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

FAT PROCESSING CHAMPIONS


Picture by Judy Higham

Most migratory birds put other animal athletes to shame, completing journeys thousands of miles long fuelled by energy from their exceptional lipid metabolism. One of these champions is the ruff sandpiper *Philomachus pugnax*, a shorebird that flies a round trip of up to 30 000 km a year between wintering grounds in Africa and nesting grounds in northern Scandinavia. As Jean-Michel Weber from the University of Ottawa explains, researchers face a problem if they want to study the metabolism of flying birds, as it requires invasive measurements which are very difficult to do once a bird is airborne. So to find out more about a sandpiper's lipid metabolism, Weber and his colleague Eric Vaillancourt took a different approach and studied the birds during shivering, which raises the metabolism but makes it much easier to take measurements (p. 1161).

The team made two sets of measurements simultaneously to examine the birds' lipid metabolism. The first set involved using a respirometer to measure the total amount of oxygen the birds used and the amount of carbon dioxide they produced. By comparing the quantities of the two gases, the team could work out which fuel the animal was using: carbohydrate, protein or fat. They found that the birds were getting over 80% of their energy from fat when they were at rest. When they lowered the temperature from 22°C to 5°C for 2 h to induce shivering, they found that the birds' oxygen consumption and carbon dioxide production went up. However the ratio of the two gases stayed the same, showing that the birds were simply using the lipids faster to give them enough energy.

The second set of measurements were to find out the rate at which the birds were breaking down lipids. Most fats consist of a glycerol backbone with three fatty acids attached, so the body has to break up the molecules and free the fatty acids that can be used for energy. By measuring the rate that glycerol enters the blood stream, scientists can measure how quickly lipids are being broken down. To measure glycerol production, Vaillancourt carried out delicate operations on the birds, inserting two catheters into two different blood vessels in their necks. They used the first catheter to inject labelled glycerol into the blood stream. By comparing the amount of labelled to unlabelled glycerol in blood samples taken from the second catheter, the team could measure the rate of glycerol production.

The team were surprised to find that the rate of lipid breakdown in the birds when they were at 22°C matched the highest rate that had ever been measured in an animal. When they dropped the temperature to induce shivering, they found that the rate of glycerol production in the blood stream stayed the same in the normal and cold conditions, showing that shivering didn't boost the rate of lipid breakdown, just the rate at which the birds used lipids for energy, which they saw from their respirometer measurements. This is probably because the rate of lipid breakdown is so high that it doesn't need to increase under colder conditions, because more than enough fatty acids are being released. The birds' record-breaking lipid metabolism is probably essential to achieve their long migrations.

10.1242/jeb.02762

Vaillancourt, E. and Weber, J.-M. (2007). Lipid mobilization of long-distance migrant birds *in vivo*: the high lipolytic rate of ruff sandpipers is not stimulated during shivering. *J. Exp. Biol.* **210**, 1161-1169.

WALLABIES HOP HARDER

For researchers intrigued by the less conventional ways animals use to get around, wallabies and kangaroos are ideal study subjects. 'They are just like pogo-sticks' says Craig McGowan of the University of Colorado, Boulder. When a wallaby is using its specialised design to bounce around on a flat surface, its ankle extensor muscle tendon acts like a spring, storing up energy for each hop. But when a wallaby is hopping up a hill, the ankle extensor still stores the same amount of energy and doesn't work any harder against gravity, so McGowan and his colleagues concentrated on the activity of the two largest muscles in the leg – a knee extensor called the vastus lateralis and a hip extensor called the biceps femoris – to see if they give wallabies the extra 'oomph' they need to bounce up hills (p. 1255).

To find out how much the wallabies' muscles were shortening and lengthening, and therefore how hard they were working

as they hopped along on level ground or up a slope, the team used a method called sonomicrometry. The team surgically implanted a pair of crystals, about 15 mm apart, into the vastus and biceps muscles. One crystal emits a high frequency sound wave to the second crystal, which transmits this information *via* a very fine wire to a receiver outside the animal. The time it took for the sound to travel between the crystals told the team how long the muscle was and whether it was lengthening or shortening. 'It's a very nice method as you're directly measuring length change', says McGowan. They also inserted tiny wires into the muscles to measure their electrical activity during hopping, telling them when the muscles were contracting.

