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RESEARCH ARTICLE

The diving bell and the spider: the physical gill of *Argyroneta aquatica*

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SUMMARY

***Argyroneta aquatica* is a unique air-breathing spider that lives virtually its entire life under freshwater. It creates a dome-shaped web between aquatic plants and fills the diving bell with air carried from the surface. The bell can take up dissolved O_2 from the water, acting as a 'physical gill'. By measuring bell volume and O_2 partial pressure (P_{O_2}) with tiny O_2-sensitive optodes, this study showed that the spiders produce physical gills capable of satisfying at least their resting requirements for O_2 under the most extreme conditions of warm stagnant water. Larger spiders produced larger bells of higher O_2 conductance (G_{O_2}). G_{O_2} depended on surface area only; effective boundary layer thickness was constant. Bells, with and without spiders, were used as respirometers by measuring G_{O_2} and the rate of change in P_{O_2}. Metabolic rates were also measured with flow-through respirometry. The water–air P_{O_2} difference was generally less than 10 kPa, and spiders voluntarily tolerated low internal P_{O_2} approximately 1–4 kPa before renewal with air from the surface. The low P_{O_2} in the bell enhanced N_2 loss from the bell, but spiders could remain inside for more than a day without renewal. Spiders appeared to enlarge the bells in response to higher O_2 demands and lower aquatic P_{O_2}.**

Key words: arachnid, gas exchange, metabolic rate, dissolved O_2, aquatic, bubble.

INTRODUCTION

The Eurasian diving bell spider *Argyroneta aquatica* (Clerck 1757) (Cybaeidae) is a unique spider that is said to live its entire life underwater. Their book lungs and tracheal system utilize air that is trapped at the surface of the water by hydrophobic hairs on the abdomen (opisthosoma) and the ventral side of the cephalothorax (prosoma) (Fig. 1A) (Levi, 1967; Messner and Adis, 1995). Moreover, they construct silk webs on underwater vegetation and create an air-filled bubble, the 'diving bell' (Fig. 1B). The air is held by surface tension between the silk fibres, and the chamber is open on the bottom. Smaller diving bells accommodate the abdomen only, but larger ones allow the whole spider to move in and out through the bottom. They fill the bell by sequentially carrying large bubbles from the surface, held with the abdomen and fine hairs on the rear legs (Fig. 1C). When moving free of the diving bell, however, the airspace on the body surface is thin (Fig. 1A). The spiders consume aquatic invertebrates and small fish, progress through many moults and, when adults reach approximately 50–100 mg in mass, lay eggs in a cocoon inside the diving bell (Fig. 1D) (Schütz and Taborsky, 2003). The hatched spiders (0.5 mg) are independent and, after spending a few days near the cocoon, emerge and construct tiny diving bells of their own. Surprisingly little is known about the gaseous conditions inside the bell, and in particular how much gas exchange occurs across the walls.

Previous experiments of Schütz et al. were designed to determine whether behavioural changes could be elicited by replacement of diving bell gas with either pure CO_2 or pure O_2 (Schütz et al., 2007). Not surprisingly, pure CO_2 caused marked activity involving surfacing, gas replenishment and diving bell building, but there was no significant effect of O_2 replacement. Masumoto et al. observed the distribution of *A. aquatica* in a pond in Japan and found that the spiders seemed to prefer areas where sphagnum occurred in association with low pH and low partial pressure of O_2 (P_{O_2}), in the region of 0.7–2.3 kPa (Masumoto et al., 1998a). In another study, Masumoto et al. carried out continuous observations of individual spiders in aquaria over several days (Masumoto et al., 1998b). Five spiders renewed the diving bell approximately 2.6 times every hour, on average, but dissolved O_2 levels were not associated with the observations. Schütz and Taborsky found that female spiders tended to build larger diving bells than males and that size of the bell was positively related to female size, but not male size (Schütz and Taborsky, 2003). They reported that the spiders surfaced approximately 1.4 times each hour, but the temperature and P_{O_2} of the water were not recorded. Messner and Adis studied the microstructure of the hydrophobic hairs of the abdomen of *A. aquatica* that contain the layer of air (Messner and Adis, 1995). However, it is not clear whether these hairs can support a true plastron gas exchanger that prevents collapse and permits unlimited respiration underwater. Although the authors implied that plastron breathing may be possible, the surface of the air layer on the body is not sufficient to satisfy their requirements completely. So they proposed that the spider must regularly surface to renew the air during warm summer months, but can remain continuously submerged in the diving bell during cold winter months. The idea that emerges from these studies is that *A. aquatica* relies primarily on air brought from the surface, and gas transfer from the water to the diving bell does not meet their needs, especially at higher temperatures.

An underwater bubble can exchange gases with the water. Ege showed that some aquatic insects were capable of using the bubbles that they took down with them as a 'physical gill' (distinct from an anatomical gill) (Ege, 1915). Gas exchange across the bubble wall

could supply much more O_2 than the original bubble contained, a function recognised by subsequent authors (De Ruiter et al., 1951; Rahn and Paganelli, 1968; Vlasblom, 1970). Because O_2 is consumed by the organism and the CO_2 produced dissolves quickly into the water, the partial pressure of N_2 (P_{N2}) must rise according to Dalton's law, which creates an outward N_2 gradient. In addition, the gases in the bubble are subjected to hydrostatic pressure from the external water and an added pressure due to surface tension in the curved air–water interface. Thus, an unsupported bubble must shrink, which results in a limited lifetime. Rahn and Paganelli modelled exchange of O_2, N_2 and CO_2 across a virtual bubble of diving insects, and concluded that O_2 from the water could potentially supply eight times the amount in the bubble, before collapsing completely (Rahn and Paganelli, 1968).

