### **INSIDE JEB**

## Water-skiing beetles get a bumpy ride



Time-lapse image of *Galerucella nymphaeae* flying on the water surface. Photo credit: Haripriya Mukundarajan.

Blink and you've missed it: Manu Prakash from Stanford University, USA, describes how one moment a waterlily beetle (Galerucella nymphaeae) is sat on the surface of a pond and the next it has vanished. 'The phenomenon is so incredibly fast that you don't see anything', says Prakash, describing the ripples that remain on the surface, which are the only evidence that the insect was ever there. Having observed the beetles' remarkable disappearing act, Prakash knew he had to find out how they pull off the stunt. 'Initially, I filmed them without confining them in my kitchen...because it is hard to find them [when they get loose] in the lab', he chuckles, recalling that dinner plates of water provided ideally sized ponds when filming. And when he saw the first movie, he knew that he was on to something exceptional. The beetles looked as though they were water skiing, but travelling at incredible speeds of up to  $0.5 \text{ m s}^{-1}$  – equivalent to a human travelling at around 500 km h<sup>-1</sup> – propelled by their wings alone, as if they were flying while remaining attached to the surface. Prakash was hooked and knew he had to learn more about the mysterious beetles' interfacial flight.

'It was incredibly difficult to image these guys', says Prakash, who worked with summer interns Thibaut Bardon and Dong Hyun Kim, and graduate student Haripriya Mukundarajan, filming the beetles' antics with a high-speed camera. Mukundarajan describes the insects' movements, saying, 'They have an elaborate way of preparing for flight', before outlining how the insects initially raise the middle pair of legs – to prevent them from impeding the wings during flight – before drying each leg and gently dipping the claw at the end of the

limb back into the water ready for departure. Once balanced on the tips of all four legs, the beetles open the wing case on their backs and beat the wings a couple of times to unfurl them before switching into flight mode, where they flap the wings in a characteristic figure-of-eight pattern at around 115 Hz to thrust themselves forward. However, instead of gliding smoothly across the glassy surface, the insects looked as if they were careering along a roller coaster as they flew across the ripple ridges that they generated as they moved. 'Almost like going on a road full of potholes', says Mukundarajan, 'Although these potholes are being generated by the insect itself', laughs Prakash.

Puzzled by the beetles' unexpectedly bumpy ride, Mukundarajan and Prakash analysed the forces acting on them as they slide across the surface and realised that the insects were playing a finely tuned balancing act between surface tension clinging to their tarsus claws and the lift generated by their wings, with surface tension keeping them firmly anchored at the surface. And when Mukundarajan assembled a series of equations that described the insects' movements, they explained how the tell-tale ripples – the only visible indication of the insects' high-speed performance – were produced. According to Prakash each wingbeat generates a force that momentarily pushes the insect down, making it bounce along the surface of the water. An additional set of waves – known as capillary gravity waves - are also generated spontaneously as the insect reaches a specific speed. The duo adds that there are only a narrow range of situations where an insect can fly along the surface of a pond and remain attached without popping off into the air. Prakash is also optimistic that the new mathematical model could explain how other exotic species skate, including marine flies that are content bobbing about on the waves.

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Mukundarajan, H., Bardon, T. C., Kim, D. H. and Prakash, M. (2016). Surface tension dominates insect flight on fluid interfaces. *J. Exp. Biol.* **219**, 752-766.

Kathryn Knight

The Company of Biologists

# Fish hearts get O<sub>2</sub> boost from carbonic anhydrase



A salmon heart blood vessel collapsed into a heart shape. Photo credit: Sarah Alderman.

Fish plumbing is contrary. As the heart is the last organ that blood passes through before it returns to the gills, and with little direct blood supply to the ceaselessly contracting muscle, there are occasions when it could be on the verge of failure. 'We know this can happen under certain conditions like exhaustive exercise in combination with hypoxia or elevated water temperature', says Sarah Alderman from the University of Guelph, Canada. Added to the challenge of keeping the heart supplied with oxygen, Alderman explains that the haemoglobin that carries oxygen in fish blood is finely tuned to blood pH: the more acidic the red blood cells, the less able haemoglobin is to carry oxygen, which could prevent the red blood cells of exercising fish from picking up oxygen at the gills if they didn't have an effective pump to remove acid from the cells and restore the pH balance.

