The Journal of Experimental Biology 211, 3790-3799
Published by The Company of Biologists 2008
doi:10.1242/jeb.018721

Haemoglobin as a buoyancy regulator and oxygen supply in the backswimmer (Notonectidae, *Anisops*)

Philip G. D. Matthews* and Roger S. Seymour

Ecology and Evolutionary Biology, School of Earth and Environmental Sciences, Darling Building, University of Adelaide, Adelaide, SA 5005, Australia

*Author for correspondence (e-mail: philip.matthews@adelaide.edu.au)

Accepted 11 September 2008

SUMMARY

Unlike all other diving insects, backswimmers of the genus *Anisops* can exploit the pelagic zone by temporarily achieving near-neutral buoyancy during the course of a dive. They begin a dive positively buoyant due to the large volume of air carried in their ventral air-stores, but rapidly enter a protracted period of near-neutral buoyancy before becoming negatively buoyant. This dive profile is due to haemoglobin found in large tracheated cells in the abdomen. Fibre optic oxygen probes placed in the air-stores of submerged bugs revealed that oxygen partial pressure (P_{O_2}) dropped in a sigmoid curve, where a linear decline preceded a plateau between 5.1 and 2.0 kPa, before a final drop. Buoyancy measurements made by attaching backswimmers to a sensitive electronic balance showed the same three phases. Inactivating the haemoglobin by fumigating backswimmers with 15% CO eliminated both buoyancy and P_{O_2} plateaus. Oxygen unloaded from the haemoglobin stabilises the air-store during the neutrally buoyant phase after a decrease in volume of between 16% and 19%. Using measurements of air-store P_{O_2} and volume, it was calculated that during a dive the haemoglobin and air-store contribute 0.25 and 0.26 μl of oxygen, respectively.

Key words: buoyancy, haemoglobin, insect, respiration.

INTRODUCTION

Many insects that forage underwater rely on a bubble of air collected at the surface to provide them with oxygen while submerged. An air-store held over the insect's spiracles can provide enough oxygen for a short dive. Under some conditions it may also act as a gill, facilitating the uptake of oxygen from the surrounding water (Ege, 1915; Rahn and Paganelli, 1968). However, carrying a volume of air sufficient for underwater respiration dramatically affects an insect's ability to stay submerged. Insects have an overall body density slightly greater than water, because the density of their tissues is about 1.05 g cm^{-3} and that of their chitinous cuticle is about 1.3 g cm^{-3} (Vincent and Wegst, 2004). This makes them slightly negatively buoyant. Thus the buoyant force of even a comparatively small bubble of air causes an insect to float. When positively buoyant, it must actively swim to submerge, and can only remain underwater by continuing to swim or clinging to the substrate. But while submerged, the volume of its bubble decreases as respiration consumes oxygen and both carbon dioxide and nitrogen diffuse out into the surrounding water. As a result the insect steadily becomes less buoyant, eventually to the point where it begins to sink and must swim to the surface to refill its air-store. This constant change in buoyancy has prevented almost all aquatic insects from successfully occupying the mid-water zone, instead limiting them to foraging on the bottom or floating at the surface.

The aquatic backswimmers (Hemiptera: Notonectidae, subfamily Anisopinae) are unique among insects as they carry a bubble of air on their abdomen while submerged and yet are capable of achieving a prolonged period of neutral buoyancy during a dive. Backswimmers are a common sight in still, often stagnant bodies of water, from farm dams to swimming pools. They can be found floating motionless in the water column, occasionally moving in rapid bursts, using their oar-like hind legs. Backswimmers are also remarkable in that they belong to one of only three insect families known to produce substantial quantities of haemoglobin, the other two being the larvae of *Gastrophilus intestinalis* (Oestridae) and *Chironomus* spp. (Chironomidae) (Weber and Vinogradov, 2001). The backswimmer produces haemoglobin in large modified fat-body cells within its abdomen (Bergtrom et al., 1976). An extensive network of tracheoles pervades each haemoglobin cell, and the air-filled tracheoles connect with the abdominal spiracles through larger tracheae (Bare, 1929). Two parallel grooves run along the length of the abdomen's ventral surface and contain both the abdominal spiracles and the air-store (Figs 1 and 2). Thus the haemoglobin cells are intimately associated with both the tracheal system and the air-store *via* the abdominal spiracles. Unlike many diving insects that maintain maximum contact between the surface of their air-stores and the surrounding water for reasons of gas exchange [e.g. *Aphelocheirus* (Thorpe and Crisp, 1947)], backswimmers cover their air-stores with a layer of hydrophobic hairs. These long hairs fringe the outer edge of each groove and, due to their hydrophobicity, arrange themselves in a layer across the air–water interface of the air-store when the backswimmer is submerged, and also stick to the water surface when the backswimmer surfaces, thus exposing the ventral spiracles directly to the atmosphere (Fig. 2).

Hungerford was the first to discover haemoglobin in backswimmers (*Buenoa margaritacea*), going on to suggest a link with their unusual lifestyle (Hungerford, 1922). He observed that backswimmers swam beneath the water's surface, where they appeared to be 'in perfect equilibrium', and considered that the haemoglobin probably acted as a supplementary oxygen store to extend their time underwater. Miller was the first to suggest that the oxygen released from the haemoglobin during a dive was linked

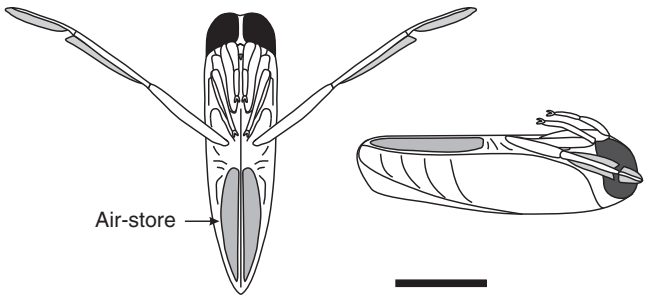


Fig. 1. Diagram of the backswimmer *Anisops deanei* from above (ventral side) and laterally. The grey shaded areas on the abdomen indicate the hair-covered grooves that contain the air-store. Scale bar is 2 mm.

to the backswimmers' buoyancy (Miller, 1964; Miller, 1966). He observed that the bright red oxyhaemoglobin present in *Anisops pellucens* at the beginning of a dive slowly changed to a dark purple once the insect had reached neutral buoyancy, indicating that oxygen was released from the haemoglobin and was contributing to respiration during the neutrally buoyant phase. In separate experiments, he fumigated them with air mixtures containing 6–25% carbon monoxide (CO), thus preventing their haemoglobin from binding with oxygen. Backswimmers treated in this manner did not enter the neutrally buoyant phase and passed directly from positive to negative buoyancy. Although these experiments clearly indicate a relationship between haemoglobin and buoyancy, they suffer from their reliance on qualitative visual estimates (e.g. haemoglobin oxygen saturation determined by comparison with colour standards), and estimation of buoyancy by examining the movements of free-swimming backswimmers.