The effectiveness of the physical gill of *A. aquatica* has not been determined, although there have been studies on gas exchange in normally terrestrial spiders that are flooded. In the first quantitative studies on physical gill function in any spider, Rovner demonstrated that survival depended on O_2 uptake from the water (Rovner, 1987). Hebets and Chapman found that some whip spiders have a proper plastron on their ventral surface that leads to the opening of their book lungs (Hebets and Chapman, 2000). Behavioural studies indicated that these spiders could remain underwater indefinitely, exchanging gas directly through the surface of the plastron. Pétillon et al. showed that certain spiders could survive 24–36 h if submerged acutely without air-filled webs and several days in a bubble surrounded by web (Pétillon et al., 2009).

The aim of this investigation was to determine the effectiveness of the diving bell as a physical gill. Gas transfer across the surface of the bubble can be modelled according to the Fick general diffusion equation, $\dot{M}_{O2}=G_{O2}(P_{O2out}–P_{O2in})$, where $\dot{M}_{O2}$ is the rate of oxygen transfer (μmol h^{-1}), G_{O2} is the diffusive O_2 conductance (μmol h^{-1} kPa^{-1}) and $P_{O2out}–P_{O2in}$ is the difference in P_{O2} (kPa) between the water and the internal air. Unlike diving insects with physical gills that cannot be separated from the body, the diving bell of *A. aquatica* can be conveniently separated every time the spider leaves. Therefore, G_{O2} can be calculated from measurements of the P_{O2} difference across the wall of an empty bell, the rate of internal P_{O2} change and the gas volume. This is accomplished using O_2-sensitive fibre-optic probes to measure P_{O2}, and injection of pure O_2 or N_2 into the chamber to determine the original volume by dilution. Once G_{O2} is known, it can be used to evaluate the $\dot{M}_{O2}$ of the spider when it enters the diving bell. The $\dot{M}_{O2}$ of the spider equals the rate of O_2 transfer through the chamber wall plus the rate of change in O_2 content of the bell, which may be either positive or negative. In this way, it is possible to determine the relationships between spider size, bubble volume and P_{O2} difference that would be required to support the metabolic demands of the species purely by diffusion from the water. The difference depends, of course, on the P_{O2} in the external water and the tolerance of the spider to low P_{O2} in the diving bell. Natural aquatic P_{O2} values are assessed from the literature and the tolerance to low P_{O2} can be measured by correlating bubble-renewal behaviour with P_{O2}.

MATERIALS AND METHODS

Specimen collection and experimental conditions

Argyroneta aquatica were collected at the edges of the Eider River near Mielkendorf (54°17′19.4″N, 10°0′49.3″E) and the Eider Ringkanal near Flemhude (54°19′19″N, 9°58′5″E) in northern Germany in June and July 2010. They were taken in containers to the Humboldt University for experimentation. The spiders were kept in holding aquaria and fed *Daphnia* and *Chironomus* larvae, which they readily consumed. They grew under these conditions and several shed their exoskeletons. Two females created cocoons within diving bells.

Experimental spiders were placed in either a four-compartment acrylic aquarium or glass jars. A few strands of water weed (*Elodea*) were placed near the surface of the water to encourage the spiders to create diving bells. The *Elodea* did not greatly affect the oxygen concentration in the water because the photosynthetic flux generated by the illumination in the lab was very low [photosynthetically active radiation (PAR) ~15 μmol s^{-1} m^{-2}; LI-190SB, LI-COR, Lincoln, NB, USA]. The water was regularly replaced with aged tap water from a large aquarium. Because we wanted to measure gas exchange under the most unfavourable conditions for respiration, the water in the chambers was stagnant for at least 18 h, enough time to create a boundary layer around the diving bell. Because convection of the water was not desired, the experiments were run without thermocirculators at room temperature [mean ± 95% confidence intervals (CI)=25.4±1.3°C]. The aquatic P_{O2} levels were therefore 'quasi-stable', being influenced very slowly by a combination of respiration and photosynthesis of aquatic organisms. However, we were able to create a broad range of P_{O2} by bubbling N_2 or O_2 into the water at least 18 h before measurements.

Oxygen measurements

Measurements of P_{O2} were made with O_2-sensitive micro-optodes with tip sizes <50 μm (sensor model PSt1, meter model TX-3 with thermal compensation, Precision Sensing GmbH, Regensburg, Germany). Optodes have the great advantage over O_2 electrodes because they do not consume O_2, do not create boundary layers in unstirred water and have a short t_{90} response time of <6 s. Two optodes could be used simultaneously by recording the outputs of Presens software on a PC, *via* a USB hub, at 1 s intervals. The optodes were calibrated with pure N_2 and atmospheric air, and P_{O2} was calculated in units of kPa from the output of percent air saturation, atmospheric pressure and saturated water vapour pressure. O_2 concentration in the bubble was calculated in mol l^{-1} at measured temperature and pressure. The volume of the diving bell was measured by injection of 50 or 100 μl of pure O_2 or pure N_2 delivered by a 100 μl gas-tight syringe (model 1710, Hamilton, Bonaduz, Switzerland). Volume was calculated by dilution from the P_{O2} values before and after addition of the gas.

