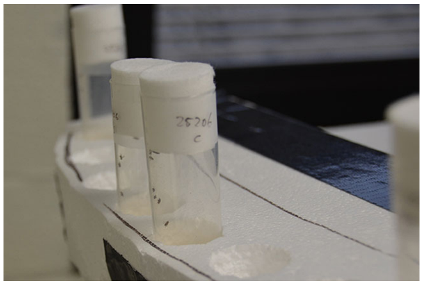
INSIDE JEB

Fruit flies strengthen leg muscles when they gain weight



Drosophila in a vial after 24 h of centrifugation. Photo credit: Ruud Schilder.

No matter how often I get on the bathroom scales, they're never going to tip back to the weight that I was when I went to university. I've piled on a few pounds since then, but my legs never seemed to notice the difference. 'It makes sense that animals should be able to "gauge" how heavy they are', says Ruud Schilder from Pennsylvania State University, USA, as most creatures have to adjust the strength of their muscles over the course of their lifetime. 'But we really do not understand well how such adjustments are achieved', he continues. Having already shown that rats can modify their muscle by producing alternative versions of a muscle protein (troponin T, which can alter the amount of force produced by a muscle) in response to weight gain, Schilder decided to find out whether fruit flies (*Drosophila melanogaster*) also alter which forms of troponin T they produce when they put on weight.

Explaining his choice of animal, Schilder says, '*Drosophila* makes for an interesting organism to study this in, as there are many molecular tools we can use to study the mechanistic details of muscle adjustments'. However, Schilder and undergraduate student Megan Raynor had to come up with a creative solution to help the insects gain weight rapidly. 'Fruit flies are fairly small, so attaching weights is not easy', chuckles Schilder. Instead, he decided to tamper with gravity by spinning the insects at high speed to increase the force pulling on their bodies; 'In other words, we tricked the fly leg muscles into thinking that they were supporting much heavier bodies', he says. However, Schilder had to cobble together an impromptu centrifuge by cannibalizing the remains of a human treadmill and an office chair to increase the flies' weight 12-fold from 0.49–0.77 mg to 5.88–9.24 mg. Spinning the flies for 24 h, Schilder and Raynor tested the strength of the flies' legs and found that the insects were better climbers and jumpers. Their leg muscles were much stronger than those of flies that had not gained weight. And when Schilder checked how stressful the insects had found the experienced – by comparing the metabolic rate of the spun insects with that of insects that had remained static – he was surprised that the fruit flies were unaffected: '[they] seemed rather unimpressed by the whole affair', he recalls.

However, when Schilder analysed which forms of troponin T (ranging from troponin T_A to troponin T_F) the flies were likely to be incorporating into their leg muscles, by looking at the different troponin T mRNA molecules (which are then converted into protein), he was impressed to see noticeable shifts in the mRNA distributions of the spun flies. One form (troponin T_A) dropped off dramatically in the legs of the bulkiest flies, while the legs of the daintiest insects produced almost as much of the troponin T_A mRNA as the unspun insects. 'The effect of weight increase on troponin T transcription depended on the actual weight load experienced by *Drosophila* leg muscles', says Schilder.

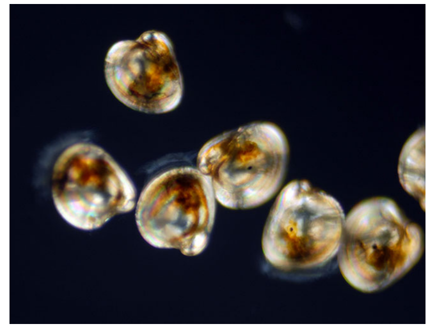
So, it seems that fruit flies have some sort of sensor that can detect when they are gaining weight that triggers leg muscle modifications, and the response is remarkably fast. Schilder also suspects that other animals may share the same mechanism, having previously discovered that rats also modify their muscles in response to weight gain. 'We don't know yet what the sensors are, but our results support the existence of an evolutionarily conserved mechanism that translates body weight variation into appropriate skeletal muscle molecular and functional responses', he says.

10.1242/jeb.170415

Schilder, R. J. and Raynor, M. (2017). Molecular plasticity and functional enhancements of leg muscles in response to hypergravity in the fruit fly *Drosophila melanogaster*. *J. Exp. Biol.* **220**, 3508-3518.

