A COMPARISON OF PHOTOSYNTHETIC ELECTRON TRANSPORT RATES IN MACROALGAE MEASURED BY PULSE AMPLITUDE MODULATED CHLOROPHYLL FLUOROMETRY AND MASS SPECTROMETRY

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The relationship between whole chain photosynthetic electron transport and PSII activity was investigated in Porphyra columbina (Montagne) (Rhodophyta), Ulva australis (Areschoug) (Chlorophyta), and Zonaria crenata (J. Agardh) (Phaeophyta). Mass spectrometric measurements of gross O₂ evolution and gross O₂ uptake were combined with simultaneous measurement of pulse-modulated chl fluorescence under a range of irradiances and inorganic carbon (Cᵢ) concentrations. At light-limiting irradiance, a good correlation between gross O₂ evolution and the electron transport rate (ETR) calculated from chl fluorescence \((F''_m - F_o)/F'_m\) was found in the optically thin species (Ulva and Porphyra). The calculated ETR was equivalent to the theoretical electron requirement in these species but overestimated gross O₂ evolution in the thicker species Zonaria. In saturating light, especially when Cᵢ availability was low, ETR overestimated gross O₂ evolution in all species. Excess electron flow could not be accounted for by an increase in gross O₂ uptake; thus neither Mehler-ascorbate-peroxidase reaction nor the photosynthetic carbon oxidation cycle were enhanced at high irradiance or low Cᵢ. Alternative explanations for the loss of correlation include cyclic electron flow around PSII that may be engaged under these conditions or nonphotochemical energy quenching within PSII centers. The loss of correlation between ETR and linear photosynthetic electron flow as irradiance increased from limiting to saturating or at low Cᵢ availability and in the case of optically thick thalli limits the application of this technique for measuring photosynthesis in macroalgae.

Key index words: inorganic carbon; Mehler reaction; photoinhibition; Porphyra columbina; primary productivity; Ulva australis; Zonaria crenata

Abbreviations: A, thallus absorptance; CCM, CO₂ concentrating mechanism; Cᵢ, inorganic carbon; ETR, electron transport rate; \(F_o\), \(F'_m\), chl fluorescence of open and closed PSII reaction centers, respectively; \(F_v\), variable chl fluorescence \((F_m - F_o)\); \(F_v/F'_m\), optimal quantum yield; \(F_o\), \(F'_m\), steady state and maximum chl fluorescence in the light, respectively; \(F''_m\), chl fluorescence immediately after darkening; \(\Phi_{CO₂}, \Phi_{O₂}, \Phi_{PSII}\), CO₂ quantum yields of CO₂ fixation, gross oxygen evolution, and effective quantum yield of PSII, respectively; PAM, pulse amplitude modulated chl fluorescence; PCO, photosynthetic carbon oxidation; PPFD, photosynthetic photon flux density; \(Q_A\), primary quinone electron acceptor of PSII; \(q_P\), nonphotochemical and photochemical quenching, respectively.

Pulse amplitude modulated (PAM) chl fluorescence methods offer an alternative to traditional gas exchange techniques for the nonintrusive analysis of photosynthetic activity in intact leaves and algae. The methods enable the selective measurement of the fluorescence yield of PSII in the presence of photosynthetic radiation and the analysis of changes in yield that reflect the partitioning of excitation energy between photochemical and nonphotochemical pathways of dissipation (Schreiber et al. 1986). Developed initially for higher plants, these techniques are also attractive for the ecophysiological study of aquatic photosynthesis. PSII quantum efficiency (\(\Phi_{PSII}\)) can be analyzed in situ without enclosing samples in chambers that can introduce artifacts by, for example, changing boundary layer conditions or inducing nutrient limitation. When the amount of light absorbed by reaction centers is also known exactly (Hartig et al. 1998, Beer et al. 1998, Beer and Björk 2000), \(\Phi_{PSII}\) can provide a comparative picture of relative rates of photosynthetic electron transport rate (ETR) through PSII under real environmental conditions. In view of the development of submersible field instruments with fiberoptic probes, interest is growing in using PAM techniques not only for making qualitative comparisons of photosynthetic performance under stress, but also for quantifying aquatic productivity. However, studies to confirm the correlation between fluorometric and gas exchange methods using direct simultaneous measurements in algae and seagrasses have only recently been initiated (Hanelt and Nultsch 1995, Beer and Björk 2000, Beer et al. 1998, 2000).