But Alderman and her colleagues, Till Harter, Tony Farrell and Colin Brauner from the University of British Columbia, Canada, also knew that fish can take advantage of a sudden drop in red blood cell pH to release oxygen rapidly at tissues - such as red muscle and the retina - when required urgently. An enzyme called carbonic anhydrase – which combines CO<sub>2</sub> and water to produce bicarbonate and acidic protons, and vice versa – lies at the heart of this mechanism. Normally there is no carbonic anhydrase in blood plasma; however, the enzyme has been found in salmon red muscle capillaries, where it facilitates the reaction of protons – that have been extruded from the red blood cell – with bicarbonate to produce CO<sub>2</sub>,

which then diffuses back into the red blood cell. The  $\mathrm{CO}_2$  is then converted back into bicarbonate and protons in the blood cell, causing the pH to plummet and release a burst of  $\mathrm{O}_2$  from the haemoglobin. Could salmon take advantage of this mechanism to boost oxygen supplies to the heart when the animals are working full out? Possibly, but only if carbonic anhydrase was accessible to blood passing through the heart.

Alderman and Jonathan Wilson began searching for the enzyme in salmon hearts. Using cobalt sulphide to stain sites where the enzyme accumulated, Alderman could clearly see the enzyme on the surface of the heart chambers. She also identified the gene that was expressed to produce the protein. Then, to be sure that carbonic anhydrase could contribute to dumping residual oxygen out of the blood in the heart, Alderman and Harter had to figure out a way of directly measuring pH inside the heart chambers to see whether the enzyme altered it. This type of experiment is usually performed using ground-up tissue in a test tube, but that wouldn't confirm that carbonic anhydrase in the heart chamber walls could alter blood pH, 'So we decided to modify the assay and use the heart itself as the reaction vessel', Alderman recalls.

Working closely together, the duo painstakingly developed a technique where they could measure the pH in the beating heart with pH probes that were thinner than a human hair. Eventually, the duo's persistence paid off and the pH in the heart plummeted as they fed CO<sub>2</sub> into the pulsating chambers. And when they added a carbonic anhydrase inhibitor (produced by Claudia Supuran) to the fluid, the pH fall slowed dramatically. Carbonic anhydrase was responsible for the drop in pH.

'The take-home message for our study is that the salmonid heart has an ace up its sleeve to help it overcome adverse conditions', says Alderman, who is now eager to find out whether the fish play this card during their marathon upstream migration to spawn.

10.1242/jeb.138974

Alderman, S. L., Harter, T. S., Wilson, J. M., Supuran, C. T., Farrell, A. P. and Brauner, C. J. (2016). Evidence for a plasma-accessible carbonic anhydrase in the lumen of salmon heart that may enhance oxygen delivery to the myocardium. *J. Exp. Biol.* **219**, 719-724.

Kathryn Knight

# Long legs drive solitarious locusts' powerful leaps



Male gregarious locust (*Schistocerca gregaria*) jumping. Photo credit: Malcolm Burrows.

Of all the biblical plagues, locusts are probably the most infamous. Stripping vegetation bare and devastating crops, an advancing swarm can decimate thousands of hectares in a single day. Explaining that locusts exist in one of two forms – the benign solitarious locust and the voracious gregarious locust -Stephen Rogers from Arizona State University, USA, says that field workers monitor how far solitarious locusts have progressed on their transformation into swarming gregarious locusts by measuring the length of the insects' hind legs: adult solitarious locusts have longer femurs than adult gregarious locusts. Recalling a discussion during a meeting when he was in Malcolm Burrows' lab in Cambridge, UK, Rogers says, 'We were mulling over things to do and we said, "We have these morphological differences that are used by the field workers, but we have no idea why they are there and what they mean for the biology of the animal". So, the team decided to get to grips with why solitarious locusts are leggier.

