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

MALE MUDSKIPPERS CARE FOR THEIR BROOD

It was the curious observation of a bubbling mudskipper burrow that led Atsushi Ishimatsu and his colleagues into some very muddy research. Analysing the gas that they had seen coming out of the burrow, Ishimatsu's team were surprised to find that there was a high concentration of oxygen inside the bubbles, despite the fact that the water in mudskipper burrows is notoriously low in oxygen. So how was the oxygen getting there? The answer most likely lay with male mudskippers, which look after fertilised eggs in their burrows, waiting for them to hatch.

As research sites go, the mudskippers' home on soft and watery mudflats is a challenge for intrepid scientists trying to learn more about them, especially when many mudflats across the world are endangered by human activity and disappearing. In the team's study site the mudskipper's breeding season also coincided with the rainy season, with torrential rain making an already muddy research site even muddier. After many years of fine-tuning, the team perfected a technique of recording the oxygen levels inside burrows out on the mudflats and measuring the movements of the male mudskippers, to find out how they care for their brood (p. 3946).

To make measurements from burrows over several days, the team made a small dome from the top of a plastic bottle, inserting an endoscope to observe the eggs inside the burrow's egg chamber and an oxygen electrode to measure oxygen levels. They also inserted electrodes under the egg chamber to measure the movements of the male mudskipper, and pressure sensors to measure the coming and going of the tides. They found that oxygen levels decreased during high tide when the burrows were submerged. However, during low tide, when the burrows were uncovered, oxygen levels increased, and the male mudskippers repeatedly entered their burrows. This suggested to the team that the males were adding gulps of air to the egg chamber, increasing the oxygen levels inside and ensuring that the embryos had enough oxygen to survive.

Adding hypoxic air to two burrows caused the males to add more gulps of air, changing the oxygen levels back to normal within one hour. Having shown that males maintained oxygen levels in the burrows, the team wanted to know whether they helped with hatching, too. Incubating eggs in air in the lab, they found that the eggs survived in air, but they died if they were left for too long in air. Submerging the eggs in water during a critical 5–6 day period during development triggered hatching in over 80% of the eggs.

Knowing that eggs must be incubated in air but submerged for hatching, the team examined their endoscope records to find that the males played a role in egg hatching too. When water covered the burrow during night-time high tides, they saw the males taking gulps of air from inside the egg chamber, and releasing them to the surface, flooding the chamber with water and inducing hatching. Night-time hatching reduces the risk of the youngsters being snatched by predators. Protected in their burrows from the environmental extremes on the surface of the mudflats, the male mudskippers care for their brood by keeping the oxygen levels up during incubation, and then allowing the burrow to flood when the eggs are ready to hatch.

10.1242/jeb.013417

Ishimatsu, A., Yoshida, Y., Itoki, N., Takeda, T., Lee, H. J. and Graham, J. B. (2007). Mudskippers brood their eggs in air but submerge them for hatching. *J. Exp. Biol.* **210**, 3946-3954.

MUSCLES AND LUNGS HELP MOVE LYMPH

With their moist, breathable skin, frogs and toads are particularly vulnerable to drying out. Their water balance isn't helped by a very leaky circulatory system, which causes them to produce up to five times more lymph for a given amount of tissue than mammals. All the lymph that oozes out is returned to the blood stream by the lymphatic system, where two pairs of dorsal lymph hearts contract rhythmically to squeeze lymph from the lymph sacs and back into the blood stream. Despite the fact that lymph gathering in the sacs builds up a pressure, this pressure is not enough to transport the lymph lying in sacs on the ventral side of the animal vertically up a few centimetres to the beating hearts. Suspecting that other mechanisms were helping the lymph on its way, Robert

Drewes, Michael Hedrick and their colleagues Stanley Hillman and Philip Withers investigated further (p. 3931).

