleagues say, 'Based on these findings, we suggest that the optometer response is utilised for course control during straight-line walking, whereas the absence of optomotor response during zigzagging is used to effectively perform the programmed walking pattern.' The team concludes by suggesting that the use of optic flow visual information by the silkmoth is controlled by two different visual pathways as they navigate odour plumes.

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Pansopha, P., Ando, N. and Kanzaki, R. (2014). Dynamic use of optic flow during pheromone tracking by the male silkmoth, *Bombyx mori*. *J. Exp. Biol.* **217**, 1811-1820.

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