Lacunar gas discharge as a measure of photosynthesis in seagrasses

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ABSTRACT: The gas filled lacunae of seagrasses receive some of the oxygen produced in photosynthesis. When the lacunar system is tapped a stream of gas is released; its flow rate is easily measured under both laboratory and field conditions. At a series of net photosynthetic rates from zero to maximum, there is a linear, zero-intercept calibration regression between volumetric gas stream observations with simultaneous oxygen production measurements. With this species-specific calibration factor all that is required to make accurate and sensitive measurements of photosynthetic rates is a graduated pipette, a razor blade and a small piece of rubber tubing.

INTRODUCTION

The difficulties and advantages of various techniques for measuring the growth rates or productivity of seagrasses have been reviewed by McRoy & McMillan (1977) and by Zieman & Wetzel (1980). The oxygen production techniques have been criticized. Zieman & Wetzel (1980) state that ' . . . it is now apparent that the oxygen technique results in quite erroneous values and should not be used'. McRoy & McMillan (1977) caution that, 'At the present time all measurements involving dissolved oxygen to estimate productivity must be considered questionable in view of the results of Hartman & Brown (1967), who demonstrated in freshwater aquatic plants that the oxygen resulting from photosynthesis is internally recycled in the lacunar spaces of the leaves . . . This problem deserves thorough study in seagrasses.' These comments have led many people to assume that the problems of determining oxygen production in other aquatic plants also apply to seagrasses.

The emergence of a stream of gas bubbles from submerged plants, particularly from cut surfaces, has been extensively reported (e.g. Arber 1920, Sculthorpe 1967, and references cited therein). Edwards & Owens (1965) showed that the rate of gas bubble loss increased with increasing irradiance or increasing oxygen supersaturation of water in communities of 2 freshwater macrophytes. Odum (1957) has correlated the release of gas with photosynthesis of a submerged *Sagittaria* community. However, Zieman & Wetzel (1980) conclude in their review that it ' . . . is clear that this relationship is highly variable and should not be used as a measure of rates of photosynthesis'. In view of these statements, it would appear that a study of oxygen production of the lacunar system in this regard is long overdue, even though earlier efforts have not been encouraging.

Lacunar gas system

Seagrasses share 2 important anatomical features with most other aquatic vascular plants: they lack stomata and the cortex of the roots, rhizomes, stems, petioles and leaves have gas-filled lacunae. The leaf chloroplasts of most seagrasses are concentrated in the unicellular epidermal layer which is thus the site of most of the oxygen produced during photosynthesis (Kuo 1983). This oxygen can be lost to the surrounding water by diffusion through the thin cuticle layer or to the lacunar spaces in the adjacent cortex. During active photosynthesis, the resulting increase in gas pressure of the leaf lacunae can lead to as much as a doubling of the leaf volume in *Thalassia testudinum* Banks ex Köning (Zieman 1974). Presumably this pressure increase is due to oxygen production. How-

ever, Kniep (1915) is quoted by Sculthorpe (1967) as having observed gas compositions of between 30 and 54 % oxygen with the balance largely nitrogen for 3 species of freshwater macrophytes. It would indeed be strange to find particularly high oxygen concentrations in a space which is in diffusive contact with an air-saturated solution.

Recent work by Roberts et al. (1984) has demonstrated the interconnection of the lacunar spaces in the leaves, petioles, stems, rhizomes and roots of *Halophila ovalis* (R. Br.) Hook f. Lacunae are continuous in the internode ending in porous diaphragms at each node. These small (0.5 to 1.0 μm) pores provide gas connectivity throughout the system. Although the connecting pores have not yet been observed, *Amphibolis antarctica* (Labill.) Sonder et Aschers. exhibits a similar lacunar morphology, and a continuous stream of bubbles is produced from any cut surface in any nodal segment of the stem or rhizome during active photosynthesis. Because this represents a stream of gas produced in intimate diffusive contact with photosynthetically active tissue, it seems sensible to attempt to use this gas release rate as a measure of photosynthetic activity. To do this, it is necessary to compare the lacunar gas release rate and the oxygen composition of this gas mixture with the rate at which oxygen is lost to the surounding water.

Thus the total oxygen produced as the sum of losses to both sinks, is measured. This overcomes the criticisms cited above, and the suitability of the rate of lacunar gas release as a measure of the total photosynthetic rate can be examined.

