COMMENTARY

Physical gills in diving insects and spiders: theory and experiment

Roger S. Seymour* and Philip G. D. Matthews[†]

Ecology and Evolutionary Biology, University of Adelaide, Adelaide, SA 5005, Australia

*Author for correspondence (roger.seymour@adelaide.edu.au)
[†]Present address: University of Queensland, Goddard Building 8, St Lucia, QLD 4072, Australia

Summary

Insects and spiders rely on gas-filled airways for respiration in air. However, some diving species take a tiny air-store bubble from the surface that acts as a primary O_2 source and also as a physical gill to obtain dissolved O_2 from the water. After a long history of modelling, recent work with O_2-sensitive optodes has tested the models and extended our understanding of physical gill function. Models predict that compressible gas gills can extend dives up to more than eightfold, but this is never reached, because the animals surface long before the bubble is exhausted. Incompressible gas gills are theoretically permanent. However, neither compressible nor incompressible gas gills can support even resting metabolic rate unless the animal is very small, has a low metabolic rate or ventilates the bubble's surface, because the volume of gas required to produce an adequate surface area is too large to permit diving. Diving-bell spiders appear to be the only large aquatic arthropods that can have gas gill surface areas large enough to supply resting metabolic demands in stagnant, oxygenated water, because they suspend a large bubble in a submerged web.

Key words: physical gill, compressible gas gill, plastron, gill factor, respiration, model, optode, scaling.

Introduction

Insects evolved from freshwater branchiopods and invaded terrestrial habitats in the Devonian (Glenner et al., 2006), when they became reliant on air-breathing (Pritchard et al., 1993). They gain O_2 from the air through a system of branching tubes called the tracheal system. The tubes open to the atmosphere through spiracles on the surface of the thorax and abdomen and lead ultimately to blind-ended tracheoles that can invade individual cells to become close to the mitochondria. Therefore, without using their circulatory systems, insects can provide for the highest metabolic rate of any known tissue, e.g. the flight muscles of a bee (Schippers et al., 2010). The reason for the success of the air-filled tracheal respiratory system is that O_2 can move by diffusion through air some 250,000 times faster than through water (Dejours, 1981). Therefore, insects eventually die if the tracheal system becomes flooded, or even if the spiracles are wetted. The situation is similar in spiders, which also evolved from air-breathing ancestors (Kamenz et al., 2008). They exchange gases in internal book lungs that connect to the atmosphere through spiracles on the abdomen. Primitive spiders have four book lungs, but more derived ones have two, and the other two have been modified into trachea, as in insects (but unrelated to them).

Despite the universal requirement of insects and spiders to breathe air, many insects have invaded water, either as eggs, larvae, pupae or adults, where they enjoy a range of resources unavailable on land (Hutchinson, 1981). Some have closed tracheal systems that gain O_2 through the cuticle of the body or in enlarged, gas-filled tracheal gills, e.g. chironomid larvae (Diptera, Chironomidae) or dragonfly larvae (Odonata); others connect their tracheal system directly to the surface of the water with a snorkel, e.g. mosquito larvae (Diptera, Culicidae); and still others carry a bubble of air on their bodies, and the spiracles open into the bubble directly, e.g. adult water beetles (Coleoptera) and water bugs (Hemiptera). These bubbles not only supply a submerged insect with O_2 from the original air obtained from the atmosphere, but also provide a gas-permeable surface that allows O_2 to diffuse from the surrounding water into the bubble. Thus the bubbles have been called physical gills to distinguish them from anatomical gills of other aquatic organisms. There are two kinds of physical gill. A compressible gas gill is a simple bubble that clings to the animal and its surface is not supported, so the pressure inside is essentially equal to the atmospheric pressure plus the hydrostatic pressure that depends on depth. An incompressible gas gill ('plastron') is a bubble that adheres to the surface of the insect, but its external surface is supported by hydrophobic structures that suspend it, so the internal pressure can be independent of depth and in fact lower than that of the atmosphere.

Physical gills occur chiefly among diving bugs and beetles, many of which spend most of their adult lives under water. They also occur among the Arachnida, including some true spiders (Araneae) and whip spiders (Amblypygi) that forage underwater or at least survive submersion (Hebets and Chapman, 2000; Pedersen and Colmer, 2012). However, the only completely aquatic spider is *Argyroneta aquatica* (Cybaeidae), which lives in a 'diving bell' made from a bubble of air held by an underwater web (Seymour and Hetz, 2011).

This Commentary discusses the physics and physiology of gas gill function from both theoretical and experimental viewpoints. Recent developments of O_2-sensitive fiber optics can provide continuous measurement of O_2 inside miniscule bubbles and allow us to compare actual patterns of gas exchange with a century of theory and modelling. For example, we show that some of the
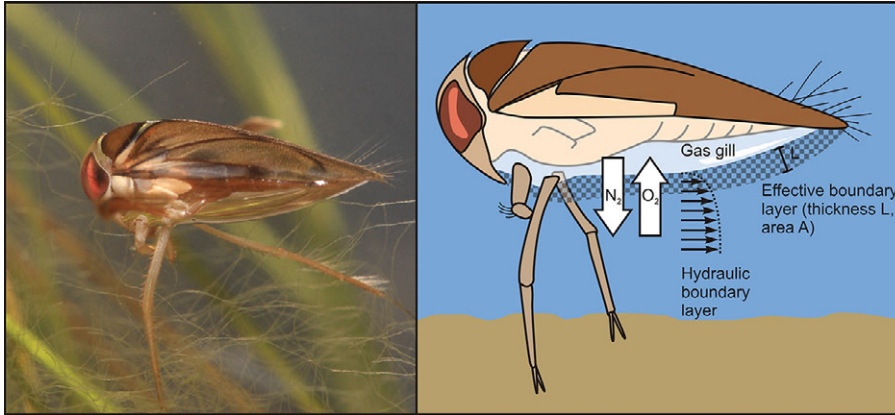
Fig. 1. A submerged corixid bug (*Agraptocorixa eurynome*) carrying a compressible gas gill on its ventral surface and the edges of its hemelytra (left) and a diagram showing the gas gill's effective boundary layer (right). The white arrows indicate the direction of O_2 and N_2 diffusion between the bubble and the surrounding water across the boundary layer. The effective boundary layer is the imaginary layer of stagnant water of thickness *L* and surface area *A* necessary to achieve all gas exchange by diffusion only. The hydraulic boundary layer illustrates the real distribution of flow velocity, which decreases toward the bubble's surface.

