INSIDE JEB

New fluid fats fuel frozen flies

Eurosta solidaginis overwinter inside galls on goldenrod plants. Photo credit: Brent Sinclair.

North American winters can be harsh, so anything that wants to make it to summer has to be pragmatic about surviving the coldest months; and insects are no exception. Katie Marshall from The University of British Columbia, Canada, explains that as the days draw in, the larvae of the goldenrod gall fly (*Eurosta solidaginis*) shut down their metabolism and development and begin producing cryoprotectants ready to freeze and survive the winter months. Intrigued by how the larvae manage their energy reserves as they repeatedly freeze and thaw, Marshall decided to investigate the energy-rich lipids (fats) stored by the overwintering insects. But when she began analysing the insect's lipid content, she stumbled upon an unexpected mystery. In addition to all of the regular lipids that occur routinely in all other terrestrial organisms, there was another class of lipids that didn't match any of the standard molecules that always crop up (p. 1580). What were they and what was their purpose?

Puzzled, Marshall showed her data to her PhD supervisor, Brent Sinclair from the University of Western Ontario, Canada, who recalls that he was initially dubious. 'First, Katie needed to prove to me that she wasn't screwing up', chuckles Sinclair. But Marshall's fiancé and pharmaceutical scientist Áron Roxin was more confident that something novel was going on. Marshall recalls that he was sceptical that she and Sinclair could be so certain of the identities of the lipids that they had identified so far. He suggested that Marshall try some analytical chemistry to find out exactly what the novel lipids were, so she began searching for chemists who could help her. 'Katie was literally wandering the halls of the chemistry department reading posters to see if anybody was using methods that might be useful', recalls Sinclair.

Teaming up with local chemists Raymond Thomas, Eric Chen and Elizabeth Gillies to analyse the mystery lipids with techniques including mass spectrometry and NMR, Marshall and Sinclair were eventually convinced that the molecules were acetylated triacylglycerols. These lipids are less energy dense than the long chain triacylglycerols that are found in all other organisms, so the duo began searching for an explanation for their presence.

Measuring the ratio of the acetylated triacylglycerols to other lipids in the overwintering insects, Marshall and Sinclair found that the novel lipid levels rocketed to 37% of the insects' lipid pool during the winter, compared with the normal triacylglycerols, which only comprised 17% of the total lipids. And when Marshall monitored how the acetylated triacylglycerol levels varied across the seasons, she found that the insects accumulated the lipids in winter and when frozen repeatedly. She also found that the lipids might even have some antifreeze characteristics. Then, Marshall tested the tissue of the goldenrod to see whether the plant was the source of the acetylated triacylglycerols, eventually concluding that the larvae must synthesise the lipids from their own resources by converting the normal long-chain triacylglycerols into the acetylated lipids. Next, Marshall contacted Jason Brown, who showed that instead of occurring in the insect's cell membrane, the acetylated triacylglycerols were mainly located in the insect's energy-storing fat body. And when she measured the lipid's melting point, she found that it was significantly lower than that of other lipids, providing a ready supply of energy for the frozen insect.

Having discovered an exciting new class of lipids that remain fluid at low temperatures to provide energy for frozen animals, Marshall and Sinclair are keen to find out how the fly larvae produce it and whether we might be able to use these new low calorie fats to tackle the human obesity crisis.

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Marshall, K. E., Thomas, R. H., Roxin, Á., Chen, E. K. Y., Brown, J. C. L., Gillies, E. R. and Sinclair, B. J. (2014). Seasonal accumulation of acetylated triacylglycerols by a freeze-tolerant insect. *J. Exp. Biol.* **217**, 1580-1597.

Kathryn Knight

Elephant seals susceptible to human disturbance

Northern elephant seals at Año Nuevo State Park. Photo credit: Tom McElroy (NMFS permit 14636-04).