The relationship between PAM chl fluorescence and photosynthetic electron transport has been examined in a number of higher plants, against two theoretical frameworks for the source of nonphotochemical energy dissipation. Assuming that excess energy was dissipated as heat through complete inactivated PSII centers, Weis and Berry (1987) demonstrated a linear correlation between the ETR calculated from fluores-
cence and from CO₂ exchange in attached *Helianthus* and *Phaseolus* leaves. Their semiempirical technique corrected changes in the quantum yield of steady state fluorescence for the species-specific amount of photochemical quenching due to reduced primary electron acceptors (Qᵥ). Similar results were found by Sharkey et al. (1988) at a range of photosynthetic rates that were obtained by varying CO₂ or photosynthetic photon flux density (PPFD). An alternative method, which assumes nonphotochemical quenching within the PSI antenna, was developed by Genty et al. (1989). By this method, the effective quantum efficiency of PSI measured at a given PPFD (ΦPSII = (Fₚ/Fₚm) is the product of the proportion of oxidized PSI reaction centers, represented by the photochemical fluorescence quenching parameter q₂ and the variable efficiency of excitation capture by those oxidized centers (Fₚ/Fₚm'). Unlike the method of Weis and Berry, there is no need to make the difficult measurement of nonphotochemical quenching of the minimal fluorescence of oxidized PSI (Fₚ) when using the popular Genty technique.

Besides careful measurement of the proportion of light absorbed by PSI, extrapolation of ΦPSII to the absolute photosynthetic ETR depends on knowledge of the specific correlation among ΦPSII and the quantum yield of O₂ evolution (ΦO₂), carbon fixation (ΦCO₂), and any other competing sinks for electrons. The correlation between ΦPSII (Genty method) and ΦCO₂ at various irradiances is linear in several C₃ species (Krall and Edwards 1990) and in leaves of C₃ plants under nonphotorespiratory conditions but can be curvilinear at low irradiance at normal levels of O₂ and CO₂ where electron flow to O₂ via the photosynthetic carbon oxidation (PCO) cycle and/or the Mehler-ascorbate-peroxidase reaction in the water–water cycle (Asada 1999) competes with carbon fixation and confounds measurements of O₂ evolution (c.f. Genty et al. 1989, 1990, 1992, Harbinson et al. 1990). Variations in the correlation between ΦPSII and the efficiency of CO₂ assimilation (ΦCO₂), as well as a high degree of variability between the calculated ETR and O₂ evolution or actual CO₂ assimilation when measured in the field (Leisner et al. 1997, Green et al. 1998), have led to the suggestion that fluorometric methods should not be used alone to indicate the absolute photosynthetic performance of intact plants (Biehler and Fock 1995, Green et al. 1998).

Several microalgae display a nonlinear relationship between the Genty ΦPSII and ΦCO₂ or ΦO₂ at either or both low and high irradiance (Geel et al. 1997, Flameling and Kromkamp 1998, Hartig et al. 1998). At moderate irradiance, ETR calculated from ΦPSII closely matches gross O₂ evolution (net - dark respiration measured after each light level) in the chlorophyte macroalga *Ulva lactuca* and *U. fasciata* (Beer et al. 2000). In contrast, *in situ* measurements of diel photosynthesis in *U. lactuca* revealed a good correlation of ETR and O₂ evolution at moderate light but higher than expected ETR in high light (Longstaff et al. 2001). In *Ulva rotundata*, diurnal declines in the optimal quantum yield of PSII (Fₚ/Fₚm) and ΦO₂ associated with midday photoinhibition are correlated from morning to noon but become uncoupled during the afternoon (Henley et al. 1991). Here, Fₚ/Fₚm recovered whereas ΦO₂ remained low, implying increased competition from O₂ for electrons. Despite the oft-cited potential for a stimulation of O₂ uptake when CO₂ fixation is limited, few studies have directly measured O₂ consumption during photosynthesis in algae to confirm this hypothesis. Further uncertainty in the correlation can be due to an incorrect estimation of the amount of light absorbed, especially in species with thick thalli or leaves (Beer and Björk 2000), to using different material for the two measurements, or simply to making the measurements under different optical configurations.

Algae differ markedly from terrestrial C₃ plants in the potential for O₂ uptake. Many algae limit RUBISCO oxygenase activity through the activity of CO₂ concentrating mechanisms (CCMs) or forms of RUBISCO with different kinetic properties than those found in higher plants (Badger et al. 1998, 2000). In seagrasses, the presence of a CCM has been correlated with a linear relationship between ETR and gross O₂ evolution (Beer et al. 1998). Furthermore, O₂ uptake during photorespiration, where it occurs, can be reduced due to incomplete phosphoglycolate metabolism and the excretion of glycolate (Husic et al. 1987). On the other hand, algae possess substantial chlororespiratory activity that reduces O₂ as electrons are passed from cytochrome b₅/f to a terminal oxidase on the thylakoid membranes (Bennoun 1982). Other competing reactions include reduction of NO₃⁻ and NO₂⁻ (Holmes et al. 1989). Therefore, it is difficult to predict the relationship between a chl fluorescence-derived measure of PSII activity and primary productivity in algae by extrapolation from physiological processes in higher plants.

