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A COMPARISON OF PHOTOSYNTHETIC ELECTRON TRANSPORT RATES IN MACROALGAE MEASURED BY PULSE AMPLITUDE MODULATED CHLOROPHYLL FLUOROMETRY AND MASS SPECTROMETRY [1]

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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 O_2 evolution and gross O_2 uptake were combined with simultaneous measurement of pulse-modulated chl fluorescence under a range of irradiances and inorganic carbon (C_i) concentrations. At light-limiting irradiance, a good correlation between gross O_2 evolution and the electron transport rate (ETR) calculated from chl fluorescence ($(F_m' - F_s)/F_m'$) 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 O_2 evolution in the thicker species *Zonaria*. In saturating light, especially when C_i availability was low, ETR overestimated gross O_2 evolution in all species. Excess electron flow could not be accounted for by an increase in gross O_2 uptake; thus neither Mehler-ascorbate-peroxidase reaction nor the photosynthetic carbon oxidation cycle were enhanced at high irradiance or low C_i. 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 C_i 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, CO_2 concentrating mechanism; C_i, 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_s, F_m', steady state and maximum chl fluorescence in the light, respectively; F_o', chl fluorescence immediately after darkening; Φ_{CO_2}, Φ_{O_2}, Φ_{PSII}, CO_2 quantum yields of CO_2 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_N, 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 (Φ_{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), Φ_{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-

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cence and from CO_2 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 (Q_A^-). Similar results were found by Sharkey et al. (1988) at a range of photosynthetic rates that were obtained by varying CO_2 or photosynthetic photon flux density (PPFD). An alternative method, which assumes nonphotochemical quenching within the PSII antenna, was developed by Genty et al. (1989). By this method, the effective quantum efficiency of PSII measured at a given PPFD ($\Phi_{PSII} = (F_m' - F_s)/F_m'$) is the product of the proportion of oxidized PSII reaction centers, represented by the photochemical fluorescence quenching parameter q_P and the variable efficiency of excitation capture by those oxidized centers (F_v'/F_m'). 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 PSII (F_o) when using the popular Genty technique.

Besides careful measurement of the proportion of light absorbed by PSII, extrapolation of Φ_{PSII} to the absolute photosynthetic ETR depends on knowledge of the specific correlation among Φ_{PSII} and the quantum yield of O_2 evolution (Φ_{O_2}), carbon fixation (Φ_{CO_2}), and any other competing sinks for electrons. The correlation between Φ_{PSII} (Genty method) and Φ_{CO_2} at various irradiances is linear in several C_4 species (Krall and Edwards 1990) and in leaves of C_3 plants under nonphotorespiratory conditions but can be curvilinear at low irradiance at normal levels of O_2 and CO_2 where electron flow to O_2 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 O_2 evolution (c.f. Genty et al. 1989, 1990, 1992, Harbinson et al. 1990). Variations in the correlation between Φ_{PSII} and the efficiency of CO_2 assimilation (Φ_{CO_2}), as well as a high degree of variability between the calculated ETR and O_2 evolution or actual CO_2 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 Φ_{PSII} and Φ_{CO_2} or Φ_{CO_2} 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 Φ_{PSII} closely matches gross O_2 evolution (net - dark respiration measured after each light level) in the chlorophyte macroalgae *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 O_2 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 (F_v/F_m) and Φ_{O_2} associated with midday photoinhibition are correlated from morning to noon but become uncoupled during the afternoon (Henley et al. 1991). Here, F_v/F_m recovered whereas Φ_{O_2} remained low, implying increased competition from O_2 for electrons. Despite the oft-cited potential for a stimulation of O_2 uptake when CO_2 fixation is limited, few studies have directly measured O_2 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 C_3 plants in the potential for O_2 uptake. Many algae limit RUBISCO oxygenase activity through the activity of CO_2 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 O_2 evolution (Beer et al. 1998). Furthermore, O_2 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 O_2 as electrons are passed from cytochrome b_6/f to a terminal oxidase on the thylakoid membranes (Bennoun 1982). Other competing reactions include reduction of NO_3^- and NO_2^- (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 $^{16}O_2$ evolution from water and $^{18}O_2$ 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 Φ_{PSII} and Φ_{O_2} *Hodeum* leaves, Biehler and Fock (1995) demonstrated clearly that the ETR of PSII measured on either side of *Triticum* leaves did not reflect the integrated photosynthetic performance of the whole leaf, with photorespiration leading to a nonlinear relationship between Φ_{O_2} and Φ_{CO_2}. 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 CO_2 concentrations in *Porphyra columbina*

