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

SEAWEEDS CRACK UP

Picture by Luke Miller

Not many organisms would choose to live on a wave-swept shore, yet many species of seaweeds cling to the rocks, battered by thousands of waves a day. A particular section of the shore, the marine intertidal zone, is especially hostile since as the tide goes in and out, it is exposed to the air and submerged underwater every day. Here the waves are strongest, and water velocities up to 35 m s^{-1} subject seaweeds to 'truly stupendous forces,' says Katharine Mach of the Hopkins Marine Station at Stanford University. Wondering how organisms survive in this environment, Mach and her colleagues wanted to find out if repeated poundings by the waves would eventually lead to seaweeds breaking due to material fatigue.

To find out what forces a seaweed can take, scientists previously used standard 'pull to break tests'; which involve fastening the seaweed in a clamp and measuring how hard they had to pull to break it. Comparing these forces with the forces exerted by waves in the field led them to predict that seaweeds shouldn't be breaking. But seaweeds encounter many waves in one day, not just one big one, and it's also clear from the piles of seaweed washed up on the shore after a big storm that they break frequently. To try and close the gap between predictions and what is actually happening, Mach took a new approach and turned to engineering theory, specifically fracture mechanics (p. 2213). 'It describes how materials cope in the presence of cracks and under fatigue, also how cracks form and then propagate before the material finally breaks,' she explains, '[we're] using engineering theory and applying it to understanding how all the forces that a seaweed experiences in one day will affect its breakage'. First the team tackled the engineering literature, bringing together theories developed for a variety of materials, especially rubbers, and putting them into a biological context. 'We wanted to standardise terms and make it something that [biologists] can use,' says Mach.

Having worked out which fracture mechanics theories were best suited to squashy biological tissues, the team chose four seaweeds to study: two species of *Mazzaella*, and one each of *Porphyra* and *Ulva* (p. 2231). 'They were a good place to start because they are relatively flat and simple, and can be considered as 2D structures, which makes things simpler from the modelling point of view', Mach explains.

The team cut sections of seaweed and placed them in grips each connected to an arm in a metal frame. Moving the top arm pulled the seaweed: the sample's change in length relative to its starting length told them how much strain it was under. The fixed bottom arm measured the forces acting on the seaweed.

Using the standard 'pull to break tests', the team tugged on the seaweeds with large continuous forces until they broke. They did this with complete samples and samples with small cracks, to measure the maximum stresses and strains the seaweeds were under, and then used their model to calculate the rate at which released strain energy contributed to making the cracks bigger. Mach explains that how cracks move through a material depends on the properties of the material; for example if it is elastic, or if it is stiff. As the crack grows, a material also releases energy as the area around the crack relaxes slightly, and this released energy goes into making the crack bigger.

Having measured the maximum stresses and strains the seaweeds could take, they next looked at how the seaweeds responded to smaller forces applied thousands of times over. Using a telemicroscope, they periodically scrutinised the progress of cracks moving through the seaweed pieces.

The team found that the seaweeds behaved differently. *Mazzaella* was the most robust of the three, tolerating cracks that tended to grow slowly in the material. It lives in the most exposed environment, so not only is it more likely to get damaged but also has to be better able to withstand damage once it has occurred. '*Ulva* was the hardest to study,' says Mach. Either it didn't crack at all or the cracks shot through the material, suggesting that once damaged, *Ulva* doesn't last long if struck by a large wave.

By knowing how strain energy is released under the different conditions, the team then predicted how long seaweeds would last in the field when subjected to wave forces. Using wave speeds and water forces measured from *Mazzaella*'s environment

they calculated that wave speeds over 8 m s^{-1} would lead to accumulated damage. Depending on how many waves a day reach that speed, many seaweeds would only last hours or days if they were damaged and subjected to a relentless pummelling. Next, it is back to the shoreline, taking measurements of wave forces to better predict how long a seaweed would last in the field.