conclusions from modelling may be misleading, particularly in relation to the boundary layer around the bubble. Scaling of body size, metabolic rate and collapsible bubble surface area reveals that uptake of O_2 from the water cannot match resting demands without considerable convection of water across the surface. Actual measurements show how backswimmers (Hemiptera, Notonectidae) balance the O_2 from their compressible bubble and the O_2 from associated haemoglobin-containing cells to achieve nearly neutral buoyancy while they forage in mid-water.

Physics of bubble gas exchange in collapsible gas gills

Nearly a century ago, Ege showed that the bubbles of aquatic insects had a respiratory significance, both by providing O_2 in the bubble at the beginning of the dive and by allowing O_2 uptake from the water (Ege, 1915). He made the first measurements of bubble gas and showed that O_2 could decrease from 21% to nearly zero, while the CO_2 fraction did not increase above approximately 2–3%. This meant that the fraction of N_2 could rise from 78% originally in the bubble to above 95%, causing it to diffuse out into the water and eventually collapse the bubble.

Ege expressed gas levels in percentages, but it is a gradient in partial pressure (measured in Pascals), not concentration, that causes gases to diffuse between water and air. Fortuitously, standard barometric pressure is ~100 kPa (101.3 kPa to be precise), so 21% or 78% of any gas equals approximately 21 or 78 kPa at sea level, but not at higher altitudes. Converting to pressure is important, because gases dissolved in water are less concentrated than in air at the same partial pressure. Also, the bubble is under additional hydrostatic pressure determined by its depth. This pressurization increases the pressure of all gases in the bubble, further enhancing N_2 loss and restricting O_2 gain.

The movement of gases between a bubble and the surrounding water is determined primarily by diffusion across a theoretically stationary layer of water around the bubble, known as the 'boundary layer' (Fig. 1). Although the concept of a boundary layer is useful in understanding diffusive exchanges between a bubble and the water, it has no discrete outer edge. The edge is generally defined by hydrodynamicists as a distance equal to 90 or 99% of the distance from the surface to freely moving water (Pinder and Feder, 1990). Water flows at all levels within the boundary layer, but the velocity at each level decreases as the stationary surface is approached, where velocity is zero (Vogel, 1981). However, if the surface is the air–water interface of a bubble, water velocity does not necessarily become zero, because it can skim along on the air to some extent. In this case, both the outer and inner limits of the hydrodynamic boundary layer are unknown. Nevertheless, all of

the literature on physical gills and this presentation in particular consider the imaginary 'effective boundary layer' as the thickness that would cause a rate of diffusion through it as if it were completely stagnant water. Thus the effective boundary layer is thinner than the hydrodynamic one.

The rate of gas flux can be approximated in terms of Fick's first law of diffusion (Piiper et al., 1971):

$$\dot{M}_G = K_G \, (A \,/\, L) \, (\Delta P_G), \qquad (1)$$

where $\dot{M}_G$ is the rate of diffusion, K_G is Krogh's coefficient of diffusion (=the product of solubility and diffusivity), A is surface area, L is thickness of the effective boundary layer and ΔP_G is the difference in partial pressure of the gas in question.