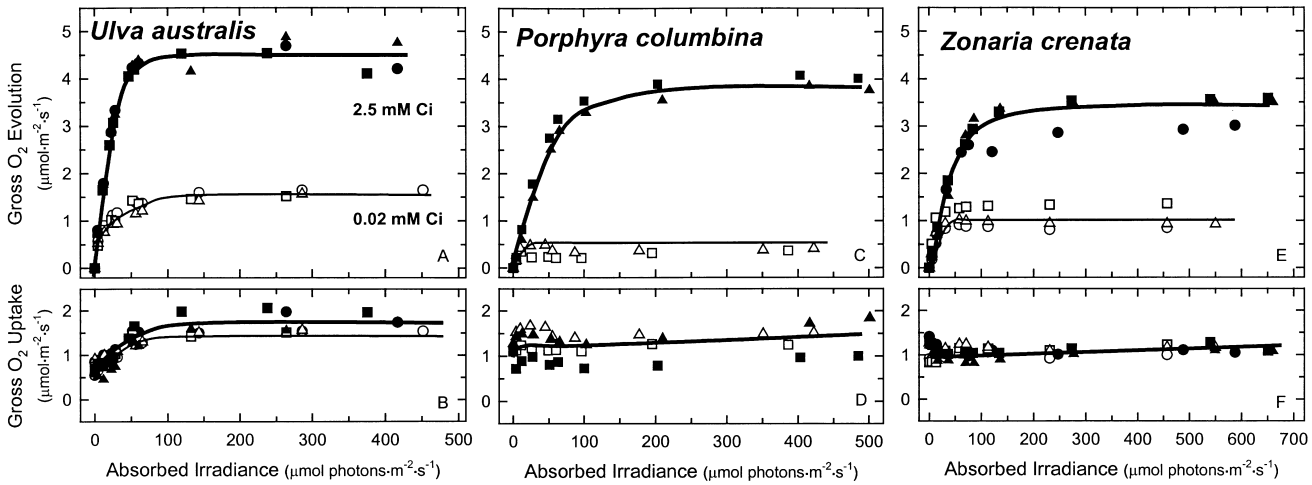

FIG. 1. Changes in gross O_2 evolution (A, C, and E) and gross O_2 uptake (B, D, and F) as a function of absorbed irradiance. Measurements were made in 20 mM BTP-buffered seawater, pH 8.0, at a natural (2.5 mM, closed symbols) or minimum (0.025 mM, open symbols) C_i. Species tested were *Ulva australis* (A, B), *Porphyra columbina* (C, D), and *Zonaria crenata* (E, F). Different symbols represent different samples.

and *Ulva australis*, congenitors of which contain CCMs, and *Zonaria crenata*, whose taxonomic family, the Dictyotales, 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 cm^2) 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 and early summer, and for each collection the gas exchange and fluorescence characteristics under various conditions were made on at least three samples per condition. Representative data sets are presented below.

Photosynthesis and chl fluorescence versus irradiance curves. Simultaneous measurements of O_2 evolution and uptake and PAM chl fluorescence were conducted in a temperature-controlled (20° C) stirred chamber attached to an isotope ratio mass spectrometer (Micromass IsoPrime EA, Manchester, UK). Thallus disks were supported over a stir bar in seawater buffered to pH 8.0 with 20 mM bis-tris propane (no nutrient enrichment). In the cases where low inorganic carbon (C_i) medium was used, C_i was removed from the seawater by sparging it overnight with CO_2-free air. The medium was vacuum degassed before being added to the chamber. $^{18}O_2$ (95% enrichment) was introduced as a bubble into the chamber, and the medium was stirred until total O_2 concentration was approximately 80% of saturation. The remaining $^{18}O_2$ bubble was removed, the chamber sealed, and a light response curve was measured. Actinic light was supplied from a halogen source through the top of the chamber via a polyfurcated fiberoptic bundle, held perpendicular to the surface of the sample. Chl fluorescence was measured using a PAM 101/103 chl fluorometer and Schott KL 1500 electronic flash lamp (H. Walz GmbH, Germany). Signals were collected through the fiberoptic bundle and visualized using a custom-designed data capture system (DasyLab32, DasyTec GmbH, Germany). Nomenclature follows van Kooten and Snel (1990).

The O_2 exchange and fluorescence measurement routine proceeded as follows. After resealing the chamber containing $^{18}O_2$-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 F_v/F_m (Schreiber et al. 1986, 1995), where F_v is the difference between the maximal fluorescence from fully reduced PSII reaction centers (F_m) and the initial fluorescence (F_o) from the antenna system of fully oxidized PSII. An increasing series of irradiances was obtained using neutral density filters, and O_2 exchange and steady state fluorescence (F_s) were measured continuously. Approximately 5 to 7 min were required to reach steady state at each irradiance, whereupon another saturating flash was applied (F_m'), followed by a 5-s period of darkness (F_o').