Oxygen consuming and evolving processes during photosynthesis can be separated by mass spectrometric measurement of ¹⁶O₂ evolution from water and ¹⁸O₂ uptake from the surrounding medium, permitting an estimate of gross rates of oxygen evolution and uptake and a true measure of whole chain electron transport (Canvin et al. 1980). When coupled with simultaneous measurement of PAM chl fluorescence, a more complete picture of the correlation of these techniques can be obtained. Whereas Genty et al. (1992) used this method to demonstrate a linear correlation between ΦPSII and φO₂, *Hodemum* leaves, Biehler and Fock (1995) demonstrated clearly that the ETR of PSII measured on either side of *Tetraselmis* leaves did not reflect the integrated photosynthetic performance of the whole leaf, with photorespiration leading to a nonlinear relationship between ΦO₂ and ΦPSII. To directly investigate the relationship between whole chain electron transport and PSII activity in macroalgae, we performed a series of simultaneous fluorescence and mass spectrometer gas exchange measurements at various irradiances and CO₂ concentrations in *Porphyra columbina*.
and *Ulva australis*, congenitors of which contain CCMs, and *Zonaria crenata*, whose taxonomic family, the Dicrondales, is reported not to exhibit CCM activity.

**MATERIALS AND METHODS**

*Plant material and sample preparation.* *Porphyra columbina* (Montagne) (Rhodophyta), *U. australis* (Areschoug) (Chlorophyta), and *Z. crenata* (J. Agardh) (Phaeophyta) were collected from the upper, middle eulittoral, and upper sublittoral zone, respectively, along the southeast New South Wales coast of Australia. Thalli were protected from direct sunlight and heating during transport to the laboratory, where they were kept in vigorously sparged containers at 18°C and 100 μmol photons·m⁻²·s⁻¹ on a 16:8-h light:dark cycle. Seawater was enriched with f/2 medium (Guillard and Ryther 1962) and changed every other day; photosynthetic measurements were made over the next 4 days. Samples for photosynthetic measurements (approx. 1 cm²) were cut from the thallus the day before use and allowed to recover from wound-induced respiration. In the case of *Ulva* and *Porphyra*, which have diffuse patterns of growth, samples were cut randomly over the thallus. *Zonaria* disks were cut from the apical margin, in areas free of epiphytes. Collections were made several times in the spring, summer, and early summer, and for each collection the gas exchange and fluorescence measurement routine proceeded as follows. After resealing the chamber containing 18O₂-enriched seawater, the sample was illuminated for 5 min with 40 μmol photons·m⁻²·s⁻¹ and then darkened. Dark respiration was measured for the next 5–7 min, followed by a saturating flash to obtain the intrinsic quantum yield Fᵥ/Fₘ (Schreiber et al. 1986, 1995), where Fᵥ is the difference between the maximal fluorescence from fully reduced PSII reaction centers (Fₘ) and the initial fluorescence (F₀) from the antenna system of fully oxidized PSII. An increasing series of irradiances was obtained using neutral density filters, and O₂ exchange and steady state fluorescence (Fₛ) were measured continuously. Approximately 5 to 7 min were required to reach steady state at each irradiance, whereupon another saturating flash was applied (Fₘ'), followed by a 5-s period of darkness (F₀').

The effective quantum yield of PSII (Φₚₛₐₐₜ = (Fₘ' − Fₘ)/Fₘ), nonphotochemical quenching (qₚ = 1 − (Fₛ − F₀)/Fₘ − F₀); Genty et al. 1989) and the proportion of reduced

| Table 1. Absorptance values as measured individually at the end of each gas exchange/fluorescence experiment and used to calculate corresponding photosynthetic rates or ETR from fluorescence on an absorbed light basis. |
|-----------------|-----------------|-----------------|-----------------|
| **Experiment**   | **Treatment**    | **Species**     | **Absorptance** |
| Light response curves | 2.5 mM Cᵢ       | *Ulva*          | 0.61, 0.54, 0.60 |
|                  |                  | *Porphyra*      | 0.59, 0.61      |
|                  |                  | *Zonaria*       | 0.71, 0.79, 0.80 |
| Cᵢ-depleted      | *Ulva*           | 0.65, 0.60, 0.65 |
|                  | *Porphyra*       | 0.56, 0.51      |
|                  | *Zonaria*        | 0.71, 0.78, 0.77 |
| Cᵢ response curves | Saturating irradiance | *Porphyra* | 0.57, 0.59 |
|                  | *Ulva*           | 0.56, 0.59      |
|                  | *Zonaria*        | 0.79, 0.76, 0.82 |
| Limiting irradiance | *Ulva*          | 0.57, 0.55      |
|                  | *Porphyra*       | 0.61, 0.53      |
|                  | *Zonaria*        | 0.78, 0.74, 0.78 |
primary PSII acceptor, \(Q_\text{P} = 1 - (F_m' - F_s')/(F_m' - F_o')\); Bilger and Björkman 1990), were calculated for each irradiance. ETR through PSII was calculated as \(\text{ETR} = \Phi_{\text{PSII}} \times \text{PPFD} \times \lambda \times 0.5\), where \(\lambda\) was the proportion of incident PPFD absorbed by the sample (see below) and the factor 0.5 accounted for the presence of two photosystems, assuming equal involvement in linear electron flow. Gross \(\text{O}_2\) evolution and \(\text{O}_2\) uptake were calculated from changes in the concentration of \(^{16}\text{O}_2\) and \(^{18}\text{O}_2\), respectively (Canvin et al. 1980). The quantum yield of \(\text{O}_2\) evolution, \(\Phi_{\text{O}_2}\), was calculated by dividing the rate of gross \(\text{O}_2\) evolution by the absorbed irradiance.

