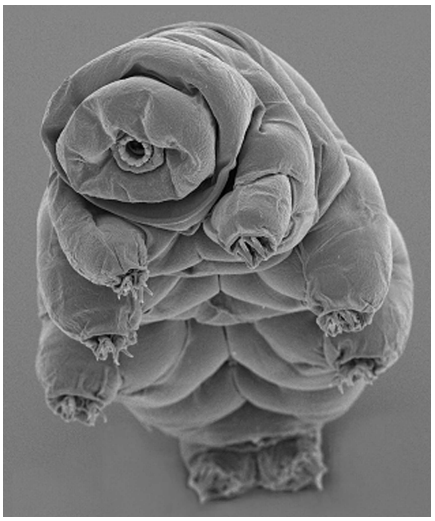
Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

CHEATING DEATH BY DEHYDRATION



Tardigrades are hardy little critters; there isn't a single niche they haven't made their own. And the secret of the microscopic creature's success? Cryptobiosis: when it's too dry to survive, the tiny animals curl up and dry out too. But instead of perishing under the stressful conditions, tardigrades fall into cryptobiosis, a state of suspended animation, ready to emerge as soon as water returns. Intrigued by the cellular mechanisms that protect the tiny creatures, Ralph Schill, Günther Steinbrück and Heinz Köhler began analysing the tardigrade's stress response by looking to see whether stress resistance proteins protect tardigrade's from death by dehydration (p. 1605).

Schill explains that there are probably over 600 species of tardigrade across the planet. But while some species survive the most extreme climates on earth, *Milnesium tardigradum* failed to thrive in the lab; at least until Schill discovered their taste for Volvic™ water. Content to live on agar made from the French mineral water, Schill finally had access to one of the few captive tardigrade colonies in the world, ready to analyse their response to dehydration stress.

Schill soon faced other technical problems. For a start, the 0.8 mm long creatures were too small to produce enough protein for detection with antibodies. Schill realised that he would have to use alternative methods relying on nucleic acids to identify whether tardigrades use heat shock proteins to protect them from stress. But tardigrade cells have the lowest nucleic acid levels of any cell known, making extraction tricky. Undeterred, he set about extracting DNA from 100 tardigrades, to find whether they carried the gene for Hsp 70, a well-known heat shock protein that protects proteins damaged by physiological stress from aggregating and causing further cellular damage.

Sure enough, the tiny creatures carried *Hsp 70* genes; in fact, they carried three. But would the animals use the genes to defend themselves from hot and stressful conditions?

Warming individual tardigrades up to 37°C, Schill tested all three isoforms' expression patterns. Tracking the mRNA levels of each gene with real time PCR, Schill realised that all three *Hsp 70* genes were activated to produce mRNA and proteins to protect the tardigrades from the sudden warm spell. But would the genes respond to other stresses too? Would desiccated tardigrades also show high levels of the Hsp 70 isoforms?

Drying out the tardigrades' Petri dish homes, Schill monitored the animals as they descended into cryptobiosis and later while they rehydrated. Extracting miniscule amounts of RNA from the animals, Schill used real time PCR to measure each isoform's mRNA level. While the first and third isoform levels fell during cryoptobiosis, the second isoform's levels more then doubled as the tiny creatures curled up and dried out. The second isoform was clearly involved in some way to protect the desiccated animals.

Delighted that he's beginning to understand the molecular basis of these robust little animals resilience, Schill knows there's still much left to learn. But he admits that he still finds cryptobiosis fascinating; 'it's amazing that animals can dry out and still be alive afterwards' he says.

10.1242/jeb.00983

Schill, R. O., Steinbrück, G. H. B. and Köhler, H.-R. (2004). Stress gene (*hsp70*) sequences and quantitative expression in *Milnesium tardigradum* (Tardigrada) during active and cryptobiotic stages. *J. Exp. Biol.* **207**, 1605-1611.

PUMPING UP A WHISPER

Humans are a superficial bunch. For us, beauty tends to be skin deep. But for many creatures, it goes much deeper. Weakfish males prepare for courtship by building up a pair of swim bladder-vibrating muscles, which contract simultaneously to serenade the ladies with a booming low croak. But weakfish aren't the only fish blessed with

an unusual swim bladder-voice; toadfish and searobins also call when the need arises. Fascinated by these fish and their extraordinary sonic muscles, Connaughton was puzzled when he read about the searobin. According to recordings made by Andy Bass from the neural centre that governs the fish's call, searobins appeared to alternate the muscles' contractions, rather than vibrating them simultaneously. Puzzled by the searobin's alternative sonic approach, Connaughton began recording the fish's call and sonic muscle activity, to find out how the fish project their voice (p. 1643).

Unfortunately for Connaughton, searobins don't reside in the waters around the Mount Desert Island Biological Lab in Maine. They had to be caught in the warmer waters off Woods Hole and transported north before Connaughton could begin recording croaks and electromyographs (EMGs) from the fish's contracting sonic muscles, to see whether the muscles worked together or alternately.

