Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry

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ABSTRACT: Photosynthetic rates of seagrasses have until recently been measured as gas exchange of chamber-enclosed leaves mainly in the laboratory, and in situ measurements under natural conditions are scarce. In this work we explore the possibility of measuring such rates by pulse amplitude modulated (PAM) fluorometry, using a newly developed underwater device. This was done by first comparing photosynthetic O₂ evolution (net photosynthesis corrected for dark respiration) with rates of electron transport (ETR) derived from fluorescence measurements of the effective quantum yield of photosystem II multiplied with the estimated photon flux of photosynthetic active radiation absorbed by this photosystem. In the field, ETRs were then measured both as rapid light curves (RLCs) and by in situ point measurements under ambient light during the day. Photosynthetic O₂ evolution showed a linear relationship with ETR within a range of irradiances for the Mediterranean seagrass Cymodocea nodosa, while the tropical Halophila stipulacea and a temperate intertidal population of Zostera marina exhibited decreasing O₂ evolution rates relative to ETRs at high irradiances. These differences are likely due to photorespiration, which is absent in C. nodosa. The molar ratio between photosynthetic O₂ evolution and ETR within the range of their linear relationship was found to be 0.3 for C. nodosa, which is close to the theoretical stoichiometric ratio of 0.25, but was higher and lower for Z. marina and H. stipulacea, respectively. Point measurements of ETR in the field showed good agreements with rates derived from RLCs for H. stipulacea and Z. marina, but values varied greatly between replicate measurements for C. nodosa at high irradiances. It is speculated that this variation was partly due to light-flecks caused by waves in the shallow water where these measurements were done. In all, this work shows that PAM fluorometry can efficiently yield photosynthetic rates for seagrasses in the laboratory, without the typical lag experienced by O₂ electrodes, as well as in situ under natural conditions which are not disturbed by enclosures.

KEY WORDS: Marine angiosperms, Photosynthesis, PAM fluorometry, Seagrasses

INTRODUCTION

Photosynthetic rates of marine angiosperms (seagrasses) are typically determined by enclosing the plants, or parts of them, in chambers and measuring either O₂ evolution or ¹⁴CO₂/H¹⁴CO⁻₃ uptake. While information on photosynthetic mechanisms has been obtained in this way (reviewed in Beer 1997), one drawback of this approach for eco-physiological studies is that the plants must either be removed from their growth site and/or be enclosed in chambers where conditions differ from those of their normal environment. Therefore, it has usually not been possible to determine rates of photosynthesis under in situ natural conditions for this plant group. Photosynthetic parameters can also be appraised by non-intrusive chlorophyll fluorescence measurements. For example, the potential (or maximal) quantum yield of electron flow through photosystem II (PSII) can be estimated in dark-adapted plants as $F_v/F_m$ (Krause & Weis 1991, see Dawson & Dennison 1996 for the application of this method for seagrasses). In addition, pulse amplitude modulated (PAM) fluorometry has made it possible to determine a similar parameter also in the light as $\Delta F/F_m\prime$, where $F_m\prime$ is the maximal fluorescence of a light-
adapted plant when all reaction centres are reduced, or closed [as caused by a short dose of saturating light], and $\Delta F = F_{m} - F$, $F$ being the fluorescence in the light when part of the reaction centres are open (see Schreiber & Björk 1993). $F/F_{m}$ can be termed the effective quantum yield ($Y$) of photosynthetic electron transport through PSII, and this parameter has been shown to vary proportionally with the quantum yield as determined either by CO$_2$ uptake in barley (Genty et al. 1989) or, within a certain range of irradiances, O$_2$ evolution in a cyanophyceae (Sundberg et al. 1997). If so, then multiplying $Y$ by the photosynthetic photon flux (PPF) absorbed by PSII (in $\mu$mol photons m$^{-2}$ s$^{-1}$) should yield rates of photosynthetic electron transport (ETR, in $\mu$mol electrons m$^{-2}$ s$^{-1}$). While PAM fluorometers thus can determine $Y$, quantitative calculations of ETR require that at least the fraction of PPF absorbed by the leaf ($AF$) is known so that the irradiance absorbed by PSII can be estimated.

The first underwater PAM fluorometer became commercially available in April 1997. Since then, a few reports have evaluated its use for measuring relative (Schreiber et al. 1997, Beer & Ilan 1998, Ralph pers. comm.) as well as absolute (Beer et al. 1998) photosynthetic rates of photosymbiont-containing marine invertebrates. In one as yet unpublished study by Beer & Björk, the photosynthetic performance of 2 tropical intertidal seagrasses was measured by PAM fluorometry. When comparing ETRs as a function of PPF with rates of O$_2$ evolution, a linear relationship was found for one of the species (Halophila ovalis) but not for the other (Halodule wrightii); in the latter, rates of O$_2$ evolution became lower relative to ETRs at high PPFs. These differences, as well as the scarcity of similar data, urged us to verify the use of PAM fluorometry for quantitative photosynthetic assessments in a wider variety of seagrasses including, for the first time, in situ underwater measurements. This was done by (1) estimating their AF values, (2) comparing the light responses of ETR with those of O$_2$ evolution and (3) performing field measurements in situ, both as rapid light curves (RLCs) and as point measurements under ambient light.

