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Implications of water flow and oxygen gradients for molluscan oxygen uptake and respirometric measurements

Sandra Irwin* and John Davenport

Department of Zoology, Ecology & Plant Science and Environmental Research Institute, University College Cork, Distillery Fields, North Mall, Cork, Ireland. *Corresponding author, e-mail: s.irwin@ucc.ie

Oxygen microenvironments adjacent to mussel (*Mytilus edulis*) and periwinkle (*Littorina littorea*) tissues were described using micro-optodes. For mussels these environments did not differ from the surrounding water body in either static or stirred conditions. Consequently no difference was seen in the MO_2 of mussels within stirred and unstirred respirometers. An oxygen extraction efficiency of 30% was recorded for mussels in stirred and unstirred conditions. Under static conditions, periwinkles, with their weak ventilatory arrangements, were not capable of ventilating the surrounding water efficiently, and their tissues were surrounded by hypoxic seawater, even in fully aerated water. The resultant build up of oxygen gradients close to the tissue surface led to measures of oxygen consumption representative of hypoxic conditions in fully aerated, unstirred respirometers. Stirring of the contents of the respirometer immediately prior to final oxygen concentration measures was insufficient to counter this effect, which could only be avoided by continuous artificial stirring of the respirometric chamber water. These findings have considerable implications for study of the oxygen consumption of aquatic animals with limited ventilatory capacity, such as gastropod molluscs.

Mussels and periwinkles were collected from the south coast of Ireland (51°41′N 83°1′W and 51°39′N 83°5′W, respectively) in Autumn 2004. In this series of experiments oxygen was measured using a temperature-compensated oxygen meter with fibre-optic oxygen microsensors (PreSens GmbH, Germany). These are optical chemical sensors (or micro-optodes) with a tip diameter of less than 50 μm that, unlike traditional amperometric oxygen electrodes, consume no oxygen during measurement. Optodes were calibrated using a conventional two-point calibration in oxygen-free water and water-vapour saturated air, and during operation, continuous recordings of oxygen were made at 1 s intervals. For investigation of oxygen micro-environments, animals were immobilized by fixing to a Perspex rod within a 2 litre experimental chamber filled with seawater (34‰). Oxygen concentrations were measured at 1 mm distance intervals from mussel tissue at both the inhalant and exhalent siphon sites (N=8), and adjacent to the gill tissue of periwinkles (N=9) under both static and flowing conditions.

Mussels used for respirometry had a mean length of 4.0 cm and a mean dry tissue weight of 0.44 g. Periwinkles had a mean shell height of 2.3 cm and a mean dry tissue weight of 0.25 g. Test animals were held in individual clear plastic closed respirometers (100 ml for mussels, 63 ml for periwinkles), each containing a 12 mm magnetic follower, isolated from the main experimental chamber by a mesh barrier. The respirometers were mounted over submersible magnetic stirrers (Rank Brothers Ltd, UK) in a constant temperature bath at 18°C. Measurement of oxygen content (% air saturation) was by means of microsensors inserted through sealed holes in the lids of the respirometers. Control respirometers without animals were used to account for any chamber effects on oxygen concentration; none were observed. The animals were divided into two groups (N=8) for each species. In the first group, the chamber was continuously stirred, and in the second, the water was static, but was stirred briefly before the final measurement of oxygen concentration was taken. Respiration rates were calculated on the basis of dry weight (determined by drying the tissue to a constant weight at 60°C) and expressed as μmol O_2 g^{-1} h^{-1} dry weight.

Oxygen concentrations (P*i*, P*e*) were determined within the inhalant and exhalant siphons of mussels under static conditions and in flowing seawater, using either filtered seawater or seawater containing algal cells (*Isochrysis galbana*) at a particle concentration >5000 cells ml^{-1} (N=6 per treatment). Two micromanipulators were used to manipulate the oxygen probes into position in the inhalant seawater flow and within the exhalant siphon. The results were used to calculate the oxygen extraction efficiency (E) of undisturbed actively filtering animals using the equation:

$$E = \left(\frac{Pi - Pe}{Pe}\right) \times 100\% \quad (1)$$

Water flow and oxygen conditions

At both inhalant and exhalant siphons of mussels, oxygen concentrations were similar to the surrounding seawater in static and stirred conditions, with no gradients or significant differences observed in measured oxygen concentration at any point within 5 mm of mussel tissue (Figure 1). The mean (SEM) oxygen concentration measured adjacent to the gill tissue of periwinkles in fully aerated seawater was 42.5 (0.2) % air saturation under static conditions. Under static conditions the oxygen concentration was seen to increase through the first few millimetres from the tissue of periwinkles, and reached levels similar to the surrounding seawater at a distance of approximately 5 mm. Steep declining oxygen concentration gradients were measured through this layer moving away from the tissue surface (Figure 2). These results demonstrate conclusively that, even in oxygen-rich seawater, marine invertebrates that are poor ventilators may experience considerably hypoxic conditions adjacent to their tissues. Facultative anaerobes such as the periwinkle are well equipped to survive such conditions and can easily adapt to rapidly changing oxygen conditions. However, one of the important implications of the steep oxygen gradients observed adjacent to periwinkles in static normoxic seawater is their potential to impact on oxygen uptake in an air saturated water body. Oxygen uptake becomes depressed under hypoxic conditions (Bayne, 1971), and static conditions impose localized hypoxia.