The effective quantum yield of PSII ($\Phi_{PSII} = (F_m' - F_s)/F_m'$), nonphotochemical quenching ($q_N = 1 - (F_m' - F_o')/(F_m - F_o)$; 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 C_i	*Ulva*	0.61, 0.54, 0.60
		Porphyra	0.59, 0.61
		Zonaria	0.71, 0.79, 0.80
	C_i- depleted	*Ulva*	0.65, 0.60, 0.65
		Porphyra	0.56, 0.51
		Zonaria	0.71, 0.78, 0.77
C_i response curves	Saturating irradiance	*Ulva*	0.56, 0.59
		Porphyra	0.57, 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 ($1 - q_P = 1 - (F_m' - F_s)/(F_m' - F_o')$; Bilger and Björkman 1990), were calculated for each irradiance. ETR through PSII was calculated as ETR = $\Phi_{PSII} \times$ PPFD $\times$ A $\times$ 0.5, where A 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 O_2 evolution and O_2 uptake were calculated from changes in the concentration of $^{16}O_2$ and $^{18}O_2$, respectively (Canvin et al. 1980). The quantum yield of O_2 evolution, Φ_{O_2}, was calculated by dividing the rate of gross O_2 evolution by the absorbed irradiance.

Photosynthesis and chl fluorescence versus C_i concentration. The responses of gross O_2 evolution, gross O_2 uptake, and PAM chl fluorescence to changes in C_i concentration were conducted by a similar procedure. Starting with buffered seawater containing low C_i, samples were illuminated for 5 min with 40 μmol photons·m^{-2}·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 O_2 exchange were recorded until the rate of photosynthesis became C_i 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 faction of light absorbed by the thallus (absorptance, A) 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 O_2 exchange and chl fluorescence. O_2 exchange data for representative samples of *Ulva*, *Porphyra*, and *Zonaria* are presented in Figure 1, plotted as a function of the absorbed irradiance (incident PPFD $\times$ A). Thallus absorptance varied among collection dates, and the following ranges were recorded:

