

Respiratory adaptations of secondarily aquatic
organisms:
studies on diving insects and sacred lotus

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(Gaertn.)

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Abstract

Compared with the free atmosphere, the aquatic environment is oxygen poor. As a result many secondarily aquatic organisms have adaptations that allow them to continue to use the atmosphere, directly or indirectly, to supply their oxygen requirements. This thesis examines how diving insects use bubbles of air collected at the surface of the water as oxygen reserves, gills and flotation devices, and how an aquatic angiosperm channels convective flows of air from its emergent leaves to its submerged organs.

1. Backswimmers (*Anisops* spp.) begin a dive positively buoyant, but rapidly enter a protracted period of near neutral buoyancy. A bubble of air held on the insect's abdomen shrinks as respiration consumes its oxygen, while at the same time highly soluble carbon dioxide dissolves into the surrounding water. The reduced air volume confers neutral buoyancy. In response to low oxygen partial pressure (PO_2) in the bubble, oxygen is released from large haemoglobin cells in the abdomen. The haemoglobin's sensitivity to falling PO_2 maintains the oxygen tension between 5.1 and 2.0 kPa. This stabilises the volume and buoyancy of the bubble. During a dive the haemoglobin and air-store supply 0.25 and 0.26 μL of oxygen, respectively.
2. The oxygen affinity of backswimmer haemoglobin determines the stability of the neutrally buoyant phase as well as its ability to satisfy the insect's respiration. An oxygen equilibrium curve (OEC) determined *in vivo* has a highly sigmoid shape and an oxygen affinity of 3.9 kPa. In comparison with OEC made *in vitro*, the *in vivo* measurements show increased cooperativity and oxygen affinity, consistent with the presence of cationic effectors. Models strongly support the accuracy of the *in vivo* OEC method.
3. It has long been assumed that a bubble of air held over the spiracles of an insect enables the uptake of oxygen from the surrounding water and thus acts as a 'gas gill'. Oxygen diffuses into a bubble of air when its PO_2 is lower than the surrounding water, but a coincident higher nitrogen partial pressure causes it to dissolve. Several models have been produced to describe the gas exchange process, but all are based on untested assumptions of gill parameters. Measurements of gas gill volume and PO_2 made on water bugs

(*Agraptocorixa eurynome*) demonstrate that both drop quickly at the beginning of a dive, but PO₂ reaches a stable level while the gas gill continues to dissolve. The importance of ventilation in maintaining an acceptable rate of oxygen consumption is also shown.

4. Interconnected gas spaces within the leaves, stems and rhizomes are a common feature of many emergent aquatic plants. Pressurised air from the leaves and culms of these plants ventilate these lacunae, flowing back to the atmosphere through efflux points. Unlike most aquatic plants, which have simple interconnected pith spaces, sacred lotus, *Nelumbo nucifera*, possess discrete gas canals which only interconnect where a leaf grows from the rhizome. Silicone casts and pneumatic tests of the gas canals reveal a complex repeating pattern of interconnections which channel air from specific regions of the leaf blade to the rhizome and out through efflux points on adjacent leaves.
5. Lotus, *Nelumbo nucifera*, possess in the centre of their leaves a specialised efflux organ which connects the gas canals in the leaves and stems with the atmosphere through the apertures of large stomata. Measurements made on excised lotus leaves and *in situ* reveal that the large stomata act as exhaust valves, opening and closing in a diurnal pattern to regulate the flow of pressurised gas from the leaf lamina and gas canals. This behaviour is shown to regulate gas flow rate and direction.

The aquatic environment offers similar respiratory challenges to both plants and insects. While the oxygen uptake and transport mechanisms evolved by these groups are markedly different, they all function according to the same physical laws. Diving insects are separated from the atmosphere while underwater, forcing them to rely on oxygen either carried with them from the surface or extracted from the surrounding water. Emergent aquatic plants have permanent access to atmospheric oxygen, but must transport it long distances from their aerial leaves and stems to their roots and rhizomes. This thesis examines the uptake and storage of oxygen by diving insects and the gas transport system of the sacred lotus.

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Publications Arising

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Matthews, PGD, Seymour, RS. (2006). Anatomy of the gas canal system of *Nelumbo nucifera*. *Aquatic Botany* **85**, 147-154

Introduction

Terrestrial organisms have returned to the aquatic environment many times since life made the evolutionary leap from water to terra firma. However, re-adapting to a life in water poses many problems, most notably concerning adequate oxygen supply. In comparison with air, water has approximately 1/30th the oxygen capacitance, 1/8000th the oxygen diffusivity, 50 times the viscosity, and 800 times the density (Dejours 1981; Schmidt-Nielsen 1990; Ward 1992). As a result, aquatic environments are poor in oxygen and offer considerable resistance to oxygen diffusion. Water-dwelling organisms can overcome these problems by using atmospheric air to supply or augment their oxygen requirements. Indeed, for many secondarily aquatic organisms, the successful occupation of an underwater niche is dependent on access to atmospheric oxygen. This thesis examines the respiratory adaptations used by secondarily aquatic organisms to supply their submerged oxygen requirements.

Insects are one of the most successful and diverse groups of organisms on the planet, occurring in every environment except the open ocean. Although insects are predominantly terrestrial, many lineages have secondarily adopted a partially or completely aquatic existence. Terrestrial insects breathe air through spiracles connected to a network of rigid tubes called tracheae, which divide into smaller and smaller tracheoles before terminating within tissues (Wigglesworth 1972). Those insects that have made the transition from terrestrial to aquatic life have retained their air-filled respiratory system (Mill 1985). While an aquatic insect with an open air-filled respiratory system risks drowning, many temporarily overcome this problem by covering their spiracles with a large bubble of air held on the ventral surface of the abdomen and/or under elytra (Parsons 1970). The air bubbles carried by diving insects have been thought to function as oxygen reserves, flotation devices and gills. In fact they are all these things, but the relative importance of each role varies between species. The aquatic bugs *Anisops deanei* and *Agraptocorixa eurynome* both carry bubbles of air on their abdomen while underwater, but use them for different purposes.