The optodes were supported by three-dimensional micromanipulators positioned above the experimental chambers. After the spiders had created diving bells, the optodes were used to measure P_{O2} of the water 5–10 mm away from the bubble and within the bubble itself. The spiders often reacted to piercing of the top of the bell, but did not respond to the optode thereafter. The usual protocol was to measure aquatic P_{O2}, insert the optode into the bell and monitor P_{O2} changes with the spider present for periods typically ranging from 20 to 50 min. Then the spider either voluntarily left the bell or was encouraged to leave by touching it with a thin wire through the bottom opening, and P_{O2} changes were again measured. During the measurement periods, aquatic P_{O2} changed negligibly. Finally, gas volume was measured. Diving bell G_{O2} was calculated from the slope of the P_{O2} curve without the spider present, according to the Fick diffusion equation. ΔP_{O2} was taken as the difference between aquatic P_{O2} approximately 5 mm away from the bell and the mean P_{O2} inside the bell during the duration of the measurement. With the spider present, the difference in the slope of the P_{O2} curve could then be used to calculate the consumption by the spider. This procedure involved calculating the

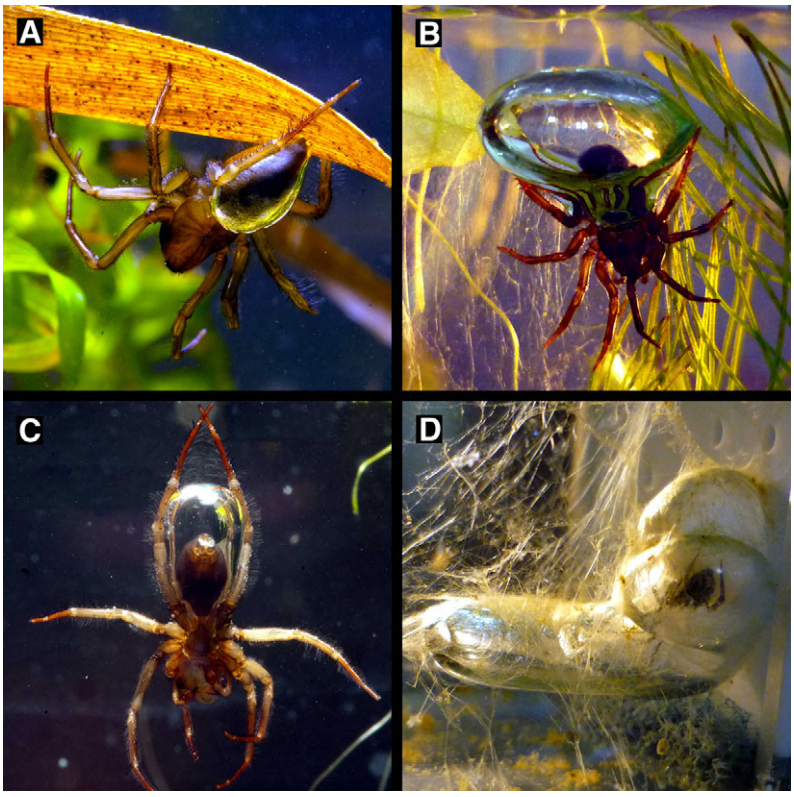

Fig. 1. *Argyroneta aquatica* with its physical gill. (A) Air clinging to the hydrophobic hairs on the abdomen of a spider away from the diving bell. (B) A small diving bell, supported by invisible web, large enough to admit the abdomen only. (C) A large bubble, captured at the surface and held on the abdomen and rear legs, is carried down to the diving bell. (D) A female in her diving bell, below the cocoon, showing the lateral extension of volume and surface area.

rate of O_2 uptake through the bell wall ($\mu mol\,h^{-1}$) from G_{O_2} and ΔP_{O_2} and the rate of change in bell O_2 content ($\mu mol\,h^{-1}$) from the ideal gas law and bell volume. To estimate the effect of adding the spider's volume to bell volume on G_{O_2}, 14 bells made by 12 spiders were analysed by adding a volume equivalent to the spider's mass to the empty bell and calculating the change in G_{O_2}. G_{O_2} increased by 7.6% (95% CI=±2.2%) and, together with the thin layer of air on the surface of the spider, it was estimated that G_{O_2} increased by approximately 10% with the spider in the bell. This correction was used to calculate $\dot{M}_{O_2}$.

A series of measurements was undertaken to determine the threshold for ventilation of the diving bell by the spiders. The diving bell P_{O_2} at which undisturbed spiders voluntarily went to the surface to obtain fresh air was recorded as the threshold. One large spider that had not made a diving bell was also opportunistically studied by carefully admitting an optode into the abdominal air bubble shortly after renewal. The slope of the P_{O_2} line could be extrapolated back to the time of surfacing to determine initial P_{O_2} and the fraction of gas renewed on the abdomen. Unfortunately it was not possible on this occasion to measure the volume of gas attached to the spider.