Marine mammals have been pottering around unhindered in the oceans for millions of years. But, more recently, another mammal – humans – has begun exploiting their aquatic environment. 'The ocean is a much noisier and busier place than marine animals have previously experienced in their evolutionary histories', says Jennifer Maresh from The University of California, Santa Cruz, USA. Maresh explains that our activities are probably disturbing the distribution of many populations in the ocean, and that will impact on the amount of effort that these predators exert to grab a meal. 'We want to know how resilient animals are when it gets harder to find food', says Maresh. However, vacuuming up prey in a particular location to find out how predators respond when food is scarce is not an option, so Maresh and her colleagues Dan Costa, Dan Crocker and Terrie Williams decided to make elephant seals work harder for their dinner in a different way. They attached neutrally buoyant blocks to the backs of commuting animals to find out how they adapt as they increase their exertions (p. 1485).

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Maresh, Samantha Simmons and Birgitte McDonald travelled to the Año Nuevo State Park just north of Monterey Bay in California, where elephant seals go to breed. Selecting 12 animals over a period of months, the scientists injected two different types of modified water into the animals (to measure their energy expenditure) and strapped a wooden block to their backs to increase their exertion by increasing the amount of water that they must push aside while swimming. In addition, they also attached GPS trackers, time-depth recorders, accelerometers and magnetometers to the animals' backs to monitor their behaviour, before driving the animals 50 km down the coast to the Hopkins Marine Station where they were released. 'Elephant seals have a tendency to return to their rookery', says Maresh, who was confident that she would be able to retrieve the priceless data when the animals returned home. The team also repeated the exercise with the same animals, but this time they removed the drag block, to find out how the animals behave and how much energy they consume under normal circumstances.

After successfully retrieving the data loggers, the team was impressed to see that the unhampered animal's movements are incredibly efficient. 'Elephant seals had low energy requirements (106.5 $\mathrm{kJ\,kg^{-1}\,day^{-1}}$), approaching or even falling below predictions of basal requirements', says Maresh. However, when the animals had to drag the blocks through the water, their metabolic rates rocketed by 65% to 175.2 $\mathrm{kJ\,kg^{-1}\,day^{-1}}$. Yet, when the team scrutinised the seals' behaviour, they were surprised that the animals had barely altered their swimming style: they did not beat their flippers wider or faster to compensate and they continued diving to the same depths and for as long as the unhindered seals. However, the animals spent 46% longer recovering at the surface after returning from a dive with the drag block and they took longer to ascend and descend. Instead of altering their swimming style to maintain efficiency, the seals stuck to their usual technique, which became increasingly inefficient.

Explaining that increasing time at the surface puts the seals at increased risk of predation by sharks and orcas, Maresh adds, 'Elephant seals are considered to be a relatively hardy species … that they were so easily affected by a disruption to their routine swimming behaviours was thus somewhat surprising', and she warns that other more sensitive species are likely to be more seriously affected by human disturbance of the sea.

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Maresh, J. L., Simmons, S. E., Crocker, D. E., McDonald, B. I., Williams, T. M. and Costa, D. P. (2014). Free-swimming northern elephant seals have low field metabolic rates that are sensitive to an increased cost of transport. *J. Exp. Biol.* **217**, 1485-1495.

Kathryn Knight

Inactivity exacerbates flies' senescence

The fruit fly *Drosophila melanogaster* on necrotic fruit. Photo credit: Stephen Roberts.

When a honey bee embarks on its foraging career, its days are numbered. The relentless schedule takes its toll and most of the insects die within 3 weeks. 'Foraging has really profound effects on longevity, flight ability and physiological performance', says Stephen Roberts from Central Michigan University, USA.

Intrigued by the ageing process – known as senescence – in insects, Roberts and his colleague Michelle Elekonich are keen to understand how flight, the most energetically expensive behaviour known, affects senescence. However, when it comes to answering complex questions about the mechanisms that govern ageing, *Drosophila* are better insects than bees for experimentally disentangling the effects of age and flight on senescence. Yet, little was known about the effects of a lifetime of activity on these ageing flies, so Roberts decided to get to grips with how different flight histories impact on geriatric fruit flies (p. 1437).