Photosynthesis and chl fluorescence versus \(\text{Ci}\) concentration. The responses of gross \(\text{O}_2\) evolution, gross \(\text{O}_2\) uptake, and PAM chl fluorescence to changes in \(\text{Ci}\) concentration were conducted by a similar procedure. Starting with buffered seawater containing low \(\text{Ci}\), samples were illuminated for 5 min with 40 \(\mu\text{mol}\) photons \(\text{m}^{-2}\text{s}^{-1}\) and darkened for respiration and \(F_v/F_m\) determination. The samples were then illuminated at low or medium irradiance until steady state was reached and fluorescence values were recorded. Exact light levels are given in the figure captions. Small quantities of 1 mM NaHCO\(_3\) were added using a syringe through a port in the closed chamber, and fluorescence and \(\text{O}_2\) exchange were recorded until the rate of photosynthesis became \(\text{Ci}\) saturated.

Light measurements, thallus absorptance, and chl content. Incident irradiance at the level of the sample (PPFD) was measured using a quantum sensor (LI-COR, Lincoln, NE) attached to an exact copy of the stirred measuring chamber. At the end of each measurement series, the fraction of light absorbed by the thallus (absorptance, \(\lambda\)) was measured at the highest irradiance used for the experiment, by comparing the irradiance measured in the absence and in presence of the sample (Henley 1992, Beer and Björk 2000, Beer et al. 2000).

RESULTS

Light response curves of \(\text{O}_2\) exchange and chl fluorescence. \(\text{O}_2\) exchange data for representative samples of \textit{Ulva}, \textit{Porphyra}, and \textit{Zonaria} are presented in Figure 1, plotted as a function of the absorbed irradiance (incident PPFD \(\times \lambda\)). Thallus absorptance varied among collection dates, and the following ranges were recorded:

![Graphs showing changes in chl fluorescence as a function of absorbed irradiance](image-url)

**Fig. 2.** Changes in chl fluorescence as a function of absorbed irradiance, measured as in Figure 1. (A–C) Changes in effective quantum yield (\(\Phi_{\text{PSII}}\)). (D–F) Changes in the proportion of reduced \(Q\). \textit{Ulva australis} (A, D), \textit{Porphyra columbina} (B, E), \textit{Zonaria crenata} (C, F). Different symbols represent different samples.
Ulva 0.54–0.65, Porphyra 0.51–0.61, and Zonaria 0.76–0.80 (Table 1). However, rates were always calculated based on the absorptance measured for that particular sample. The maximum rate of gross O₂ evolution in buffered seawater at 2.5 mM Cᵢ ranged from 3 to 5 mol photons m⁻² s⁻¹ among the three species tested, with the highest rates measured in Ulva. Uptake was slightly higher at high light in Porphyra but relatively unchanged from the dark value in Zonaria. Sparging the medium with CO₂-free air reduced the Cᵢ concentration to approximately 20 to 30 M. In all species under these conditions, gross O₂ evolution was reduced, but the rates of gross O₂ uptake remained indistinguishable from those measured at 2.5 mM Cᵢ. Net O₂ evolution in Ulva and Zonaria was approximately zero (not shown) but was slightly negative in Porphyra. As expected, photosynthesis became light saturated at a lower irradiance in Cᵢ-depleted medium.

At normal Cᵢ (2.5 mM) concentrations, the effective quantum yield of charge separation in PSII declined with increasing irradiance (Fig. 2, A–C). Although the rate of decline slowed as light-saturated photosynthesis was reached, the maximum reduction occurred at an irradiance four to five times higher than saturation. Semi-log plots of Φₛₛᵢ versus irradiance (not shown) revealed two first-order processes in the decline, as previously observed for Ulva exposed for increasing periods of time to a set irradiance (Franklin et al. 1992). The change was quite extreme in each case, with a Φₛₛᵢ of 0.1 or below at an incident irradiance of approx. 680 μmol photons m⁻² s⁻¹ (200–300 μmol photons m⁻² s⁻¹ absorbed). This irradiance was approximately a third of the level normally encountered in the field at the position where the Ulva and Porphyra samples were originally collected, and Φₛₛᵢ measured in this habitat was typically below 0.1 during midday low tides on sunny days (personal observation). Despite the extreme reduction, Φₛₛᵢ rose again immediately when the light was reduced. Calculation of qₑ, a measure of the degree of dissipation of excess energy as heat, confirmed that the decline in Φₛₛᵢ was photoprotective (data not shown). At low Cᵢ concentration, Φₛₛᵢ fell far more rapidly, in keeping with a lower level of irradiance required for saturation. The extent of reduction at high irradiance was similar to that measured at 2.5 mM Cᵢ for Ulva and Porphyra but greater for Zonaria, and at the highest irradiance no variable fluorescence was observed until the light level was reduced.