Despite the rarity of insect haemoglobin, there have been surprisingly few studies on its function from a whole-animal perspective. Subsequent studies on backswimmer haemoglobin have focused more on the biochemical aspects of synthesis (Bergtrom et al., 1976), structure (Bergtrom, 1977; Osmulski et al., 1992; Vossbrinck et al., 1993) and oxygen-binding properties (Wells et al., 1981). These studies examined the functioning of haemoglobin *in vitro*, extracting it from large numbers of homogenised backswimmers. Only Wells and colleagues discussed the relationship between the oxygen affinity of haemoglobin and its physiological role (Wells et al., 1981). Their *in vitro* analysis of *Anisops assimilis* haemoglobin showed a very steep oxygen equilibrium curve, with oxygen released readily only at low oxygen partial pressure (P_{O_2}). They hypothesized that the backswimmer's respiration would cause the P_{O_2} and volume of its positively buoyant air-store to decrease during a dive. With the P_{O_2} of the air-store reduced, the haemoglobin could then unload its oxygen, temporarily stabilising the volume of the now neutrally buoyant air-store. However, the insect's buoyancy, the air-store P_{O_2} and the relative contribution of the haemoglobin to submerged respiration were not measured.

Progress in this area has been impeded by an inability to measure the gas composition and volume of the air-store in these small insects. However, this study, which expands on previously published experiments (Matthews and Seymour, 2006), uses new techniques to quantitatively assess the *in vivo* function of haemoglobin in the backswimmer *Anisops deanei* (Brooks 1951). Fibre optic oxygen probes were used to directly measure the partial pressure of oxygen within the air-stores of submerged bugs, while measurements of buoyancy were made on tethered backswimmers using a sensitive electronic balance. These measurements were then used to determine the role of haemoglobin in buoyancy control and respiration.

MATERIALS AND METHODS

The backswimmers used were identified as *Anisops deanei* according to Andersen and Weir (Andersen and Weir, 2004), and were collected from ponds at the North Terrace campus and Waite campus of Adelaide University, Adelaide, South Australia. They were kept in the laboratory in a 31.5 l aquarium illuminated by a fluorescent aquarium light on a 12 h:12 h light:dark cycle. Water

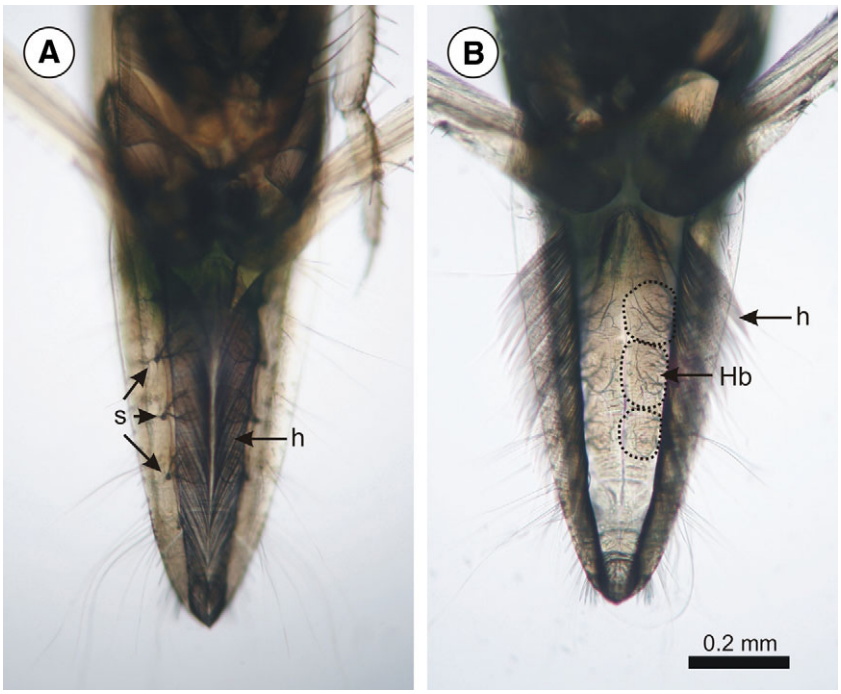


Fig. 2. The ventral abdominal surface of first instar *Anisops deanei*, submerged (A) and at the surface (B). The hydrophobic hairs (h) completely cover the air-store when submerged and expose it at the surface. The spiracles (s) connected to the tracheoles that invade the haemoglobin cells (Hb) are visible.

temperature was maintained at 20°C by circulating water from a temperature-controlled water bath (Thermomix 1442 D, B. Braun, Melsungen, Germany) through a glass coil submerged in the aquarium. The backswimmers were fed on a diet of locally caught live mosquito larvae, cladocerans and copepods. All experiments were carried out at 20°C.

Determination of body density

The density of *Anisops deanei* was determined according to Archimedes' principle. Fifteen specimens were collected from outdoor ponds and transported to the laboratory, where they were transferred into vials containing cotton wool soaked in chloroform, and killed. They were gently blotted with tissue paper to remove any water, weighed to 1×10^{-5} g, and then transferred into individually numbered 5 ml vials. Each vial was filled with water, and a small square of soft nylon mesh was inserted to prevent the insect from floating to the surface. All air adhering to the backswimmers' bodies and in their tracheal systems was removed by placing the uncapped vials in a desiccator jar connected to a vacuum pump (H. I. Clements Pty, Sydney, Australia). A suction pressure of –80 kPa was applied to the desiccator jar, which was then sealed for 16 h. After this period any bubbles adhering to the backswimmers were removed by gently tapping the vials. The negatively buoyant insects were then sucked into a 1 ml disposable syringe and transferred under water to a custom-made weighing pan hanging in a 4 cm deep Petri dish of water below an AE 163 balance (Mettler, Greifensee, Switzerland). Their submerged weight was determined to 1×10^{-5} g. Body volumes were then determined according to the formula:

$$V = (W_1 - W_2)\rho_{H_2O}\,, \tag{1}$$

where V is volume (cm^3), W_1 is the insect's weight in air and W_2 is its weight underwater (g), and ρ_{H_2O} is the density of pure water at the temperature used in the measurement ($g\,cm^{-3}$). The density of the backswimmers ($\rho_b\,g\,cm^{-3}$) was then calculated by:

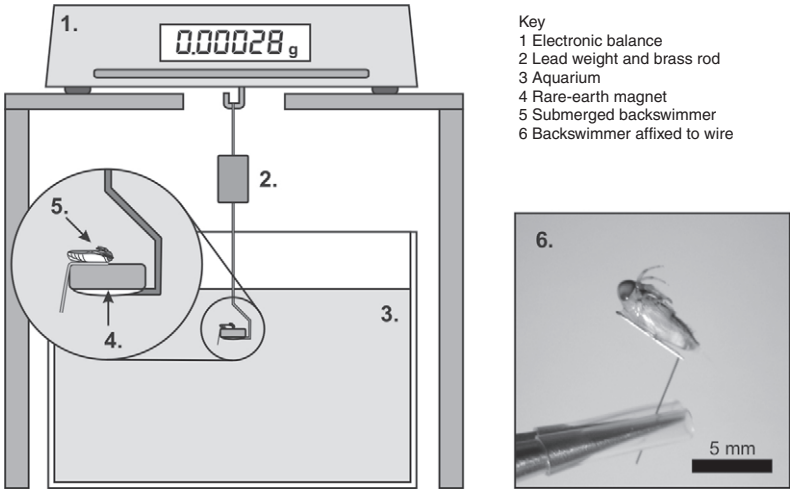
$$\rho_b = \frac{W_1}{V}. \tag{2}$$

Determination of initial air-store volume

The volume of air carried from the surface of the water by free-swimming backswimmers was measured by removing the gas and measuring the volume directly (Scholander and Evans, 1947). An upright funnel with nylon mesh blocking its throat was submerged inside a 1 l beaker filled with air-equilibrated water. A backswimmer was dried on blotting paper, weighed on an AE 163 electronic balance, then placed within the funnel and allowed approximately 15 min to settle and spontaneously dive. A 'capture tube' was made from a 4 ml test-tube that was filled with kerosene and inverted in a second beaker of water, such that the buoyant kerosene remained in the tube. After the backswimmer submerged, the funnel was partially lifted out of the first beaker. The suction created by the water draining from the funnel held the insect transiently against the nylon mesh. The capture tube was transferred to the funnel by holding a thumb over its opening, and quickly placed over the still-submerged insect. The backswimmer's hydrophobic air-holding surfaces were flooded with kerosene on contact, displacing the air, which floated to the top of the test-tube. The bug was then crushed against the wall of the capture tube with a spatula, forcing the remaining air from the tracheal system. The trapped air bubble was drawn into a length of 0.86 mm internal diameter polyethylene tubing and then expelled into an inverted 0.5 cm internal diameter glass cup filled with kerosene. With a micromanipulator under a dissecting microscope, the bubble was then transferred to a horizontally mounted micrometre burette constructed and operated according to the design of Scholander and Evans (Scholander and Evans, 1947).

