INSIDE JEB

Softly, softly, mosquitoes outwit human victims

Photomontage of a blood-fed mosquito taking off. Photo credit: Florian Muijres.

Stealth and haste are often at the heart of most successful raids, and the forays of famished female mosquitoes are no different. 'Female mosquitoes need a blood meal to develop their eggs', explains Florian Muijres, from Wageningen University, The Netherlands, adding that zipping in and out after a quick sip without attracting the attention of the unfortunate victim is essential if the insect is to survive. 'One critical manoeuvre is the escape take-off after blood-feeding', says Muijres. The fully loaded insect should push off quickly from the surface of its victim to get away swiftly, but a softer and gentler take-off would be less likely to arouse the attention of a disgruntled host. Knowing that humans are particularly vulnerable to mosquito bites, which can transmit deadly disease, Muijres, Jeroen Spitzen (Wageningen University) and Sofia Chang (University of California, Berkeley, USA) scrutinised the voracious insect's escape technique to find out how it manages to evade scrutiny when fully laden.

'The experimental setup was rather complex', admits Muijres, describing how Chang had to ensure that the insects were well fed on human blood before take-off trials, in addition to coordinating three cameras trained on the insect's launch pad to capture every aspect of the departure in minute detail. 'To get everything to work properly required a lot of preparation, precise work and a steady hand', chuckles Muijres.

As most insects simply hurl themselves into the air with a hefty push of the legs, Chang, Muijres and Wouter van Veen were surprised to find that the mosquitoes began beating their wings about 30 ms before the final push-off, using an extraordinarily high wingbeat frequency of ∼600 beats s^{-1} compared with ∼200 beats s^{-1} used by other similarly sized insects. And when the team calculated how hard the mosquitoes pushed down on their victim over the course of a take-off, they realised that the insects took advantage of their exceptionally long legs, extending them gently while pushing down slowly over the 30 ms take-off, leaving the unsuspecting injured party none the wiser of the departure. In addition, when Chang and Muijres calculated the wings' contribution to the take-off, they were impressed that they contributed 60% of the force needed to lift a well-fed mosquito off its victim.

Next, the team compared the mosquitoes' take-off performance with that of more stocky fruit flies to find out whether the mosquitoes had compromised the speed of their evasive action for a light-footed getaway. Calculating the take-off force generated by a sturdy fruit fly, the team was surprised that the take-offs weighed in at almost four times the forces exerted by the nimble mosquitoes; sufficient to attract the attention of a human. However, when the team compared the two insects' take-off speeds, the mosquitoes were every bit as speedy (∼0.24 m s^{-1}) as their heavy-footed counterparts. Mosquitoes are not penalised by their softly-softly strategy, and are every bit as swift as less subtle insects. And when Bart Biemans compared the size of the mosquito and fruit fly leg muscles, the mosquito leg muscles were smaller, 'because they need to produce a lower force at push-off', says Muijres.

Having discovered the secret behind the mosquito's stealthy departure, Muijres and his colleagues are keen to find out whether other blood-sucking pests use the same strategy to outwit their victims and whether the irritating insects' landings are as graceful as their departures.

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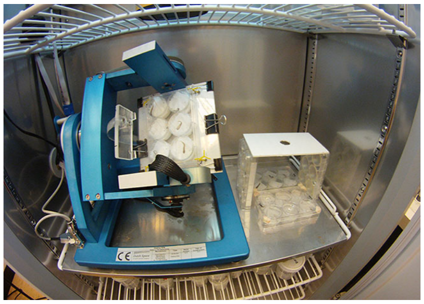
Muijres, F. T., Chang, S. W., van Veen, W. G., Spitzen, J., Biemans, B. T., Koehl, M. A. R. and Dudley, R. (2017). Escaping blood-fed malaria mosquitoes minimize tactile detection without compromising on take-off speed. *J. Exp. Biol.* **220**, 3751-3762.

Kathryn Knight

Fish feel gravity



Mangrove rivulus fish experiencing microgravity in a random positioning machine. Photo credit: Andy Turko.