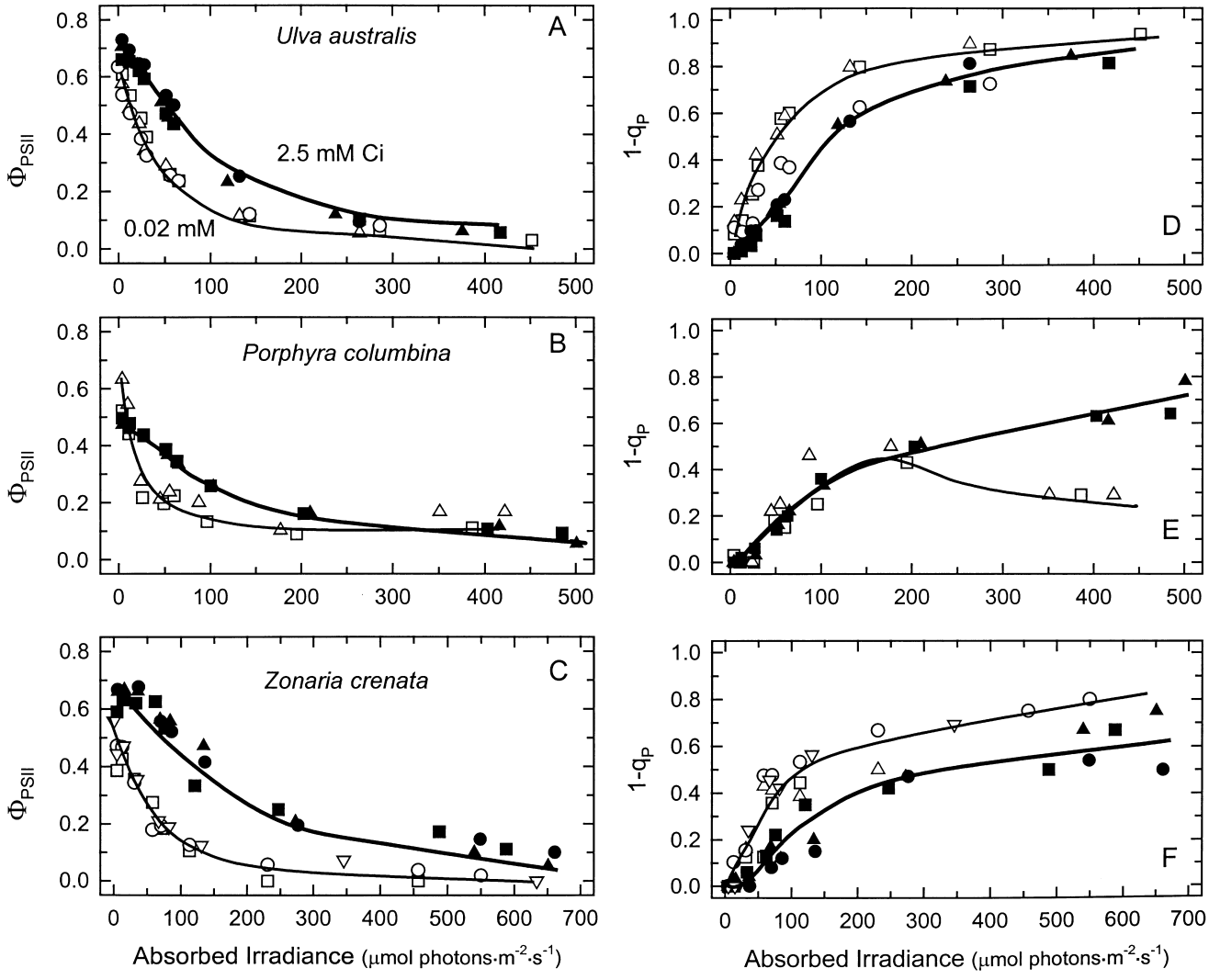

FIG. 2. Changes in chl fluorescence as a function of absorbed irradiance, measured as in Figure 1. (A–C) Changes in effective quantum yield (Φ_{PSII}). (D–F) Changes in the proportion of reduced Q. *Ulva australis* (A, D), *Porphyra columbina* (B, E), *Zonaria crenata* (C, F). Different symbols represent 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 O_2 evolution in buffered seawater at 2.5 mM C_i ranged from 3 to 5 μmol photons·m^{-2}·s^{-1} among the three species tested, with the highest rates measured in *Ulva*. With increasing irradiance, gross O_2 uptake in *Ulva* increased until photosynthesis became light saturated. Uptake was slightly higher at high light in *Porphyra* but relatively unchanged from the dark value in *Zonaria*. Sparging the medium with CO_2-free air reduced the C_i concentration to approximately 20 to 30 μM. In all species under these conditions, gross O_2 evolution was reduced, but the rates of gross O_2 uptake remained indistinguishable from those measured at 2.5 mM C_i. Net O_2 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 C_i-depleted medium.

At normal C_i (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. Semilog plots of Φ_{PSII} 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 Φ_{PSII} 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 Φ_{PSII} measured in this habitat was typically below 0.1 during midday low tides on sunny days (personal observation). Despite the extreme reduction, Φ_{PSII} rose again immediately when the light was reduced. Calculation of q_N, a measure of the degree of dissipation of excess energy as heat, confirmed that the decline in Φ_{PSII} was photoprotective (data not shown). At low C_i concentration, Φ_{PSII} 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 C_i 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 C_i medium than at 2.5 mM C_i (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 C_i concentrations, but in contrast to the other species, the Q pool appeared to be less reduced at high light in C_i-depleted medium.

When sufficient C_i was present in the medium and the irradiance was low, the relationship between Φ_{PSII} and Φ_{O_2} in *Ulva* and *Zonaria* was slightly curvilinear, with a decrease in Φ_{O_2} at a constant Φ_{PSII} (Fig. 3). At higher irradiance and values of Φ_{PSII} less than approximately 0.6 (0.4 in the case of *Zonaria*), the relation-

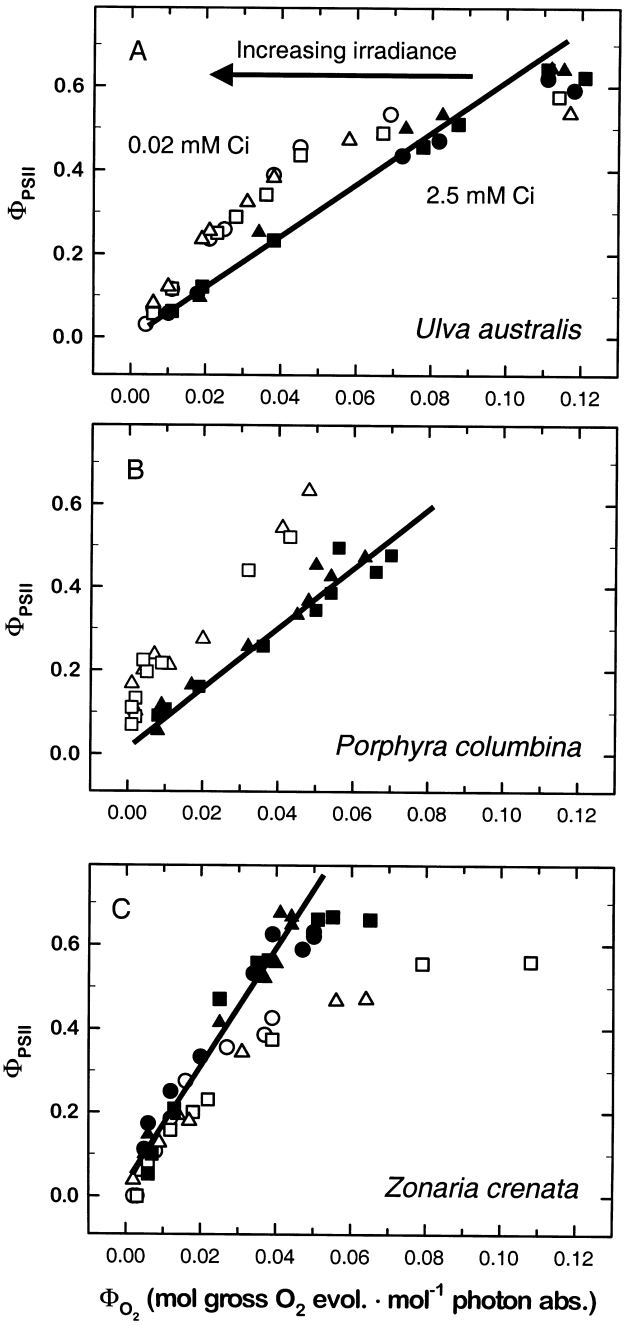

FIG. 3. Comparison of the effective quantum yield of PSII (Φ_{PSII}) and the quantum yield of O_2 evolution (mol O_2 evolved per mol photon absorbed by the thallus) at a natural (closed symbols) or low (open symbols) C_i concentration. *Ulva australis* (A), *Porphyra columbina* (B), and *Zonaria crenata* (C). Different symbols represent different samples.