Respirometry

The metabolic rates of the spiders used in our experiments were verified by a second method involving the measurement of the carbon dioxide release rate ($\dot{M}_{CO_2}$; $\mu mol\,h^{-1}$) in a flow-through respirometry system. Spiders that had been starved for at least 3 days were placed individually into 2 ml Eppendorf vials. The lid of each vial was equipped with a 2 mm hole to allow gas exchange. The bottom of the vial was filled with 0.25 ml of degassed, acidified, distilled water in order to prevent dehydration of the spiders. The vial was placed into a thermostatted respirometer chamber made of brass and the spiders were allowed to settle for at least 3 h. The chamber was kept constant at 25°C (95% CI=±0.1°C) by a custom-built Peltier thermostat. $\dot{M}_{CO_2}$ was measured with a differential infrared gas analyser (URAS 14, 0–100 ppm, ABB Analytical, Frankfurt, Germany) at a flow rate of $50\,ml\,min^{-1}$, as previously described (Hetz, 2007). Calibration was performed before and after each run with the calibration cuvettes included with the URAS. The spider's position in the vial was inspected visually. When the spiders had moved and changed their position, that part of the data set was not used to calculate mean resting $\dot{M}_{CO_2}$. Individual runs lasted between 3 and 5 h. Gas exchange in the resting spiders was continuous. $\dot{M}_{CO_2}$ dropped after 1 h and remained almost constant for the rest of the experiment. The last 2 h of the data were used to calculate the $\dot{M}_{O_2}$ by multiplying the mean $\dot{M}_{CO_2}$ by 1.41, the respiratory exchange ratio for fat metabolism in starved animals.

All spiders were dried of surface water with a tissue and weighed to 0.1 mg on an analytical balance (model 1201 MP2, Sartorius AG, Göttingen, Germany) within a day of other measurements.

Statistics

Statistics are presented as means ± 95% CI or standard errors of the slope (SE_b). Ordinary least-squares regressions on log-transformed data were used to fit power equations. Analysis of covariance (ANCOVA) was used to compare regressions (Zar, 1998).

RESULTS

Spiders readily adjusted to captivity and most created diving bells immediately in the top 5–10 cm of water. Diving bells were constructed on a framework of web between the sides of the aquaria and vegetation (Fig. 1B,D). Working from below, spiders first deposited web to the top and sides of the chamber by touching the spinnerets to the framework and building up a layer of web. They then went to the water surface, obtained a large bubble between the abdomen and their rear legs (Fig. 1C), and entered the chamber from

below. This enlarged the gas space, and a naked meniscus protruded from the bottom of the diving bell. Then webbing was placed around the lower sides of the chamber and the process was repeated. Bubbles taken from the surface were much larger than the gas layer on the abdomen and cephalothorax when the spider was otherwise moving normally underwater (*cf.* Fig. 1A,C).

Diving bell volume, gas conductance and boundary layer

Volume (V) and G_{O_2} were measured in 14 empty diving bells made by 11 spiders (Fig. 2). The equation relating the two variables is $G_{O_2}=0.075V^{0.665}$. Because the bells are roughly spherical, and the surface area of a sphere is related to its volume raised to the power of 0.67, it appears that G_{O_2} depends only on surface area (A), not thickness (L). G_{O_2} is proportional to A/L in a linear diffusion model. For a spherical model, the effective boundary layer thickness can be calculated from G_{O_2} (μmol h^{-1} kPa^{-1}), Krogh's coefficient of oxygen diffusion in water (K_{O_2}; μmol h^{-1} kPa^{-1} cm^{-1}), inner radius (r_i) and outer radius (r_o) of the boundary layer, according to eqn 8 in Seymour (Seymour, 1994). K_{O_2} at 25°C is taken as 1.04×10^{-3} μmol h^{-1} kPa^{-1} cm^{-1} according to data for pure water (Seymour, 1994), a value similar to that used by Rahn and Paganelli (Rahn and Paganelli, 1968). Thus, the calculated effective boundary layer in stagnant water around the 14 diving bells averaged 0.83 mm (95% CI=±0.11 mm).

Respiration rate

At the mean temperature of 25°C, $\dot{M}_{O_2}$ (μmol h^{-1}) of *A. aquatica* increased with body mass (M; g) according to the allometric equation $\dot{M}_{O_2}=6.61M^{0.897}$ (Fig. 3). $\dot{M}_{O_2}$ measured indirectly from spiders in diving bells was slightly higher than values from more direct flow-through respirometry (ANCOVA, with Bonferroni adjustment, P=0.048). We do not know the reason for the small but significant discrepancy. Our allometric equation is similar to that derived from several species of spider by Terblanche et al. (Terblanche et al., 2004), $\dot{M}_{O_2}=5.75M^{0.817}$; 25 mg spiders consume 0.24 μmol h^{-1} with our equation and 0.28 μmol h^{-1} with theirs. Our data are also similar to oxygen uptake in the range of 0.17 to 0.27 μmol h^{-1} in two species of spider, *Pardosa lugubris* (Lycosidae) and *Marpissa muscosa* (Salticidae), weighing between 22 and 30 mg (Schmitz, 2004).