There are several reasons why this equation is not accurate for a bubble, including the assumptions that A is flat, L is known and there is no regional variation in P_G in either the gas or aquatic phases. Nevertheless, the equation is useful in understanding the relative exchanges of O_2 and N_2, because both take the same pathway. The partial pressure difference is the 'driving force' for diffusion, and the movement of each gas is nearly independent of movements of the other. Dalton's law of partial pressures describes the fact that the total pressure of a gas is the sum of the partial pressures of each gas in the mixture. The total pressure inside a submerged bubble is determined by the sum of three pressures: the atmospheric barometric pressure, the hydrostatic pressure due to depth and the pressure derived from the surface tension of the bubble. At sea level, barometric pressure is 101.3 kPa. Hydrostatic pressure increases 9.8 kPa per meter of depth in freshwater. The surface tension of a 1 mm diameter bubble is 0.3 kPa and decreases in larger bubbles. Because diving insects are in shallow water and typically have bubbles larger than 1 mm, it is apparent that atmospheric pressure has the overwhelming effect on total pressure in the bubble. Pure dry air is 78% N_2, 21% O_2 and 1% trace gases (mainly argon; CO_2 is only 0.039%). A bubble taken to 1 m depth at 20°C therefore contains 108.8 kPa total pressure, made up of P_{N_2}=84.9 kPa, P_{O_2}=22.8 kPa, P_{trace}=1.9 kPa and P_w=2.3 kPa (P_w is the saturated vapor pressure of water and is constant at a given temperature). The partial pressures of dissolved gases in equilibrium with the atmosphere are independent of depth and are: P_{N_2}=77.2 kPa and P_{O_2}=20.8 kPa. Thus simply taking a naked bubble of air from the surface to 1 m depth causes an outward pressure difference for N_2 (84.9–77.2=7.7 kPa) and O_2 (22.8–20.8=2.0 kPa). Both gases continue to diffuse into the water until the bubble disappears completely. If the water body is large, the addition of gas to it does not significantly affect its dissolved gas tensions.
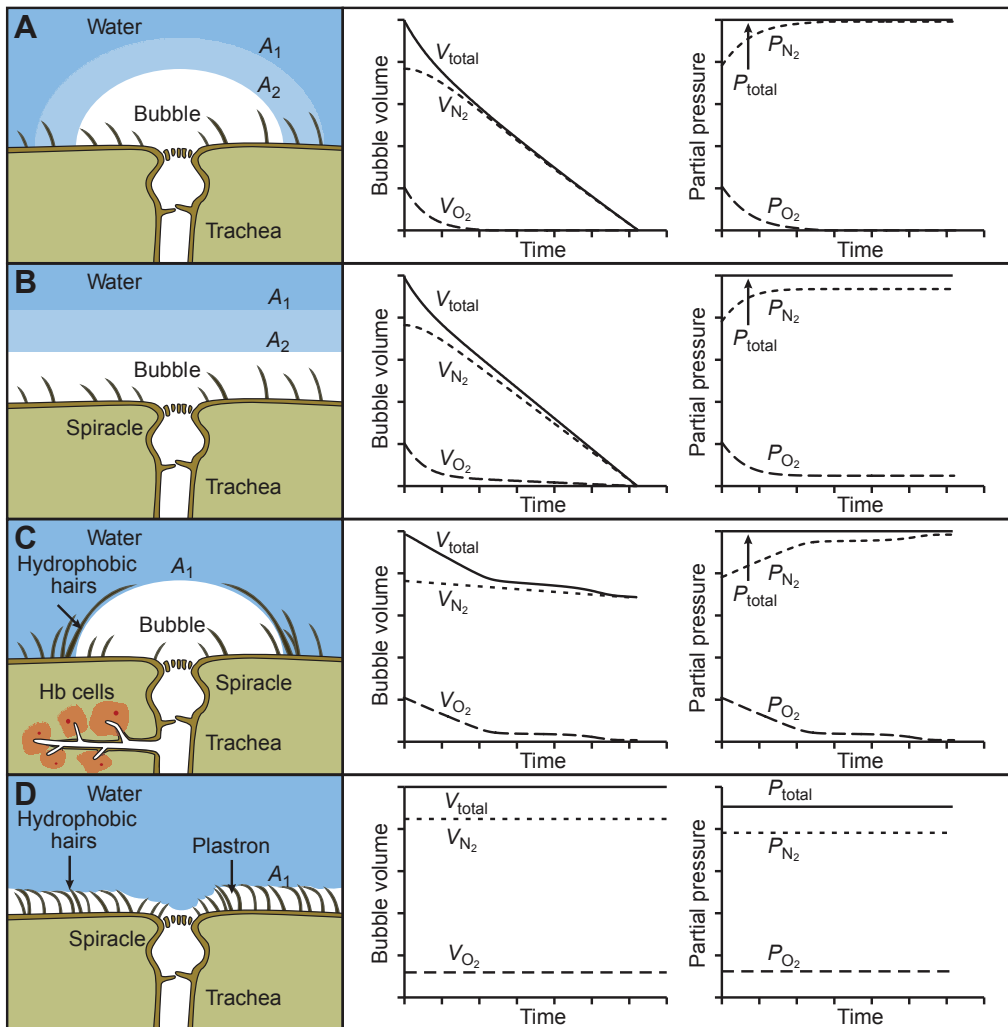
Fig. 2. Theoretical changes in bubble volume and total pressure (solid lines) in relation to gas volume and partial pressure (dashed lines) within compressible and incompressible (plastron) gas gills. (A) A compressible gas gill with a decreasing surface area A (where $A_2<A_1$) cannot have a stable P_{O_2} during the dive as a decreasing area for O_2 uptake prevents equilibrium with the insect's rate of O_2 consumption. (B) The P_{O_2} within a compressible gas gill with a constant area (where $A_1=A_2$) can reach equilibrium, theoretically supplying O_2 from the surrounding water until it dissolves completely due to N_2 loss. (C) Backswimmers (*Anisops* spp.) store O_2 bound by haemoglobin (Hb) within cells in their abdomen. This O_2 is released during a dive to temporarily stabilize the P_{O_2} and volume of their air bubble to achieve a period of near-neutral buoyancy. (D) The incompressible bubble of the plastron can resist the hydrostatic pressure, allowing the P_{O_2} in the bubble to remain below that in the surrounding water while P_{N_2} remains in equilibrium. Thus the total pressure under the plastron is less than the atmospheric plus hydrostatic pressure.

When an insect takes a bubble from the surface, the same conditions apply initially, but because the bubble is connected to the animal's tracheal system, O_2 is consumed from the bubble and P_{O_2} decreases. This quickly reverses the outward O_2 diffusion to an inward one. As P_{O_2} in the bubble decreases, P_{N_2} increases practically to the same extent, according to Dalton's law. The reason for this is that the CO_2 produced by the insect's respiration is rapidly taken up by the water, because CO_2 is approximately 28 times more soluble in water than O_2 (Rahn and Paganelli, 1968). The P_{CO_2} measured in the bubbles of several species of diving insects is greater than that of the water (ca. 1–3 kPa), causing outward diffusion, but the P_{CO_2} in the bubble quickly becomes independent of dive duration (Ege, 1915). Therefore, P_{CO_2} can be considered to be a constant, like P_w.

There are two models of the dynamics of volume and pressure changes in a collapsible gas gill that depend on whether the surface area A is constant or changing. If the edges of the bubble can move, then area can decrease as volume decreases (Fig. 2A), but if they are fixed, then area can remain almost constant as the bubble gets thinner (Fig. 2B). A decreasing area predicts a non-linear decline in P_{O_2} until it reaches zero (Chaui-Berlinck and Bicudo, 1994; Rahn and Paganelli, 1968). A constant area predicts that P_{O_2} stabilizes at a level dependent on the surface area and the O_2 uptake rate of the insect (Rahn and Paganelli, 1968).