At first Connaughton made recordings from the sonic muscle on the fish's right side. For every two vibrations produced by the oscillating swim bladder, the muscle only twitched once. And when he recorded from the left side's muscle, he found the same 2:1 pattern again. But when he recorded EMGs from both muscles simultaneously, each swim bladder oscillation corresponded to a contraction from one, or other, of the muscles. The muscles were contracting alternately, at a relatively low frequency of 100 Hz to produce the fish's 200 Hz call.

But had the fish traded off their voice's volume in favour of their low frequency solution to a high frequency problem? Surely the amplitude of searobin vibrations produced by single muscular contractions would be half the amplitude if both muscle's contracted simultaneously. Connaughton recorded the amplitude of the acoustic waveforms generated by each searobin muscular contraction to see whether the fish had softer voices.

As Connaughton suspected, the amplitude of the vibrations was less than if both muscles contracted simultaneously; but it wasn't cut by half. He realised that each individual vibration was being amplified somehow, so decided to take a closer look at the fish's acoustic waveforms.

Watching the vibration's traces, Connaughton noticed that both the contraction and relaxation phases of the sonic muscle's twitch generated a vibration in the swim bladder. During the first few cycles of a croak, the fish's voice was weak, until the relaxation vibration began interfering constructively with the contraction vibration from the same muscle. Suddenly the fish's voice gained strength as the vibrations produced by that muscle interfered constructively to boost the amplitude produced by a single muscle. So these resourceful fish have come up with a constructive solution to pump up a weak whisper's volume.

10.1242/jeb.00982

Connaughton, M. A. (2004). Sound generation in the searobin (*Prionotus carolinus*), a fish with alternate sonic muscle contraction. *J. Exp. Biol.* **207**, 1643-1654.

FLAPPING FORCES

Humans have always been fascinated by flight. As Ty Hedrick says 'over the last century we've become pretty good at flight on a large scale', so now we defy gravity with propellers and jets. But understanding the forces that keep our feathered friends aloft; that's a different matter. Hedrick explains that until recently, only one study had measured the forces acting on a bird in flight directly,when a team in Germany fitted accelerometers to a pigeon. But that was 20 years ago, and the technology only allowed them to analyse one wing beat. Since then, no one had succeeded in directly measuring the net forces acting on a flying bird, until Hedrick and his colleagues, Jim Usherwood and Andy Biewener, fitted accelerometers to cockatiels. By convincing the birds to fly steadily in a wind tunnel while the team videoed the bird's flight, Hedrick and his colleagues have collected the first instantaneous measurements of the net forces that keep birds aloft (p. 1689).

For Hedrick, the birds were the least of his problems; cockatiels are enthusiastic fliers. The difficulties were in synchronising the video and accelerometer data. Hedrick explains that he needed to know the position of the bird's body at each instant of the wing beat, so that he could correct the accelerations measured on the body as the bird twisted, tilted and moved, ultimately measuring the true aerodynamic forces. Hedrick remembers that the first acclerometer trace 'was full of crazy peaks', and nothing like the smooth traces he was expecting. But after laboriously correcting the acceleration data according to the bird's orientation and movements, all the peaks vanished leaving Hedrick with a realistic record of the accelerations and forces the bird experienced. 'Now we knew we could do this, and it was going to work' says Hedrick.

After putting the birds through their paces at speeds ranging from 1 m s^{-1} to 13 m s^{-1}, Hedrick noticed that the cockatiel's flight pattern was smoothest around their cruising speed of 7 m s^{-1}. At low and high speeds, the lift produced by each upstroke was a fraction of the downstroke's force, so the birds were bounced along by each wing beat. But at intermediate speeds, the down and upstrokes were better matched, giving the birds a smoother ride.