Air-store volume and buoyancy

Changes in air-store volume and buoyancy were determined by logging the weight of a submerged backswimmer attached by a magnet to the AE 163 electronic balance (Fig. 3). The balance was placed on top of a 55 cm high frame over a bench, and a thin brass rod was suspended vertically from a hook attached to the balance arm. A 30 g lump of lead was attached half-way down the length of the rod to compensate for the weight of the removed weighing pan and to reduce lateral movement. The end of the rod was bent into an L shape. A nickel-coated rare-earth magnet, 10 mm in diameter by 3 mm, was fixed horizontally to the L with epoxy resin adhesive (Araldite, Selleys Pty, Padstow, NSW, Australia). A plastic container filled with water was positioned beneath the insect holder and raised and lowered with a scissor jack. The container was raised until the magnet was submerged 2.5 cm below the water surface. This was done 20 min before measurements, to allow the level of the meniscus around the rod to stabilise. The balance and frame were then enclosed in a tent of plastic sheeting to exclude



Key
1 Electronic balance
2 Lead weight and brass rod
3 Aquarium
4 Rare-earth magnet
5 Submerged backswimmer
6 Backswimmer affixed to wire

Fig. 3. Apparatus used for measuring the buoyancy of a submerged backswimmer.

draughts. Finally, the balance was remotely tared using a computer connected through a 012 digital interface (Mettler).

Pieces of 0.1 mm diameter iron wire, approximately 16 mm long, were bent at right angles half-way along their length. One arm of the wire was then doubled back to form a U shape. Backswimmers were narcotised with CO_2, gently blotted on tissue paper, and weighed. The U part of the wire was then dipped in a drop of cyanomethacrylate adhesive (Selleys Pty) and fixed lengthwise along the back of the immobilised backswimmer, with the unbent arm oriented posteriorly and perpendicularly away from it (Fig. 3). The unbent arm of the wire was then inserted into a sheet of foam, thus holding the backswimmer on its back for 10 min while the adhesive set. During this time the backswimmer was placed either in air or in a 60 ml glass syringe ventilated with 15% CO, 20% O_2 and 65% N_2 standard temperature and pressure (STP) at a rate of 63 ml min^{-1} from a gas-mixing apparatus custom-built from mass flowmeters (model GFC171; Aalborg Instruments and Controls, Orangeburg, NY, USA) regulated by a PC running control software through a D/A converter (ProfessorDAQ™ and PowerDAQ™ PD2-AO, United Electronic Industries, Walpole, MA, USA). Backswimmers treated with CO were submerged in 20°C water that had been aerated with the same gas mixture; otherwise the water used was air equilibrated.

While CO binds readily to haemoglobin, it also has the potential to inhibit aerobic metabolism by binding to cytochrome oxidase (Ball et al., 1951). However, a pilot study demonstrated that oxygen partial pressure in the air-stores of CO-treated backswimmers initially declined in the same manner as in control insects, thus demonstrating aerobic respiration. For CO to depress aerobic respiration it must reduce the number of available cytochrome oxidase enzymes below that required to support a particular level of aerobic activity. So while CO may have depressed the backswimmers' maximum possible rate of oxygen consumption, it would not have depressed their resting metabolic rate. This is in agreement with previous studies that show insects are remarkably tolerant of CO. For example, in Miller's study on *Anisops*, the dive durations of backswimmers exposed to 6%, 12% and 25% CO were not significantly different as would be expected if aerobic respiration was depressed (Miller, 1966). Studies on stick insects and beetles show they can survive for over 30 days in 20% CO and for up to 10 days in atmospheres containing 80% CO (Baker and Wright, 1977). And at the extreme, adult silkworm hearts exposed to 4 atm (~404 kPa) of CO pressure continue to show aerobic function, with complete aerobic inhibition achieved at 5 atm (~505 kPa) (Harvey and Williams, 1958). We therefore conclude that 15% CO did not inhibit aerobic metabolism.

Using the unbent wire arm as a handle, the backswimmer was moved to the water-filled container beneath the balance with a pair of anti-magnetic pointed tweezers. It was half-submerged head first at approximately 25 deg. until the hairs fringing the abdominal air-store caught on the surface tension of the water. It was then fully submerged. While still submerged, the backswimmer was released 1 cm away from, and slightly above, the magnet. The magnet readily attracted and held the insect, orienting it in the normal swimming position (horizontally, with its ventral surface uppermost). The combined weight of the submerged backswimmer, its air-store, and the wire and glue, was recorded every 2 s. Once the backswimmer attempted to surface, it was recovered from the magnet, and the recording stopped. The balance was left to settle and then re-tared. The glue and wire were then easily peeled off the backswimmer, allowing it to be weighed separately underwater.

Air-store volume calculation

The air-store volume at each 2 s interval was calculated according to the formula:

$$V_{air} = -1(W_{tot} - W_w - W_i)\,, \quad (3)$$

where V_{air} (ml) is the volume of air carried by the backswimmer, W_{tot} (g) is the total submerged weight of the insect with attached wire and glue, W_w (g) is the submerged weight of the wire and glue, and W_i (g) is the submerged weight of the backswimmer. The hydrostatic pressure of water pushes a submerged bubble upward with a force equal to the mass of water it displaces. Therefore, the apparent negative weight of a submerged air bubble is proportional to its volume when multiplied by –1. As W_i was not measured directly it was calculated using the backswimmer's weight in air and the previously measured mean density:

$$W_i = W_l - \left(\frac{W_l}{\rho_b}\right)\rho_{H_2O}\,. \quad (4)$$

Air-store P_{O_2} measurement

Changes in air-store P_{O_2} during a dive were measured before and after a backswimmer had been exposed to CO. The bug was narcotised in pure CO_2 gas for 3 min and then weighed. While still immobilised it was then affixed to a glass microscope slide on its back, using a drop of cyanomethacrylate adhesive. The insect was then left in air to recover for 10 min before measurements began.

A 2 cm deep Petri dish filled with air-equilibrated water at 20°C was placed on the stage of a dissecting microscope. The bug was half-submerged in the water until the long hydrophobic hairs fringing the abdominal grooves were caught on the surface tension of the water. The bug was then completely submerged, with a bubble of air held over the abdomen beneath the hairs. A syringe-mounted optical oxygen probe with a <50 μm diameter tip attached to an oxygen meter (TX3, PreSens GmbH, Regensburg, Germany) was then manoeuvred into the air-store using a micromanipulator. P_{O_2} was then logged at 1 s intervals until the backswimmer attempted to surface, at which point recording was stopped and the animal was removed from the Petri dish. For the CO treatment, this procedure was repeated using the same backswimmer, but with the 10 min recovery period performed in a 60 ml glass syringe ventilated with 15% CO, 20% O_2 and 65% N_2 STP at a rate of 63 ml min^{-1} from the gas-mixing apparatus. The CO-treated bug was then submerged in water that had been aerated with the same gas mixture, and measurement of air-store P_{O_2} was carried out during the dive as described above.