Backswimmers (*Anisops* and *Buenoa* spp., Notonectidae) are the only truly pelagic insects, completing their entire life cycle floating in the water column (Bare 1929). They surface periodically to refresh a small air-store held beneath a fringe of hairs on their abdomen. Immediately after submerging the bugs are positively buoyant, but they quickly enter a period of near neutral buoyancy. This ability is

linked to the presence of large quantities of haemoglobin within their abdomen (Miller 1964; Miller 1966; Wells *et al.* 1981).

Previous studies on backswimmers have tended to focus on the biochemical aspects of their haemoglobin. In this thesis I approach the relationship between buoyancy regulation and the oxygen-binding properties of the backswimmer's haemoglobin from a whole animal perspective, examining how changes in air-store volume and oxygen partial pressure (chapter 1) relate to the oxygen-binding properties of haemoglobin determined *in vivo* (chapter 2).

The idea that a bubble of air can function as a gas gill was first proposed by Comstock (1887, in Thorpe 1950), who noticed *Corixia* bugs fanning water over their bubbles when submerged. In his seminal paper, Ege (1915) showed that the water bugs *Corixia* and *Hyphidrus* could remain submerged for longer than the initial oxygen content of their bubble should allow. He attributed this finding to oxygen diffusing from the water into the bubble. Two numerical models have since been proposed to describe the diffusion of oxygen and nitrogen between a submerged bubble carried by an insect and the surrounding water (Chauy-Berlinck and Bicudo 1993; Rahn and Paganelli 1968). However, no one has yet measured changes in oxygen partial pressure within a functioning gas gill, and for this reason neither the models of Chauy-Berlinck and Bicudo nor Rahn and Paganelli can be critically assessed. Indirect observation of oxygen gain by recording dive time is not sufficient to understand completely the functioning of compressible gas gills.

Waterboatmen (*Agraptocorixa*, Corixidae) are dorso-ventrally flattened bugs which carry a large bubble of air covering the entire ventral surface. Measurement of changing oxygen content within their bubble, in conjunction with simultaneous measurement of buoyancy (as a measure of bubble volume) and insect ventilatory activity, provide a clear picture of gas gill function (chapter 3).

The freshwater environment holds many advantages for plants. Apart from the obvious benefit of water, aquatic environments are often rich in nutrients. But to access this resource, secondarily aquatic plants, like aquatic insects, have had to overcome problems caused by the low oxygen content and high diffusive resistance of water.

Low oxygen content and slow diffusion in still water favour the production of anoxic layers. Many higher plants root in the aquatic substrate, which is especially prone to anoxia. Submersed sediments with high organic content support abundant

communities of microbes which deplete available oxygen (Santruckova *et al.* 2001). Low oxygen levels are intrinsically problematic for macrophytes, but an anoxic environment also lowers the redox potential of the sediment, leading to the production of toxic levels of reduced chemicals such as Fe^{2+} and Mn^{2+} (Grosse *et al.* 1996b; Santruckova *et al.* 2001). This situation is worsened by anaerobes producing methane and other metabolites that can accumulate to harmful levels (Armstrong *et al.* 1996). The solution to all these problems is to ensure that any buried roots or rhizomes receive an abundant supply of oxygen (Colmer 2003; Grosse *et al.* 1996b), either directly from photosynthesis or from the atmosphere. Consequently, most secondarily aquatic plants possess continuous airspaces, usually termed lacunae, within their stems, leaves and rhizomes, and these allow for the movement of oxygen and other gases.

Diffusion of gas through air is approximately 240,000 times faster than through water, but it is not as effective as bulk flow in rapidly moving large volumes of gas over long distances. Bulk flow of gases occurs from a region of high pressure to a region of low pressure. For aquatic plants to take advantage of bulk flow, they must possess a flow-through system of lacunae with separate entry and exit points as well as a means of generating a pressure gradient across them. It has been known since the mid-1800s that the leaves of many emergent and floating-leaved wetland plant species are capable of generating a pressure flow to aerate their submerged organs. Currently, pressurised ventilation is known to occur among families of floating-leaved species including the *Nelumbonaceae* (Dacey 1987; Mevi-Schutz and Grosse 1988), *Nymphaeaceae* (Dacey 1980; Dacey 1981) and *Menyanthaceae* (Grosse *et al.* 1991), as well as many wetland grasses (Bendix *et al.* 1994; Hwang and Morris 1991; Teal and Kanwisher 1966; White and Ganf 1998; White and Ganf 2000) and trees (Buchel and Grosse 1990; Curran *et al.* 1996). Within this diverse range of growth types, several different methods of generating pressure flow have been identified.

The gas transport systems of most freshwater plants are simple, with the porous lacunae of leaves, stems, culms and rhizomes all interconnected. Thus, gas flows from the pressurising aerial organs and out through the nearest efflux point. The sacred lotus, *Nelumbo nucifera*, is unusual as it has discrete gas canals running through its leaves, petioles and rhizomes, while every leaf possesses a specialised efflux organ. The interconnections between the gas canals of *N. nucifera* are

examined (chapter 4) and the significance of the stomata comprising the efflux organ in regulating gas flows is tested (chapter 5).