

Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.



A FISHY TALE

When it comes to catching prey or escaping predators, top speed can be vital. The study of maximal speed swimming in fish, dolphins and whales is fraught with difficulties: there are no equivalents to racing greyhounds and horses, which regularly and predictably achieve near-maximal speeds. It is also often unclear whether free-swimming animals are cheating, using bow waves from the observation vessels. It is therefore brave for a study to approach the physical limits of swimming speed, and exciting when its conclusions are moderately robust and relatively straightforward.

Gil Iosilevskii and Danny Weihs re-visit fish and cetacean swimming, using a simple approach that models a tail's movement as a wagging wing. Using best-guess empirical observations, and the hydrodynamic theory of lift, drag and stall, Iosilevskii and Weihs explore the limits to swimming speeds for animals such as dolphins, tunas and mackerel sharks. They also consider cavitation, which presents a limit to speed rarely considered in the biological literature. When a fluid experiences a pressure below the vapour pressure, it cavitates, forming a pocket of gas within the water. This phenomenon is undesirable: if the pocket collapses, there is sufficient energy to cause damage; if the region of gas is maintained beyond the fin, performance crashes.

The authors consider three potential limiting factors to speed. The first is simply down to the power requirements, which change according to scale: the drag-producing surfaces scale approximately with the length of the swimmer squared, but the muscle volume – and hence the muscle power – scales with length cubed. Thus, larger fish are less speed-limited by power because of their greater muscle bulk. Precise predictions are more difficult

because of the uncertainty of the value of mass-specific power: the authors used values ranging from 10 to 160 W kg⁻¹, noting that this value will be species and conditions dependent.

The second and third potential limiting factors to swim speed are both based on stall. Stall for swimming fish occurs when the flow over the low-pressure surface separates and is associated with an increase in drag (bad) and decrease in thrust (very bad). Typical stall occurs when the fluid arrives at the aerofoil at too high an angle, causing a sudden reduction in thrust. For a given maximum tail beat frequency, there is a maximum body drag that can be overcome and a maximum swimming speed before the required angle of attack becomes too great and stall is inevitable. The precise speed limit relies on another difficult-to-measure physiological parameter: tail beat frequency. *If* a swimmer could increase this frequency *then* higher speeds should be achievable before this form of stall occurs.

Unlike the previous two limiting factors, the third, cavitation, cannot be avoided by higher powers or frequencies. Top swimming speed before cavitation occurs when the tail is wagging neither too quickly nor too slowly. By making reasonable assumptions about a fish's hydrodynamic properties, the authors conclude that cavitation provides a real speed limit to larger swimmers near to the water surface. Further below the surface, cavitation is less of a problem as it takes lower pressures to produce the vapour pockets. Smaller fish, meanwhile, are more likely to be speed-constrained by power.

While marine propeller design has been influenced by issues of cavitation for many years, it is exciting to think that fish and cetaceans may also have been developing under the selective pressures of not only power and efficiency but also cavitation avoidance. With this insight, further relationships between behaviour, fin design, physiology and hydrodynamics are ripe for discovery.

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Iosilevskii, G. and Weihs, D. (2007). Speed limits on swimming of fishes and cetaceans. *J. R. Soc. Interface* doi: 10.1098/rsif.2007.1073.

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FLY BEHAVIOUR



GHOST IN THE MACHINE?

Anyone who's ever worked on animal behaviour knows that animals, irrespective of whether they are vertebrates or invertebrates, do whatever they please, whenever they please, often completely ignoring the experiment that you've spent weeks painstakingly designing. Much of this spontaneous behaviour generated by animals is at best confusing because it doesn't seem to be linked to any external stimulus – under identical conditions animals often respond in entirely different ways. This variability is often attributed to 'noise' within the nervous system: for example, variation in the pattern of neural and muscle activity when carrying out a specific action. The implication is that in the absence of such noise, the animal's behaviour would lack this variability. A recent paper by Alexander Maye and his colleagues from Germany and the USA set out to determine whether noise in the nervous system really is sufficient to account entirely for the spontaneity of animal behaviour.

To assess this spontaneity they recorded the turning behaviour of fruit flies, *Drosophila melanogaster*, flying whilst attached to a torque monitor in a homogenous arena free of any external cues. When the flies attempted to turn either left or right they generated rapid changes, or spikes, in the yaw torque. By monitoring these spikes and the time between them, the team determined the spontaneous changes in direction the flies made. They compared the behaviour of the flies in the homogenous arena to flies in an arena with a single black stripe, upon which they could fixate, or in an arena with a uniformly textured surface, which allowed the flies to fly straight but not fixate.