Effect of temperature and aquatic P_{O_2} on voluntary dive duration

The dive durations of backswimmers in an aquarium maintained at different temperatures and P_{O_2} were measured. Backswimmers naturally seek out conspecifics and form aggregations in the wild (Bailey, 1987). It was observed that backswimmers placed individually into the aquarium took far longer to settle than when at least one other backswimmer was present. Thus in order to obtain the most consistent and repeatable measurements, dive durations were measured when the backswimmers were kept in pairs.

A 15.8 l aquarium was vertically partitioned with a black sheet of plastic perforated at the top and bottom. A coil of glass tubing flushed with water from a temperature-controlled water bath was placed in the small compartment between the end wall of the aquarium and the plastic sheet, to maintain the aquarium water at

the desired temperature. Compressed air or nitrogen was blown through an air stone next to the coil to control the P_{O_2} of the water. Aquatic P_{O_2} was monitored using an optical oxygen probe submerged in the aquarium connected to the TX3 oxygen meter. The back and sides of the dive chamber were covered with black plastic sheeting, leaving the front of the aquarium open for observation. Backswimmers were introduced to the large compartment in pairs and given 30 min to acclimatise, after which six consecutive dive events performed by each backswimmer were observed and timed using a stopwatch. Dive times were recorded in water P_{O_2} treatments of 100%, 80%, 40% and 25% air saturation at 20°C, as well as in normoxic water at 10, 15, 20, 25 and 30°C.

Statistical analysis

All statistics were performed using the Microsoft Excel™ add-in StatistiXL version 1.6 (www.statistixl.com). Data are given as the mean of N samples ±95% confidence interval (CI).

RESULTS

Determination of density

The mean backswimmer weight was 12.43±0.18 mg (N=15). Female backswimmers were significantly (Student's t-test P=0.043) heavier than males (with means of 14.96 and 11.16 mg, respectively), but no significant difference was found between their densities. The mean gas-free body density (ρ_b) of *A. deanei* was determined to be 1.0784±0.0074 g cm^{-3}. This value was used in all calculations. Submerged backswimmer weight calculated using this value is expected to lie within ±8.5% of the actual submerged weight (i.e. within 95% CI limits).

Mechanisms of air-store volume regulation

The volume of air collected at the surface of the water is regulated largely by the abdominal grooves on the backswimmer's abdomen and the long hairs that fringe them (Fig. 2). When submerging the backswimmers for experimentation, it was necessary to lower the bug into the water until its abdominal hairs spread onto the surface tension of the water before submergence. Over-large bubbles occurred if the backswimmer was submerged too quickly, and flooded grooves occurred if it was submerged too slowly. Observation of backswimmers diving in aquaria also revealed the importance of the abdominal hairs when surfacing. Backswimmers refilled their air-stores in two distinct ways. The first involved reversing, abdomen first, towards the surface, touching the tip of the abdomen to the surface for a fraction of a second, and then diving rapidly. This behaviour, which was the most frequently observed, allowed a small quantity of fresh air into the air-store and a quick return to buoyant equilibrium. The second surfacing behaviour involved the backswimmers placing the entire ventral surface of their abdomen against the water's surface. This caused the hairs to stick to the surface tension of the water, forming a dark border resembling eyelashes on each side of the abdomen, while exposing the abdominal grooves to the atmosphere (Fig. 2). The backswimmers rested against the water's surface for a little over 1 s before rapidly diving with quick strokes of their oar-like hind legs. This resulted in the hairs snapping back into place over the air-filled grooves. Once submerged, it took approximately 20 s before the insects again approached neutral buoyancy. Occasionally, after refilling its air-store in this manner, a backswimmer submerged carrying too much air. Inbetween swimming to keep submerged, the backswimmer would vigorously attempt to wipe the excess air from its abdomen using its hind legs. Dislodging minute bubbles of air reduced the insect's buoyancy and allowed it to assume its normal position in the water column. If it was unsuccessful, it returned to the surface to obtain a more suitable air volume.

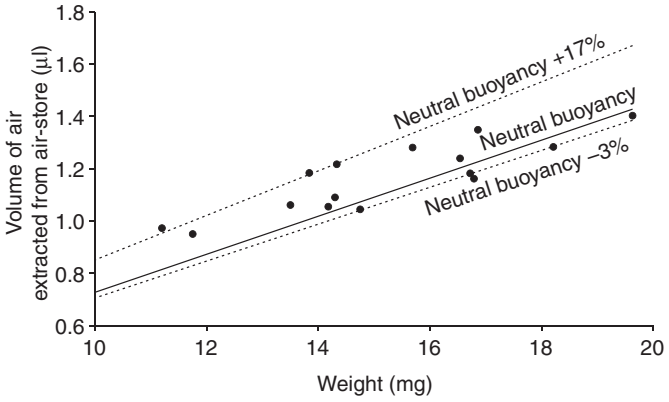


Fig. 4. The volume of air extracted from a backswimmer immediately after diving voluntarily. The variation in collected air volume is affected by length of dive and amount of struggling during capture, but lies between 19.4% above to 4.9% below neutral buoyancy.

Initial air-store volume in free dives

The time between the backswimmer submerging and its capture in kerosene varied between approximately 3 and 15 s. The mean volume of air extracted from the submerged bugs was 1.153±0.068 µl (N=15). This is equivalent to the backswimmers carrying 0.07759±0.00304 ml g^{-1} or a volume of air 6.7±4.2% larger than required for neutral buoyancy (Fig. 4). The maximum air volume measured (as a percentage of the backswimmer's body weight) was 19.4% larger than that required for neutral buoyancy, while the smallest was 4.9% less than that required for neutral buoyancy, as calculated using the mean gas-free body density (ρ_b) of *A. deanei*.

Air-store volume and buoyancy in experimental dives

The initial volume of air carried by each backswimmer was calculated from the first stable weight once the balance had recovered from the disturbance of having the backswimmer attached. To verify that the initial air-store volume was determined accurately, the percentage decrease in initial volume that had occurred by the time the backswimmer began vigorous surfacing attempts (observed in the data from the electronic balance as rapid spikes in weight) was calculated. This volume decrease was due primarily to consumption of oxygen, which constitutes approximately 20% of the initial volume of air, and a smaller amount of nitrogen diffusion into the water. Thus a backswimmer attempting to surface and replenish its oxygen stores must do so after a decrease in air-store volume of just over 20%. In 10 control and six CO-treated backswimmers, decreases in air-store volume were 24.8±3.6% and 26.8±5.7%, respectively. A two-tailed t-test found no difference between the initial air-store volumes of control and CO-treated backswimmers, and so these data were pooled. There were a few records where volume appeared to drop more than 35%, and these were considered to represent an underestimation of initial air volume, resulting from a shift in the tared weight of the balance, a change in the meniscus effect on the support shaft or loss of part of the bubble, and these data were excluded from the analysis. Mean initial air-store volume was 0.11401±0.01259 ml g^{-1} and 31.5±7.3% larger than the calculated volume required for neutral buoyancy (Fig. 5).