Observations of the real collapsible gas gill on the ventral surface of water boatmen, *Agraptocorixa eurynome* (Hemiptera,

Corixidae), indicate that a constant-area model is appropriate for this species, because the edges of the bubble are mainly fixed (Matthews and Seymour, 2010) (Fig. 3). Records of bubble P_{O_2} during water boatmen dives apparently plateau, but this may in part be related to active regulation of the effective thickness of the boundary layer by leg movements that ventilate the surface of the bubble. Quiescent water boatmen produce P_{O_2} values that are well below the critical P_{O_2} at which $\dot{M}_{O_2}$ is forced to decline, and the dives are clearly unsustainable.

Similar optode measurements are available from the diving-bell spider, *A. aquatica* (Seymour and Hetz, 2011). This unique spider constructs a bell-shaped web underwater and fills it by repeated trips to the surface to collect large air bubbles that cling between its body and rear legs. The size of the diving bell can be adjusted such that P_{O_2} can decrease, remain stable or rise, depending on the balance between metabolic rate and surface area (Fig. 4A). There is evidence that the volume of the bell can be behaviorally adjusted to match increased respiration of larger spiders and further enlarged during feeding and egg brooding, and possibly in response to changes in ambient P_{O_2}. The physical gill of the bell is clearly capable of satisfying all of the metabolic demands of the spider, whereas the air-film on the surface of the spider's thorax and abdomen is not. Optodes reveal rapidly decreasing P_{O_2} to levels at which the spider renews the film at the surface every 15–20 min (Fig. 4B). The rate of decrease is approximately –800 Pa min^{-1} and
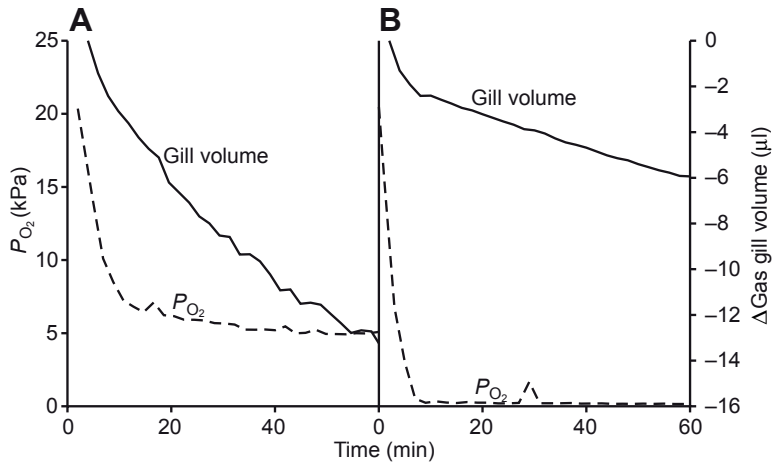
Fig. 3. Measurements of the P_{O_2} (dashed lines) and volume (solid lines) of gas gills carried by submerged water boatmen (*Agraptocorixa eurynome*). (A) Vigorous ventilation of the gas gill using their hind limbs maintains a high P_{O_2} within the bubble but causes it to dissolve rapidly. (B) The P_{O_2} within an unventilated gas gill quickly drops below the insect's critical P_{O_2} of 2.1 kPa while the rate at which the gill dissolves is also reduced. Reproduced from Matthews and Seymour (Matthews and Seymour, 2010) with the publisher's permission.

aligns well with observations of the 20 min duration of underwater foraging of raft spiders, *Dolomedes fimbriatus* (Araneae, Pisauridae), which have similar abdominal air-films (Pedersen and Colmer, 2012).

Buoyancy and haemoglobin

A submerged insect can temporarily solve its O_2 supply problems by using a bubble of air, but the buoyancy of the bubble presents its own problems. Insects have a body density only slightly greater than the density of water, meaning that they have a natural tendency to sink. However, carrying even a small quantity of air (>8% of the body volume) is sufficient to make the insect float (positively buoyant). Large bubbles may supply more O_2, but require more swimming effort to dive. Furthermore, the insect's buoyancy is not constant over the course of a dive, but decreases as O_2 and N_2 are lost. The problem of buoyancy restricts most diving insects to clinging to objects underwater or floating on the surface, and only a few occupy a free, mid-water existence. These are small, aquatic bugs called backswimmers (*Anisops* and *Buenoa* spp., Hemiptera, Notonectidae) that have evolved a unique solution to both of these problems (Matthews and Seymour, 2008). Unlike almost all other insects, they possess large quantities of the respiratory pigment haemoglobin (Hb) concentrated within special cells in their abdomen. Backswimmers use their Hb to bind atmospheric O_2 when they surface and then release it during the following dive. This has two important consequences. First, carrying O_2 bound to Hb means that it is removed from the gas phase and does not contribute to the volume of the bubble. As such, backswimmers can begin a dive carrying a smaller, less buoyant bubble while still possessing a substantial reserve of O_2. Second, as the O_2 the insect is respiring is both supplied by the bubble and released by the Hb, the decrease in bubble volume attributable to the insect's respiration is greatly reduced (Fig. 2C). Thus, by releasing O_2 from their Hb stores at a rate close to their respiration rate, backswimmers temporarily stabilize the volume of their bubble and enter a phase of near-neutral buoyancy. As a result, predatory backswimmers are able to float mid-water while hunting their zooplankton prey.

