Inside JEB 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

BARNACLE GLUE CURES LIKE BLOOD CLOTS

Barnacles are a big problem for boats. Adhering to the undersides of vessels, carpets of the crustaceans can increase fuel consumption by as much as 25%. Ship owners would love to know how to stop these hitchhikers gluing on, but before you can learn how to disrupt an adhesive, you have to understand the curing process. Curious about many aspects of the crustacean's lifestyle, Dan Rittschof from Duke University decided to find out how barnacle adhesive polymerises. 'The process must be related to something because glue isn't *de novo*,' says Rittschof, so he wondered what else coagulates under water and came up with two answers: blood and semen. With a colossal body of blood clotting literature to draw on, Rittschof decided to follow his evolutionarily inspired theory to see whether barnacle glue polymerisation is really an extreme example of scab formation (p. 3499).

Rittschof teamed up with Gary Dickinson and the first thing that Dickinson had to do was work out how to collect the unpolymerised glue and keep it fluid. Building on 30 years of Rittschof's experience and Beatriz Orihuela's expertise at growing and reattaching barnacles, Dickinson learned to gently lift polymerised glue away from the pores that secrete the adhesive and quickly collect the minute drops as they oozed from the shell. Working in the cold room to slow the polymerisation process, Dickinson had only 5 min before each sample polymerised and the glue set solid.

Next the team had to convince themselves that the viscous secretion was glue and not some other body fluid. Dickinson found that the fluid polymerised rapidly and was packed full of protein, just like barnacle glue. Next Dickinson teamed up with Kathy Wahl to use atomic force microscopy to compare the molecular structures of naturally cured glue (from stuck-down barnacles) and his polymerised samples. The two samples were virtually indistinguishable and Dickinson could clearly see tangled webs of fibres in his glue drops, similar to the tangled fibres in blood clots.

But this evidence was still far from proving that barnacle glue cures by the same process as blood clots. Dickinson and Rittschof needed to identify the key proteins that polymerise the cement. Knowing that blood clots are formed when enzymes, known as trypsin-like serine proteases, trigger a cascade of events that culminates in the formation of the long fibres found in blood clots, Dickinson and Rittschoff began searching for the protease in the unpolymerised glue. Separating the glue's components on a gel, Dickinson could see the tell-tale pattern of bands that suggested that a trypsin-like serine protease was present. And when Dickinson added an inhibitor (to inactivate the protease) to a fresh sample of glue, the sample didn't set.

Having convinced themselves that the glue contained a trypsin-like serine protease, the team began to search for other blood-clot-like proteins in the barnacle's secretions. Teaming up with Joseph Bonaventura and Irving Vega, Dickinson chopped each glue component into minute fragments, measured their sizes with mass spectrometry and matched the fragment pattern to known protein sequences. Amazingly, one of the glue proteins was remarkably similar to human factor XIII: a human blood clotting factor that cross-links clot fibres to form a scab. In fact, some regions of the human and barnacle proteins were completely identical. Dickinson and Rittschof had stumbled across the crucial protein that cross-links the glue fibres to cure barnacle cement and it was very similar to factor XIII, an essential human blood-clotting factor.

Rittschof admits that he is shocked that he has been unable to disprove his hypothesis. 'It seems likely that barnacle glue polymerisation is a specialised form of wound healing,' he says and suspects that many other marine animals that rely on glue to get a grip may use the same polymerisation mechanism.

10.1242/jeb.038927

Dickinson, G. H., Vega, I. E., Wahl, K. J., Orihuela, B., Beyley, V., Rodriguez, E. N., Everett, R. K., Bonaventura, J. and Rittschof, D. (2009). Barnacle cement: a polymerization model based on evolutionary concepts. *J. Exp. Biol.* **212**, 3499-3510.