There is a very good reason why the largest creatures reside in our oceans. 'The buoyant support of water can explain why whales are so much larger than elephants', says Andy Turko, from the University of Guelph, Canada. In contrast, the skeletons of terrestrial animals are moulded by the effects of gravity: they can detect and adjust to changes as they gain or lose weight. Which begs the question: can fish skeletons also detect and respond to gravity? 'Some researchers have assumed that they would never have needed to evolve the ability to sense or respond to gravity', says Turko; terrestrial animals – including the whale's ancestors – were believed to have evolved the sense only after they left their fish cousins in the water. However, Turko and his colleagues Patricia Wright and Doug Fudge wondered whether the ability to sense gravity is more ancient. Knowing that amphibious fish, such as the mangrove rivulus (*Kryptolebias marmoratus*), routinely experience the effects of gravity when they clamber onto land, Wright, Fudge and Turko wondered whether the tiny fish can stiffen their skeletons when their weight increases after leaving the water.

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Fixing on the 1 mm long gill arch as the most suitable bone in the fish's body to test, Turko and Fudge kept some of the fish on dry land for 1 and 2 weeks, while another group of fish was allowed to remain in water, before comparing the stiffness of their gill arches. Impressively, the gill arch bones of the fish that were out of water were 60% stiffer than those of the submerged fish after 1 week and the effects of gravity persisted for more than 2 weeks after the fish's return to water. However, the team wasn't entirely convinced that they could attribute the bone's increased stiffness to the effect of gravity alone: were they becoming stiffer as a result of drying? Turko and Wright realised that they would have to simulate weightless conditions while the fish were out of water before they could be convinced that gravity was the culprit.

Recalling that Roger Croll, Frank Smith and Matthew Stoyek, from Dalhousie University, used a random positioning machine to simulate the effects of low gravity on developing zebrafish embryos, Wright sent Turko to Dalhousie to test whether the fish's bones became stiffer when they were effectively weightless while out of water. After a week of gyrating the air-exposed fish in microgravity – to simulate weightlessness out of water – and measuring the stiffness of their gill arches, the bones were as flexible as those of the fish that were swimming free. So the bones had not become stiffer because they were drying out. Fish that climb out of water onto land are able to sense the effects of gravity and increase the stiffness of their bones to bear the extra weight.

But how were the high-and-dry fish modifying their bones to increase their stiffness? Extracting proteins from the bones of fish that had spent 2 weeks out of water, Dietmar Kültz, from the University of California, Davis, USA, was impressed to see an extraordinary (ninefold) increase in one protein, type X collagen, which is known to be involved in bone growth. 'To see such a dramatic increase was exciting evidence that gravity exposure causes bone growth', says Turko.

Having found that fish are able to sense the effects of gravity, Turko suspects that this ability may help fish to adapt their skeletons to withstand the stresses and strains of swimming. He also suggests that our ancient fish ancestors may have been able to stiffen their skeletons in response to weight gains when they first pulled themselves out of the water. 'This may mean that something considered a major challenge during the invasion of land by vertebrates (supporting their own body weight) may not actually have been that challenging of an evolutionary problem', says Turko.

10.1242/jeb.171223

Turko, A. J., Kültz, D., Fudge, D., Croll, R. P., Smith, F. M., Stoyek, M. R. and Wright, P. A. (2017). Skeletal stiffening in an amphibious fish out of water is a response to increased body weight. *J. Exp. Biol.* **220**, 3621-3631.

Kathryn Knight

Size matters, even for Jack-of-all-trades lizards

A steppe-runner lizard on a coarse sand runway. Photo credit: Philip Bergmann.