10.1242/jeb.008433

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Mach, K. J., Hale, B. B., Denny, M. W. and Nelson, D. V. (2007). Death by small forces: a fracture and fatigue analysis of wave-swept macroalgae. *J. Exp. Biol.* **210**, 2231-2243.

KILLIFISH: CHAMPION ANOXIA SURVIVORS

The annual killifish *Austrofundulus limnaeus* lives life on the edge. The Venezuelan ponds where it lives rapidly evaporate in the dry season, killing off the adults and exposing their mud-buried embryos to extended periods of drought. Not that drought is the first of the embryo's problems: microbes living in the soil use up all the oxygen. 'Not only are they coping with life without water, but also with life without oxygen,' says Jason Podrabsky from Portland State University, who has investigated with his colleagues the root of the embryos' extreme anoxia tolerance (p. 2253).

The key to the embryos' survival is that they go into periods of dormancy, or diapause, twice during their development, even if conditions are ideal. 'Their environment is very harsh and unpredictable, so diapause occurs as a part of their natural developmental program,' says Podrabsky. Unlike most annual killifish, *A. limnaeus* embryos skip diapause I at four days of development, but enter diapause II at around 24 days old and diapause III near the end of development. During diapause the metabolism slows down greatly, so could this be the key to surviving anoxia?

To assess anoxia tolerance during development, the team collected fertilised embryos from 4 to 32 days old and put them in embryo medium at 25°C before sealing the jars and bubbling nitrogen through them to remove all the oxygen. They then kept a close eye on the developing embryos, opening the jars when they started dying and counting how many of them were still alive. They found that the later embryos were exposed to anoxia, the longer they survived: 4-day-old embryos suffered 50% mortality after 20 days of anoxia, while 32-day-old embryos, in diapause II, suffered 50% mortality after more than 60 days anoxia. This is the longest anoxia survival of any vertebrate at 25°C.

The team knew from the brine shrimp, another anoxia tolerant animal that enters diapause, that there is a window of opportunity after diapause where the animal can shut down again even if it has restarted its development. To find out if this window existed in the killifish embryos, they collected embryos just out of diapause II and tested their anoxia tolerance. They found that embryos exposed to anoxia 4 days after the end of diapause were still very tolerant, but lost this tolerance when they were tested 8 days

after, showing that post-diapause safety cushion in the killifish is about 4 days.

But what about changes in metabolism? The team took embryos of different developmental stages and flash froze them to preserve their metabolites. After mashing them up and extracting their metabolites, which they analysed using chromatography and mass spectrometry, they found that anoxic embryos produced lactate, succinate and the amino acid alanine, which are end-products of anaerobic metabolism. This suggests that the embryos survive by relying on glycogen stores and reducing their metabolic rate, but that they haven't developed an alternative strategy for dealing with anoxia. Embryos that accumulated lactate at the slowest rate also survived the longest.

They also found that anoxic embryos produced lots of the neurotransmitter GABA, which is known to protect neurons in other anoxia tolerant species, such as turtles and the crucian carp. Podrabsky explains that it's possible that GABA is protecting the traditionally anoxia-sensitive brain and heart tissue in diapause II embryos. For now, the killifish embryo can elbow its way onto the roll-call of champion vertebrate anoxia survivors.