Maye and his colleagues proposed several hypothetical mechanisms that could account for the observed behavioural

spontaneity. Each hypothetical mechanism makes specific predictions about the temporal patterns of yaw spikes that the flies produce. They began by testing whether noise is sufficient to produce the patterns of inter-spike intervals (ISIs) that they observed under all three conditions. If noise is sufficient to generate the spontaneous behaviour of the flies, then the ISIs should not differ from a random process. However, the ISIs generated by the flies under all three conditions were significantly different from those of a random process, leading the team to conclude that noise could not be sufficient to explain the flies' spontaneous behaviour.

Next, Maye and colleagues tested whether the flies' behaviour could be generated by a process in which each subsequent step is only dependent on their current state – a 'memoryless' process – which had been previously used as a model for spontaneous behaviour. This possibility was also eliminated by comparing the sequences of ISIs from the flies under the three different conditions with randomly shuffled sequences of ISIs. Another model that assessed the fluctuations within the ISI time series suggested that there were strong non-linear relationships contained within the flies' behaviour that could partially account for the behavioural patterns. In non-linear systems, the responses to one set of inputs can't be used to predict those to a different set of inputs. Finally, using computer-modelling techniques usually applied to weather forecasting, the team showed that non-linearity was not sufficient to fully capture the flies' behaviour: the non-linearity also needed to operate under specific conditions.

This study convincingly shows that non-linear processes and not random noise accounts for the spontaneity of the flies' behaviour. Yet, as the authors themselves point out, the source of this non-linearity within a fly's brain remains unclear. With such a clear behavioural paradigm, however, it should be possible to tease apart the components of the neural circuitry involved, and that's just what Maye and his colleagues suggest they will do next.

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Maye, A., Hsieh, C.-h., Sugihara, G. and Brembs, B. (2007). Order in spontaneous behavior. *PLoS ONE* 2, e443. doi:10.1371/journal.pone.0000443.

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MOSQUITO LEGS



SHE'S GOT LEGS...

As annoying as mosquitoes can be, most biologists harbour a healthy respect for them. Just think of the adaptations a mosquito must possess to steal blood from a large, powerful mammal or bird: carbon dioxide and infrared sensors for stalking a host, mouthparts for stealthily piercing skin and probing for a capillary, and injected anaesthetics and anticoagulants for avoiding detection and keeping the blood flowing.

It's not surprising then that most research on mosquito biology has focused on their blood-sucking and associated disease-spreading abilities. But new research from Chengwei Wu and colleagues suggests that mosquitoes possess adaptations on their legs and feet that are also worthy of our study. Flies and geckos have received a lot of attention lately because of their ability to walk on vertical or even overhanging surfaces. The mechanisms by which they do this is an active area of research and has already inspired the development of novel dry adhesives.

Mosquitoes don't tend to walk or run on vertical surfaces like flies and geckos, but they have no trouble landing on a smooth vertical surface like a wall, even after doubling their body weight with a blood meal. Wu and colleagues wondered if mosquitoes make use of the same tricks that flies and geckos use to stay stuck. By examining the fine structure of mosquito feet with a scanning electron microscope, the researchers found that the feet possess hundreds of hockey-stick-shaped setae that are indeed similar to the tiny bristles used by both geckos and flies.

Clinging to walls and ceilings is not all that mosquitoes can do with their legs. After a female mosquito has successfully acquired a blood meal and converted it into eggs, she sets off to find a pool of standing water where she can lay them. Laying eggs

in water is a tricky business for an insect – the eggs must be placed in the water without the animal getting stuck and drowning. Damselflies avoid this problem by standing on nearby vegetation and dipping only their ovipositors into the water. Mosquitoes, however, land directly on the water surface and remain afloat in a posture that is reminiscent of how water striders ‘walk’ on water – with only their legs touching the surface. When the researchers examined the fine structure of mosquito legs, they found that they possess tiny scales that are somewhat similar to the microsetae found on the legs of water striders. In water striders, these spindly projections result in a much higher effective hydrophobicity, or water repellency, than could be accomplished by simply coating them with a hydrophobic compound such as wax.