Diving bell volume and spider size

Argyroneta aquatica constructed diving bells of widely variable volumes. Small ones permitted the spider to admit only the abdomen, with the thorax and legs in the water (Fig. 1B). Others could accommodate the entire animal. The volumes of all web-supported diving bells are shown in Fig. 4. With knowledge of the relationship between volume and G_{O_2} (Fig. 2), and an allometric equation for spider metabolic rate (Fig. 3), one can calculate the isobars for the difference in P_{O_2} between the water and the chamber when $\dot{M}_{O_2}$ of the spider equals the instantaneous $\dot{M}_{O_2}$ through the bell wall (Fig. 4). As a spider grows and increases its metabolic rate, a larger diving bell would be necessary to maintain a constant P_{O_2} difference across the wall. It appears that the bells made during our study would produce differences from below 5 kPa to approximately 20 kPa in stagnant water. This aligns well with observations of the direction of change in P_{O_2} in the bells of different resident spiders, depending on external P_{O_2}, bell size and spider mass. Some smaller occupied bells produced decreasing P_{O_2}, indicating that the physical gill was not keeping up with metabolic rate; larger bells showed the opposite.

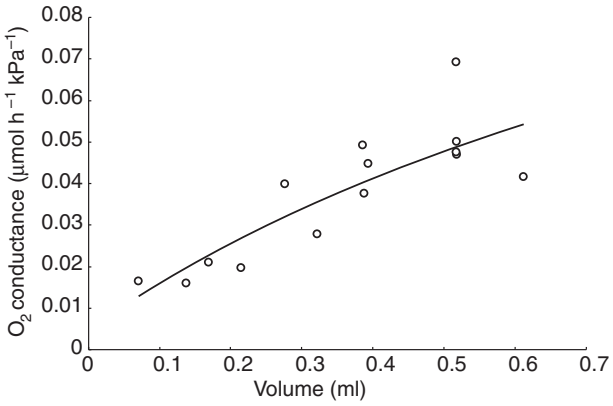

Fig. 2. Relationship between volume (V) and O_2 conductance (G_{O_2}) in unoccupied diving bells of *A. aquatica*. The power equation is: $G_{O_2}=0.075V^{0.665}$ (R^2=0.79, SE_b=0.10, N=14 diving bells).

Threshold for air renewal

Records were obtained for the minimum P_{O_2} that caused refreshing of the diving bell with air from the surface. Thirteen renewal events from two spiders averaged 2.3 kPa (95% CI=±0.6 kPa). Four other spiders did not return to the surface at all when P_{O_2} was above 2.0–4.6 kPa. These results show that resting spiders tolerate a minimum P_{O_2} below 5 kPa. One female with a cocoon in the diving bell renewed the air when P_{O_2} dropped to 3.5 kPa (Fig. 5A), and another with a cocoon renewed at 6.8 kPa. It is noteworthy that $\dot{M}_{O_2}$ appears to become diffusion limited at P_{O_2} below approximately 4 kPa, because the trace starts to curve upward (Fig. 5B).

Changes in P_{o_2} of the abdominal bubble

It was generally not possible to measure the volume or P_{O_2} in the abdominal bubbles of spiders outside of the bell because the air film was very thin and the spiders moved away when touched. However, we were successful with one large (103 mg) female spider and measured the rate of decrease in P_{O_2} after two trips to the surface (Fig. 5B). The rate of P_{O_2} decrease was –0.80 kPa min^{-1} with a P_{O_2} difference of up to 8 kPa. By extrapolating the linear decrease to the time of renewal, it was possible to determine the fraction of gas

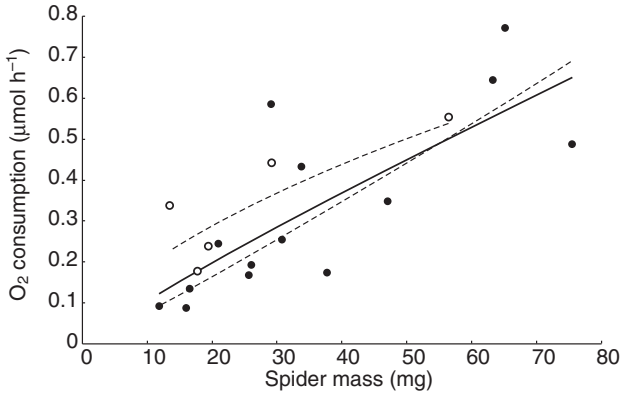

Fig. 3. Rates of oxygen consumption (μmol h^{-1}) by inactive *A. aquatica* at 25°C measured directly by flow-through respirometry (closed circles) and indirectly by P_{O_2} changes, O_2 conductance and volume measurements (open circles). An allometric equation is set to all data points in relation to body mass (M; g): $\dot{M}_{O_2}=6.61M^{0.897}$ (R^2=0.57, SE_b=0.19, N=18 individuals). The dashed curves are power equations set to the two data sets.