Since many of the muscles in the pelvis and connected to the hind legs of frogs and toads run very close to lymph sacs, it's possible that a muscular 'pump' could squeeze the sacs to help move lymph. To find out if this was the case, the team operated on anaesthetised cane toads and bullfrogs to insert recording wires into the leg and pelvic muscles and pressure gauges into their lymph sacs. They recorded the muscle activity and lymph sac pressure as the animals rested in a container 12–24 h after surgery. They found that pressure changes in the lymph sacs always correlated with muscle activity, and that the pressures generated were enough to move lymph vertically to the lymph hearts. One muscle called the piriformis moved the urostyle, a thin bone made up of fused vertebrae at the base of the spine. As the urostyle bended and flexed, it acted like a pump handle, changing the volume and pressure of the pubic lymph sac and contributing to vertical lymph movement.

In a second group of cane toads, the team severed the tendons of some of the muscles that attached to the skin beneath the thighs and the pelvic region, finding that 10 days later around 20 times more lymph had collected in one of the sacs. This confirmed that the movements of these muscles are important for helping to move the lymph out of the sacs.

The team suspected, however, that muscle movements weren't the whole story (p. 3940). There are fewer muscles associated with the front legs in frogs and toads, so it was unlikely that muscle movements would help move lymph in the front half of the body. They already knew that lung movements and muscle movements were coordinated, so suspected that lung movements also helped move lymph to the lymph hearts. As before, they inserted pressure gauges into the lymph sacs of anaesthetised frogs and toads. They also inserted a tube into the lungs, so that they could artificially inflate and deflate the lungs, and then measure what happened to the pressure in the lymph sacs. They found that as the lungs expanded and shrank, the pressure in the lymph sacs surrounding the lungs changed too: as the lungs inflated, the pressure in the sacs increased, and when the lungs deflated, the pressure in the sacs went down.

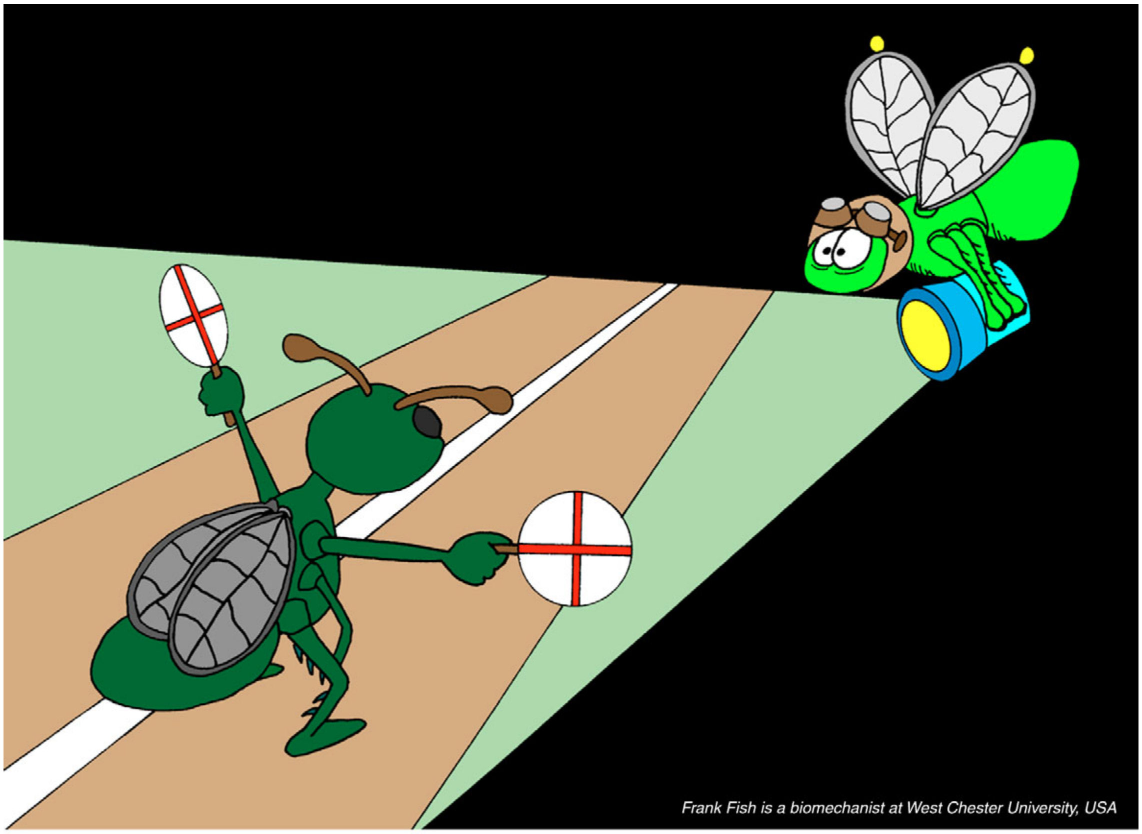
Having shown that filling the lungs with air influenced lymph sac pressure, the team were interested to know what happens in awake animals. The team carried out another operation to insert pressure gauges into a forelimb lymph sac and in a sac overlying the lungs. As the amphibians recovered in their tank, the team monitored pressure in the sacs. They found that when the animals breathed out, the pressure in the sac above the lungs went down, probably because it expanded in size to compensate for the decrease in lung size. At the same time, the pressure in the forelimb sac went down, and lymph was sucked vertically from this sac into the sac overlying the lungs. When the amphibians breathed in, filling their lungs with air, the team suspect that lymph in all the sacs surrounding the lungs was squeezed towards the lymph hearts, which then pumped the lymph back into the blood stream.

