

## INSIDE JEB

## Dragonfly haemolymph looks more like ancestors'



An eight-spotted skimmer moulting from the aquatic nymph into the air-breathing adult. Photo credit: Philip Matthews.

Observing metamorphosing tadpoles pull themselves from the water is a rite of passage shared by budding naturalists the world over. Yet, frogs are not the only animals to pass their early life stages immersed in water. Dragonflies spend the majority of their lives submerged before emerging as aerobic adults, trading in their water-breathing gills for trachea that deliver oxygen to every tissue in the body. However, Philip Matthews from the University of British Columbia (UBC), Canada, explains that the dragonflies and frogs have converged on the same aquatic lifestyle choice from different origins. Dragonfly larvae re-evolved the ability to breathe water from terrestrial ancestors, in contrast to amphibian tadpoles, which spent the whole of

their evolutionary history immersed. And Matthews was puzzled; air-breathing animals carry high CO<sub>2</sub> concentrations in their body fluids, while water-breathing species have lower internal concentrations of CO<sub>2</sub> thanks to the high solubility of the gas in water. Would the body fluids of water-breathing dragonfly nymphs resemble those of their aerial ancestors or the species that they re-joined in water?

Describing trapping dragonfly nymphs and adults on the UBC campus, Matthews says, 'For the adult dragonflies, you need to be fast', recalling how he, Master's student Dan Lee and undergraduate Raman Ubhi captured the adults with insect nets while the nymphs were easier to collect with dip nets from ponds. Back in the lab, Lee skilfully collected samples of haemolymph (the insect equivalent of blood) from the adults and nymphs at various stages of development, to measure the total amount of CO<sub>2</sub> – which can occur in several different forms – in the insects' bodies. 'We had to develop our own equipment to measure the CO<sub>2</sub> that was released from our tiny 5 µl haemolymph sample', says Matthews, remembering how Lee worked closely with a glass blower to build the bespoke system. In addition, Lee worked with Martin Gutbrod and Fernando Ferreras from PreSens, Germany, who had built a prototype CO<sub>2</sub> probe, to measure the partial pressure of the gas (the pressure exerted by the CO<sub>2</sub> molecules in a liquid) in the haemolymph of early dragonfly nymphs. However, Matthews needed convincing that the partial pressure readings were accurate, so Lee also measured the CO<sub>2</sub> partial pressure of crayfish haemolymph. 'This gave us

confidence in the values we recorded in dragonfly nymphs', says Matthews, as he could compare the new crayfish measurements with values in the literature.

Despite their aquatic lifestyle, which should have resulted in low CO<sub>2</sub> levels, the total CO<sub>2</sub> concentration in the haemolymph of the dragonfly nymphs was three times higher than that of crayfish and rainbow trout. 'When we saw this, we were very excited', says Matthews, adding, 'This suggests that they aren't expelling CO<sub>2</sub> as easily as one would expect while breathing water'. And when Lee compared how the dragonflies' total CO<sub>2</sub> levels changed as the insects prepared to depart the water, the total CO<sub>2</sub> levels increased to approach those experienced by the air-breathing adults: '[which] suggests that they might be preparing to become air breathers (or becoming poorer water breathers) during their final instar', says Matthews.

So, water-breathing dragonfly nymphs have relatively high levels of CO<sub>2</sub> in their bodies compared with those of other water-breathing creatures, with the consequence that the transition that they undergo – to high total CO<sub>2</sub> levels – as they shift to an aerial lifestyle is less dramatic than it is for emerging tadpoles. And Matthews is eager to find out why aquatic dragonfly nymphs differ so much from other aquatic residents. 'There's so much more to do', he says.

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