RIB CRUNCH THEORY COULD EXPLAIN CICHLID BUZZ

No one is quite sure how many different species of cichlid there are, but the great lakes in the African Rift Valley have some of the highest numbers of cichlid species in the world. How these fish have evolved

into so many distinct species in the same waters has intrigued scientists for decades. Eric Parmentier from the University of Liège, Belgium, explains that two major forces had been thought to drive this dramatic example of evolution: modification of the jaw in response to different feeding environments and diets; and sexual selection where females choose males with specific colourings and patterns. However, a third evolutionary force could be at work: sound production. Parmentier explains that cichlids often make sounds during courtship and in combat, and that individual cichlid species could have evolved different sound production mechanisms to distinguish themselves from other species. But to discover whether or not sound production is a true evolutionary force, Parmentier and his colleagues had to find how cichlids make sounds (p. 3395).

Analysing the acoustics of the sounds produced by the Nile tilapia (*Oreochromis niloticus*), Parmentier and his student Nicolas Longrie quickly ruled out the possibility that the sounds were being generated by the fish grinding their teeth or as a result of the fish's swimming action. And when the team stimulated the fish to make the sound, they were able to change the sound's intensity by slightly deflating the fish's swim bladder. They realised that the swim bladder was involved in producing the sounds, but it wasn't clear how. Parmentier decided to take a closer look at the way the fish move to see if they could find out more.

Teaming up Quentin Mauguit, Parmentier and Longrie encouraged the fish to make the sounds naturally by placing a male and a female in each half of a gravel-bottomed tank divided by a transparent partition; the male could scoop out a nest without being disturbed by the female. Next Longrie introduced an intruder into the nesting male's side of the tank, and began recording and filming the nest builder's warning buzzes. Using high-speed cameras (250–500 frames s^{-1}) to visualise movements that would not be clear to the naked eye, the team could see that the fish's pectoral fins rotated backwards as they made the sound. Could this fast fin movement contribute to a sound produced by the swim bladder?

Knowing that the bones of the pectoral and pelvic girdle are linked to the moving fins, Parmentier took a look inside the buzzing fish with X-rays. Collaborating with Sam Van Wassenbergh from the University of Antwerp, Longrie filmed the skeleton's movements with X-rays as the fish sounded off. Correlating the movements with different phases of the buzz profile, the team could see that during the first phase of a buzz the pelvic girdle and scapular bones moved backwards while one of the bones at the base of the anal fin moved forward, crunching the ribs together.

Suspecting that muscles in the fish's body were contracting to crunch the ribs against the swim bladder and generate the sound, Parmentier teamed up with Pierre Vandewalle and identified a muscle, which they named the *vesica longitudinalis*, that could pull the pelvic girdle and scapula backwards to compress the ribs and swim bladder to generate the sound.

Parmentier is quick to add that his rib-crunching hypothesis is currently just a theory. 'The hardest thing is to find a way to definitively cut this muscle to prove the mechanism,' he says. But he is optimistic that he will eventually find a way to prove the theory and learn more about the role of sound production in cichlid evolution.

10.1242/jeb.038950

Longrie, N., Van Wassenbergh, S., Vandewalle, P., Mauguit, Q. and Parmentier, E. (2009). Potential mechanism of sound production in *Oreochromis niloticus* (Cichlidae). *J. Exp. Biol.* **212**, 3395-3402.

INFECTED CRABS BREATHE EASY DURING EXERCISE

As the current swine flu pandemic attests, infectious diseases are a fact of life for all creatures. Atlantic blue crabs are no exception and play host to numerous bacteria. Lou Burnett, a physiologist at the Grice Marine Laboratory in Charleston, USA, explains that crabs fighting infections suffer from the crab equivalent of a stuffy nose. Bacteria mixed with hemocytes – crabs' white blood cells – form clumps that get trapped in the gills, become incorporated into the crabs' exoskeleton and are eventually sloughed off during the next molt. 'It's good that gills can help crabs get rid of bacteria, but this comes at the cost of interfering with respiration,' Burnett says, as the clogged-up gills can make it difficult for crabs to get enough oxygen. He decided to investigate whether crabs fighting a bacterial infection can still cope with increased physical activity without getting out of breath (p. 3428).