Once the wallabies had recovered from their operations, they went to work on level or inclined treadmills. Comparing the muscles' electrical activity between level and incline hopping, the team found that the vastus and biceps muscles were active for the same amount of time during both level and incline hopping. However, when they examined changes in the muscles' lengths, they found that both muscles were working harder as the wallabies bounced uphill, but in different ways.

The biceps muscle shortened more during uphill hopping, showing that it was contracting and producing more force to help move the wallaby against gravity. The vastus muscle behaved differently: during level hopping, it lengthened more than it shortened, acting like a shock absorber. Hopping uphill, the muscle lengthened less, meaning that it could absorb less energy. Instead, it acted as a knee stabiliser to counteract some of the very high forces being produced by other muscles at the hip and the knee.

Interested to know how much work the biceps and vastus were contributing, the team put their data, plus data from wallabies hopping on force plates, into a mathematical model which estimates how much force each muscle contributes. Their calculations showed that both the muscles were working harder to help the wallabies hop up the slope, but weren't working as hard as their size suggested they would. McGowan suspects that other muscles in the hips also work harder to help wallabies hop up hills.

10.1242/jeb.02764

McGowan, C. P., Baudinette, R. V. and Biewener, A. A. (2007). Modulation of proximal muscle function during level *versus* incline hopping in tammar wallabies (*Macropus eugenii*). *J. Exp. Biol.* **210**, 1255-1265.

SOME SNAILS LEARN BETTER THAN OTHERS

It's not just some humans that find it easier to learn than others, snails too can be memory champions or scatterbrains. The key question is why some snails are better at forming long-term memories than others: it might help researchers understand how long-term memories form, too. Etsuro Ito and his Japanese and Canadian colleagues scrutinised memory champions and scatterbrains – or good and bad learners – in the pond snail (*Lymnaea stagnalis*), to find out how good snails are at forming memories, and what might affect their formation (p. 1225).

First the team had to train their snails to remember a specific event. They used a technique called conditioned taste aversion, where snails are fed a yummy treat – sucrose or carrot juice – followed by a horrible taste, in this case bitter potassium chloride (KCl) solution. If the snails have learned, they will avoid the same sweet treat in the future, knowing that it is followed by a bitter aftertaste.

To see how well the snails had learned, the team tested their trainees 9 min 30 s after training, counting how many bites they took of sucrose solution. The team found that 42% of the snails were good learners, not feeding on sucrose. The remainder fed on the sucrose solution, showing that some snails had remembered the bitter aftertaste, while others had not.

Having shown that some of the snails could form memories, the team next wanted to test if the memories were long-term. Testing both the good and poor learners again seven days later, they found that only the good learners remembered their training, showing that the memories were long-term. Even though the memories had lasted a week, there was always the possibility that the memory could be wiped out. This is called extinction, and the team wanted to know if their training was resistant to extinction or not, and if the snails had formed strong memories. They gave trained snails extra extinction training to try and make them forget the link between the sucrose and bitter KCl: each snail dined on sucrose, without KCl, on three separate occasions. Testing the snails' response to sucrose 1 h and 24 h later, the snails still rejected the sweet substance, showing that the memory had resisted extinction.

But when were the memories forming? The team knew that memories would form sometime during or directly after training, and would involve protein synthesis and altered gene activity in the brain. Immediately after training, the team cooled a group of snails down to 4°C for 30 min, testing them again once they had re-warmed them to 20°C. None of the snails had remembered their training, suggesting that memories were formed in this 10 min period. If the team delayed cooling until 10 min after training, the snails remembered their training, supporting this idea.

Knowing that some snails learn better than others, and that memories are formed in the first 10 min after learning, researchers will be able to focus their attention on this crucial time window, which might give them clues as to what separates the good learners from the bad.