At a given irradiance, Ulva and Zonaria samples generally had a more highly reduced Q pool in depleted Cᵢ medium than at 2.5 mM Cᵢ (Fig. 2, D–F). Under both conditions, maximum reduction occurred at high irradiance and was greater than 80% in the case of Ulva but between 60 and 80% in Zonaria. A similar degree of reduction at low irradiance occurred in Porphyra at both Cᵢ concentrations, but in contrast to the other species, the Q pool appeared to be less reduced at high light in Cᵢ-depleted medium.

When sufficient Cᵢ was present in the medium and the irradiance was low, the relationship between Φₛₛᵢ and Φₒₑ in Ulva and Zonaria was slightly curvilinear, with a decrease in Φₒₑ at a constant Φₛₛᵢ (Fig. 3). At higher irradiance and values of Φₛₛᵢ less than approximately 0.6 (0.4 in the case of Zonaria), the relation-
ship became linear and intercepted the axes near the origin. Greater curvilinearity was more apparent at low concentrations of \( C_i \), but no curvilinearity was observed for \( \text{Porphyra} \) in either case. Whereas the slope of the linear decline was similar for \( \text{Ulva} \) and \( \text{Porphyra} \) at 2.5 mM \( C_i \), \( \text{Zonaria} \) appeared to maintain a relatively higher \( \Phi_{	ext{PSII}} \) at a given \( \Phi_{	ext{O}_2} \). At low \( C_i \), the slope of the linear relationship was similar among species but was higher (\( \text{Ulva} \) and \( \text{Porphyra} \)) or lower (\( \text{Zonaria} \)) than at 2.5 mm \( C_i \). Thus it appeared that \( \Phi_{	ext{PSII}} \) became relatively greater than \( \Phi_{	ext{O}_2} \) under \( C_i \) limited conditions in the former species.

The rate of electron transport through PSII was calculated from \( \Phi_{	ext{PSII}} \) and the amount of absorbed irradiance, with the assumption that absorbed light was equally distributed between photosystems (Fig. 4). Values of \( \Phi_{	ext{PSII}} \) less than 0.080 were not used for the calculation, because it was difficult to distinguish such low fluorescence signals from background noise. ETR became light saturated at a similar irradiance as \( \text{O}_2 \) evolution in \( \text{Ulva} \) and \( \text{Zonaria} \) but in \( \text{Porphyra} \) continued to increase. As predicted from Figure 2, A–C, but in contrast to the difference in gross \( \text{O}_2 \) evolution between \( C_i \)-replete and -depleted conditions (Fig. 1, A, C, and E), ETR did not decline remarkably in \( \text{Ulva} \) or \( \text{Porphyra} \) at low \( C_i \) but was one third the rate at high \( C_i \) in \( \text{Zonaria} \).

A linear relationship between ETR and gross \( \text{O}_2 \) evolution or the actual rate of linear electron flow was

![Fig. 4](image1.png)

**Fig. 4.** Changes in ETR as a function of absorbed irradiance. Measurements were made in 20 mM BTP-buffered seawater, pH 8.0, at a natural (closed symbols) or minimum (open symbols) \( C_i \) concentration. \( \text{Ulva australis} \) (A), \( \text{Porphyra columbina} \) (B), and \( \text{Zonaria crenata} \) (C). Different symbols represent different samples.

![Fig. 5](image2.png)

**Fig. 5.** Comparison of the PSII electron transport rate calculated from \( \Phi_{	ext{PSII}} \) and actual rate of gross \( \text{O}_2 \) evolution at a natural (closed symbols) or minimum (open symbols) \( C_i \) concentration. \( \text{Ulva australis} \) (A), \( \text{Porphyra columbina} \) (B), and \( \text{Zonaria crenata} \) (C). Different symbols represent different samples.
obtained in all species tested at irradiances below light saturation and when sufficient $C_i$ was available (Fig. 5). Given that four electrons are required to oxidize two molecules of $H_2O$ to form one molecule of $O_2$, a good match was obtained between the calculated and observed rates in Ulva and Porphyra, with 3.3 and 4 electrons per $O_2$ evolved estimated from the regression, respectively. In Zonaria, the calculated ETR was almost twice that required for the observed rate of $O_2$ evolution, at 7.7 electrons per $O_2$. At higher irradiances, the linear relationship began to deviate in Porphyra and Zonaria such that there was apparently more electron flow through PSII than could be accounted for by $O_2$ evolution. When $CO_2$ was limiting, the deviation occurred at the lower saturating irradiance in Ulva and Porphyra, but no additional deviation was observed in Zonaria.

