BRIEF COMMUNICATIONS

Diving insects boost their buoyancy bubbles

Underwater backswimmers use their haemoglobin to help them stay stationary while waiting for prey.

Backswimmers (Notonectidae) are common diving insects found around the world that exploit the mid-water zone for predation — they breathe by using an air bubble collected at the surface. Here we show that backswimmers achieve prolonged periods of neutral buoyancy by using oxygen stored in their haemoglobin to stabilize the volume of the bubble as they breathe from it. This enables them to maintain their position in the water column without continually swimming.

Many air-breathing aquatic insects dive with air bubbles that either supply oxygen directly or function as a 'gas gill' that obtains dissolved oxygen from the water^{1,2}. But bubble volume decreases during a dive because of oxygen uptake by the insect and diffusion of carbon dioxide and nitrogen into the surrounding water. Most diving insects are therefore limited to floating at the water's surface or clinging to submerged bodies.

Backswimmers are remarkable in that they are the only insects to inhabit mid-water environments as adults and are also the only ones to have haemoglobin throughout their entire life cycle³. Although it has been suggested that haemoglobin must play a role in buoyancy control^{4–6}, this has not been directly confirmed. By using new technology involving a sensitive electronic balance and fibre-optic

Hold your breath: the backswimmer *Anisops deanei* uses an air bubble to survive underwater.

oxygen sensors (for details of methods, see supplementary information), we have been able to measure changes in buoyancy and oxygen partial pressure (p_{O_2}) within the bubble of the Australian backswimmer *Anisops deanei*.

After the bubble is captured, buoyancy and p_{O_2} decrease linearly; these then plateau until the insect starts to swim to the surface to renew the bubble (Fig. 1). The insect obtains oxygen mainly from the bubble during the

initial linear phase, but from its haemoglobin during the plateau phase. The plateau begins at a p_{O_2} of 4.3 kilopascals (kPa) and ends at 3.3 kPa (5 replicates), which corresponds to a haemoglobin–oxygen affinity (P_{50}) of 3.9 kPa — similar to that measured in *A. assimilis*⁶. Adding 15% carbon monoxide to the breathing gas prevents the insect's haemoglobin from combining with oxygen and eliminates the plateau (Fig. 1), indicating that oxygen released from haemoglobin stabilizes p_{O_2} within the bubble for about 4 min. Note that backswimmers do not use their bubbles as gas gills⁶, so nitrogen loss from the bubble is minimal.

Neutral buoyancy is achieved only when the buoyant force of the bubble balances the submerged weight of the insect. Neutral buoyancy is not attained immediately upon diving: we found that when backswimmers begin a dive, they carry a bubble that is 17% larger (s.e. = 3%, $n = 10$) than would be required for neutral buoyancy. This overinflation is related to the properties of *A. deanei* haemoglobin: its high oxygen affinity prevents it from releasing its oxygen until the p_{O_2} has dropped from 20.6 kPa to about 4.3 kPa, which is equivalent to a 16% reduction in the initial volume of the bubble. The initial volume therefore provides the maximum amount of oxygen in the bubble at the start of the plateau period. Unrestrained backswimmers must swim against positive buoyancy initially, and this activity causes a steeper decrease in p_{O_2} than in our experiments, where the insects' metabolic rates were lower because they were motionless.

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Figure 1 | Partial pressure of oxygen inside a bubble and the change in the bubble's volume while attached to the insect *Anisops deanei* during a dive. Oxygen released from haemoglobin stabilizes bubble p_{O_2} (red line) and volume (purple line) after 4 min in the motionless insect. Fluctuations in buoyancy (purple line) at 8 min indicate attempts to swim to the surface. Exposure to 15% carbon monoxide before a dive prevents the insect's haemoglobin from binding and releasing oxygen (blue line), eliminating the p_{O_2} and buoyancy plateaux. Inset, experimental setup showing *A. deanei* secured on a submerged platform attached to a balance; an optical setup probe is inserted into the abdominal air bubble.