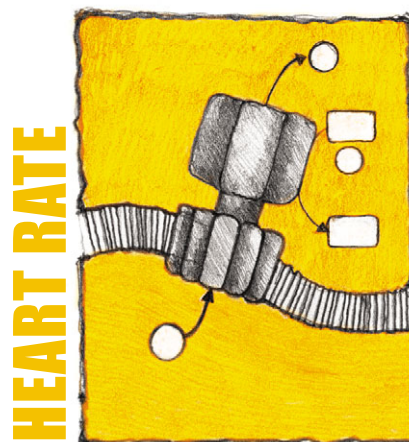
To test whether mosquito legs possess similar super-hydrophobicity, the researchers measured the contact angle of water droplets that they placed on the legs, which provides a quantitative measure of the affinity of a water droplet for a surface. On a hydrophobic surface, a water droplet beads up into an almost perfect sphere, which translates into a high contact angle between the droplet and the surface. Hydrophilic surfaces are better at overcoming surface tension and tend to spread a water droplet out, resulting in a lower contact angle. Wu and colleagues found the contact angle of droplets on mosquito legs to be about 153°, close to the value obtained for water strider legs. They also measured the force that a single mosquito leg could bear before it penetrated the water’s surface and found it to be not as high as for water striders’ legs but still 23 times larger than the animal’s body weight. The ability of mosquito legs to support these kinds of loads explains how female mosquitoes can so readily land on and take off from water.

Although water striders don’t stick to walls, and flies usually drown when they land on water, this study demonstrates that wall-clinging and water-walking are not mutually exclusive adaptations in insects.

10.1242/jeb.001123

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BEATING THE COLD

Ectothermic animals don’t thrive in the cold: low temperature slows down the heart rate, which tends to decrease its output and makes animals slow and sluggish. However, some fish maintain an active lifestyle in the cold. Upon prolonged exposure to low temperature, these fish recruit compensatory mechanisms that bypass the depressive effect of cold temperature on heart rate, enabling the heart to continue to beat at a high rate and precluding an active lifestyle.

The fish heart beats independent of external stimulation due to the tightly regulated firing of action potentials (APs) from a specialized set of heart muscle cells called the pacemaker. This regular firing results from coordinated ion movements in the muscle cells. The cold-induced, compensatory increase in heart rate could be due to alterations in humoral and neural regulation of cardiac pacemaker activity and/or modification of the ionic currents underlying the regular AP firing in the pacemaker.

Jaako Haverinen and Matti Vornanen of the University of Joensuu, Finland, were interested in determining if and how thermal compensation of the rainbow trout (*Oncorhynchus mykiss*) heart occurs at the level of the primary pacemaker cells. Because the exact location of the pacemaker cells in the fish heart is not known, the researchers first located the pacemaker region of the fish heart by systematically impaling spontaneously contracting fish hearts with sharp microelectrodes to record APs. These have a specific shape depending on which heart region they are recorded from. Using this technique, and examining the heart tissue, the team discovered that the primary pacemaker in the rainbow trout heart is located in a small ring of tissue between

the sinus venosus, the first chamber of a fish’s heart, and the atrium.

Armed with this knowledge, the team then enzymatically isolated individual pacemaker cells to find out if cold compensation occurred at the cellular level. Using patch-clamping, the team recorded APs from isolated pacemaker cells from both 4°C (cold)- and 18°C (warm)-acclimated fish at the common temperature of 11°C and found that the duration of pacemaker APs was shorter and the intrinsic pace-making rate higher in cold-compared to warm-acclimated trout, showing that at least part of the compensatory increase in heart rate is inherent to the pacemaker cells.

Finally, to understand which ionic mechanisms were responsible for the cold-induced changes in pacemaker AP shape and frequency, the team compared APs recorded from spontaneously contracting pacemaker tissue preparations from warm- and cold-acclimated fish. They added chemical blockers specific to two systems: sarcoplasmic reticulum calcium ion (Ca²⁺) cycling and the movement of potassium ions (K⁺) through channels called delayed rectifier K⁺ channels. Both of these ionic mechanisms are implicated as being important for cardiac pacemaking in mammals and are enhanced in cold-acclimated trout.

The team found that blocking sarcoplasmic reticulum Ca²⁺ cycling did not modify pacemaker AP shape or frequency in cold-acclimated tissue. This, they argue, eliminates the possibility that this mechanism underlies the compensatory increase of heart rate. By contrast, the team discovered that the movement of K⁺ increased in the cold and could be important for increasing heart rate. When they blocked the K⁺ channel at the common temperature of 11°C, pacemaker AP frequency and duration decreased more in warm-acclimated preparations than in cold-acclimated ones. The team explains that the greater flow of K⁺ in pacemaker cells of cold-acclimated trout should theoretically shorten AP duration, increase AP discharge frequency and thus increase heart rate.

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Haverinen, J. and Vornanen, M. (2007). Temperature acclimation modifies sinoatrial pacemaker mechanism of the rainbow trout heart. *Am. J. Physiol.* **292**, R1023-R1032.

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