INSIDE JEB

# Mystery of how aquatic insects breathe in aquifers solved

A *Paroster macrosturtensis* beetle. Photo credit: Karl Jones and Mark Haase.

You can't always count on finding water above ground in Australia. Some rivers flow through the sand beneath their beds and arid calcite crusts in Western Australia seal off water trapped in permeable rocks beneath. Yet, far from being sterile isolated pools, these calcrete aquifers are teaming with life. 'It can be a really bustling metropolis down there', chuckles Karl Jones from the University of Adelaide, Australia, describing how these subterranean waterways are home to hundreds of species of tiny diving beetle. But no one knew how the diminutive insects breathe. 'Terrestrial diving beetles generally go to the surface and collect a bubble of air, take it underwater and consume oxygen from there', says Jones. But with no obvious source of bubbles for the subterranean dwellers, Jones, Steven Cooper from the South Australian Museum and Roger Seymour, also from the University of Adelaide, were curious to know just how the intriguing mini-beasts breathe.

'Collecting the beetles meant driving halfway across the country to the Western Australian Goldfields, says Jones, recalling the gruelling journey. Once the team had set up an impromptu lab at the Sturt Meadows station, they went fishing for beetles in boreholes drilled into the aquifer. 'We used the bottom half of a fishing rod with a small plankton net attached to the line. We dropped the net into the aquifer water, dragged it up and down a bit and then pulled it up with the animals collected in a small vial at the bottom', he says. 'The local farmers must have thought we were crazy', he laughs. Having collected *Paroster macrosturtensis*, *P. mesosturtensis* and *Limbodessus palmulaoides*, the team set about painstakingly measuring the oxygen concentration in the water immediately next to each insect's body.

Jones explains that when aquatic insects on the surface of the planet absorb oxygen directly from water through their skin, the level of oxygen in the layer of water adjacent to their body is lower than that in the surroundings. Wondering whether the aquifer beetles could absorb oxygen directly from the water, the trio cautiously manoeuvred a tiny oxygen sensor against the body of submerged beetles and then carefully shifted the sensor away, in 50 µm steps at first and then in 100 and 200 µm steps as they recorded the oxygen concentration. 'It was very fiddly and delicate work', recalls Jones. When the team reconstructed the oxygen profile, it was clear that the oxygen levels in the water adjacent to the beetles were significantly reduced. 'The beetles use cutaneous respiration', says Jones.

Then the team asked themselves whether the beetles' limited supply of oxygen through their skin could account for their diminutive size; 'All known species are less than 5 mm long', explains Jones. To test their idea, the team reduced the amount of oxygen available to *P. macrosturtensis*, one of the larger species, from ∼20% down to ∼5%, and monitored the beetles' reactions. Initially, the insects scampered around, but as the oxygen declined, they moved less and less. The team realised that as the oxygen levels dropped, the amount of oxygen and spare energy available to power the beetles' activity fell dramatically: '*P. macrosturtensis* has no excess capacity within its respiratory system to maintain its metabolic rate at lower oxygen levels', says Jones.

So, breathing oxygen across their skin from the surrounding water naturally limits the size of these reclusive beetles and Jones is keen to discover whether other species of subterranean aquifer beetles depend on oxygen absorbed through their skin or have evolved alternative forms of respiration that allow them to live permanently underwater.

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**Jones, K. K., Cooper, S. J. B. and Seymour, R. S.** (2019). Cutaneous respiration by diving beetles from underground aquifers of Western Australia (Coleoptera: Dytiscidae). *J. Exp. Biol.* **222**, jeb196659.

**Kathryn Knight**
**kathryn.knight@biologists.com**

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