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 *Porphyra* in either case. Whereas the slope of the linear decline was similar for *Ulva* and *Porphyra* at 2.5 mM C_i, *Zonaria* appeared to maintain a relatively higher Φ_{PSII} at a given Φ_{O_2}. At low C_i, the slope of the linear relationship was similar among species but was higher (*Ulva and Porphyra*) or lower (*Zonaria*) than at 2.5 mm C_i. Thus it appeared that Φ_{PSII} became relatively greater than Φ_{O_2} under C_i limited conditions in the former species.

The rate of electron transport through PSII was calculated from Φ_{PSII} and the amount of absorbed irradiance, with the assumption that absorbed light was equally distributed between photosystems (Fig. 4). Values of Φ_{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 O_2 evolution in *Ulva* and *Zonaria* but in *Porphyra* continued to increase. As predicted from Figure 2, A–C, but in contrast to the difference in gross O_2 evolution between C_i-replete and -depleted conditions (Fig. 1, A, C, and E), ETR did not decline remarkably in *Ulva* or *Porphyra* at low C_i but was one third the rate at high C_i in *Zonaria*.

A linear relationship between ETR and gross O_2 evolution or the actual rate of linear electron flow was

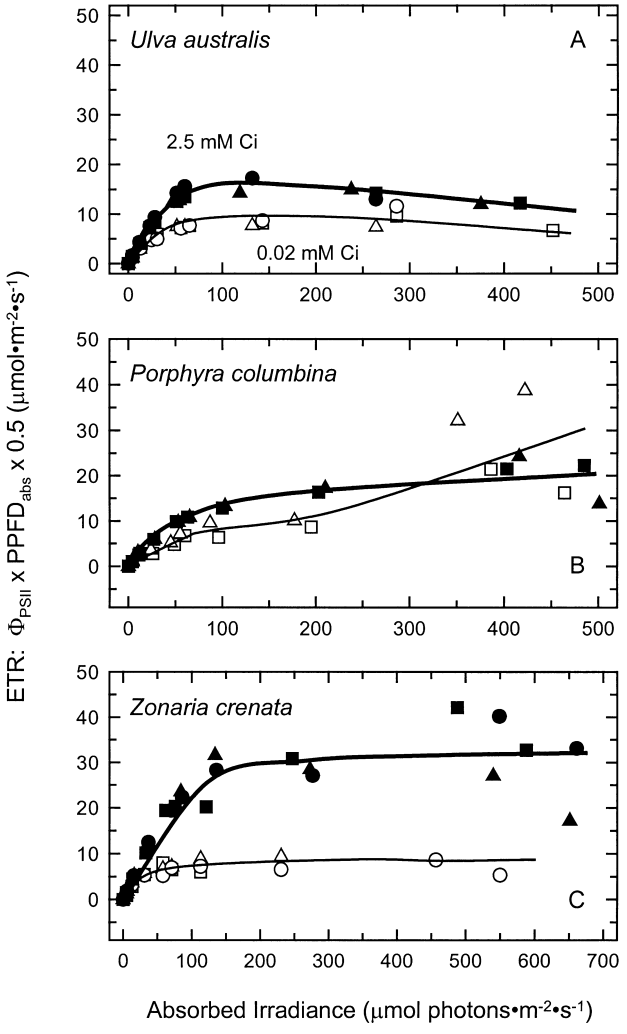

FIG. 4. Changes in ETR as a function of absorbed irradiance. Measurements were made in 20 mM BTP-buffered seawater, pH 8.0, at a natural (closed symbols) or minimum (open symbols) C_i concentration. *Ulva australis* (A), *Porphyra columbina* (B), and *Zonaria crenata* (C). Different symbols represent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