Kathryn Knight

Turbulent oyster larvae at risk of starvation



Two-week-old oyster larvae viewed under polarized light. Photo credit: Heidi Fuchs.

The numbers are staggering: for every oyster that makes it onto a dinner plate, 10 million siblings perished as larvae, and the factors that lead to such shocking statistics fascinate Heidi Fuchs from Rutgers, The State University of New Jersey, USA. Having realised early in her career that water turbulence can determine where the larvae of bottom-dwelling species settle, Fuchs discovered recently that oyster larvae swim harder – propelled by microscopic hairs (cilia) covering two fleshy oval wings that protrude out of the larva's shell – when they are tumbling in strong currents: '[which] made me wonder about the energetic cost to the larvae and how much they would have to eat to make up for it', says Fuchs. Could agitated larvae consume enough in choppy conditions to sustain their turbulent lifestyle?

Despite their minute size, Fuchs reckons that oyster larvae (*Crassostrea virginica*) are some of the easiest plankton to work with; 'We get them delivered overnight from a hatchery', she explains. However, measuring the oxygen consumption of the minute 2-week-old molluscs and observing their swimming manoeuvres as they swirled around in water simulating eddies in a gentle stream through to smashing storm breakers was technically challenging. 'Fortunately, Adam Christman perfected some techniques in advance and my graduate student, Jackie Specht, was able to assist',

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Journal of Experimental Biology (2017) 220, 3399-3401

says Fuchs, recalling how Specht took charge of the oxygen measurements that would tell them how much energy the larvae consumed, while she monitored the larvae's swimming movements. The duo also added microscopic algae (*Isochrysis galbana*) to the swishing water during some of the trials, to investigate how much the turbulence affected the larvae's ability to feed.

Monitoring the larvae, the team could see that although the tiny youngsters spent more time swimming downward in the roughest conditions, on the occasions when they swam upward, their energy consumption doubled, even though their energy use became more efficient – thanks to the turbulence counteracting the effects of gravity. And the larvae that were provided with an algae diet used more energy than the unfed larvae as they beat their propulsive cilia to produce a feeding current, in addition to propelling themselves through the water.

However, Fuchs and Diane Adams were most surprised at the impact that the churning water had on the larvae's ability to feed. Although the young oysters bumped into more algae in the choppiest conditions, they grasped hold of less food. 'Our interpretation is that the water motion… made it more difficult for the cilia to handle or retain food particles. We suspect that the larvae can increase their ciliary activity for either swimming or feeding, but they cannot do both at full capacity at the same time, so in strong turbulence they focus most efforts on swimming', says Fuchs.

In fact, the larvae were so seriously impaired that they were unable to consume enough food to survive in the most turbulent waters, no matter how many algae were available. Fuchs says, 'Our observations suggest that many of these larvae could be lost to turbulence-induced starvation', admitting that she is surprised by how badly the young oysters are affected. However, she suspects that smaller (younger) larvae may be at less risk of starvation, as they have not developed their sense of balance and should conserve more energy by spending less time swimming against the turbulent conditions.

10.1242/jeb.170407

Fuchs, H. L., Specht, J. A., Adams, D. K. and Christman, A. J. (2017). Turbulence induces metabolically costly behaviors and inhibits food capture in oyster larvae, causing net energy loss. *J. Exp. Biol.* **220**, 3419-3431.

Kathryn Knight

Fastest whales and dolphins reinforce diaphragms

Killer whales, one of the species investigated in the study. Photo credit: Wayne Vogl.

Every diving animal that depends on air to breathe faces the challenge of crushing pressures compressing every tissue in their bodies, including the lungs, as they descend. Elastic tissues, such as arteries, which expand to accommodate pressure surges above the surface, become vulnerable to compression as soon as an air breather leaves the surface. 'A few years ago, we discovered that the mechanical properties of fin whale arteries were different from those of typical terrestrial animals', says Margo Lillie, who – with colleagues, Robert Shadwick and Wayne Vogl from the University of British Columbia (UBC) Canada, and others – discovered that the arteries are remarkably resistant to collapse, which may protect them during a dive. However, the trio also realised that the distinctive bobbing swimming style of whales and dolphins – where they beat their tails up and down – might also place arteries under increasing pressure if the animals compress their abdomen with each downward tail beat. Intrigued by the possibility that the abdomens of these diving mammals may be under more pressure than had been realised, the team wondered whether the divers' diaphragms might be reinforced in some way to withstand the additional abdominal pressure generated during each downward tail beat.