10.1242/jeb.008441

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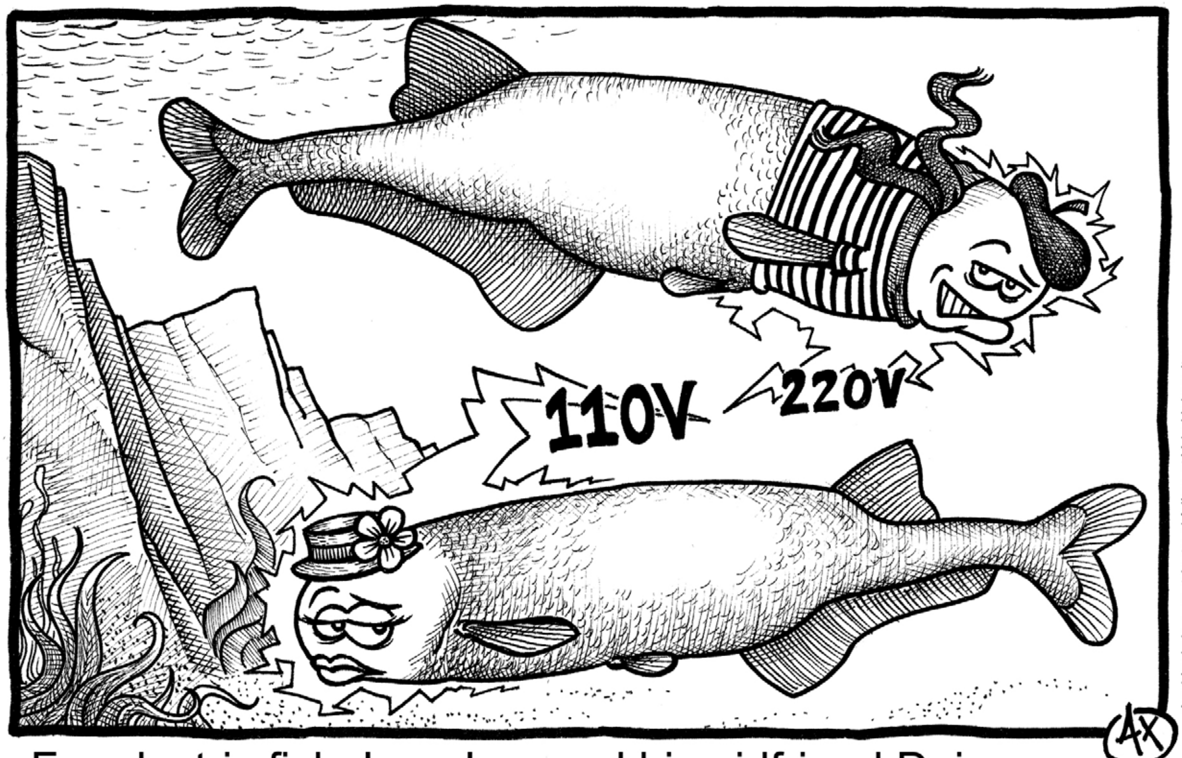
AN ELECTRIFYING COURTSHIP



Axel Innis is a postdoctoral fellow at Yale University

For electric fish Jean-Luc and his girlfriend Daisy, cultural differences often proved difficult to overcome.

In the murky waters of their African home, mormyrid electric fish (*Brienomyrus brachyistius*) use pulses from their electric organ to sense their surroundings, find prey and communicate with each other. The waveform of a fish's pulse is like an electronic 'fingerprint', telling others their species, sex and social status. On the other hand the sequence and pattern of pulses tells other fish a great deal about an animal's behaviour and its motivation, for example if it is aggressively defending its territory or courting another fish.

Researchers hit a problem when they wanted to find out more about the fishes' courtship signals, because electric fish are fiendishly difficult to breed in captivity. However Ryan Wong and Carl Hopkins tempted four pairs of fish to court each other by carefully creating ideal breeding conditions in the lab (p. 2244). Using a combination of video recording and computer software that distinguished between the electric organ pulses from individual fish, the team classified nine behaviours and 11 pulse sequences that occurred during both normal interactions and courtship. For example during courtship, males typically lunge at females, follow them around the tank and circle head to tail with them before spawning. Certain pulse sequences were more common during courtship, where male fish serenade females with lower frequency 'creaks' and higher frequency 'rasps'. The team also found that males and females took part in duets, alternating sequences of rasps and staccato 'bursts'. The next step will be to decode the fishes' transmissions and unravel their meaning.

10.1242/jeb.008458

Wong, R. Y. and Hopkins, C. D. (2007). Electrical and behavioral courtship displays in the mormyrid fish *Brienomyrus brachyistius*. *J. Exp. Biol.* **210**, 2244-2252.

Laura Blackburn
laura@biologists.com