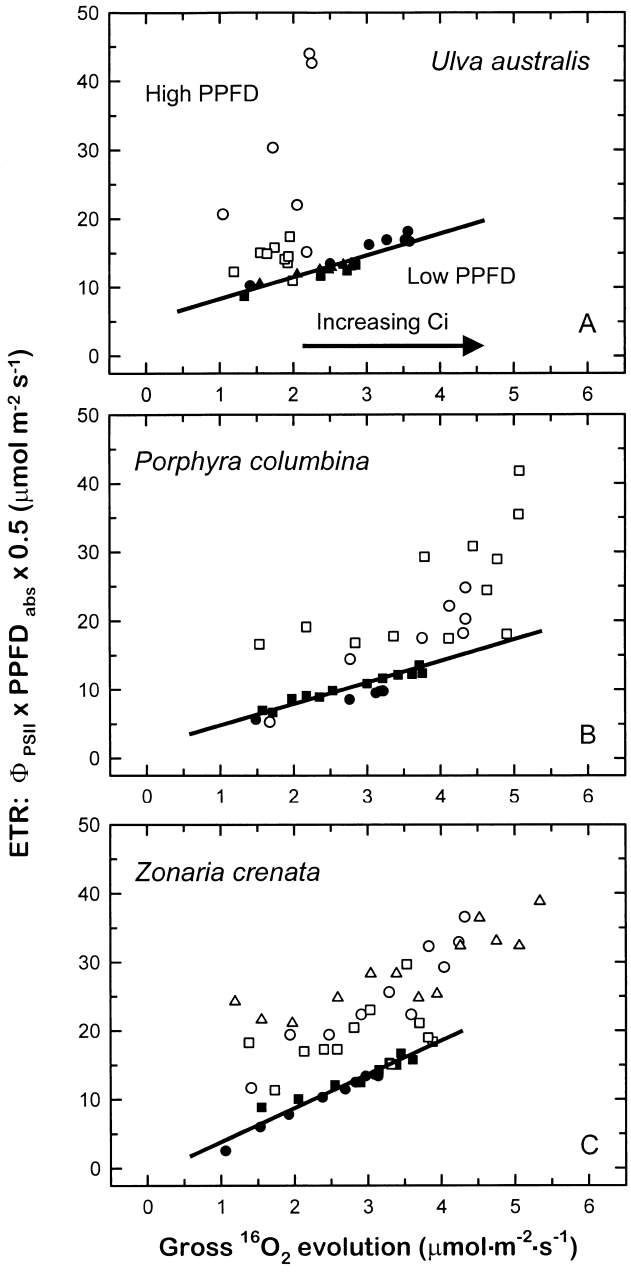

FIG. 5. Comparison of the PSII electron transport rate calculated from Φ_{PSII} and actual rate of gross O_2 evolution at a natural (closed symbols) or minimum (open symbols) C_i concentration. *Ulva australis* (A), *Porphyra columbina* (B), and *Zonaria crenata* (C). Different symbols represent different 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 verse irradiance relationship at 2.5 mM C_i. In *Ulva*, C_i-saturated rates of gross O_2 evolution at 50 μmol photons·m^{-2}·s^{-1} were similar to those measured previously (Fig. 1), but at 390 μmol photons·m^{-2}·s^{-1} rates were lower. At the same time 1-q_P 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 in low light (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 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 Φ_{PSII} measured in low light was linearly correlated with Φ_{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 Φ_{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, Φ_{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 Φ_{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

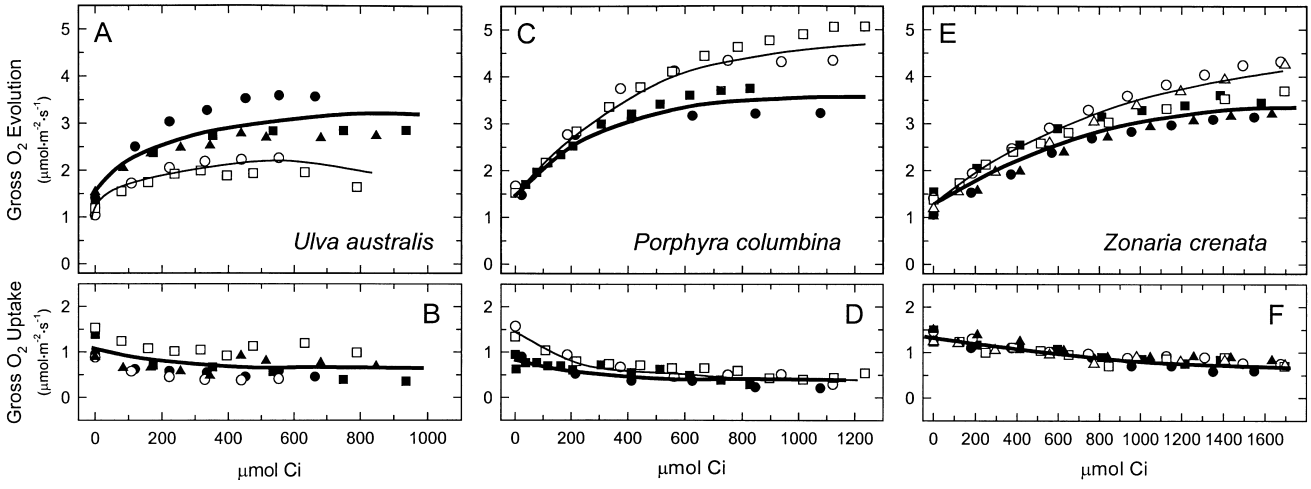

FIG. 6. Changes in gross O_2 evolution (A, C, and E) and gross O_2 uptake (B, D, and F) as a function of inorganic carbon concentration. Measurements were made in 20 mM BTP-buffered seawater, pH 8.0, at 90 or 685 μmol photons·m^{-2}·s^{-1} incident PPFD (closed or open symbols, respectively). *Ulva australis* (A, B), *Porphyra columbina* (C, D), and *Zonaria crenata* (E, F). Different symbols represent different samples.