Changes in $O_2$ exchange and chl fluorescence in response to $C_i$ concentration. To examine further the importance of light saturation on the relationship between chl fluorescence and gross $O_2$ evolution, $C_i$ response curves were measured at a limiting and a saturating irradiance (Fig. 6) selected from the photosynthesis versus irradiance relationship at 2.5 mM $C_i$. In Ulva, $C_i$-saturated rates of gross $O_2$ evolution at 50 $\mu$mol photons m$^{-2}$s$^{-1}$ were similar to those measured previously (Fig. 1), but at 390 $\mu$mol photons m$^{-2}$s$^{-1}$ rates were lower. At the same time $q_F$ declined with increasing $C_i$ concentration in low light but remained above 0.7 in high light (not shown), indicating that the combined stress of high light and low $C_i$ led to a greater probability of photoinhibition (Weis and Berry 1987). $O_2$ uptake was lower than recorded in the light response curves (Fig. 1). In Porphyra, $C_i$-limited and -saturated rates of gross $O_2$ evolution were similar to those measured previously (Fig. 1), but slightly higher in high light. In Zonaria, rates of gross $O_2$ evolution at $C_i$ saturation were 17%–25% higher than measured previously (Fig. 1) for low and high light, respectively. In all species, $O_2$ uptake was higher when $C_i$ was limiting. No differences between the two irradiances tested were found in Ulva and Zonaria, but there was slightly greater $O_2$ uptake at high light in Porphyra. In contrast to Ulva, the reduction status of $Q_o$ declined with increasing $C_i$ concentration at both low and high irradiance in both Porphyra and Zonaria.

With increasing amounts of $C_i$, the rise in $\Phi_{PSII}$ measured in low light was linearly correlated with $\Phi_{O_2}$ (Fig. 7). The slope of the relationship was identical for Ulva and Porphyra but higher for Zonaria. Although lines plotted for the latter two species passed close to the origin, it was offset to a high $\Phi_{PSII}$ in Ulva. At high light, neither parameter responded significantly to changing $C_i$. Although the low efficiencies in Porphyra and Zonaria measured in high light were similar to those predicted by the relationship determined in low light, $\Phi_{PSII}$ was lower in high light treated Ulva, again suggesting photoinhibition under $C_i$ limitation.

Comparison of the calculated ETR and gross $O_2$ evolution (Fig. 8) showed that ETR continued to give a relatively good estimate of gross $O_2$ evolution at low irradiance, even when $C_i$ was limiting for photosynthesis. The discrepancy between actual and calculated rates observed in Figure 5 were even more apparent at constant high PPFD, where a greater ETR was calculated from $\Phi_{PSII}$ than was necessary to account for the observed evolution of $O_2$. The relationship was either linear (Zonaria), curvilinear (Porphyra), or random (Ulva).

**DISCUSSION**

Overall, the rates of gross $O_2$ evolution measured here in seawater were similar to those reported for high light-acclimated *U. rotundata* (Osmond et al. 1993), *U. lactuca* (Beer et al. 2000), intertidal *P. perforata* (Herbert and Waaland 1988), and *Z. turneriana* (Taylor et al. 1999) (Figs. 1 and 6). Beer et al. (2000) reported a close

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**Fig. 6.** Changes in gross $O_2$ evolution (A, C, and E) and gross $O_2$ uptake (B, D, and F) as a function of inorganic carbon concentration. Measurements were made in 20 mM BTP-buffered seawater, pH 8.0, at 90 or 685 $\mu$mol photons m$^{-2}$s$^{-1}$ incident PPFD (closed or open symbols, respectively). *Ulva australis* (A, B), *Porphyra columbina* (C, D), and *Zonaria crenata* (E, F). Different symbols represent different samples.
linear correlation between ETR and \( \text{O}_2 \) evolution (net corrected for dark respiration) in \textit{U. lactuca} at irradiances up to 36\% of growth irradiance and at various \( \text{Ci} \) concentrations at 13\% growth irradiance, light levels that were presumably less than saturating for photosynthesis. Calculated molar ratios of \( \text{O}_2 \) evolved/ETRs were close to the theoretical maximum of 0.25. In \textit{U. australis} and \textit{P. columbina}, we also observed close to the theoretical ETRs for \( \text{O}_2 \) evolution at subsaturating irradiance but higher than expected ETRs under conditions where there was more light available than required for maximum photosynthesis (Figs. 5 and 8). The irradiance at which the deviation occurred depended on \( \text{Ci} \) concentration, and under these conditions, the potential for electron flow to other acceptors, like \( \text{O}_2 \), should be maximal.

Fluorescence-based ETRs in excess of gross \( \text{O}_2 \) evolution have been observed previously in some algae.
and sea grasses (Henley et al. 1991, Geel et al. 1997, Beer et al. 1998, Flaming and Kromkamp 1998). The assumption is usually made that these discrepancies reflect enhanced photosynthetic activity, because laboratory studies of higher plants have indicated that the quantum yield of CO₂ fixation could be accurately measured by fluorometry, so long as photosynthesis was kept to a minimum by limiting the availability of O₂ (Sharkey et al. 1988, Krall and Edwards 1990, Harbingon et al. 1990, Seaton and Walker 1990). Results in the field and with more diverse species have been less equivocal as to the role of photosynthesis. The quantum yield of CO₂ fixation and PSII varied linearly at 21% O₂ with PPFD or temperature in the moss Bryum argenteum, which would be presumed to have photorespiration, but not in Umbilicaria aprina, a green algal lichen. This was unexpected, because lichens typically do not have high rates of PCO activity (Green et al. 1998).