MATERIALS AND METHODS

Plant material. Three seagrass species were used in this study. One of them, an eastern Mediterranean population of Cymodocea nodosa (Ucria) Aschers., was found growing in shallow water outside the Limnological and Oceanographic Institute, Haifa, Israel (32° 50' N, 34° 57' E). While laboratory O$_2$ exchange versus fluorometric measurements were done at the institute, field fluorometry was carried out in situ at ca 0.5 m depth (in November 1997). A second species investigated was Halophila stipulacea (Forsk.) Aschers. from the northwestern coast of the Gulf of Aqaba, the Red Sea. Laboratory experiments were carried out at the Inter-University Institute in Eilat, Israel (31° 35' N, 34° 54' E), and field measurements were done at ca 6 m depth just outside the institute (in October 1997). The third seagrass used was an intertidal population of the temperate Zostera marina L. from the White Sea, northwestern Russia. Laboratory measurements were done at the Kartesh Zoological Station east of Chupa, some 40 km south of the polar circle (66° 20' N, 33° 40' E), while field work was carried out on air-exposed plants during low tide in a nearby bay (in late September 1997).

Determining fractions of absorbed light (AF). AF values of the different seagrasses were determined in the following way: leaves were placed on top of photosynthetic active radiation (PAR) quantum sensors connected either to a Li-Cor (USA) LI-189 (for Zostera marina in the air), or to the light meter of the fluorometer described below (for the other 2 species during submersion). The irradiance from a fibre-guided halogen light source (Schott, Germany) reaching the sensors (Lr) through 1 to 4 layers of leaves was recorded. The Lr values were then plotted against the number of leaf layers, and the linear correlation was calculated. The slope of this line ($a$) and the $\gamma$-intercept ($\beta$) were determined by regression analysis, and AF was calculated as $1 - \exp(\beta a)$. Reflectance from the upper leaf surfaces was estimated as the difference between the irradiance extrapolated to zero leaves [$\exp(\beta a)$] and the incident irradiance actually measured when no leaves were present. No significant difference could be found between these 2 values in these seagrasses, and it was assumed that the AF values obtained therefore adequately described the fraction of light absorbed by the leaves.

Comparing O$_2$ evolution and electron transport. 'Gross' photosynthetic O$_2$ evolution rates (net photosynthesis corrected for dark respiration) were determined for ca 3 cm long leaf sections (or whole leaves for Halophila stipulacea) using a Hansatech (UK) O$_2$ electrode set-up. The leaves were inserted into the electrode chamber in the shape of an upside-down 'U'. Light, at various irradiances, was supplied by a dual-fibre optically guided halogen lamp (Schott, Germany) in a way that provided a seemingly uniform distribution along both sides of the leaf. Temperatures during these measurements were kept close to the ambient ones: 20°C for Cymodocea nodosa, 23°C for H. stipulacea and 7°C for Zostera marina.

Simultaneous to the O$_2$ measurements, chlorophyll fluorescence parameters were measured using the PAM fluorometer Diving-PAM (Walz GmbH, Ger-
ETR were either calculated as described Fig. 1. (a) Time course of photosynthetic evolution (GPS, left y-axis) after downloading the measured data and electron transport rate (ETR, right y-axis) at various irradiances (indicated as pm01 photons m\(^{-2}\) s\(^{-1}\)) and (b) the corresponding GPS versus ETR, in a leaf of Cymodocea nodosa. Measurements were done at 20°C.

Field fluorometry. Field measurements with the Diving-PAM were done both on air-exposed (Zostera marina) and submerged shallow (Cymodocea nodosa, in choppy waters, by snorkelling), as well as deeper (Halophila stipulacea, accessed by SCUBA diving) growing, plants. Rapid (within 5 min) light response curves (RLCs) were generated by irradiating parts of leaves with the actinic (photosynthesis-causing) light source of the Diving-PAM at pre-set PPFs. This was done by clipping part of an intact leaf to a modified 'dark leaf clip' with the shutter open, and then attaching the tip of the instrument's main light guide to it just before starting a RLC. (The clip was modified so as to allow for water flow around the leaf segment measured through small drilled holes.) Values of ETR were calculated as described above.