Together, the team collected over 20 whale and dolphin diaphragms over a 5 year period. 'We relied heavily on a network of people who collect the carcasses of animals that stranded on the shore and we also got diaphragms collected as part of a Canadian biological sampling programme and a commercial whaling operation in Iceland', says Lillie. Although it was possible to ship most of the organs back to Vancouver, Lillie, Shadwick, Vogl and Stephen Raverty from the Animal Health Centre, Canada, had to travel to Iceland to investigate the colossal 1.5 m-wide fin whale diaphragms.

'Compared to the diaphragms of terrestrial animals, the cetacean diaphragms looked so complex and they vary from one species to another, so it took me a while to start seeing patterns', admits Lillie. However, it eventually became clear that the animals' diaphragms were reinforced with a network of stiff collagen fibres. 'There is a lot of variation in the amount of collagen on the surface of the diaphragm', says Lillie; 'There was hardly any on the beluga and minke diaphragms, while the Dall's porpoise diaphragm was covered with it', she adds. Suspecting that the amount of reinforcement was related to the strength of the species' tail beats – and therefore their swimming speed – Lillie was pleased when she realised that the diaphragms of the fastest swimming species – the Dall's and harbour porpoises – were covered in the highest proportion of collagen (almost 60%), while the slowest species – the belugas, and fin and minke whales – had the lowest proportion of collagen (∼10%).

'We were surprised how closely the amount of collagen correlated with speed. It means that collagen deposited on the diaphragm could be necessary to withstand increased abdominal pressures associated with swimming', says Lillie. And when they compared the whale and dolphin's diaphragm structures with those of other diving mammals (seals and sea lions), which probably do not compress their abdomens when beating their tails from side to side, there was no evidence of the extensive collagen reinforcement. Reflecting on the discovery, Lillie says, 'The evolutionary decision to swim by moving the flukes up and down instead of side to side appears to have altered the design of the diaphragm', and now she and Shadwick are keen to find out how the increase in abdominal pressure has affected the return flow of blood through veins to these animals' hearts.