To investigate how crabs are affected by infections, Burnett teamed up with Lindy Thibodeaux and his wife, immunologist Karen Burnett. First, they caught Atlantic blue crabs in Charleston Harbor, took them back to the lab, and screened them for infections to identify healthy crabs. To create a port to infect the animals, they drilled a tiny hole in each crab's carapace and sealed it with rubber. After injecting the crabs with either saline or a sub-lethal dose of bacteria they waited an hour for the infection to take hold. The team then placed the crabs on a treadmill, where they scurried along at a speed of 8 m min^{-1} for half an hour.

With the gills of the infected crabs getting clogged up with bacteria, how did the crabs cope with exercise? By placing the treadmill in a flow-through respirometry chamber, the team was able to measure how much oxygen the crabs used before, during and after their brisk walk. To their surprise, they found that a crab's oxygen uptake plummeted by 30% to 40% shortly after receiving a bacterial injection, suggesting that there is a dramatic drop in metabolic rate when an immune response is launched to clear an infection from the crab's system. 'This bacterially induced metabolic depression was totally unexpected,' says Burnett.

The team expected crabs fighting off an infection to suffer from impaired oxygen delivery from their gills, causing infected crabs to switch to anaerobic respiration during exercise. To investigate this, they measured lactic acid build up by taking blood samples from the crabs before, during and after their bout of treadmill exercise. But the crabs surprised them once again: they weren't relying on anaerobic respiration. 'We didn't see higher lactate levels in the infected crabs' hemolymph,' Burnett says.

Do healthy and infected crabs fuel their exercise differently? To find out, the team flash-froze crabs in liquid nitrogen, pulverised the frozen crab carcasses to powder, and measured the levels of metabolites important to energetics in the pulverised crab tissue. Yet again, they found no differences between infected crabs and their healthy counterparts. 'The only difference was that infected crabs had metabolic depression before the exercise, which persisted during exercise,' Burnett says. 'But they were still able to fuel their muscle movements in the same way as healthy crabs.'