MATERIALS AND METHODS

Experimental material. Samples of *Amphibolis antarctica*, *Posidonia australis* Hook f. and *Halophila ovalis* used in the laboratory were either collected the morning of the experiment or held in running seawater for a few days before use. All of these species are available in the shallow water adjacent to the University of Western Australia Marine Biological Laboratory. *A. antarctica* is tough and can often be pulled out of the sediment with its rhizome and hairless roots intact. *P. australis* and *H. ovalis* are more delicate and the sediment must be blown or fanned away from the roots. Care was exercised to collect material as free from epiphytes as possible with generally less than 25 % of the leaf surface having visible epiphyte cover. The amount of plant material was measured in terms of leaf area or number of leaf heads or dry weight (constant weight at 105 °C).

Gas collection from the seagrass lacunar system. The petiole, stem or rhizome of a seagrass sample was cut through cleanly with a new razor blade, and connected to a 3-way luer-lock stopcock with a short section of silicon rubber tubing. One outlet of the stopcock was connected to a graduated pipette and the second served to withdraw gas samples with a hypodermic syringe. Alternatively, some plant segments were connected directly to a pipette with a silicon tubing section and gas samples were taken directly by puncturing the tubing with a needle and syringe. The connections were as secure as possible, but as the gas collection was made with nearly zero pressure difference, the tubing served only to guide the gas stream to the measuring pipette. Silicon rubber tubing was chosen as the most suitable material: it is soft; has good elastic properties; is available in a good range of internal diameters and wall thicknesses; and the usual formulations are non-toxic. The inside diameter of the tubing was slightly smaller than the stem diameter and the wall thickness was inversely related to the delicacy of the plant material. Where possible, the cut and connection were made in air to prevent flooding and blocking of the lacunar system, although underwater connections during active photosynthesis rarely cause problems.

In the laboratory, water was drawn up into the collection pipette with a syringe by putting the open end in a test tube of water and filling the pipette to the zero point. The vertical distance between the zero point of the pipette and the free water surface in the test tube determines the small negative pressure (2 to 8 cm water) on the cut stems. In the field situations, gas was collected in the pipette by direct water displacement at ambient pressure.

As many plant elements as desired can be connected to the same pipette by providing some sort of gas collection manifold. We have used up to 8 *Amphibolis antarctica* stems on 1 pipette with a 'tree' of 7 'Y' connectors joined by short sections of silicon tubing. With an intact system, the distal rhizome section with stems and leaves attached can be connected to a single pipette so that the rhizome serves naturally as a manifold. However, the laboratory technique should reflect the type of sample that can readily be obtained and used in field situations.

Test equipment. Laboratory experiments were conducted by incubating seagrass samples in glass jars. The jars were covered with a clear plastic lid that contained 3 holes to admit an oxygen electrode, an irradiance meter sensor and the silicon tube connecting the sample to the collection pipette. Oxygen concentration was measured with an Orion Research, model 97-08, oxygen electrode. Irradiance was measured with a Biospherical Instruments Inc., model QLS-100, radiometer. The jar was completely filled with seawater and kept well mixed with a magnetic stirring

bar. The jar stood in a water bath whose temperature was maintained at 21.0 ± 1.0 °C by constant flushing with tap water. The seawater in the jar was changed after each photosynthetic rate determination in order to keep the range of ambient oxygen concentration constant and as close to the saturated value as possible. The experiments were conducted under natural light between mid-morning and mid-afternoon under completely cloudless skies. Neutral density screens were used to obtain a range of light intensities necessary to give a complete range of photosynthetic rates.

Gas samples of 1.0 or 0.5 cm³ were collected in disposable plastic syringes and analysed on a Carle Analytical Gas Chromatograph fitted with thermistor sensors and a 91.4 × 0.635 cm stainless steel column packed with 60/80 molecular sieve 5A. The gas chromatograph was operated at 60 °C with the helium carrier gas at a flow rate of 65 cm³ min⁻¹ to analyse for oxygen and nitrogen content in the gas mixture.

RESULTS

Lacunar gas production, as a function of time of day, exhibited features characteristic of plant photosynthesis. The rates rose with increasing light intensity to a morning maximum, dropped back to a broad shoulder from midday to late afternoon, then fell as the light intensity decreased. Scattered clouds had little effect from mid-morning to mid-afternoon but at lower light intensities, the shading signature of each cloud was distinct in the record. Thus the gas release rate as a function of time was a sensitive and responsive indicator of the photosynthetic rate. These observations are similar to those reported by Zieman & Wetzel (1980).

Oxygen released into the lacunae represented between 7 and 8 % of the total oxygen production. Results for the release of oxygen both to the surrounding water and the lacunal space by *Amphibolis antarctica* during photosynthesis are shown in Table 1. The data represent 3 different seagrass collections and are thus representative of the reproducibility of the methods as well as population sampling; variability is minimised by the use of 8 stems in each case.