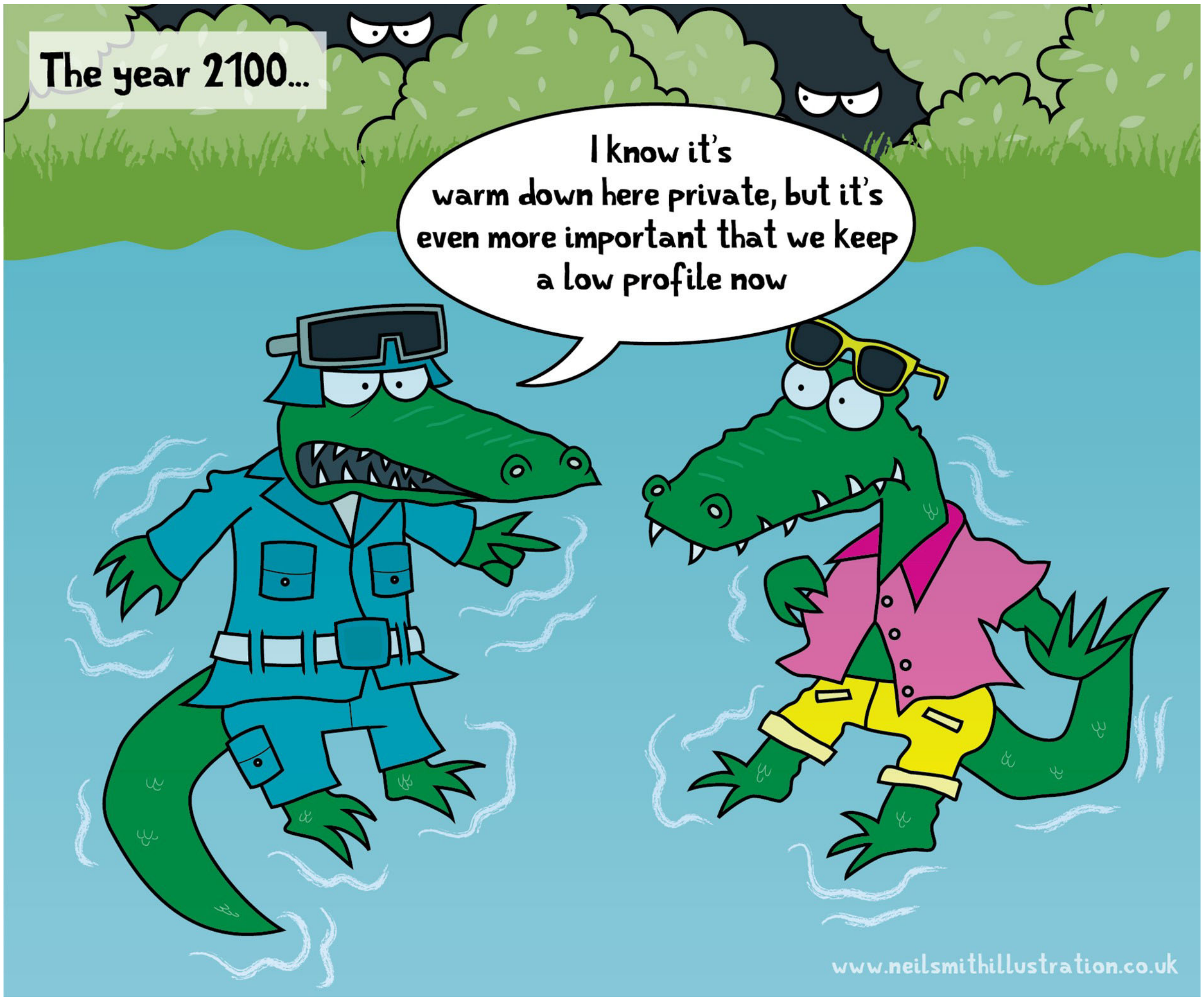
10.1242/jeb.170399

Lillie, M. A., Vogl, A. W., Raverty, S., Haulena, M., McLellan, W. A., Stenson, G. B. and Shadwick, R. E. (2017). Controlling thoracic pressures in cetaceans during a breath-hold dive: importance of the diaphragm. *J. Exp. Biol.* **220**, 3464-3477.

Kathryn Knight

Journal of Experimental Biology (2017) 220, 3399-3401

Hot crocs can't hide for as long



It's a natural response to run and hide when you feel threatened; even young crocodiles dive for cover when another creature shows an interest in devouring them. But Craig Franklin from The University of Queensland, Australia, is concerned that startled croc youngsters may not be able to remain safely submerged for as long as they do now as the climate continues warming. When so-called 'cold blooded' (ectothermic) animals get warmer, they consume oxygen faster as their metabolism increases, possibly restricting how long frightened crocodiles can remain submerged when avoiding a threat. Teaming up with Essie Rodgers, Franklin measured how long scared juvenile crocodiles can remain submerged at current river water temperatures and the temperatures that crocodiles are predicted to encounter in 2100 to find out how these reptiles may fare in the future.

After adapting the young crocodiles to modern day (28°C water) or future (34°C) climate scenarios for 2 months, Rodgers startled the animals with a gentle tap on the back and timed how long they remained submerged. Impressively, the crocodiles that were adapted to current climate conditions were content to remain submerged for 18.5 min, extending to over an hour if they felt particularly harassed after performing four consecutive dives. However, the hot water crocodiles could only remain under water for 9 min after a single tap on the back and the more threatened animals only stayed down for 28 min. Rodgers also measured the animals' heart rates and oxygen consumption to try to understand why the hot water animals' refuge tactics were so impaired, and found that the crocodiles that had adapted to 2100's predicted warmer temperatures were unable to lower their metabolism as much as the cooler crocodiles, burning through oxygen at a faster rate, forcing them to return to the surface sooner than their cooler cousins.

'This finding suggests predator avoidance dives may be shortened if water temperatures continue to increase in marine and freshwater habitats', warn Rodgers and Franklin, who are concerned that crocodile youngsters will become more vulnerable to predators as they are likely to have to surface more frequently if the temperature continues rising.

10.1242/jeb.170423

Rodgers, E. M. and Franklin, C. E. (2017). Physiological mechanisms constraining ectotherm fright-dive performance at elevated temperatures. *J. Exp. Biol.* **220**, 3556-3564.

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