Teaming up with master's student Steven Lane, Roberts monitored how three populations of flies that had experienced different amounts of flight activity through their lives fared as they aged. The team allowed the first population to fly whenever they wished, but imposed a complete flight ban on the second group by loosely stuffing the insects' jar home with light wedding-veil gauze, only allowing the insects to walk around the interior. The third population was also free to fly, but their jar was strapped to a vortex shaker that was programmed to give the insects a brief nudge at random times over the day, frequently forcing them to take flight. Then, Lane analysed the insects' flight performance, weight and metabolism as they aged – starting with 15 day old youngsters, moving up to 35 day old middle-aged insects and concluding with 65 day old geriatrics – to find out how they had senesced.

Comparing the three populations, the team could see that insects that had been allowed to fly whenever they wished fared reasonably well. Testing the insects' ability to take to the air in low density air (where half of the nitrogen had been replaced with helium), they found the majority flew well in middle age and 30% were still able to take to the thin air as geriatrics. However, when the team tested the flight performance of flies that had been forced to fly throughout their lives, they had essentially burned out. By 35 days their metabolic rate had plummeted by 57% and by 65 days none of them were able to get off the ground in the helium-supplemented air. Roberts explains that this dramatic decline was predictable because of wear and tear and the insects' increased exposure to toxic oxygen by-products produced by their hectic lifestyles.

However, the big surprise came when the team investigated the insects that had been prevented from flying. Instead of benefiting from their leisure, the couch potatoes paid a high metabolic price for their sloth. 'Their flight ability was compromised the earliest and the most out of all of the treatment groups', says Roberts. By 65 days, none of the flies could get off the ground in the test atmosphere. However, unlike immobile humans, they had not gained weight.

'We didn't know what we were going to see with the no-flight group', says Roberts, who explains that it was possible that inactivity might have slowed the ageing process, but evidently it had not. In fact, it exacerbated the insect's decline. He says, 'Behaviour can have profound effects on an organism's future; what it has done in the past has profound effects on how good its life may be in the future.'

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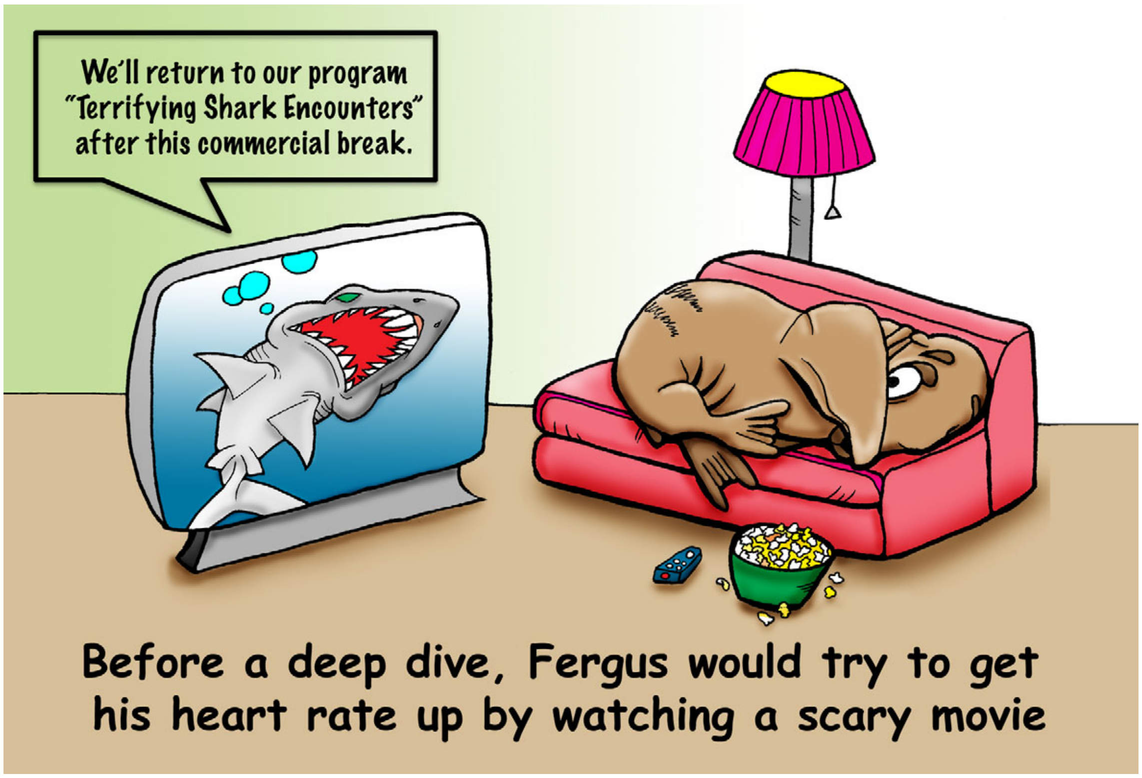
Lane, S. J., Frankino, W. A., Elekonich, M. M. and Roberts, S. P. (2014). The effects of age and lifetime flight behavior on flight capacity in *Drosophila melanogaster*. *J. Exp. Biol.* **217**, 1437-1443.