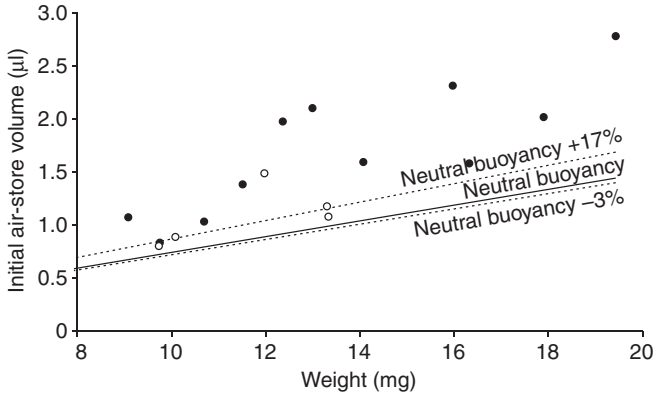


Fig. 5. The relationship between the weight of the backswimmer and the initial volume of air carried at the beginning of a forced dive. Filled circles indicate data from control backswimmers, and open circles indicate data from CO-treated backswimmers. Neutral buoyancy occurs along the solid line, where the air-store volume balances the insects' negative buoyancy.

CO significantly reduced the time taken for backswimmers to begin surfacing behaviour, with CO-treated backswimmers attempting to surface after only 372±57 s compared with 490±58 s in control bugs (one-tailed *t*-test: control dive duration > CO dive duration, P=0.006). As well as reducing dive duration, the manner in which air-store volume decreased during a dive was also affected. Both control and CO-treated backswimmers began a dive with a steep initial decline in air-store volume. In control treatments, this was then followed by a period of relative stability, with only a minor decrease in volume over several minutes, until a final drop triggered a burst of activity in the backswimmers as they attempted to surface (Fig. 6). In CO-treated backswimmers, this extended plateau was eliminated and air-store volume dropped continuously.

Air-store P_{O_2}

Tethered backswimmers usually remained inactive while submerged, only using their oar-like hind limbs once the P_{O_2} of their air-store had dropped to very low levels (<2 kPa). Changes in air-store P_{O_2} during submergence were measured using untreated backswimmers (N=10) and backswimmers both before and after CO exposure (N=6). Immediately upon submerging, the air-store P_{O_2} of untreated backswimmers decreased in a linear manner (Fig. 7).

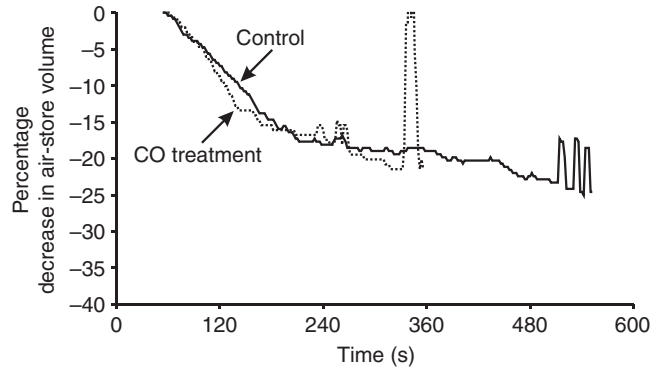


Fig. 6. Typical traces showing percentage decrease in air-store volume in untreated (solid line) and 15% CO-exposed (dotted line) backswimmers. Spikes in volume at the end of each trace were produced by the energetic movements of the backswimmers as they attempted to surface.

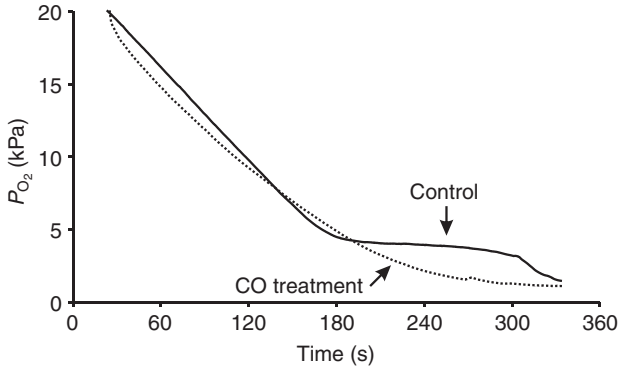


Fig. 7. Typical changes in the P_{O_2} of a submerged backswimmer's air-store, before (solid line) and after (dotted line) exposure to 15% CO.

But once the P_{O_2} of the air-store fell below 5 kPa, the rate of oxygen decrease slowed considerably, so that for several minutes the P_{O_2} remained between 3 and 5 kPa. Eventually the oxygen level again declined, stabilising at a minimum value of approximately 0.5 kPa (Fig. 7). By this stage the backswimmer began swimming movements, as if trying to reach the surface. Exposing the backswimmers to CO eliminated the P_{O_2} plateau. At the beginning of the dive the P_{O_2} decreased linearly as before, but continued to decline asymptotically towards the same minimum air-store P_{O_2} recorded at the end of the control dives (Fig. 7). These patterns were observed in all backswimmers from which measurements were obtained.

The progressive changes in air-store P_{O_2} were divided into three phases (P1, P2 and P3) according to their relative rates of decline. P1 corresponds to the initial linear P_{O_2} decrease, followed by the plateau (P2), which terminates in a final P_{O_2} drop (P3). The precise change point between P1 and P2 was defined as the point on the P_{O_2} trace where the initial rate of air-store P_{O_2} change ($\dot{P}_{O_2}$) decreased by half (Fig. 8, point a). The transition from P2 to P3 was

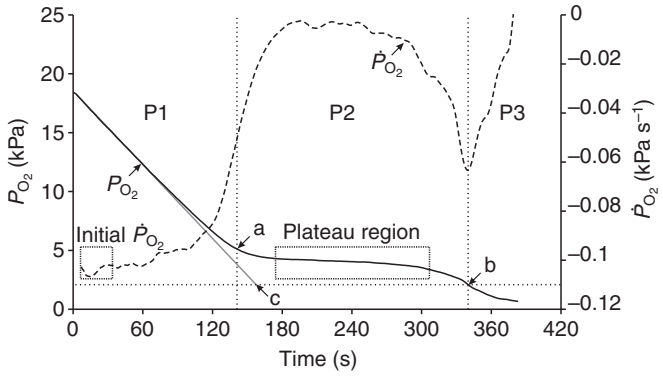


Fig. 8. Three phases of the air-store's P_{O_2} during a dive (solid black line), defined by the rate of P_{O_2} change ($\dot{P}_{O_2}$, dashed line). The initial steep P_{O_2} decline in phase 1 (P1) enters a plateau phase (P2) once P_{O_2} has decreased by half its initial rate (point a). The arbitrary end of P2 (point b) is marked by the maximum rate of decrease in P_{O_2}. The low air-store P_{O_2} forces the backswimmer to surface during P3. The effective oxygen contributions of the air-store and haemoglobin were calculated by assuming that in the absence of haemoglobin the air-store would supply oxygen until point c, while the oxygen contributed by the haemoglobin extends the dive time to point b. The initial P_{O_2} was taken from the first stable 20 s of the trace (first dotted box), while the plateau region was defined as the middle two-thirds of the P_{O_2} trace between P1 and P3 (second dotted box).