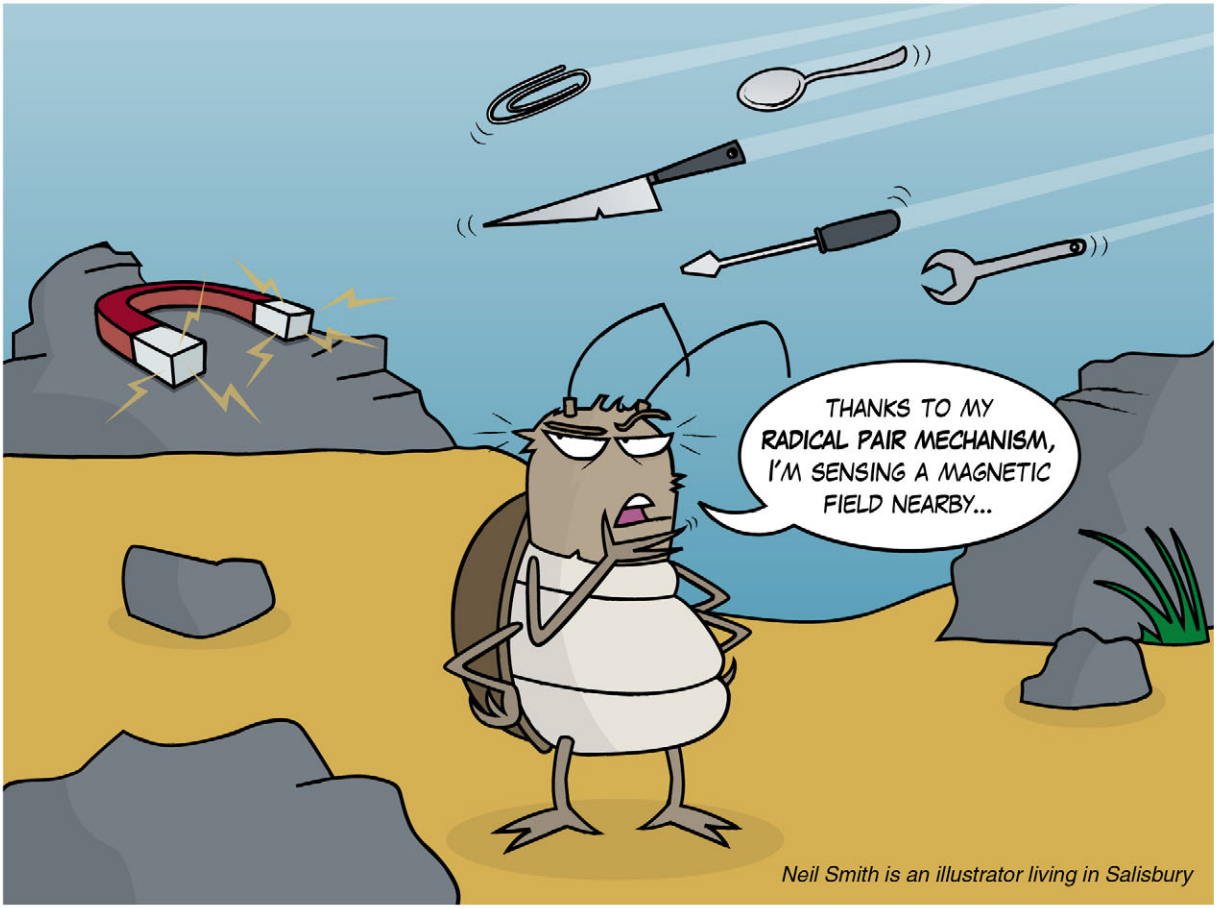
Burnett notes that they put the crabs through their paces in well-oxygenated water, and that crabs are likely to experience tougher conditions in their natural environment. 'We suspect that the crabs won't perform as well in real-world conditions,' he says. 'We're now planning to test their resilience at different temperatures and oxygen levels.'

10.1242/jeb.038919

Thibodeaux, L. K., Burnett, K. G. and Burnett, L. E. (2009). Energy metabolism and metabolic depression during exercise in *Callinectes sapidus*, the Atlantic blue crab: effects of the bacterial pathogen *Vibrio campbellii*. *J. Exp. Biol.* **212**, 3428-3439.

Yfke Hager

COCKROACHES USE RADICAL PAIR MECHANISM TO DETECT MAGNETISM



Restricted to our own suite of five senses, it is sometimes hard to imagine what the world is like for creatures that detect phenomena beyond our own capabilities. How many birds and insects negotiate the world by sensing the magnetic field that bathes our planet is a complete mystery to us. However, it is becoming apparent that there are two ways in which animals can detect magnetic fields: through ferromagnetic iron particles embedded in tissue or through pairs of molecules with unpaired electrons (known as radical pairs) that are associated with a light sensitive photoreceptor. Knowing that weak radio waves can jam an animal's radical pair magnetic sense, Martin Vácha and his colleagues from Masaryk University, Czech Republic, decided to see whether they could also 'deafen' American cockroaches' magnetic senses with radio waves to find out which magnetic mechanism they may be using (p. 3473).

Filming cockroaches in a magnetic field as they rotated the field back and forth by 60 deg., Vácha, Tereza Puzová and Markéta Kvícalová monitored the insects' activity levels after the magnetic field rotated and found that the cockroaches turned around more during, and after, the field moved. But when the team tried 'jamming' the insect's magnetic sense with radio waves, the cockroaches did not rotate in response to the magnetic field's movement. The team was able to 'deafen' the cockroaches' magnetic sense with radio waves, suggesting that the insects use a radical pair mechanism to detect magnetic fields.

10.1242/jeb.038935

Vácha, M., Půžová, T. and Kvíčalová, M. (2009). Radio frequency magnetic fields disrupt magnetoreception in American cockroach. *J. Exp. Biol.* **212**, 3473-3477.

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