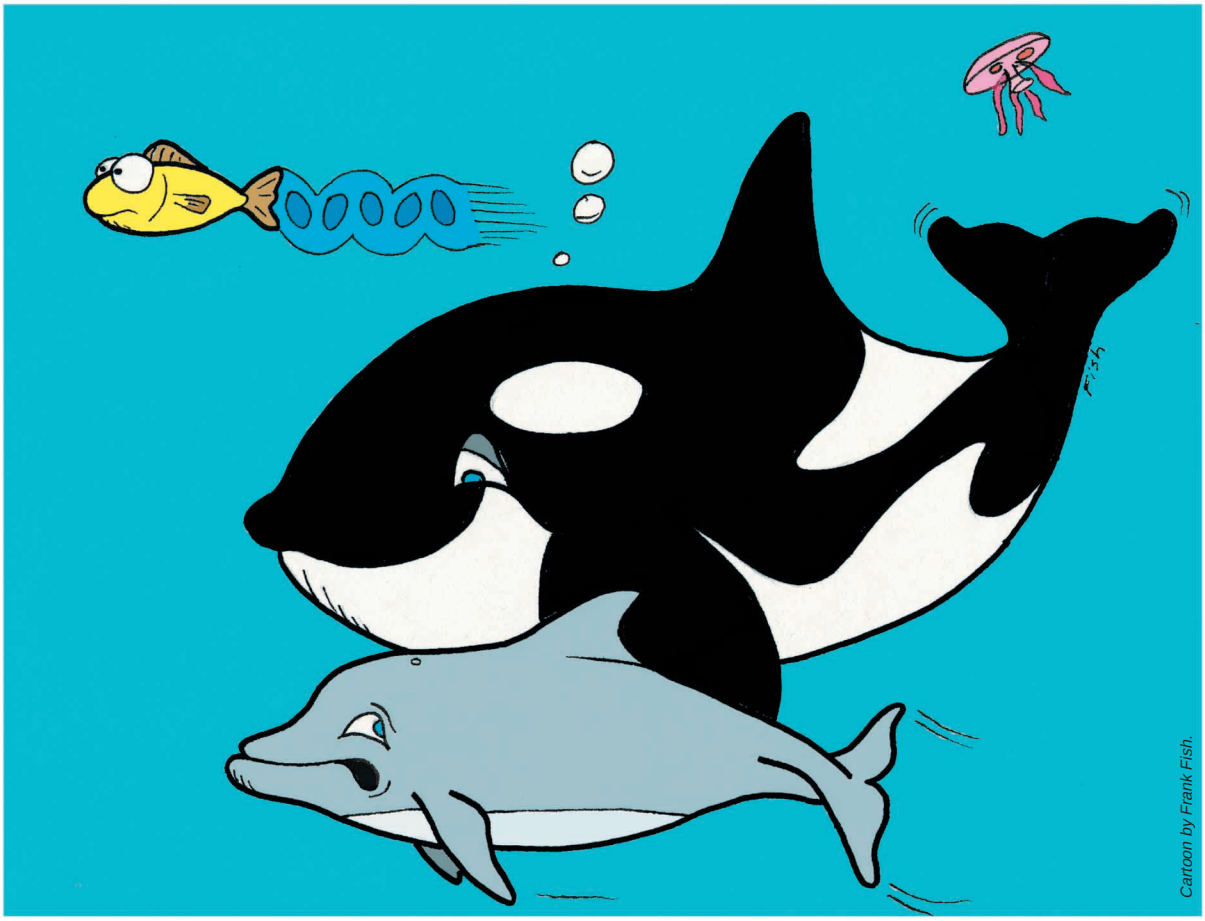
Hedrick adds that although it was widely accepted that the downstroke generates a significant amount of lift during a wing beat cycle, there was some debate about the upstroke's contribution to aerodynamic forces. Was the upstroke generating lift, or simply a way of returning the wing back to its starting point? The Harvard team's results suggest that the upstroke's main contribution to flight is simply to return the wing to the top of the cycle, while minimising the effects of drag.

Knowing that the upstroke appears to contribute little to the aerodynamic forces of bird flight, the team wondered whether the upstroke comes with an energetic penalty; after all it takes energy to lift the wing's weight. The accelerometer data showed Hedrick and his colleagues that although the birds used all of the kinetic energy of the downstroke producing aerodynamic force, the same was not true for the upstroke, the energy was not recovered from the wing for later use. In fact the upstroke cost the cockatiels a significant 14% of their energy in flight.

10.1242/jeb.00980

Hedrick. T. L., Usherwood, J. R. and Biewener, A. A. (2004) Wing inertia and whole-body acceleration: an analysis of instantaneous aerodynamic force production in cockatiels (*Nymphicus hollandicus*) flying across a range of speeds. *J. Exp. Biol.* **207**, 1689-1702.

CETACEAN WAKE



BY SHEDDING VORTICITY FROM MY FLUKES AT THE RIGHT STROUHAL NUMBERS, I CAN INCREASE EFFICIENCY AND SURVIVABILITY. WELL, BETTER A WAKE THAN A FUNERAL.

When Jim Rohr and Frank Fish decided to get to grips with cetacean swimming, heading out into the ocean wasn't a viable option. But heading down to the aquarium was. Most major aquaria house a few of the large mammals, trained to perform and entertain, and the team knew these animals would be ideally prepared for participating in their swimming tests to record the first comprehensive collection of swimming efficiency parameters for a large number of whales and dolphins (p. 1633).

Rohr explains that a cetacean's efficiency can be related to its tail beat amplitude, frequency, and swimming speed. These parameters can be combined to calculate an index known as the Strouhal number, with efficient swimmers recording Strouhal numbers between 0.2 and 0.4. Cetaceans were thought to fall well within this range, with Strouhal numbers between 0.25–0.35, but would this hold up to the test when Rohr and Fish put seven cetacean species through their paces?

The team began videoing animals ranging from bottlenose and spotted dolphins, up to killer whales and pilot whales, swimming over a range of speeds in their aquaria homes. After capturing over 260 swimming sequences, Rohr and Fish calculated Strouhal numbers for each sequence and plotted their distributions. But instead of falling within the predicted range of values, many of the mammals' Strouhal numbers fell below the predicted range, with 74% recording Strouhal numbers from 0.2–0.3 when swimming most efficiently. Also, the distribution of the animal's Strouhal numbers suggested that vorticity control by their tails is important for the animals' swimming performance.

10.1242/jeb.00981

Rohr, J. J. and Fish, F. E. (2004). Strouhal numbers and optimization of swimming by odontocete cetaceans. *J. Exp. Biol.* **207**, 1633-1642.

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