Table 1. Duration, P_{O_2} and rate of P_{O_2} change ($\dot{P}_{O_2}$) of the first and second air-store phases

		Mean	95% CI
Duration of phase (s)	Phase 1	243	48
	Phase 1+2	514	94
	Phase 2	271	55
	Ratio of 1:2	0.96	0.19
P_{O_2} at phase change (kPa)	Phase 2 (start)	5.10	0.31
	Phase 2 (middle)	3.79	0.16
	Phase 2 (end)	2.01	0.18
$\dot{P}_{O_2}$ (kPa s^{-1})	Phase 1	–0.087	0.021
	Phase 2 (middle)	–0.008	0.002
	Ratio of 1:2	12.35	2.46

N=16; CI, confidence interval.

then defined as the point where the rapid increase in $\dot{P}_{O_2}$ at the end of the plateau began to slow (Fig. 8, point b). Using these definitions, P1 and P2 were of nearly equal duration (243±48 and 271±55 s, respectively), with P2 beginning at 5 kPa and ending at 2 kPa (Table 1). P2 showed a marked decrease in $\dot{P}_{O_2}$ relative to P1, with the P_{O_2} declining 12 times more slowly during the plateau period (the middle two-thirds of P2) than at the beginning of the dive. Oxygen released by the haemoglobin maintained the P_{O_2} at a mean value of 3.79 kPa during this plateau (Table 1).

Effect of temperature and aquatic P_{O_2} on voluntary dive duration

Aquatic P_{O_2} was found to have no significant effect (nested ANOVA P=0.260) on dive time (Fig. 9) with the dive time at 100% air-saturation (mean 275±36 s) only slightly greater than that at 25% (248±19 s). Water temperature showed a significant effect (nested ANOVA P=0.000) on dive duration. Dive time increased with decreasing water temperature, with the line of least-squares regression having a slope of –12.453 s °C^{-1} and R^2=0.969 (Fig. 10).

DISCUSSION

A backswimmer's dive can be divided into three phases (P1–3, Fig. 8). P1: the dive begins with the backswimmer positively buoyant and it must swim to remain submerged. During this phase the insect's buoyancy gradually decreases monotonically. P2: the 'neutrally buoyant phase' follows, during which the backswimmer floats almost weightless in the water column. P3: in the final phase, a rapid drop from neutral to negative buoyancy causes the backswimmer to surface and repeat the cycle (Miller, 1966). Measurements of air-store P_{O_2} showed the same, three-phase pattern, where a steep, constant decline in air-store oxygen is followed by an extended stable period before a rapid, final drop. This pattern can be explained by the oxygen-binding characteristics of the backswimmer's haemoglobin. At the beginning of every dive the insect's respiration consumes oxygen largely from within the air-store alone, causing a constant decline in P_{O_2} (P1). Eventually, decreasing P_{O_2} causes the haemoglobin to unload its oxygen. This oxygen supplements the oxygen being consumed from the air-store, thus reducing the rate at which P_{O_2} declines (P2). But once bound oxygen becomes depleted, the P_{O_2} drops for a final time and the air-store becomes severely hypoxic (P3). The P_{O_2} traces from CO-exposed bugs had none of these phases and instead showed a continuous asymptotic decline in P_{O_2} (Fig. 7).

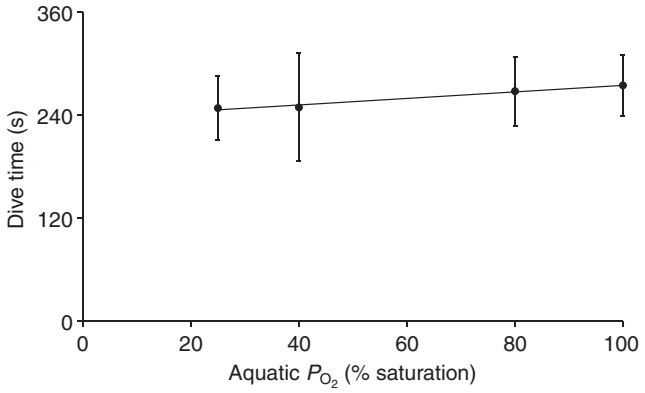


Fig. 9. Relationship between aquatic P_{O_2} on voluntary dive time at 20°C. Line shows least-squares regression. Error bars indicate 95% CI of mean dive times. *N*=8 backswimmers per treatment, six dives recorded from each animal (*N*=32 in total).

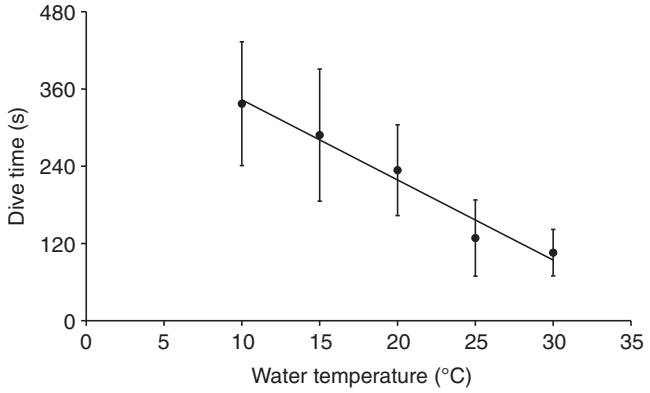


Fig. 10. Effect of water temperature on voluntary dive time. Line shows least-squares regression (–12.45°C+467.8). Error bars indicate 95% CI, *N*=8 backswimmers per treatment, six dives recorded from each animal (*N*=40 in total).

There are important differences between voluntary dives of free backswimmers and the pattern of changes in buoyancy and P_{O_2} in tethered dives, when the insects were inactive. The relative durations of the three phases of a dive must be affected by changes in the backswimmer's activity during a dive. Under natural conditions, insects must swim to remain submerged during the positive buoyancy of P1. This activity would increase their respiration rate and reduce the duration of this phase. However, the duration of P2 would not be expected to change, as swimming activity is greatly reduced. The third phase involves activity in both cases, but free insects are able to reach the surface within a few seconds.

Dive durations of forcibly submerged backswimmers were affected both by the method of submergence and by the method used to define surfacing activity. Animals glued to wire and held submerged on a magnet began actively attempting to surface after 490±58 s (control) and 372±57 s (CO treated). In comparison, bugs glued to microscope slides were considered to 'surface' once the P_{O_2} in their air-stores reached 2 kPa. By this determination control dives lasted 510±94 s and only 330±120 s following exposure to CO. Both methods showed reduced dive durations following CO exposure. However, as shown by the large confidence intervals, the variability of the air volumes carried by forcibly submerged insects resulted in variable surfacing times.

Air-store volume and buoyancy

The air-store carried by backswimmers serves as both an oxygen reserve and a buoyancy device. For a bubble of air to allow a backswimmer to become neutrally buoyant, (a) it must have a constant volume to confer a stable buoyancy, and (b) the stable volume must produce a buoyant force that is matched to the backswimmer's weight. These conditions cannot occur in a simple air bubble, as the constant decline in P_{O_2} caused by the insect's respiration would cause the bubble's volume and buoyancy to drop continuously. Backswimmers temporarily overcome this problem as oxygen is released from their haemoglobin cells in response to declining P_{O_2}. This stabilises the P_{O_2} and, therefore, the volume of their air-store. But because haemoglobin only responds to P_{O_2} and not to the total volume of the air-store, it alone cannot guarantee that the stable volume will confer neutral buoyancy. For this to occur, the insect must collect a specific volume of air so that neutral buoyancy coincides with the P_{O_2} necessary to cause the haemoglobin to unload its oxygen. This constrains the volume of air that a backswimmer can collect from the surface.