Rogers explains that solitarious locusts and gregarious locusts are genetically identical and the solitarious insects only transform into threatening gregarious locusts when thrust into the company of others, so they have to be reared in individual cages in the lab while the gregarious animals are raised communally. Rogers, Joanna Riley and Caroline Brighton then built an insect athletics stadium to measure the length of the insects' leaps and they were impressed

to see the leggy solitarious locusts outstrip their stumpier gregarious counterparts, recording impressive 1.1 m-long leaps, in comparison to the gregarious locusts' modest 0.85 m-long bounds. And when Riley and Brighton filmed the take-offs with a high-speed camera, they could see the solitarious locusts hurl themselves forward at speeds that were 23% faster (3.26 m s<sup>-1</sup>) than the gregarious locusts' 2.65 m s<sup>-1</sup> take-off – although the solitarious insects pay a higher price for their impressive leaps, consuming twice as much energy per jump as the gregarious locusts.

So, the solitarious locusts' longer legs propelled the insects faster and farther, in contradiction of what was already known about spring-loaded leaps. Burrows and Greg Sutton had previously shown that recoil speed due to elastic energy stored in the limb was the major factor that affected the speed of spring-assisted leapers, not leg length. So why were the solitarious locusts packing so much more of a punch than their shorter limbed cousins?

Rogers looked inside the locusts and all became clear. Measuring the dimensions of the leg, he realised that the solitarious locusts' hind limbs were longer to accommodate the bulging muscle that is required to wind up the elastic energy storage structures. And when Rogers scrutinised these springs – known as semi-lunar processes – on either side of the locust's knee joint, they were 25% less stiff than the gregarious locusts' and the muscle tendon was also less stiff, allowing the two structures to store more elastic energy. He suspects that solitarious locusts invest more in their powerful leaps to evade danger when a threat looms, while gregarious locusts prefer to plod along en masse. 'You could even argue if you are a super athlete in a locust swarm, and you stand out, it is a bad thing', laughs Rogers, who suggests that getting ahead of the crowd is no advantage for gregarious locusts.

10.1242/jeb.138982

Rogers, S. M., Riley, J., Brighton, C., Sutton, G. P., Cullen, D. A. and Burrows, M. (2016). Increased muscular volume and cuticular specialisations enhance jump velocity in solitarious compared with gregarious desert locusts, *Schistocerca gregaria*. *J. Exp. Biol.* 219, 635-648.

Kathryn Knight

# WOW! Nobody uses a grappling hook to turn like Paratarsotomus macropalpis, Dave... MACROPALDIS You can say that again Bob! MITE GRAND PRIX: LIVE WWW.neilsmithilustration.co.uk

### Record-breaking mite uses grappling hook tight turns

Looney Tunes would have you believe that the fastest animal in California is the aptly named Road Runner, closely pursued by its nemesis, Wile E. Coyote, but Jonathan Wright from Pomona College, USA, would like you to consider a much smaller creature. 'Mites attain some of the fastest relative speeds documented in the animal kingdom', say Wright and his colleagues, and when the team filmed their local mite, Paratarsotomus macropalpis, scuttling across hot concrete on a driveway close to the college campus, they were in for a treat. The tiny animals racked up top speeds of up to 0.26 m s<sup>-1</sup>. Scaling the speeds relative to the mite's diminutive body size, the team was truly astonished to see one

youngster hit a blistering 323 body lengths (BL)  $s^{-1}$ , leaving the previous world record holder – an Australian tiger beetle – in the dust at a trifling 171 BL  $s^{-1}$ .

Impressed by the mite's agility, the team went on to scrutinise the arachnid's footwork, recording the mites' stride frequency at a remarkable 135 Hz, which they say is the highest value reported for any weight-bearing muscle. And when they analysed the mites' turning performance, they discovered that the mites used two strategies: a high-speed turn at approximately 795 deg s<sup>-1</sup>, where they pivot around the third inner leg as the claw at the end of the limb attaches to the surface like a grappling hook; and a

slower turn at around 567 deg s $^{-1}$ , where they scuttle around a corner with the outer legs taking longer strides than the inner legs. Investigating the mites as they sprinted from a standing start, Wright and colleagues were also impressed to see the animals hit a respectable 0.15 m s $^{-1}$  in just 15–20 ms, pulling accelerations of 7.2 m s $^{-2}$  as they took off and decelerations of 10.1 m s $^{-2}$  when they came to a standstill.

10.1242/jeb.139006

Rubin, S., Young, M. H.-Y., Wright, J. C., Whitaker, D. L. and Ahn, A. N. (2016). Exceptional running and turning performance in a mite. *J. Exp. Biol.* **219**, 676-685.

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