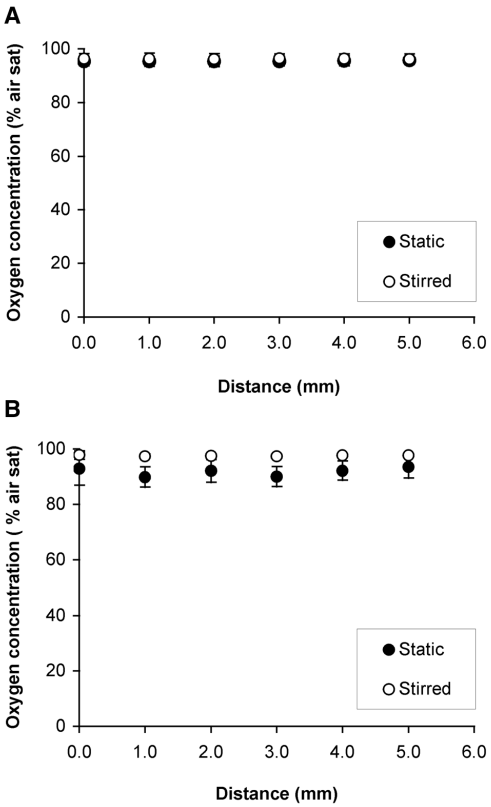

Figure 1. Oxygen concentrations (mean ±SEM) in seawater surrounding mussels, adjacent to (A) the inhalant siphon; and (B) the exhalent siphon, in static and stirred conditions.

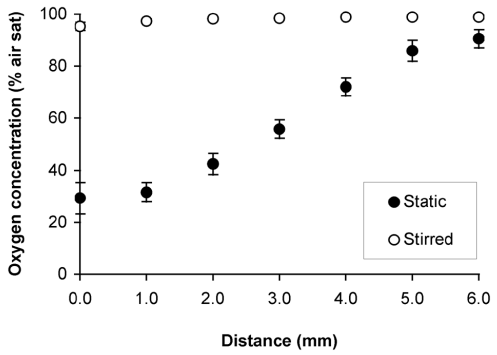

Figure 2. Oxygen concentrations (mean ±SEM) adjacent to, and at 1 mm intervals away from periwinkle gill tissue in static and stirred seawater.

Water flow and oxygen uptake rate

The average dry weight oxygen consumption (MO_2) of mussels in unstirred respirometric chambers was 20.7 (1.7) μmol O_2 g^{-1} h^{-1} DW and in stirred chambers was 21.6 (1.3) μmol O_2 g^{-1} h^{-1} DW. When the seawater in the chambers was mixed briefly before a final reading was taken, the corresponding MO_2 was 21.0 (1.3) μmol O_2 g^{-1} h^{-1} DW. No significant effect of stirring of respirometric chambers was observed ($F_{21,2}$=1.63, $P>0.05$). These results indicate that normal cilliary ventilation of the mussels is sufficient to maintain irrigation of the gills with oxygenated water and to allow for normoxic oxygen consumption, as suggested by Taylor & Brand (1975). By contrast, a significant difference was seen between the results obtained using stirred and unstirred respirometers with periwinkles ($F_{21,2}$=0.13, $P<0.05$). The measured MO_2 of periwinkles in unstirred respirometric chambers was 20.4 (3.4) μmol O_2 g^{-1} h^{-1} DW; following mixing was 19.9 (±2.0) μmol O_2 g^{-1} h^{-1} DW, and in continuously stirred chambers was 25.3 (±1.0) μmol O_2 g^{-1} h^{-1} DW. The latter is similar to values reported in previous studies carried out in normoxic conditions (McMahon & Russel-Hunter, 1978). These results demonstrate that for this weakly ventilating species, oxygen uptake is depressed by as much as 20% in unstirred respirometers, to levels indicative of hypoxic stress (McMahon & Russel-Hunter, 1978). Brief mixing of the water in the respirometer chamber at the conclusion of measurements served only to provide a true measure of oxygen consumption within the entire chamber. In the absence of this mixing, the measure of oxygen concentration within the chamber is arbitrary, and representative only of the oxygen concentration at the sensor tip, in a water body where oxygen gradients have developed, unless irrigation has been provided by the animal's own ventilatory activity. The effect of mechanical stirring of the respirometry chambers throughout experimentation was to replace this behaviour with mechanical irrigation, thus increasing the oxygen available to the periwinkles and sustaining oxygen uptake rates at normoxic levels.

Direct measurement of oxygen extraction efficiency (E) in mussels showed that it did not differ significantly between flow treatments when food was withheld from the mussels (static: 34.4%; flow: 33.2%; T=0.16, df=5, $P>0.05$). Furthermore, oxygen extraction efficiency was significantly reduced (by approximately half) when food was made available to the animals (starved: 33.2%; fed: 17.0%; T=6.29, df=5, $P<0.01$). Previous workers using indirect determination of mussel oxygen extraction efficiency, report values in air-saturated water of between 5 and 12%. Very high values have been measured in the laboratory in response to increased food availability and there is no evidence that they can be sustained over long periods (Widdows et al., 1979). Feeding and metabolism are linked in mussels, and evidence to date, collected indirectly, supports an exponential increase in extraction efficiency with increasing seston concentration. The present study demonstrates that the effect of complete, prolonged food deprivation (i.e. seston concentration of zero) is in fact an increase in oxygen extraction efficiency, most likely as a consequence of reduced pumping rate.

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