We did not see any evidence to support enhanced electronic flow to O₂ for the three species tested here. In U. australis, gross O₂ uptake did increase slightly as PPFD rose to light saturation, but the calculated ETR continued to reflect actual whole chain electron transport whereas photosynthesis was light limited. Maximum gross O₂ uptake rates in all species were on the order of 25%–30% of the maximum rate of O₂ evolution (Fig. 1), similar to those reported for other microalgae (Weger et al. 1989, Badger et al. 1998). Furthermore, these rates were insensitive to CO₂ concentration and comparable with C₃ higher plants with low rates of PCO activity (Badger 1985). In comparison, C₃ plants typically have O₂ uptake rates of 30%–50% of the CO₂-saturated rate of electron transport (Canvin et al. 1980, Gerbaud and Andre 1980). Overall then, it is unlikely that the higher than expected ETRs calculated from fluorescence data at low CO₂ or high irradiance represented an increase in PCO or Mehler activity.

By direct comparison with higher plants, it might be expected that the extent to which oxygen uptake rises at limiting CO₂ in various algae may be dependent on the presence or absence of effective CCMs. However, comparisons of light-stimulated O₂ uptake among different micro- and macroalgal species under various extents of CO₂ limitation show that limiting CO₂ either in the presence or absence of a CCM does not appear to greatly stimulate O₂ uptake (Badger et al. 1998). For the species examined here, U. australis and Porphyra have been shown to have characteristics consistent with the presence of active O₂ uptake: the presence of a pyrenoid in the chloroplast and a CCM (see Badger et al. 1998). For Zonaria, there is less direct evidence on which to base conclusions. A member of the Dictyotales, it has many discoid pyrenoid-less chloroplasts per cell, a feature that would be consistent with the lack of a CCM (Badger et al. 1998). The reasons for the small stimulation of O₂ uptake at limiting CO₂ may be due to a combination of factors, including evolutionary changes in the RUBISCO oxygenase kinetic properties, the rapid inactivation of RUBISCO at limiting CO₂, and the presence of variable amounts of CCM activity (Badger et al. 1998).

Because we have seen no evidence for significantly enhanced O₂ uptake at high irradiance or low CO₂, in our experiments, the discrepancies in calculated ETR we have observed are not likely to be due to electron flow to O₂. Several other possibilities exist. Formulation of Φₚₛᵢᵢ by the Genty method, as the product of reaction center “openness” and excitation capture efficiency by open centers, assumes that nonradiative dissipation occurs in the light-harvesting antenna. Alternatively, nonphotochemical quenching may be occurring in the PSII reaction center (Krause and Weis 1991), altering the proposed relationship between photochemical and fluorescence yield (Schreiber et al. 1995). Upon acidification of the thylakoids in saturating light, Ca²⁺ can be lost from the water oxidation complex. Subsequent charge separation at Ca-depleted centers is proposed to be followed by charge recombination between Qₐ and P₆₈₀, with a release of energy as heat and consequent quenching of fluorescence (Schreiber et al. 1995). Antenna-based nonphotochemical quenching in algae is generally associated with reversible conversion of the xanthophyll cycle pigments violaxanthin/antheraxanthin and zeaxanthin or diadinoxanthin/diatoxanthin. Rhodophyta are generally thought to lack xanthophyll cycle activity (Hager 1980), and the xanthophyll cycle is slow in U. rotundata (Franklin et al. 1992) and U. australis (personal observation), despite the fact that these species occupy very high light environments. Thus, alternative quenching mechanisms cannot be ruled out.

A further explanation for higher than expected ETRs in high light is cyclic flow around PSII from the quinone acceptor Qₐ (or pheophytin) via cyt b₅₅₉, and chl Z to P₆₈₀. Using a “pump and probe” fluorescence technique, which uses weak probe flashes to measure the change in quantum yield of fluorescence that is excited by a single turnover pump flash, Falkowski et al. (1986) and Praslin et al. (1996) demonstrated an uncoupling between water splitting activity in PSII and Φₚₛᵢᵢ under conditions where the plastoquinone pool became highly reduced (e.g. saturating light), with greater than expected ETR. The pump and probe technique differs significantly from the saturating pulse method used here in that single turnover flashes do not necessarily excite maximum fluorescence yield (Fₘ'). This can result in an underestimation of Φₚₛᵢᵢ compared with the Genty method, because the photochemical yield generated by the single turnover flash is influenced by the redox status of the plastoquinone pool (Schreiber et al. 1995). Although the results of the two techniques are not directly comparable, general models for a photoprotective cyclic pathway of electron flow involving cyt b₅₅₉ are relevant to this study (reviewed by Stewart and Brudvig 1998). In the case where electrons cannot be resupplied to P₆₈₀, from the water oxidation complex (donor side inhibition), the strong oxidant P₆₈₀₊ can be re-reduced by cyt b₅₉₃, preventing the oxidation of...
nearby elements of the reaction center. In the case where there is an excess of reducing equivalents on the acceptor side of PSII, donation from Pheo$^{-}$ to cyt$\text{b}_{559}$ can relieve that pressure and prevent the recombination of Pheo$^{-}$ with P$\text{b}_{559}$ to form triplet state P$\text{b}_{580}$ and highly reactive singlet O$_2$. During acceptor side inhibition, this “clutch” mechanism (Prasil et al. 1996) would provide additional photoprotective capacity for keeping PSII active without gross O$_2$ evolution or net CO$_2$ fixation. In cases where xanthophyll cycle activity is limited or nonexistent (Franklin and Larkum 1996), cyclic flow in PSII could be essential for protecting those species living in highly variable light environments.