The water-saturated air above the surface of a pond at 20°C is 20.5% oxygen, corresponding to a partial pressure of 20.77 kPa at 1 atm (101.3 kPa). Therefore, an insect's respiration can reduce the volume of a newly refilled air-store by a maximum of 20.5%, assuming all oxygen is consumed. Consequently, for a positively buoyant air-store to be reduced to neutral buoyancy requires that the initial air-store volume is less than 20.5% larger than that required for neutral buoyancy, as this reduction in volume would reduce the P_{O_2} of the air-store to 0, thus making the dive unsustainable. The exact volume of air collected at the surface must be matched to the oxygen-binding properties of the backswimmer's haemoglobin, as the haemoglobin stabilises the air-store's volume over a specific range of P_{O_2} values. The plateau phase (P2) of the air-store begins at a P_{O_2} of 5 kPa and ends at 2 kPa. During this phase the haemoglobin readily unloads its oxygen into the air-store, maintaining the P_{O_2} of the middle two-thirds of P2 at 3.79±0.16 kPa. Assuming that the initial air-store began with a P_{O_2} of 20.77 kPa, and occupied 20.5% of the initial air-store volume, then these P_{O_2} changes at the beginning, middle and end of the plateau correspond to decreases in initial air-store volume of 16.0%, 17.2% and 19.0%, respectively. From this it can be seen that an initial air-store volume about 17% larger than that required for neutral buoyancy would be necessary to result in neutral buoyancy at the P_{O_2} levels at which haemoglobin releases its oxygen. Because of the relatively high oxygen affinity of the haemoglobin, it is therefore necessary for the bug to be positively buoyant for a considerable period at the beginning of a dive.

The mean volume of air collected by free-diving bugs was 6.7±4.2% larger than that required for neutral buoyancy (Fig. 4), compared with the forcibly submerged insects, which were calculated to begin the dive with a mean air-store volume 31.5±7.3% larger than that required for neutral buoyancy (Fig. 5). The air volumes collected by free-swimming backswimmers provide an insight into the behavioural mechanisms of buoyancy control used by these insects. The measured volume of air did not always equate to the initial volume, as the backswimmers usually tried to escape being captured in kerosene after diving and consequently must have decreased the volume of their air-stores by consuming oxygen. Since this was unavoidable, the largest air volumes (i.e. 16%–19%) are the most representative of the initially collected volumes of air. This supports the assertion that backswimmers should select initial air volumes approximately 17% larger than neutral buoyancy. This is further supported by the measured range of air-store volumes collected from free-diving backswimmers. Assuming all backswimmers collected a volume of air 17% larger than that required for neutral buoyancy and were then caught after consuming varying proportions of their oxygen stores (from none to all), then the volumes should vary around neutral buoyancy over a range equivalent to the initial 20.5% volume of oxygen in the air-store, i.e. from 17% above neutral buoyancy to 3.5% below it. This is comparable to the actual range of air volumes, which varied from 19.4% above neutral buoyancy to 4.9% below (Fig. 4).

Gas exchange with the surrounding water

A bubble of air held under water dissolves due to the hydrostatic pressure of the water increasing the partial pressures of the gases it contains above those in the surrounding water. If respiration is reducing the P_{O_2} within this bubble, then the partial pressure of nitrogen (P_{N_2}) increases according to Dalton's law, further increasing the gradient driving nitrogen loss. As backswimmers control their buoyancy by regulating the volume of their air-store, any volume change due to this nitrogen diffusion must be minimised. Unlike other diving insects that can use their air-stores as gills (e.g. Ege, 1915; Vlasblom, 1970), backswimmers achieve this by limiting the area of their air-store in contact with the surrounding water. Not only are the grooves containing the air-store narrow, but also they are fringed with hydrophobic hairs, which arrange themselves across the air–water interface (Fig. 2), further reducing the area available for diffusion. The efficiency of this arrangement in limiting diffusion is evident when examining the effect of aquatic P_{O_2} on dive duration. Dive duration was not significantly affected by P_{O_2} (Fig. 9), indicating minimal exchange of oxygen between the air-store and water. Similar experiments by Miller, Vlasblom, and Wells and colleagues support this finding (Miller, 1966; Vlasblom, 1970; Wells et al., 1981). As the Krogh's diffusion coefficient for nitrogen in water at 20°C is 2.19 times smaller than that for oxygen (Rahn and Paganelli, 1968), it is concluded that the volume of nitrogen lost during an average 4 min dive in air-equilibrated water is inconsequential.

Oxygen store volume

The only oxygen available to a submerged backswimmer is what it carries from the surface in its air-store and that bound to its haemoglobin. The volume of oxygen contributed by each of these two stores, as well as the backswimmer's oxygen consumption rate ($\dot{V}_{O_2}$), can be determined from the P_{O_2} traces. This requires that the respiration rates of the inactive backswimmers are constant during

Table 2. Backswimmer $\dot{V}_{O_2}$ and effective oxygen contributions of the air-store and haemoglobin calculated from P_{O_2} traces

	Mean	95% CI	*N*
$\dot{V}_{O_2}$ (µl h^{-1})	3.8	0.8	15
Calculated time to reach 2 kPa without Hb (s)	260	50	15
Time to reach 2 kPa with CO treatment (s)	330	120	6
Time to reach 2 kPa with Hb (s)	510	94	15
Difference in time to reach 2 kPa with and without Hb (s)	250	55	15
V_{O_2a} (µl)	0.26	0.02	15
V_{O_2Hb} (µl)	0.25	0.06	15
Ratio of V_{O_2a} to V_{O_2Hb}	1.2	0.3	15

CI, confidence interval; $\dot{V}_{O_2}$, oxygen consumption rate; V_{O_2a}, effective oxygen content of air-store; V_{O_2Hb}, effective oxygen content of haemoglobin.

measurement and any diffusion of oxygen into the air-store from the surrounding water is insignificant. Because the volume of air held by the submerged backswimmers was unknown during P_{O_2} measurement, it was approximated from the mass-specific mean initial air-store volume of forcibly submerged insects (114.01 µl g^{-1}). Under these conditions the constant respiration of the insect would cause the P_{O_2} of the air-store to drop steadily to 0 kPa. The time (t, h) taken to reach 0 kPa is:

$$t = \frac{P_{O_2}}{\dot{P}_{O_2}}, \quad (5)$$

where P_{O_2} is the initial air-store oxygen tension (kPa), and $\dot{P}_{O_2}$ is the rate of P_{O_2} change (kPa h^{-1}) obtained from the first 20 s of P_{O_2} measurement. The initial air-store volume (V_a, µl) includes an amount of oxygen (V_{O_2}) equivalent to:

$$V_{O_2} = V_a \frac{P_{O_2}}{P_B}. \quad (6)$$

It follows, then, that the backswimmer's $\dot{V}_{O_2}$ (µl h^{-1}) is:

$$\dot{V}_{O_2} = \frac{V_{O_2}}{t}. \quad (7)$$

The mean volume of oxygen in the air-store was 0.28±0.02 µl, while the mean predicted time taken to consume it was 301±52 s, giving a mean $\dot{V}_{O_2}$ of 3.84 µl h^{-1}. This is in close agreement with the mean predicted metabolic rate of a typical arthropod at rest (2.89 µl h^{-1}) calculated as $\dot{V}_{O_2}$=127.7$M_b^{0.853}$ at 20°C, where M_b is body mass (g) (Lighton et al., 2001). The greatest potential error in these calculations arises from the approximation of the initial volume of air carried by the backswimmer.