Plastron breathers

The problems of collapsing bubbles and positive buoyancy were solved by some insects (e.g. *Aphelocheirus aestivalis*, Hemiptera, Aphelocheiridae) that use an incompressible gas gill (plastron) that is permanently present on their surface (Fig. 2D). In the strict sense, the plastron has to be supported by hydrophobic hairs or other

structures that push against the air–water interface to prevent the gill from collapsing (Balmert et al., 2011; Flynn and Bush, 2008). In species living near the surface, the plastron has only to push to a maximum of approximately 20 kPa in order to offset the loss of O_2, so the total internal pressure drops to as low as approximately 80 kPa. P_{N_2} in the bubble and dissolved in the water are equal, so no N_2 is lost. The bubble is therefore permanent and the insect has never to return to the surface. Many insects and spiders have hydrophobic surfaces, usually with hairs, that are sometimes called 'plastrons', but it seems unlikely that any of them can withstand a
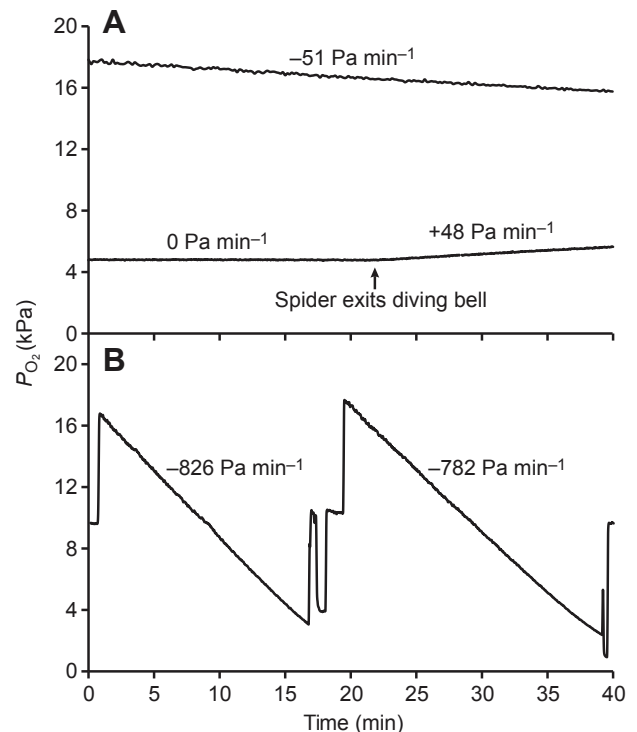

Fig. 4. Patterns of change of bubble P_{O_2} in the diving-bell spider, *Argyroneta aquatica*. (A) Upper line: decrease in P_{O_2} in an occupied diving bell (P_{O_2} of water=15.9 kPa); lower line: stable P_{O_2} in an occupied diving bell until ~22 min, when the spider vacated and P_{O_2} began to rise (P_{O_2} of water=14.6 kPa). (B) P_{O_2} measured in the body surface film of a spider away from the bell after two trips to the surface to renew the gas (P_{O_2} of water=9.8 kPa, as indicated when the optode was away from the spider). The depression at 18 min is an artifact. Data are from a study by Seymour and Hetz (Seymour and Hetz, 2011).
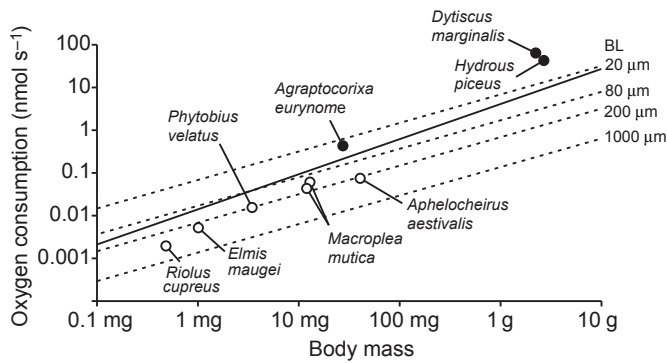
Fig. 5. The effect of insect body size on demand and supply of O_2 through a physical gill. The solid line for demand is derived from a regression of resting metabolic rate of insects at 20°C (Chown et al., 2007). The dashed lines for supply are based on gill surface area and effective boundary layer (BL) thickness. Because the supply lines are generally lower than the resting demand lines, especially in larger insects, it is necessary for the gill to be ventilated to reduce BL thickness. The lines for supply are calculated from Eqn 1, assuming a calculated bubble surface area and four arbitrary BL thicknesses. Bubble surface area is calculated as half the surface of a sphere of a volume equal to the mass divided by an insect density of $1.078 \, \mathrm{g \, ml^{-1}}$ (Matthews and Seymour, 2010). The calculations also use the following constants: Krogh's coefficient of diffusion of O_2 of $3.11 \times 10^{-7} \, \mathrm{\mu mol \, cm^{-1} \, s^{-1} \, kPa^{-1}}$, and a P_{O_2} difference of 19 kPa across the BL. Superimposed are individual data from five species of plastron insects (open circles) that are known to survive indefinitely underwater (Kölsch and Krause, 2011; Thorpe and Crisp, 1949) and compressible gas gills from a diving bug (Matthews and Seymour, 2010) and two diving beetles (Di Giovanni et al., 1999) (filled circles). Plastron insects are small, have allometrically low metabolic rates and produce BL thicknesses above $100 \, \mathrm{\mu m}$; however, diving bugs and beetles are larger, have high metabolic rates and would require very thin BLs for gas gill function. The latter must rapidly ventilate the gill surface or rely primary on O_2 brought from the surface.

drop in internal pressure sufficient to make the gill permanent. Theoretically, the total pressure of gas in the plastron is lower than in compressible gas gills, and the levels of O_2 and N_2 are constant if O_2 uptake rate is constant. Actual conditions in the plastron have not yet been measured dynamically, because the insects are too small. In fact, it is significant that they are all small. For a plastron to function, the surface area on the body must be adequate to meet the metabolic demand for O_2, and the ratio of surface area to O_2 demand is more favourable in small animals. The following section shows why this is true.