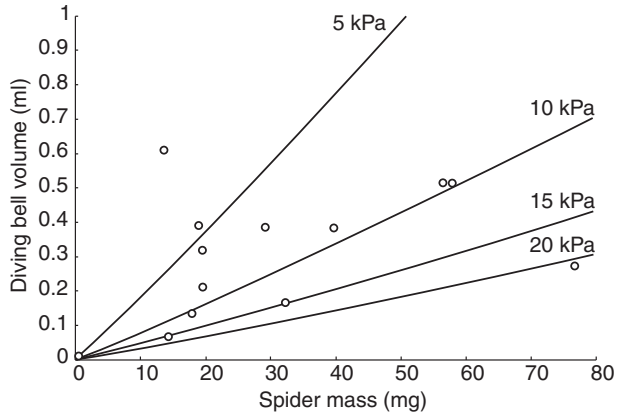

Fig. 4. Volume of diving bells made by 12 *A. aquatica* spiders in relation to their mass. Volume was measured by injected gas dilution, except for the smallest spider (near the origin) for which it was estimated from diameter. The lines are predicted P_{O_2} differences between the water and chamber air, based on the relationship between V and G_{O_2} (Fig. 2) and spider resting metabolic rate (Fig. 3).

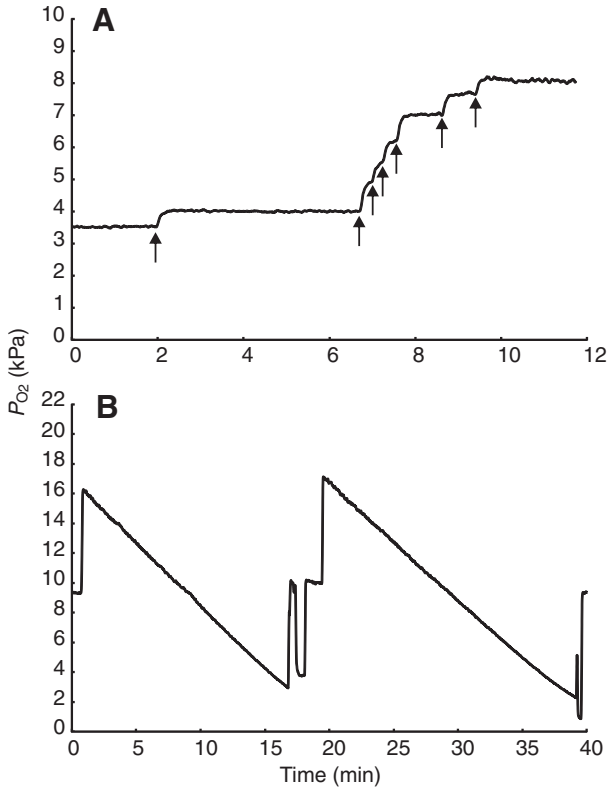

Fig. 5. (A) Record of a female *A. aquatica* adding air to a diving bell containing a cocoon involving seven successive trips to the surface (arrows). (B) Record from the air on the abdomen of a 103 mg spider away from the diving bell. The spider made two trips to the surface, followed by steep declines in P_{O_2} on its body. P_{O_2} values of approximately 9 kPa represent the water. Other abrupt changes in the trace at 17 and 39 min are artefacts. Note the reduction in slope at low P_{O_2}.

renewed at the surface by comparing the initial P_{O_2} in the bubble with atmospheric P_{O_2}. The mean renewal was 87%. Because the calculation assumes that there was no oxygen in the bubble before renewal, the real fraction is slightly higher.

DISCUSSION

Effectiveness of the physical gill

This study reveals how well the diving bells of *A. aquatica* function as physical gills. Resting water spiders can use the surface of the bell to meet their metabolic oxygen requirements by diffusive O_2 uptake from the water, and they do not have to resort to replenishment with atmospheric air in moderately oxygenated water. The surface of the diving bells constructed voluntarily by the spiders limited the P_{O_2} difference between the water and the bell to less than 10 kPa in most cases (Fig. 4). Only one spider (77 mg, Fig. 4) required an unreasonably high difference of over 20 kPa to satisfy resting metabolic rate. It is clear that the surface area of the gas on the abdomen alone (Fig. 1A) is insufficient to achieve equilibrium of O_2 demand with supply from the water. The rapid decline in P_{O_2} apparent in Fig. 5B (–0.80 kPa min^{-1}) is more than 10 times faster than the worst-case spider with a bell in Fig. 4 (–0.07 kPa min^{-1} for the 77 mg spider).

Although the spiders in this study are smaller than those investigated by Schütz and Taborsky (Schütz and Taborsky, 2003), their data for body mass and diving bell volume predict similar results. Their adult females weighed approximately 100 mg and produced diving bell volumes of approximately 1 ml, which would be expected to produce a P_{O_2} difference of approximately 8 kPa at the metabolic rates of this study (Fig. 4). Their adult males weighed approximately 155 mg and had diving bell volumes of approximately 0.7 ml, producing a P_{O_2} difference of approximately 15 kPa. Thus their data also indicate that the diving bell acting as a physical gill would support resting spiders without frequent air renewal. This probably has a selective advantage, because trips to the surface and back involve considerable effort, alert potential prey and expose the spider to aquatic and aerial predators (Schütz and Taborsky, 2003).