Many animals take the surfaces across which they move in their stride: some slow down if a surface is less to their liking, while others relish the same material and surge forward. 'Species sometimes use different approaches for dealing with changes in the surfaces that they are running on', says Philip Bergmann, from Clark University, USA, who was intrigued by how steppe-runner lizards cope when running on surfaces ranging from fine sand to gravel. However, he adds, 'No one had really taken a systematic approach, looking at substrates composed of different particle sizes… and no one had studied the effects of the shape of the particles on locomotion'. As steppe-runner lizards have no specialised adaptions that allow them to favour one surface over another and they encounter a wide range of surfaces in their natural homes, Bergmann and Kyle Pettinelli decided to test the lizards' sprinting prowess on surfaces ranging from 25 µm fine glass beads and sand to coarse gravel and 4 cm pebbles to find out how this running Jack of all trades adapts to different conditions.

Filming the lizards as they scampered along flat sand and gravel runways, Bergmann saw that some lizards were less enthusiastic athletes than others and required more encouragement to pull out their fastest performances. However, when he compared the lizards' highest speed sprints, they ran fastest on the coarse sand ($\sim$1.1 m s^{-1}), increasing their stride rate rather than taking longer strides, in contrast to the lizards that sprinted on silt and pebbles, which ran at speeds just below 1 m s^{-1}. The lizards also seemed to prefer running on natural surfaces rather than similarly sized smooth glass beads, and rarely recorded speeds of over 0.75 m s^{-1} when running on the glass beads.

Wondering why the lizards were more competent on the less uniform natural surfaces, Marian Crockett and Erika Schaper compared the density of the glass beads, sand and gravel, and the stability of the surfaces that they produce, and found that the coarse sand should shift least beneath the lizards' feet as they sprinted over it.

'Substrate particle size affects both how fast the lizards can run on them and how they move', says Bergmann, who admits that he was surprised that the lizards performed so much better on coarse sand when they were thought to run competently on most surfaces. However, he suspects that the stable and even surface that is provided by the coarse sand may allow the lizards to produce their top performances, 'unlike gravel, which makes for a bumpy and uneven surface'.

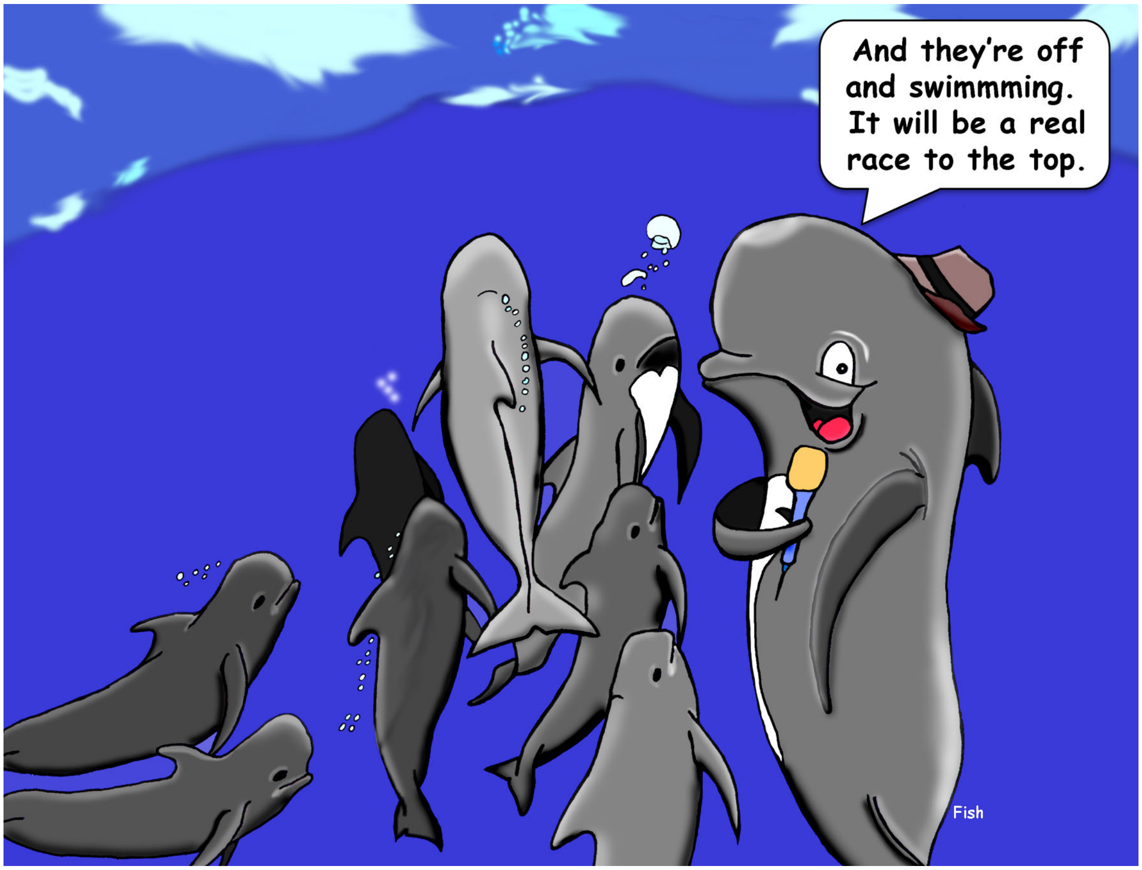
Having shown that particle size matters, even for an animal that does not seem to have any specialised adaptions for one particular surface over another, Bergmann is now keen to discover how sprinting lizards cope on more realistic terrains where sand, silt and gravel are mixed, and how they adapt when running up sand and gravel hills.