Effects of body size on gas gill dimensions
Ege estimated from rates of O_2 consumption and bubble size that large insects such as dytiscid beetles (Coleoptera, Dytiscidae) could not supply enough O_2 to maintain resting metabolic rate, but small ones might (Ege, 1915). He concluded that at higher temperatures

and during activity, the supply would be even less able to balance demand. However, analyses based on modelling leave one with the idea that as long as the bubble exists and has close to the original surface area, it can satisfy at least the resting metabolic demand of the insect, and that many times the amount of O_2 in the original bubble can be supplied by the physical gill. This idea is certainly misleading and stems from underestimation of the boundary layer thickness in models. We can demonstrate this by comparing real data for resting metabolic rates of insects and estimates of O_2 transfer across bubbles of different areas and boundary layer thicknesses, according to Eqn 1. We assume that $\dot{M}_{O_2}$ would become limiting if P_{O_2} in the bubble decreases below 2 kPa where $\dot{M}_{O_2}$ begins to drop (the 'critical' P_{O_2}), which is a reasonable value (Matthews and Seymour, 2010; Thorpe and Crisp, 1947). Thus, maximum ΔP_{O_2} becomes 19 kPa. Bubble surface area is taken as half the area of a sphere of a mass equal to that of an insect and a density of $1.08 \, \mathrm{g \, ml^{-1}}$. This is a rough calculation, because insects are not spherical, but elongate. With the allometric relationship of $\dot{M}_{O_2}$ and body mass in resting insects, these assumptions permit predictions of bubble boundary layer thicknesses that would support resting metabolism (Fig. 5). The modelling demonstrates that body mass affects $\dot{M}_{O_2}$ and surface area with different allometric slopes. Resting metabolic rates at approximately 20°C increase with body size according to the equations $\dot{M}_{O_2} = 14.1 M_b^{0.82}$ in insects (Chown et al., 2007) and $\dot{M}_{O_2} = 5.75 M_b^{0.82}$ in spiders (Terblanche et al., 2004), where M_b is body mass. Surface area generally increases with volume raised to the power of 0.67 in similarly shaped objects, which is considerably less than the power of 0.82 for metabolic rate. Therefore a 10-fold increase in body size results in a 29% decrease in the ratio of A to $\dot{M}_{O_2}$. This confirms Ege's view that gas gills are less advantageous in larger insects. Indeed, the only insects that are known to use a plastron and stay underwater indefinitely are small (Hinton, 1976; Thorpe and Crisp, 1947; Thorpe and Crisp, 1949). Even then, plastron breathers have metabolic rates averaging 38% of predicted rates for most insects their size (Table 1).

For larger insects to satisfy respiratory demands with a gas gill, the thickness of the effective boundary layer must decrease. This would predict a boundary layer of approximately $80 \, \mathrm{\mu m}$ for a 2 mg insect and $35 \, \mathrm{\mu m}$ for a 1 g insect, just to meet resting demands (Fig. 5). But what are the effective boundary layer thicknesses in real insects and spiders? One approach is to calculate boundary layer thickness from individual species of true plastron insects that are known to be able to remain indefinitely in water (Table 1). This indicates thicknesses ranging between 119 and $795 \, \mathrm{\mu m}$. Thick effective boundary layers align with other direct estimates from the literature: $830 \, \mathrm{\mu m}$ around the diving bell of *A. aquatica* (Seymour and Hetz, 2011), $400–1000 \, \mathrm{\mu m}$ near trout eggs and larvae (Ciuhandu et al., 2007), more than $1500 \, \mathrm{\mu m}$ in unstirred water over amphibian skin (Pinder and Feder, 1990) and 300 to over $1000 \, \mathrm{\mu m}$ near membranes in unstirred experimental setups (Barry and

Table 1. Respiratory variables of insects that rely on plastron gas exchange underwater

Species	Mass (mg)	Resting $\dot{M}_{O_2}$ (pmol s^{-1})	% Predicted	Area (cm^2)	BL thickness (μm)
Riolus cupreus	0.47	2.0	27	0.0126	376
Elmis maugei	1.00	5.2	38	0.014	159
Phytobius velatus	3.36	15.4	42	0.08	307
Macroplea mutica	12.70	59.9	55	0.12	119
Aphelocheirus aestivalis	40.00	74.4	27	1	795

Oxygen consumption rate ($\dot{M}_{O_2}$) is compared with allometric predictions for insects in general (Chown et al., 2007). Boundary layer (BL) thickness is calculated from Eqn 1. Data are from Thorpe and Crisp (Thorpe and Crisp, 1947; Thorpe and Crisp, 1949).

Diamond, 1984). It is striking that the boundary layers around real gas gills are much thicker than previous modelling studies assumed: 20 μm (Rahn and Paganelli, 1968) or 8–30 μm (Chaui-Berlinck and Bicudo, 1994). Such thin layers turn out to be necessary for the models to produce reasonable results (i.e. keeping ΔP_{O_2} less than 19 kPa). To achieve them in still water, gas gills need to be ventilated.

Requirement for gas gill ventilation

The ability of the physical gill to exchange gas is inversely related to the effective boundary layer thickness (Fig. 5), and insects must ventilate the surface to reduce the thickness. Some species position themselves in flowing water, for example insects with plastrons thrive in streams rather than ponds (Thorpe, 1950). Insects in stagnant water can either swim through it or wipe the bubble surface. For instance, the collapsible gas gill of water boatmen, *A. eurynome*, is highly dependent on gas gill ventilation (Matthews and Seymour, 2010). With limb movements, water boatmen can survive for long periods, because P_{O_2} stabilizes at approximately 3.2 kPa, which is above the critical P_{O_2} (Fig. 3A). Without movements, P_{O_2} drops to nearly zero and is clearly unsustainable (Fig. 3B). The diving bell of *A. aquatica* has such a large surface area that it does not require ventilation (Seymour and Hetz, 2011).