This shows that breathing has two important roles in frogs and toads. When oxygen levels in the blood drop and carbon dioxide levels rise, breathing rate will go up to restore the balance of gases in the blood. However the role of the lungs in moving lymph means that breathing rate will also change in response to lymph pooling, helping squeeze lymph to the lymph hearts and on into the blood, maintaining blood volume.

10.1242/jeb.013623

Drewes, R. C., Hedrick, M. S., Hillman, S. S. and Withers, P. C. (2007). Unique role of skeletal muscle contraction in vertical lymph movement in anurans. *J. Exp. Biol.* **210**, 3931-3939.
Hedrick, M. S., Drewes, R. C., Hillman, S. S. and Withers, P. C. (2007). Lung ventilation contributes to vertical lymph movement in anurans. *J. Exp. Biol.* **210**, 3940-3945.

LOW LIGHT, BAD FLIGHT



SINCE ADDING LANDING LIGHTS, THERE WAS A DRAMATIC DROP IN MISHAPS DURING FINAL APPROACH AT NIGHT

It takes a pair of highly sensitive eyes to see well at night, although unfortunately for the nocturnal sweat bee, evolution messed up. These bees don't have the same eye type as other nocturnal insects, such as moths. In moth eyes, light is gathered from many different lenses and focussed on one photopigment containing structure called the rhabdom, increasing light sensitivity. Sweat bees rely on the same eye type as their day-active relatives instead, where the light from individual lenses is channelled separately onto individual rhabdoms. In the inky blackness of the Panamanian jungle, sweat bees likely rely on neural mechanisms in the brain that sum the signals coming from the eyes in space and time, making the visual signal stronger. At the darkest light levels, however, the bees' flight is likely to be much less precise.

To find out how light levels affect flight ability, Jamie Theobald and his colleagues illuminated the area surrounding 8 bees nests with infrared light, and then recorded their 3D flight trajectories using two cameras as the bees tried to land after foraging trips (p. 4034). The team found that in lighter conditions, the bees flew shorter and quicker routes to the nest entrances, whereas in darker light conditions, return trips were a mixture of both quick and slow. They found that in the darkest conditions, the bees flew passes as they approached the nest; sometimes they were successful on the first pass and found their target, while other times they flew many passes to reach their nest entrance. The nearer the nest, the slower they flew. By living in these dark conditions, the bees probably enjoy reduced predation and competition from other bees, but the light they fly in is so dim that they are likely flying to the limit of their visual ability, meaning that they often miss their target completely.

10.1242/jeb.013573

Theobald, J. C., Coates, M. M., Wcislo, W. T. and Warrant, E. J. (2007). Flight performance in night-flying sweat bees suffers at low light levels. *J. Exp. Biol.* **210**, 4034-4042.

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