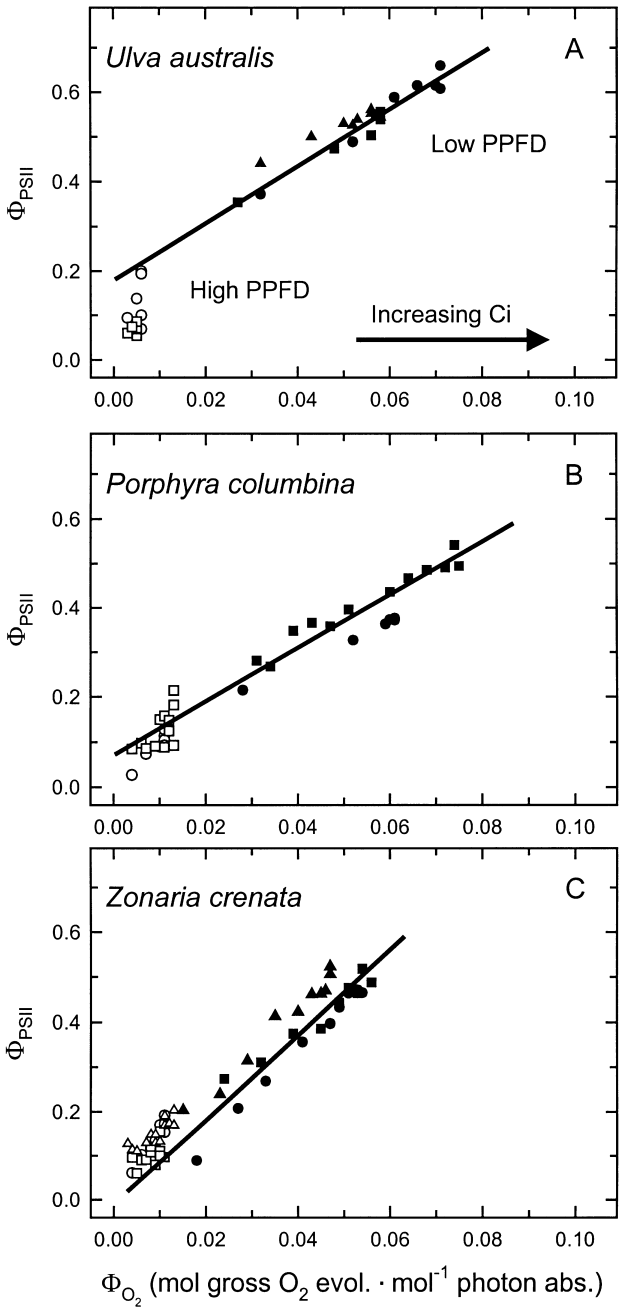

FIG. 7. Comparison of the effective quantum yield of PSII (Φ_{PSII}) and the quantum yield of gross O_2 evolution (mol O_2 evolved per mol photon absorbed by the thallus) at 90 or 685 μmol photons·m^{-2}·s^{-1} incident PPFD (closed or open symbols, respectively). *Ulva australis* (A), *Porphyra columbina* (B), and *Zonaria crenata* (C). Different symbols represent different samples.