Bubble longevity

Unlike plastron gas gills, collapsible gas gills are not supported by any surface, so the internal pressure practically equals the atmospheric plus water pressure. Therefore, the bubble must lose gas and eventually shrink to nothing. How long this takes and the amount of O_2 taken up from the water while it occurs has been subject to some debate. With estimates of K_{O_2} and K_{N_2}, Ege estimated that approximately 11.8 times the amount of O_2 would diffuse into the bubble as originally contained in it, before all of the N_2 was gone and the bubble disappeared, giving a 'gill factor' of 12.8 (=11.8+1) (Ege, 1915). Rahn and Paganelli used different values of K_{O_2} and K_{N_2}, and arrived at a gill factor of 8.3, which they considered to be a constant, and Chaui-Berlinck et al. used still different values of Krogh's constants in their model, but concluded that the gill factor is not a constant (Chaui-Berlinck et al., 2001; Rahn and Paganelli, 1968). The gill factor is, in any case, the theoretical maximum O_2 supply from a bubble. Real bubbles do not come close to this, because the animal renews the bubble long before it collapses completely (Hutchinson, 1981). Nevertheless, measurements of total O_2 uptake and bubble volume at any point in the dive can indicate the gill factor by extrapolation. For instance, the graph for water boatmen strikes zero bubble volume at a gill factor of 7.5, assuming a constant bubble surface area (Matthews and Seymour, 2010). Analysis of bubble volume and O_2 consumption rate of the diving-bell spider indicates that the maximum gill factor is approximately eight for the average spider inside the average bell that shrinks in volume and surface area (Seymour and Hetz, 2011).

Future work

Optodes are currently available that would permit investigations of the P_{O_2} of the tiny volume of gas inside the physical gills of plastron insects to test models of plastron respiration (Rahn and Paganelli, 1968; Thorpe and Crisp, 1947). Total pressure inside the plastron gas space could also be measured with micro-pressure transducers to test theories of plastron function (Flynn and Bush, 2008; Thorpe and Crisp, 1947). Experiments at low temperature could see whether insects and spiders without strong plastrons could

nevertheless gain O_2 from water over extended periods. Dead notonectids, which do not have a permanent plastron, can retain a gas-film on their surfaces for longer than 120 days (Balmert et al., 2011). Gas gill function may therefore be sufficient to tide over some insects under ice-covered ponds through winter. The survival of diving-bell spiders during winter might be explained by this adaptation (Messner and Adis, 1995).

It would be interesting to explore other cases of insects that use underwater air bubbles. For instance, the curious African stream beetle *Potomodytes tuberosus* (Coleoptera, Elminthidae) is able to maintain a permanent bubble in shallow, fast-flowing water by reducing the pressure inside the bubble with the Bernoulli principle, in which water flowing past the surface reduces the external pressure and offsets the effect of hydrostatic pressure (Stride, 1955). By so doing, the bubble does not collapse, and the boundary layer must be very thin. There are also some lepidopteran pupae that exist in torrential streams, bound within cases filled with air of unknown origin (Nielsen, 1950). The diversity of aquatic insect gas exchange is likely to hold other examples of evolutionary solutions to the problem of respiring underwater.

List of symbols and abbreviations

A	surface area
BL	boundary layer
K_G	Krogh's coefficient of diffusion of a gas
L	distance
M_b	body mass
$\dot{M}_G$	molar rate of flow of a gas
ΔP_G	difference in partial pressure of a gas
V_G	volume of gas

Glossary

Book lungs
  Internal gas exchange organs of arachnids, consisting of broad, hemolymph-filled leaves alternating with air-spaces that are connected to the atmosphere through a small hole.

Boundary layer (effective, hydraulic)
  A layer of fluid (water or air) next to a surface in which the velocity of fluid is less than that in the surrounding bulk medium. The effective boundary layer is the thickness of a layer in which velocity is theoretically zero, and the diffusion rate across the stagnant layer is equivalent to that which occurs naturally by a combination of diffusion and convection between the free medium and the surface. The hydraulic boundary layer occurs in that thickness in which velocity is less than an arbitrary 99% of that in the free medium.

Convection
  The movement of a substance by being carried in a moving fluid (water or air).

Diffusion
  The movement of a substance down a gradient in concentration or partial pressure without bulk movement of the medium.

Fick's first law of diffusion
  A mathematical expression describing that the rate of gas diffusion depends on the product of the gas partial pressure difference and the gas conductance (a measure of the ease of diffusion through a barrier).

Gas gill (compressible, incompressible)
  A gas space (bubble) on the external surface of an animal that permits respiratory gas exchange through its surface. If the bubble is free and unsupported, it is compressible and continually collapses. If the bubble is supported (by a plastron), it is incompressible and potentially permanent.

Gill factor
  The ratio of the potential total amount of O_2 delivered by a collapsible gas gill and the amount originally present upon renewal at the water surface. The amount delivered is that originally in the gas gill plus the amount taken up from the water before total collapse of the bubble. Theoretical

gill factors are approximately eight, meaning that seven times the amount of O_2 can be taken up from the water as originally present after renewal.

Partial pressure

The pressure (measured in Pascals) exerted by each gas of a mixture independently of the others. The sum of the partial pressures equals the total pressure of the mixture. Thus barometric pressure of dry atmospheric air at sea level is ~101.3 kPa=21 kPa P_{O_2}, 79 kPa P_{N_2}, plus 1.3 kPa trace gases. The partial pressures of gases dissolved in water are equal to the partial pressures of the gases in air when in equilibrium, but the concentrations of dissolved gases are much lower in water than in air.

Plastron

A layer on the surface of an animal or plant containing gas and protected against collapse by hydrophobic supporting structures that press against the air-water interface.

Snorkel

A tube connecting a gas store on an insect to the surface of the water to admit atmospheric O_2.

Spiracles

The openings of the tracheal system of insects or spiders. There may be several through the exoskeleton, exposed or under other structures, and their apertures may be controlled by spiracular valves.

Trachea

The larger tubes of the tracheal system.

Tracheal gills

External extensions of the tracheal system of insects that are closed, but covered with a broad, thin chitinous barrier that nevertheless allows gas exchange with the water.

Tracheal system

A branching system of air-filled tubes that permit O_2 and CO_2 exchange between the atmosphere and cells of insects and spiders. The system opens to the atmosphere at the spiracles and ends in the tracheoles.

Tracheoles

The smallest, blind-ended tubes of the tracheal system that approach or enter cells.