The extent that metabolic rate rises with activity is not known, but it is conceivable that it could rise to levels unsupportable by gas gill function. Spiders forced to exercise can increase metabolic rate to approximately four to 10 times above rest (Seymour and Vinegar, 1973; Schmitz, 2005). It may be significant that *A. aquatica* enlarge the diving bell under conditions when metabolic rate would be expected to rise. We observed that spiders that caught and paralysed prey items would sometimes return to the diving bell and enlarge it by laying down more web and adding air before pulling the prey into the chamber for consumption. The spiders with cocoons also enlarged the diving bell progressively as the brood developed (Fig. 1D). In one case, this produced a P_{O_2} difference of 11.1 kPa, and more O_2 entered the brood chamber than was used by both the brood and the female, as P_{O_2} was increasing. Unfortunately we do not know the respiration rate of the females or their broods. Further study of behavioural modification of diving bell size in relation to external P_{O_2}, activity and breeding would be fruitful.

Tolerance of low P_{O_2}

Resting spiders within the diving bells were remarkably tolerant of low P_{O_2}. Spiders withstood values of approximately 2–3 kPa before voluntary air renewal, which reveals that moderate aquatic hypoxia is possible without air renewal. In the field sites at the edges of the Eider River, where we collected the spiders, P_{O_2} is above approximately 16 kPa and the temperature is approximately 13°C during the summer, but P_{O_2} decreases up drainage ditches away from the river (Scholz and Trepel, 2004). Such conditions would

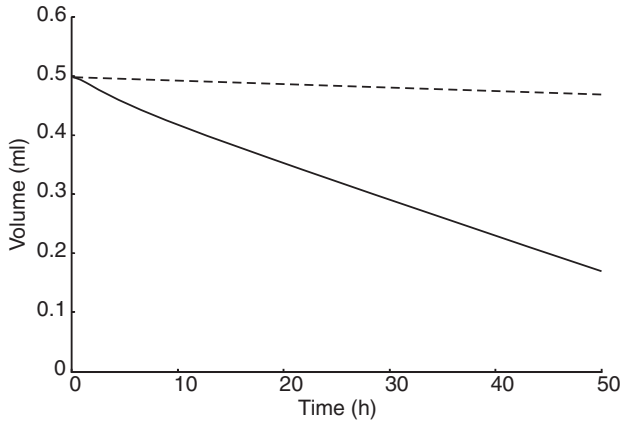

Fig. 6. Model of changes in volume of a diving bell, assuming an initial volume of 0.5 ml at 100 mm depth. Volume decreases of an empty bell (dashed line) and with a 50 mg spider (solid line) are shown. The model incorporates empirical data on the relationships between volume and conductance and the metabolic rate of the resting spider.

not require renewal of diving bell O_2 from the surface in the short term. Nevertheless, renewal may be necessary in other locations, possibly in acidic ponds (Masumoto et al., 1998a), in highly eutrophic waters (Seyyar and Demir, 2009) or at high altitudes (Walder, 1995). It has been implied that air renewal is necessary during the summer, but not winter, presumably because metabolic rate is higher in summer (Messner and Adis, 1995; Schütz and Taborsky, 2003), but the present experiments show that the physical gill can supply all requirements, even in stagnant water and at abnormally high (22–31°C) temperatures. If the water is reasonably oxygenated, the only limit to gas gill function is the longevity of the diving bell.

Diving bell longevity

The longevity of bubbles and the fraction of O_2 delivered from the original bubble and the water of diving insects has been modelled since the time of Ege (Ege, 1915). The patterns of gas changes in the bubbles differs between model systems, with Rahn and Paganelli (Rahn and Paganelli, 1968) predicting a drop in P_{O_2} to a temporary plateau where O_2 uptake from the water practically balances consumption by the insect, and Chaui-Berlinck et al. (Chaui-Berlinck et al., 2001) predicting that P_{O_2} must drop continuously, without a plateau, as the bubble shrinks. Apparently model results depend on the assumptions concerning surface areas and volumes, and actual measurements from diving Corixidae support the Rahn and Paganelli (Rahn and Paganelli, 1968) model (Matthews and Seymour, 2010).

Following the principles of gas transfer from the bubbles of diving insects (Ege, 1915; Rahn and Paganelli, 1968), it is possible to model the rate of volume decline from diving bell gas conductance and metabolic rate of *A. aquatica* measured in this study. The rate is assumed to comply with the Fick diffusion equation and is therefore equal to the product of conductance and the differences in partial pressure between the diving bell and the water. The total pressure in the diving bell is the atmospheric pressure plus the hydrostatic pressure of water at depth and the additional pressure caused by surface tension of the air–water interface. Partial pressures of O_2 and N_2 are calculated assuming that the N_2 fraction includes Ar and other trace gases. Water vapour pressure is assumed to be saturated and constant. A mole of all gases is assumed to occupy $24.47L$ at 25°C. CO_2 is ignored, because it readily dissolves in water and does not build up appreciably in the gas phase (Rahn and Paganelli, 1968). G_{O_2} is measured in relation to diving bell volume according to the equation $G_{O_2}=0.075V^{0.665}$ (Fig. 2). G_{N_2} is assumed to equal $G_{O_2}/2.19$, according to diffusivities and capacitances of the two gases in water (Rahn and Paganelli, 1968). Longevity depends on a number of factors, including diving bell size, depth in the water and rate of respiration by the spider. Here we present calculated values of longevity reasonably expected for an average 0.5 ml diving bell at 100 mm depth, first empty and then with a 50 mg spider chosen arbitrarily to result in a difference across the wall of approximately 8 kPa (Fig. 4).