Kathryn Knight

Sea lions raise heart rate before deep dives



When aquatic mammals and birds plumb the depths, they have only the oxygen reserves that they carry on board to sustain them; yet, some animals can remain immersed for tens of minutes on a single lungful of air. All divers have evolved a suite of physiological responses to eke out their oxygen reserves, including increasing oxygen stores in venous blood and lowering the heart rate (bradycardia) to conserve oxygen stored in the blood. Birgitte McDonald and Paul Ponganis from the Scripps Institution of Oceanography, USA, explain that although the cardiac response of diving phocid seals (such as grey seals and Weddell seals) have been studied extensively, less was known about the cardiac responses of the otariids, such as fur seals and sea lions. As California sea lions are good divers, McDonald and Ponganis headed to San Nicolas Island, off the coast of Southern California, to measure the heart rates of the diving animals to find out how they cope with submersion (p. 1525).

The duo captured five females and attached ECG electrodes, a pressure sensor, accelerometers and a radio transmitter to each animal; they then released the sea lions to venture off foraging. After retrieving the data loggers several days later, the team could see that the animals had two distinct heart activity patterns, depending on the dive depth. In short shallow dives lasting less than 2 min, the heart rated dropped rapidly during the descent, remained constant while the animal was at depth and increased rapidly as the seal ascended. However, when the seals undertook lengthier dives that exceeded 3 min and went as deep as 300 m, they raised their heart rate while at the surface prior to the dive before rapidly reducing their heart rate during the initial descent and slowing the heart rate decrease as they neared the bottom. The seals' heart rates then increased slightly while they foraged at depth, before gently increasing during the initial ascent and increasingly rapidly just before the seals returned to the surface. And when the sea lions remained submerged for more than 6 min, their heart rate fell as low as 6 beats min^{-1}.

The team suspects that the deep-diving sea lions use the pre-dive period when their heart rates are high to maximise their venous oxygen reserves in preparation for the long submersion, and they suggest that the very low heart rates experienced at the bottom of the dive might force the animals to consume oxygen stored in the muscle and protect them from absorbing too much nitrogen. The duo also points out that deep-diving emperor penguins use the same strategy, suggesting that this might be a common tactic used by endotherms that dive carrying a lungful of air.

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McDonald, B. I. and Ponganis, P. J. (2014). Deep-diving sea lions exhibit extreme bradycardia in long-duration dives. *J. Exp. Biol.* **217**, 1525-1534.

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