In order to investigate the constancy of the ratio describing oxygen partitioning between lacunar space and the surrounding water at various photosynthetic rates, the seagrass collections referred to in Table 1 were exposed to various light intensities. The regression and light saturation data presented in Fig. 1 and 2 were collected from the same experiments. There is a very close correlation between oxygen released to the water with that released from the lacunar system. The line, y = 0.612 + 5.18x, where y represents the oxygen lost to the water in mg h⁻¹ and x represents the gas

Table 1. *Amphibolis antarctica.* Comparison of oxygen production in the lacunar system with that of the surrounding water at saturating light intensities and at an ambient temperature of 21.0 ± 1.0 °C. All tabulated rates represent regression coefficients associated with sample correlation coefficients >0.99

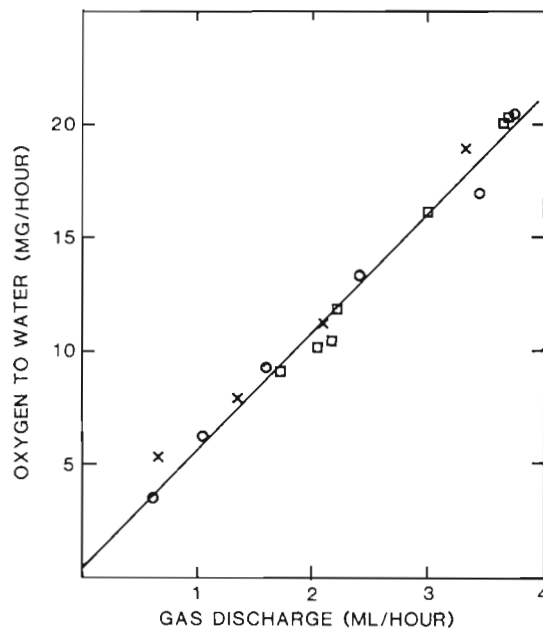| Amount of seagrass* | | % Oxygen in lacunar gas* | Oxygen production* (mmol h⁻¹) | |
Number of leaf heads (stems)	Dry weight (g)		Lacunar space	Surrounding water
21 (8)	4.85	31.8	0.0432	0.571
22 (8)	4.90	31.7	0.0451	0.557
22 (8)	4.84	31.9	0.0451	0.521

* Single samples

Fig. 1. *Amphibolis antarctica.* Comparison of lacunar gas discharge rate and the rate at which oxygen is lost to the surrounding water over a complete range of photosynthetic rates. Three sets of data (x, ○, □) are presented representing 3 samples of 8 stems each. The line is a type 1 least squares fit to the plotted data

release rate in cm³ h⁻¹, is a very good description of the data in Fig. 1 (correlation coefficient = 0.99). The regression coefficient, 5.18 mg cm⁻³, predicts with good precision the oxygen (in mg) lost to the surrounding water for each cm³ of gas released from the lacunae. The value of y = 0.612 at x = 0 is not significantly different from zero. Thus, using a calibration curve of the type shown in Fig. 1, it is no longer necessary to make both measurements, as one can be
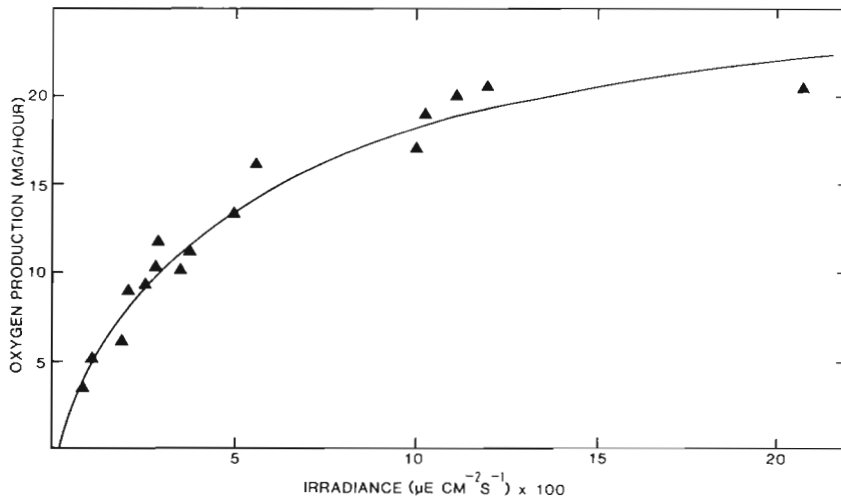
Fig. 2. *Amphibolis antarctica*. A light saturation curve with photosynthetically available sunlight. The least squares fitted curve, p = 27.3 I/(522 + I), p = photosynthesis, I = irradiance, is based on lacunar gas release data, and the plotted data represents the oxygen lost to the surrounding water

predicted from the other with a precision that substantially exceeds our ability to measure the amount of photosynthetically active plant material involved.