For an empty diving bell of pure air in air-equilibrated water, the volume loss depends only on the total pressure in the diving bell (P), which is elevated above atmospheric pressure in proportion to $\rho g h$ (where ρ is density of the water, g is acceleration due to gravity and h is depth at the bottom meniscus), and the pressure due to the curvature of the diving bell surface according to the law of Laplace, $P=2T/r$ (where T is surface tension and r is the radius). The depth of 100 mm produces 981 Pa and the radius of 4.92 mm produces 30 Pa, making a total of 1.010 kPa. The volume of such a bubble would decrease slowly, requiring 503 h (21 days) to reach half the original volume (Fig. 6, dashed line).

With the spider present, the model shows that, after a stabilization period of approximately 10 h, there is a fairly linear decrease in volume that reaches half the original at 37 h (Fig. 6, solid line). As P_{O_2} decreases in the bubble, P_{N_2} increases, according to Dalton's law. Despite uptake of O_2 from the water that eventually approaches the rate of spider respiration, the bell collapses as N_2 is continually lost to the water. The curve is also related to a decreasing G_{O_2} and G_{N_2} as the diving bell shrinks, but constant $\dot{M}_{O_2}$ of the model spider.

The model shows that 4.19 μmol O_2 are present in the bell at complete renewal. Over 24 h, the O_2 content drops to 1.25 μmol, so 3.35 μmol O_2 have been removed from the diving bell. Over the same period, the 50 mg spider consumes 10.79 μmol O_2 (0.45 μmol $h^{-1}\times 24$ h), which means that 7.44 μmol O_2 diffuses from the water into the bell. Thus the physical gill provides approximately 70% of the total O_2 required by the spider over 24 h. This fraction increases as the bubble collapses, and the model indicates that gas uptake from the water would approach 87% of the total when the bubble disappears. This accords with models of Rahn and Paganelli (Rahn and Paganelli, 1968) that set the limit for gas gill O_2 uptake at seven times the O_2 present in the fresh gas gill (7/8=0.875), and agrees with empirical estimates of the maximum effectiveness of corixid gas gills of 88% (Matthews and Seymour, 2010). It also agrees with Rahn and Paganelli's model 1, in which surface area progressively decreases and P_{O_2} continually falls. The concordance between values based on empirical measurement and purely theoretical models may be illusory, however, because numerical and analytical models with different assumptions indicate that the fraction may in fact not be a constant (Chaui-Berlinck and Bicudo, 1994; Chaui-Berlinck et al., 2001). The models are moot in any case, because the spiders could not tolerate complete collapse.

If the model assumes that diving bell is held at constant volume, aquatic respiration can continue indefinitely, as has been shown theoretically for diving insects with plastrons (Ege, 1915; Rahn and Paganelli, 1968). However, we know that the bell is collapsible and cannot act as a plastron, because the web cannot support the necessary subatmospheric pressure of 90.5 kPa, as required by the model.

The results suggest that there should be no need for diving bell renewal for more than a day, even under the warm conditions of our measurements. The high frequencies of air renewal observed by Masumoto et al. (Masumoto et al., 1998b) and Schütz and Taborsky (Schütz and Taborsky, 2003) must have been associated with low P_{O2} in the aquarium water or high rates of activity that elevated the metabolic rate, but data on these aspects are lacking. In our experience with resting spiders, air renewal was never observed except under low aquatic P_{O2} or in exceptionally small bells.

Boundary layer thickness

The mean effective thickness of the boundary layer of 0.83 mm is considerably greater than that assumed (0.02 mm) for insect gas gills by Rahn and Paganelli (Rahn and Paganelli, 1968) and the range (0.008–0.03 mm) assumed by Chaui-Berlinck et al. (Chaui-Berlinck et al., 1994). It is not clear why these small values were chosen for their models. We were able to measure the difference in P_{O2} as the optode approached the diving bell on a scale much wider than 0.02 mm. The layer around the gas gill of stationary corixid bugs (*Agraptocorixa eurynome*) is approximately 0.3 mm (P. D. G. Matthews, personal communication) and the layer is approximately 0.4 mm in the eggs, and 1 mm to the skin of larvae, of trout (*Oncorhynchus mykiss*) (Ciuhandu et al., 2007). Much thicker boundary layers are evident in micro-O_2 electrode profiles near submerged larvae of the beetle *Phaeoxantha klugii* (Zerm et al., 2004), but the results may have been affected by O_2 uptake by the electrode, an effect not produced by optodes.

Conclusions

This study demonstrates that the diving bell of *A. aquatica* functions as a physical gill, exchanging enough O_2 from the water to satisfy the requirements of resting spiders at least, even at high temperature and in stagnant water. Frequent replenishment with air from the surface is necessary only in severely hypoxic water or in exceptionally small bells. Bell size is highly variable and can be adaptively matched to ambient P_{O2} and metabolic demand to minimize trips to the surface, an objective with considerable ecological significance. However, replenishment is necessary over longer terms because of N_2 loss from the diving bell. For this reason, it is unlikely that bells would persist over winter in ice-covered water.

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