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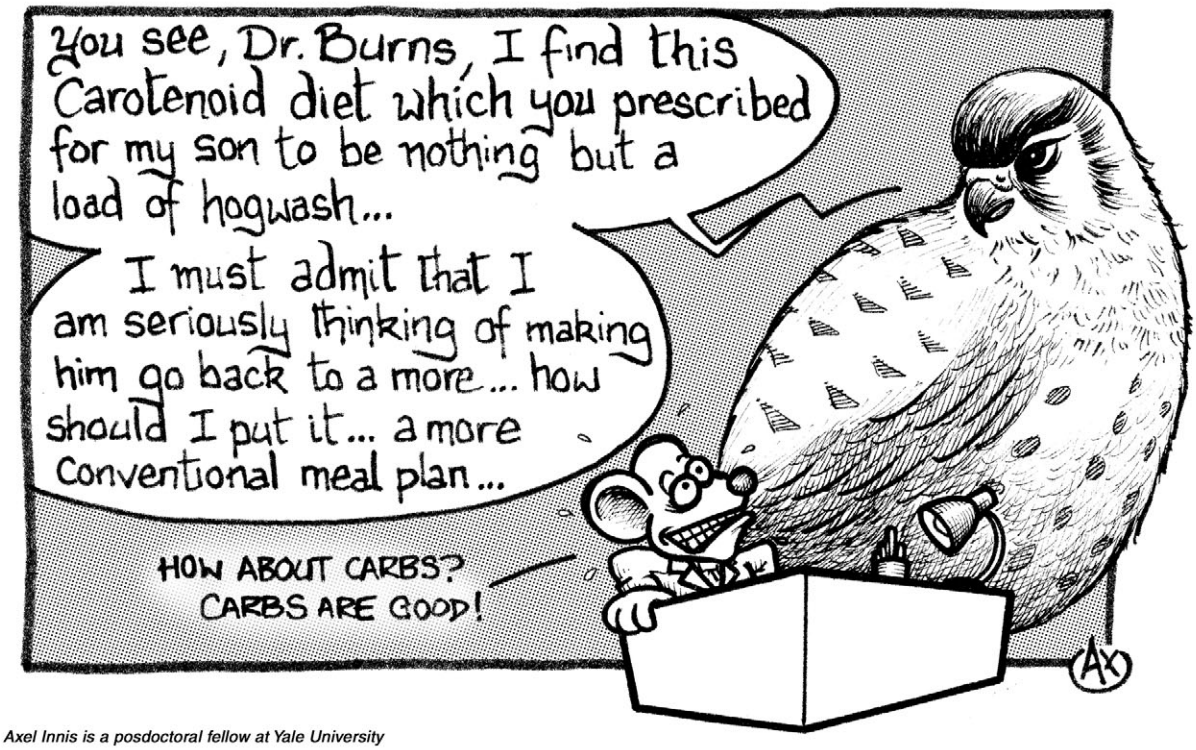
Sugai, R., Azami, S., Shiga, H., Watanabe, T., Sadamoto, H., Kobayashi, S., Hatakeyama, D., Fujito, Y., Lukowiak, K. and Ito, E. (2007). One-trial conditioned taste aversion in *Lymnaea*: good and poor performers in long-term memory acquisition. *J. Exp. Biol.* **210**, 1225-1237.

DO KESTRELS NEED CAROTENOIDS?



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Animals' cells are constantly under siege from damaging reactive oxygen species and they need anti-oxidants to help mop them up. When this clean-up system is overwhelmed, animals suffer from oxidative stress, which reduces their fitness. Carotenoids are one important group of anti-oxidants, which animals must obtain from their diet; however recent studies have suggested that the anti-oxidant role of carotenoids might not be as important as previously thought. To find out whether this was the case in young kestrels, David Costantini from the University of Rome, Sapienza, and his colleagues investigated whether supplementing the young birds' diet with carotenoids would increase their ability to deal with reactive oxygen species (p. 1238).

The team supplemented the diet of 7–8 day old kestrels with carotenoids, and then measured the carotenoid levels in the blood, finding that levels went up after supplementation. However, when they measured the blood levels of reactive oxygen species, mainly a type called hydroperoxides, and the overall anti-oxidant activity in the blood, they found that supplements caused no change. This shows that young nestlings can mop up free radicals, and don't need a dietary boost of carotenoids to help them do this.

10.1242/jeb.02765

Costantini, D., Fanfani, A. and Dell'Omo, G. (2007). Carotenoid availability does not limit the capability of nestling kestrels (*Falco tinnunculus*) to cope with oxidative stress. *J. Exp. Biol.* **210**, 1238-1244.

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