References

Balmert, A., Florian Bohn, H., Ditsche-Kuru, P. and Barthlott, W. (2011). Dry under water: comparative morphology and functional aspects of air-retaining insect surfaces. *J. Morphol.* **272**, 442-451.

Barry, P. H. and Diamond, J. M. (1984). Effects of unstirred layers on membrane phenomena. *Physiol. Rev.* **64**, 763-872.

Chaui-Berlinck, J. G. and Bicudo, J. E. P. W. (1994). Factors affecting oxygen gain in diving insects. *J. Insect Physiol.* **40**, 617-622.

Chaui-Berlinck, J. G., Bicudo, J. E. P. W. and Monteiro, L. H. A. (2001). The oxygen gain of diving insects. *Respir. Physiol.* **128**, 229-233.

Chown, S. L., Marais, E., Terblanche, J. S., Klok, C. J., Lighton, J. R. B. and Blackburn, T. M. (2007). Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. *Funct. Ecol.* **21**, 282-290.

Ciuhandu, C. S., Wright, P. A., Goldberg, J. I. and Stevens, E. D. (2007). Parameters influencing the dissolved oxygen in the boundary layer of rainbow trout (*Oncorhynchus mykiss*) embryos and larvae. *J. Exp. Biol.* **210**, 1435-1445.

Dejours, P. (1981). *Principles of Comparative Respiratory Physiology.* Amsterdam: Elsevier/North-Holland Biomedical Press.

Di Giovanni, M. V., Pirisinu, Q., Giangiuliani, G., Goretti, E. and Pampanella, L. (1999). Oxygen consumption in two aquatic Coleoptera species: *Hydrous piceus* and *Dytiscus marginalis. Ital. J. Zool. (Modena)* **66**, 329-332.

Ege, R. (1915). On the respiratory function of the air stores carried by some aquatic insects (Corixidae, Dytiscidae and Notonecta). *Z. Allg. Physiol.* **17**, 81-124.

Flynn, M. R. and Bush, J. W. M. (2008). Underwater breathing: the mechanics of plastron respiration. *J. Fluid Mech.* **608**, 275-296.

Glenner, H., Thomsen, P. F., Hebsgaard, M. B., Sørensen, M. V. and Willerslev, E. (2006). The origin of insects. *Science* **314**, 1883-1884.

Hebets, E. A. and Chapman, R. F. (2000). Surviving the flood: plastron respiration in the non-tracheate arthropod *Phrynus marginemaculatus* (Amblypygi: Arachnida). *J. Insect Physiol.* **46**, 13-19.

Hinton, H. E. (1976). Plastron respiration in bugs and beetles. *J. Insect Physiol.* **22**, 1529-1550.

Hutchinson, G. E. (1981). Thoughts on aquatic insects. *Bioscience* **31**, 495-500.

Kamenz, C., Dunlop, J. A., Scholtz, G., Kerp, H. and Hass, H. (2008). Microanatomy of Early Devonian book lungs. *Biol. Lett.* **4**, 212-215.

Kölsch, G. and Krause, A. (2011). Oxygen consumption of the aquatic leaf beetles *Macroplea mutica* and *Macroplea appendiculata* is low and not influenced by salinity. *Physiol. Entomol.* **36**, 111-119.

Matthews, P. G. D. and Seymour, R. S. (2008). Haemoglobin as a buoyancy regulator and oxygen supply in the backswimmer (Notonectidae, Anisops). *J. Exp. Biol.* **211**, 3790-3799.

Matthews, P. G. D. and Seymour, R. S. (2010). Compressible gas gills of diving insects: measurements and models. *J. Insect Physiol.* **56**, 470-479.

Messner, B. and Adis, J. (1995). Es gibt nur fakultative Plastronatmer unter den tauchenden Webespinnen. *Deutsche Entomol. Z.* **42**, 453-459.

Nielsen, A. (1950). The torrential invertebrate fauna. *Oikos* **2**, 176-196.

Pedersen, O. and Colmer, T. D. (2012). Physical gills prevent drowning of many wetland insects, spiders and plants. *J. Exp. Biol.* **215**, 705-709.

Piiper, J., Dejours, P., Haab, P. and Rahn, H. (1971). Concepts and basic quantities in gas exchange physiology. *Respir. Physiol.* **13**, 292-304.

Pinder, A. W. and Feder, M. E. (1990). Effect of boundary layers on cutaneous gas exchange. *J. Exp. Biol.* **154**, 67-80.

Pritchard, G., McKee, M. H., Pike, E. M., Scrimgeour, G. J. and Zloty, J. (1993). Did the first insects live in water or in air? *Biol. J. Linn. Soc. Lond.* **49**, 31-44.

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

Schippers, M.-P., Dukas, R. and McClelland, G. B. (2010). Lifetime- and caste-specific changes in flight metabolic rate and muscle biochemistry of honeybees, *Apis mellifera. J. Comp. Physiol. B* **180**, 45-55.

Seymour, R. S. and Hetz, S. K. (2011). The diving bell and the spider: the physical gill of *Argyroneta aquatica. J. Exp. Biol.* **214**, 2175-2181.

Stride, G. O. (1955). On respiration of an aquatic African beetle, *Potamodytes tuberosus* Hinton. *Ann. Entomol. Soc. Am.* **48**, 344-351.

Terblanche, J. S., Klok, C. J., Marais, E. and Chown, S. L. (2004). Metabolic rate in the whip-spider, *Damon annulatipes* (Arachnida: Amblypygi). *J. Insect Physiol.* **50**, 637-645.

Thorpe, W. H. (1950). Plastron respiration in aquatic insects. *Biol. Rev. Camb. Philos. Soc.* **25**, 344-390.

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.

Thorpe, W. H. and Crisp, D. J. (1949). Studies on plastron respiration: IV. Plastron respiration in the Coleoptera. *J. Exp. Biol.* **26**, 219-260.

Vogel, S. (1981). *Life in Moving Fluids: The Physical Biology of Flow.* Princeton, NJ: Princeton University Press.