10.1242/jeb.171215

Bergmann, P. J., Pettinelli, K. J., Crockett, M. E. and Schaper, E. G. (2017). It's just sand between the toes: how particle size and shape variation affect running performance and kinematics in a generalist lizard. *J. Exp. Biol.* **220**, 3706-3716.

Kathryn Knight

Long-finned pilot whales opt for high-cost dives



Efficiency is a priority for any animal that dives for its dinner. ‘It is crucial [for divers] to adopt strategies that minimize their oxygen consumption and therefore maximize their foraging time at depth’, says Kagari Aoki, from the University of St Andrews, UK, and the University of Tokyo, Japan. However, Aoki and her colleagues had a hunch that pilot whales may buck this trend. While other toothed whales opt for a leisurely low-cost descent and ascent in order to conserve oxygen supplies for use while foraging at depth, short-finned pilot whales have been reported to prefer a fast-dash approach. Intrigued by the seemingly profligate strategy of the short-finned pilot whales, Aoki and Patrick Miller attached depth and motion tags to 18 long-finned pilot whales off the coast of Norway to learn more about the animals’ antics beneath the waves.

Recording over 150 h of data and analysing 140 dives exceeding 250 m, Aoki and colleagues found that, on average, the whales reached depths of 444 m in dives lasting approximately 9 min, with one whale descending 617 m and the longest dive lasting over 13.5 min. And when the team calculated the average speed of the whales’ ascent, Aoki and her colleagues were surprised that it was significantly higher (2.7 m s^{-1}) than the expected speed (1.2–1.5 m s^{-1} based on the animals’ estimated masses). Wondering whether the whales were taking advantage of a slick skin to accelerate their rapid ascent or were swimming particularly hard, the team calculated the whales’ drag coefficient and found that it was no different from that of other species: the whales must be swimming harder and consuming more energy during their ascent. In addition, the team noticed that the whales’ ascent slowed as they became increasingly buoyant near the end of their return to the surface, slightly reducing their most efficient swimming speed.

‘Our results indicate that long-finned pilot whales maintain high diving metabolic rates during deep foraging dives’, says Aoki, who suggests that animals might be using a ‘spend more, gain more’ hunting strategy, where they expend more energy swimming faster in order to consume more calories than other cetacean species.

10.1242/jeb.171231

Aoki, K., Sato, K., Isojunno, S., Narazaki, T. and Miller, P. J. O. (2017). High diving metabolic rate indicated by high-speed transit to depth in negatively buoyant long-finned pilot whales. *J. Exp. Biol.* **220**, 3802-3811.

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