The oxygen carried by a backswimmer within its air-store and haemoglobin is never completely exhausted during a dive, because low P_{O_2} stimulates the insect to surface before its air-store becomes completely anoxic. The amount of oxygen consumed before the air-store P_{O_2} drops below this hypoxia threshold is the effective oxygen supply. The threshold was designated as 2 kPa because this P_{O_2} coincided with an increase in activity of the tethered backswimmers and is also the critical P_{O_2} of many insects – the point at which the resistance of their tracheal system begins to limit the rate of oxygen diffusion and they can no longer maintain a constant $\dot{V}_{O_2}$ (Greenlee and Harrison, 2004). The time taken for the P_{O_2} trace to reach 2 kPa (Fig. 8, point b) is the sum of the time taken to consume the effective oxygen supply of both the air-store and haemoglobin individually. In the absence of haemoglobin the time taken to consume the air-store's oxygen alone would be proportional to the backswimmer's $\dot{V}_{O_2}$. Therefore, the time taken to consume the air-store's effective oxygen supply was found by extrapolating the initial rate of P_{O_2} decline to 2 kPa (Fig. 8, point c). This time was then multiplied by the previously calculated $\dot{V}_{O_2}$ to determine the effective oxygen content of the air-store (V_{O_2a}; Table 2). The difference in the time taken to reach point b and C (t) is equivalent to the length of time the dive is sustained by oxygen released from the haemoglobin. Thus the effective oxygen contribution of haemoglobin (V_{O_2Hb}; Table 2) is found by:

$$V_{O_2Hb} = \dot{V}_{O_2} t. \quad (8)$$

Thus the air-store and haemoglobin possess virtually equal oxygen stores of 0.26±0.02 and 0.25±0.06 µl, respectively. A two-tailed t-test revealed no significant difference (P=0.814) between them. However, the oxygen contribution of the air- store is likely to be an over-estimation because the measurements were made on insects that were forcibly submerged, causing them to carry larger volumes of air than if they had been free to surface and fill their air-store. The mean air volume collected from freely submerging backswimmers was 32% smaller than the volume carried by forcefully submerged insects. An air-store of this size would contribute only 42% of the total oxygen respired during a dive, the remaining 58% being supplied by the haemoglobin. This suggests that under natural conditions the backswimmers rely more on the oxygen supplied by their haemoglobin to sustain their respiration while submerged and less on their air-stores. Miller estimated that *Anisops pellucens* derived up to 75% of the oxygen consumed during a dive from haemoglobin, 25% from the air-store, and a negligible amount from the surrounding water (Miller, 1966).

LIST OF ABBREVIATIONS

P_{N_2}	nitrogen partial pressure
P_{O_2}	oxygen partial pressure
$\dot{P}_{O_2}$	rate of P_{O_2} change within the air-store
STP	standard temperature and pressure
V	volume
V_{air}	volume of air carried by a backswimmer
V_{O_2a}	effective oxygen content of air-store
V_{O_2Hb}	effective oxygen content of haemoglobin
$\dot{V}_{O_2}$	rate of oxygen consumption
W_1	insect's weight in air
W_2	insect's submerged weight (measured)
W_i	insect's submerged weight (calculated)
W_{tot}	total submerged weight of backswimmer with attached wire and glue
W_w	submerged weight of wire and glue
ρ_{H_2O}	density of water
ρ_b	backswimmer's body density

REFERENCES

Andersen, N. M. and Weir, T. A. (2004). *Australian Water Bugs: Their Biology and Identification*, 344pp. Collingwood, Victoria: CSIRO Publishing.

Bailey, P. C. E. (1987). Abundance and age-specific spatial and temporal distribution in two waterbug species, *Anisops deanei* (Notonectidae) and *Ranatra dispar* (Nepidae) in three farm dams in South Australia. *Oikos* **49**, 83-90.

Baker, G. M. and Wright, E. A. (1977). Effects of carbon monoxide on insects. *Bull. Environ. Contam. Toxicol.* **17**, 98-104.

Ball, E. G., Strittmatter, C. F. and Cooper, O. (1951). The reaction of cytochrome oxidase with carbon monoxide. *J. Biol. Chem.* **193**, 635-647.

Bare, C. O. (1929). Haemoglobin cells and other studies of the genus Buenoa (Hiemiptera, Notonectidae). *Univ. Kansas Sci. Bull.* **18**, 265-349.

Bergtrom, G. (1977). Partial characterisation of haemoglobin of the bug, *Buenoa confusa*. *Insect Biochem.* **7**, 313-316.

Bergtrom, G., Gittelman, S., Laufer, H. and Ovitt, C. (1976). Haemoglobin synthesis in *Buenoa confusa* (Hemiptera). *Insect Biochem.* **6**, 595-600.

Ege, R. (1915). On the respiratory function of the air-stores carried by some aquatic insects (Corixidae, Dytiscidae, Notonecta). *Z. Allg. Physiol.* **17**, 81-125.

Greenlee, K. J. and Harrison, J. F. (2004). Development of respiratory function in the American locust *Schistocerca americana* I. across-instar effects. *J. Exp. Biol.* **207**, 497-508.

Harvey, W. R. and Williams, C. M. (1958). Physiology of insect diapause. XII. The mechanism of carbon monoxide-sensitivity and-insensitivity during the pupal diapause of the Cecropia silkworm. *Biol. Bull.* **114**, 36-53.

Hungerford, H. B. (1922). Oxyhaemoglobin present in the backswimmer, *Buenoa margaritacea Bueno. Can. Entomol.* **54**, 262-263.

Lighton, J. R. B., Brownell, P. H., Joos, B. and Turner, R. J. (2001). Low metabolic rate in scorpions: implications for population biomass and cannibalism. *J. Exp. Biol.* **204**, 607-613.

Matthews, P. G. D. and Seymour, R. S. (2006). Diving insects boost their buoyancy bubbles. *Nature* **441**, 171.

Miller, P. L. (1964). Possible function of haemoglobin in *Anisops*. *Nature* **201**, 1052.

Miller, P. L. (1966). The function of haemoglobin in relation to the maintenance of neutral buoyancy in *Anisops pellucens* (Notonectidae, Hemiptera). *J. Exp. Biol.* **44**, 529-543.

Osmulski, P. A., Vossbrinck, C. R., Sampath, V., Caughey, W. S. and Debrunner, P. G. (1992). Spectroscopic studies of an insect hemoglobin from the backswimmer *Buenoa margaritacea* (Hemiptera, Notonectidae). *Biochem. Biophys. Res. Comm.* **187**, 570-576.

Rahn, H. and Paganelli, C. V. (1968). Gas exchange in gas gills of diving insects. *Respir. Physiol.* **5**, 145-164.

Scholander, P. F. and Evans, H. J. (1947). Microanalysis of fractions of a cubic millimeter of gas. *J. Biol. Chem.* **169**, 551-560.
Thorpe, W. H. and Crisp, D. J. (1947). Studies on plastron respiration. II. the respiratory efficiency of the plastron in Aphelocheirus. *J. Exp. Biol.* **24**, 270-303.
Vincent, J. F. V. and Wegst, U. G. K. (2004). Design and mechanical properties of insect cuticle. *Arthropod Struct. Dev.* **33**, 187-199.
Vlasblom, A. G. (1970). The respiratory significance of the physical gill in some adult insects. *Comp. Biochem. Physiol.* **36**, 377-385.
Vossbrinck, C. R., Osmulski, P. A. and Debrunner, P. G. (1993). Characterization of hemoglobin from the backswimmer *Buenoa margaritacea* (Hemiptera: Notonectidae) reveals high electrophoretic heterogeneity. *Insect Biochem. Mol. Biol.* **23**, 421-429.
Weber, R. E. and Vinogradov, S. N. (2001). Nonvertebrate hemoglobins: functions and molecular adaptations. *Physiol. Rev.* **81**, 569-628.
Wells, R. M. G., Hudson, M. J. and Brittain, T. (1981). Function of the hemoglobin and the gas bubble in the backswimmer *Anisops assimilis* (Hemiptera: Notonectidae). *J. Comp. Physiol.* **142**, 515-522.