In addition to the light response curves, point measurements under natural light were carried out on 20 to 30 different leaves in the 3 different seagrass beds at various times of day. The 'leaf distance clip' was used for this purpose, and the tip of the Diving-PAM's PAR sensor was also attached to this clip so that it recorded the incident irradiance adjacent to the leaf, and at the same angle of downwelling light as experienced by the leaf, at the time of each separate measurement. Values of ETR were either calculated as described above after downloading the measured data from the Diving-PAM's memory to a computer, or were calculated by the Diving-PAM itself in cases where the correct AF had been pre-programmed into the Diving-PAM's memory point termed 'ETR factor' (instead of the pre-set value of 0.84).

**RESULTS**

Values of AF for the different seagrass leaves are shown in Table 1. These values were all lower than the default 'ETR factor' of 0.84 set for the Diving-PAM, and they were used when calculating ETRs. The differences in AF values among the species are mostly due to different thicknesses and chlorophyll contents of the leaves.

Fig. 1. (a) Time course of photosynthetic O₂ evolution (GPS, left y-axis) and electron transport rate (ETR, right y-axis) at various irradiances (indicated as µmol photons m\(^{-2}\) s\(^{-1}\)) and (b) the corresponding GPS versus ETR, in a leaf of Cymodocea nodosa. Measurements were done at 20°C.
Average rates of photosynthetic $O_2$ evolution (GPS) versus electron transport (ETR) in leaves of *Cymodocea nodosa* (measured at 20°C). Data points are average of 3 to 6 measurements ± SE for each of 4 leaves at various irradiances (0 to 300 μmol photons m$^{-2}$ s$^{-1}$, increasing toward the right in the figure). The points were fitted to a straight line, $r^2 = 0.93$.

Table 1. Average ($±$ SD, n = 5) fractions of incident light absorbed by a leaf (AF) of the different seagrasses.

<table>
<thead>
<tr>
<th>Species</th>
<th>AF</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cymodocea nodosa</em></td>
<td>0.72 ± 0.11</td>
</tr>
<tr>
<td><em>Halophila stipulacea</em></td>
<td>0.50 ± 0.03</td>
</tr>
<tr>
<td><em>Zostera marina</em></td>
<td>0.44 ± 0.02</td>
</tr>
</tbody>
</table>

Fig. 3. (a) Time course of photosynthetic $O_2$ evolution (GPS, *o*, left y-axis) and electron transport rate (ETR, *a*, right y-axis) at various irradiances (indicated as μmol photons m$^{-2}$ s$^{-1}$) and (b) the corresponding GPS versus ETR, in a leaf of *Halophila stipulacea*. Measurements were done at 23°C.

Fig. 2. Average rates of photosynthetic $O_2$ evolution (GPS) versus electron transport (ETR) in leaves of *Cymodocea nodosa* (measured at 20°C). Data points are average of 3 to 6 measurements ± SE for each of 4 leaves at various irradiances (0 to 300 μmol photons m$^{-2}$ s$^{-1}$, increasing toward the right in the figure). The points were fitted to a straight line, $r^2 = 0.93$.

the 2 measurements was the lag of a few minutes for obtaining steady-state $O_2$ evolution rates following each alteration of the irradiance, while the ETRs showed immediate responses. Plotting the $O_2$ data versus ETRs (Fig. 1b) showed a linear relationship between these 2 parameters. Similarly, the average photosynthetic rates recorded at steady-state $O_2$ evolution varied linearly ($r^2 = 0.93$) with average values of ETR for 4 different leaves under different irradiance levels up to 300 μmol photons m$^{-2}$ s$^{-1}$ (Fig. 2). The coefficients of variation were higher for the $O_2$ than for the ETR data. The molar ratio of ETR and $O_2$ evolution was ca. 0.3, which is close to the theoretical value of 0.25 (4 mol electrons mol$^{-1}$ $O_2$ evolved in photosynthesis).

Time courses of $O_2$ evolution and ETRs, and the corresponding relationships between the 2 measurements, for *Halophila stipulacea* (Fig. 3) and *Zostera marina* (Fig. 4) show trends similar to those found for *Cymodocea nodosa* in that a lag was present in the response time of the $O_2$ measurements, while more stable fluorescence-based ETR values were obtained immediately upon changing the irradiance level. However, unlike the linear relationship observed for *C. nodosa*, *H. stipulacea* (Fig. 5) and, especially, *Z. marina* (Fig. 6) showed reduced rates of steady-state $O_2$ evolution relative to ETRs at high irradiances (toward the right in the figures). Also here, the coefficient of variation was lower for the ETRs than for the $O_2$ evolution. Within the linear range of these relationships (i.e. at low light), the molar ratio of ETRs to rates of photosynthetic $O_2$ evolution was 0.12 for *H. stipulacea* and 0.5 for *Z. marina*.