The comparable relation for *Halophila ovalis* is y = 0.0434 + 1.270x with a correlation coefficient of 0.94. Again at x = 0, the y intercept is not significantly different from zero.

Ten observations at the gas release volume taken at 2 min intervals from a single shoot of *Posidonia australis* are described by a least-squares fitted straight line with the regression accounting for 99.9 % of the variance of the dependent variable, and with the gas release rate equal to 1.29 $cm^3 h^{-1}$. The precision of this result is representative of all our results for both *Amphibolis antarctica* and *Halophila ovalis*.

The total amount of oxygen produced varies with light intensity, and reaches a saturation value that is typical for the relation of photosynthesis with irradiance (Fig. 2). Maximum photosynthetic activity in *Amphibolis antarctica* is reached at approximately 50 % of full sunlight. A comparable light saturation curve was obtained with *Halophila ovalis*. At lower light intensity, the photosynthetic rate increased with increasing irradiance. At higher light intensities, the curve indicates that photosynthesis was maximal at about 50 % of full sunlight, but unlike *A. antarctica* there was photoinhibition at higher light intensities. These features are qualitatively and quantitatively similar to those described by Drew (1979) for *Halophila stipulacea* (Forsk.) Aschers.

The curve in Fig. 2 is a least-squares fit of a hyperbola which can be used as a description of such data (Caperon 1967). Using careful measurements and appropriate curve fitting techniques, the curve should not intersect the origin. The light intensity at zero net oxygen production can provide useful information on respiration rates at low light intensities. This light

intensity value is very small with most plants and it is evident that our results are not exceptional in this regard (see Caperon 1967 for a more extensive discussion of this subject).

DISCUSSION

Considerable difficulty has been experienced in using gas exchange techniques for photosynthetic measurements in the past. This has been largely due to the technical difficulties experienced by others in dealing with lacunar gas exchange. These difficulties may be completely overcome by attaching a glass tube to a cleanly cut stem and measuring changes in gas release volume. Indeed, a technique is now available for monitoring the photosynthetic performance of aquatic vascular plants which could be the envy of plant ecologists who deal with algae or terrestrial plants. This technique is sufficiently precise and responsive for use in physiological studies in the laboratory. It is sufficiently convenient and simple to make field ecology with seagrasses a pleasure.

The delicacy and small size of *Halophila ovalis* impose some technical difficulties which were not experienced with *Amphibolis antarctica* and *Posidonia australis*. More care is required in obtaining a secure connection of the cleanly cut stem to the rubber tubing without damaging or blocking the lacunar system. At the same time more connections are required to provide enough plant material (leaf surface area) to obtain relatively rapid changes in dissolved oxygen concentrations. Decreasing the volume of the surrounding water offers some relief, but to obtain results of the same quality as that for *A. antarctica*, we would have had to design a special incubation chamber to ensure

good light exposure, good mixing and to accommodate the light, oxygen and temperature probes. The lower correlation coefficient reflects some of these difficulties. Although the relation is good enough to support the use of gas release rate as a predictor of photosynthetic rate, it could be substantially improved by bringing the level of precision of the dissolved oxygen measurements equal to those of the gas release rates.

There is the question of whether or not the stem and leaf part severed from the root-rhizome component is photosynthetically intact. We can think of no reason why it should not be. Cut stems held overnight for additional experiments gave the same results on the second day. The photosynthetic rate for a cut section in the rhizome is the same as that of the connected stems alone after separation for both *Amphibolis antarctica* and *Halophila ovalis* (*Posidonia australis* has not been examined). In the absence of any information to the contrary, it must be assumed that the photosynthetic rate of intact seagrasses is as high as that of the cut stems, because even with the pressure relief from stem cutting, the lacunar oxygen sink accounts for only 7 to 8 % of the oxygen loss. With an intact lacunar system, pressure will build up to a point where there is a rupture or where oxygen lost from the lacunae by diffusion equals the supply; in either case, the photosynthetic rate is esseentially the same, and in any case, the difference is less than 7 or 8 %.

Any marked change in the oxygen solubility of the surrounding water will change the oxygen partitioning between the immediate environment and the lacunar space and may require correction or a new calibration (Fig. 1). Thus marked differences in oxygen concentration, temperature or salinity may require at least a one-or-two point calibration check. Similar caution is needed if substantial changes in the extent of epiphytic growth occurs.

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