The hypothesis that cyclic flow is occurring in our measurements when electron requirements for photosynthesis become saturated is generally supported by changes in the ratio of $\Phi_{\text{PSII}}$ to $\Phi_O$ relative to $C_i$ (Fig. 3). In the presence of 2.5 mM C$_i$, we observed a generally linear relationship between $\Phi_{\text{PSII}}$ and $\Phi_O$ over most of the PPFD range, with a slightly greater loss of $\Phi_O$ relative to $\Phi_{\text{PSII}}$ in Ulva and Zonaria at low irradiance. However, the relationship changed in all species under low C$_i$, becoming more curvilinear and indicating an even greater loss of $\Phi_O$ relative to $\Phi_{\text{PSII}}$ in Ulva and Porphyra as PPFD rose (higher ratio of $\Phi_{\text{PSII}}$ to $\Phi_O$). When tested as a function of changing C$_i$ concentration at a constant low or high light (Fig. 7), the relationship was generally always linear, but the ratio of $\Phi_{\text{PSII}}$ to $\Phi_O$ was equal to that measured at low C$_i$ concentration for Ulva and Zonaria (Fig. 3). The hypothesis is also supported by the correlation of excessive ETR and a highly reactive singlet O$_2$. During acceptor side inhibition, this “clutch” mechanism (Prasil et al. 1996) would provide additional photoprotective capacity for keeping PSII active without gross O$_2$ evolution or net CO$_2$ fixation. In cases where xanthophyll cycle activity is limited or nonexistent (Franklin and Larkum 1996), cyclic flow in PSII could be essential for protecting those species living in highly variable light environments.

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Finally, the calculation of ETR depends on knowledge of the proportion of incident irradiance absorbed by PSII; thus, higher than expected ETR may be the result of incorrect measurement of absorbance, as discussed by Beer and coworkers (Beer et al. 1998, 2000, Beer and Björk 2000), or due to changes in the distribution of excitation energy between PSI and PSII. Zonaria thalli are relatively thicker than Ulva and Porphyra, with a multilayer nonphotosynthetic medulla covered by a single layer photosynthetic cortex on either side. It is possible that our technique for measuring absorbance was more applicable to optically thin species, where all layers are photosynthetic. However, the discrepancies observed in Zonaria were not consistent between the two types of photosynthesis experiments (Figs. 5 and 8). Furthermore, the average absorbance value obtained for Zonaria was only slightly less than that typically measured for equally thick higher plant leaves (Evans 1996). Our absorbance measurements agree with those published previously for related algal species (Lüning and Dring 1985, Henley and Ramus 1989, Markager 1993), obtained by various techniques. We have ignored backscatter from the thallus surface, but under a perpendicular light beam, this is likely to be not more than 5% (Lüning and Dring 1985) and perhaps even less than 1% (Frost-Christensen and Sand-Jensen 1992). It is possible that absorbance might change over the course of a measurement. At high irradiance, chloroplasts in a number of algae are known to move to low absorption positions. On the other hand, a PSII absorption cross-section might have changed during the course of the experiment, in response to increased plastoquinone reduction (Fork et al. 1991), rendering our assumption of a 50:50 distribution of irradiance between PSII and PSI erroneous. We were not able to directly monitor changes in PSII cross-section during the experiment, but we did make the absorbance measurement at the highest irradiance attained in the photosynthesis versus irradiance curves or at the same irradiance used for the C$_i$ response curves. This would limit errors to an underestimate of ETR at low irradiance, which is not the case. Further experiments are required to answer this question but do not alter the fact that algae do not appear to have consistent relationships between ETR and gross O$_2$ evolution as typically measured.

**Conclusions**

From our simultaneous measurements of gross O$_2$ exchange and PSII charge separation by pulse amplitude chl fluorometry, we have found that ETRs calculated from $\Phi_{\text{PSII}}$ accurately reflected whole chain electron transport under fairly limited situations: in species with optically thin thalli under light-limited conditions. When photosynthesis is light saturated, especially when C$_i$ availability is low, chl fluorescence greatly overestimated the actual gross rate of O$_2$ evolution. Although we can confirm the suggestion of Beer and co-workers (Beer et al. 1998, 2000 Beer and Björk 2000) that PAM fluorometry can provide a practical quantitative measurement of photosynthesis in thin-bladed species, the environmental conditions under which this applies also need to be carefully and individually assessed. Whereas chl fluorescence is a good measure of PSII activity, the nature of that activity in algae can be quite variable and thus the use of $\Phi_{\text{PSII}}$ to derive estimates of algal productivity should be treated with great caution.

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