Photosynthetic ETR responses to irradiance derived from the actinic halogen light source of the Diving-PAM (i.e. the RLCs) measured in the field during midday yielded apparently typical photosynthesis-irradiance relationships for all 3 species (Figs. 7–9). The results of *in situ* point measurements under natural
light, superimposed on the RLCs, show that average ETRs measured between 09:00 and 13:30 h, and thus under various ambient light conditions, agreed well with these curves. There was, however, a large variation between the measurements at high light for *Cymodocea nodosa* (Fig. 7) as reflected by the high standard deviations of the mean values. Plotting the individual measurements revealed that there was no correlation between the single ETR values and the irradiance at which each of them had been measured. On the other hand, no such spread of the individual measurements was found a little earlier in the day when the irradiance was <100 µmol photons m⁻² s⁻¹ due to clouds. For this seagrass, which grew at relatively high midday irradiances, light saturation occurred at ca 600 µmol photons m⁻² s⁻¹ and the maximal photosynthetic ETRs were high. *Halophila stipulacea* growing under much dimmer light at 6 m depth (Fig. 8) showed close to saturating ETRs at ca 250 µmol photons m⁻² s⁻¹, but rates continued to increase slightly at higher irradiances. A low light saturation point of ca 150 µmol photons m⁻² s⁻¹, and rates decreasing at higher irradiances, was found for the temperate *Zostera marina* (Fig. 9) investigated under a low-light season.

**DISCUSSION**

Direct linear or curvilinear relationships between photosynthetic rates as measured with the O₂ electrode and those measured as ETRs with the Diving-PAM were found for all 3 species investigated. A linear correlation was found for *Cymodocea nodosa*, a seagrass which has previously been shown to possess a C₄-like CO₂ incorporation pattern (Beer & Waisel 1979), and which lacks apparent photorespiration as appraised by its O₂-insensitive photosynthetic rates (cf. Beer 1989). Contrary to this, rates of O₂ evolution decreased relative to ETRs in the high rate range for the other 2 species. While all rates of O₂ evolution were calculated as 'gross' rates by correcting for dark respiration from the measured rates of net O₂ exchange, photorespiration could not be accounted for here. As the relative decrease in O₂ evolution occurred at high irradiances, and since no such decrease was found for the species lacking apparent photorespiration, it seems very likely that photorespiration was
Fig. 6. Average rates of photosynthetic O₂ evolution (GPS) versus electron transport (ETR) in leaves of Zostera marina (measured at 7°C). Data points are average of 3 to 10 measurements ± SE for each of 4 leaves at various irradiances (0 to 250 μmol photons m⁻² s⁻¹, increasing toward the right in the figure). The points were fitted to a second degree polynomial function, \( r^2 = 0.95 \).

Fig. 7. Rapid light curve of photosynthetic electron transport rates (ETR) versus incident photosynthetic photon flux (PPF) measured in the field at noon-time (+), and average (n = 20 to 35) in situ point measurements of ETR under ambient light (○), ± SD, taken at (from left to right) 10:15, 12:00, and 13:00 h, for a Cymodocea nodosa population growing at 0.5 m depth at a temperature of 20°C. Each point measurement is also given for 12:00 (+) and 13:00 h (+).
ter between repetitive measurements in Cymodocea nodosa at 13:00 h was not due to variations in PPF at each individual measurement and, therefore, probably partly reflects average physiological responses to the sunlight flickering which was visibly caused by the choppy water surface. Such effects have been reported before for both terrestrial plants (Pearcy 1990) and algae. For the latter, it was shown that light flicks could enhance (Wing & Patterson 1993) or reduce (Kubler & Raven 1996) photosynthetic rates as compared to those at steady light. On the other hand, no such scatter was observed earlier under a cloud cover or at lower irradiances for the other plants.

Based on the results of the above trials, it is concluded that PAM fluorometry is suitable for determining photosynthetic rates of seagrasses both in the laboratory and in situ. The Diving-PAM further allows for such measurements to be done underwater to depths of 50 m. Because PAM fluorometry measures only photon-driven electron transport (which gives rise to O₂ evolution, but also to photorespiration and other possible O₂-consuming reactions involving electron flow through PSII), it cannot be applied by itself if energy or gas exchange budgets are to be determined since these depend also on diurnal rates of dark respiration. It can, however, be used in experiments where responses of the photosynthetic system per se are sought. This is exemplified in the low light saturation point and maximal ETR of Zostera marina growing at low irradiances and a low temperature as compared to the subtropical Cymodocea nodosa growing in well-lit warmer waters. In continuation of an eco-physiological study of tropical seagrasses (Björk et al. 1997), we have also used the method for determining photosynthetic responses of intertidal seagrasses to desiccation and re-submersion (Björk & Beer unpubl.).

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