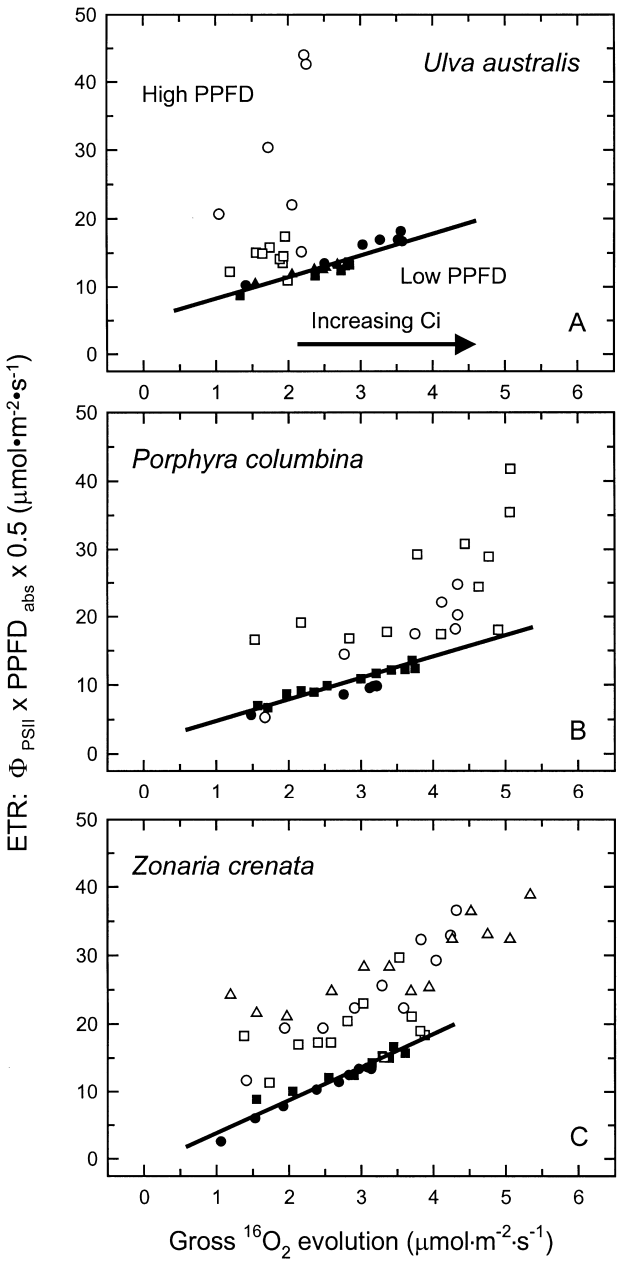

FIG. 8. Comparison of the PSII electron transport rate calculated from Φ_{PSII} and actual rate of gross O_2 evolution at 90 or 685 μmol photons·m^{-2}·s^{-1} incident PPFD (closed or open symbols, respectively). *Ulva australis* (A), *Porphyra columbina* (B), and *Zonaria crenata* (C). Different symbols represent different samples.

linear correlation between ETR and O_2 evolution (net corrected for dark respiration) in *U. lactuca* at irradiances up to 36% of growth irradiance and at various C_i concentrations at 13% growth irradiance, light levels that were presumably less than saturating for photosynthesis. Calculated molar ratios of O_2 evolved/ETRs were close to the theoretical maximum of 0.25. In *U. australis* and *P. columbina*, we also observed close to theoretical ETRs for 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 C_i concentration, and under these conditions, the potential for electron flow to other acceptors, like O_2, should be maximal.

Fluorescence-based ETRs in excess of gross 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, Flameling and Kromkamp 1998). The assumption is usually made that these discrepancies reflect enhanced photorespiratory activity, because laboratory studies of higher plants have indicated that the quantum yield of CO_2 fixation could be accurately measured by fluorometry, so long as photorespiration was kept to a minimum by limiting the availability of O_2 (Sharkey et al. 1988, Krall and Edwards 1990, Harbinson 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 photorespiration. The quantum yield of CO_2 fixation and PSII varied linearly at 21% O_2 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 electron flow to O_2 for the three species tested here. In *U. australis*, gross O_2 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 O_2 uptake rates in all species were on the order of 25%–30% of the maximum rate of O_2 evolution (Fig. 1), similar to those reported for other microalgae (Weger et al. 1989, Badger et al. 1998). Furthermore, these rates were insensitive to CO_2 concentration and comparable with C_4 higher plants with low rates of PCO activity (Badger 1985). In comparison, C_3 plants typically have O_2 uptake rates of 30%–50% of the CO_2-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 C_i 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 CO_2 in various algae may be dependent on the presence or absence of effective CCMs. However, comparisons of light-stimulated O_2 uptake among different micro- and macroalgal species under various extents of CO_2 limitation show that limiting CO_2 either in the presence or absence of a CCM does not appear to greatly stimulate O_2 uptake (Badger et al. 1998). For the species examined here, *Ulva* and *Porphyra* have been shown to have characteristics consistent with the presence of active C_i 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 O_2 uptake at limiting CO_2 may be due to a combination of factors, including evolutionary changes in the RUBISCO oxygenase kinetic properties, the rapid inactivation of RUBISCO at limiting CO_2, and the presence of variable amounts of CCM activity (Badger et al. 1998).

Because we have seen no evidence for significantly enhanced O_2 uptake at high irradiance or low C_i, in our experiments, the discrepancies in calculated ETR we have observed are not likely to be due to electron flow to O_2. Several other possibilities exist. Formulation of Φ_{PSII} 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, Ca^{2+} can be lost from the water oxidation complex. Subsequent charge separation at Ca-depleted centers is proposed to be followed by charge recombination between Q_{A-} and P_{680+} 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 Q_B (or pheophytin) via cyt b_{559}, 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 Prasil et al. (1996) demonstrated an uncoupling between water splitting activity in PSII and Φ_{PSII} 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 (F_m'). This can result in an underestimation of Φ_{PSII} 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 b_{559} are relevant to this study (reviewed by Stewart and Brudvig 1998), In the case where electrons cannot be resupplied to P_{680+} from the water oxidation complex (donor side inhibition), the strong oxidant P_{680+} can be re-reduced by cyt b_{559}, 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 b_{559} can relieve that pressure and prevent the recombination of $Pheo^-$ with P_{680+} to form triplet state P_{680} 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 Φ_{PSII} to Φ_{O_2} relative to C_i (Fig. 3). In the presence of 2.5 mM C_i, we observed a generally linear relationship between Φ_{PSII} and Φ_{O_2} over most of the PPFD range, with a slightly greater loss of Φ_{O_2} relative to Φ_{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 Φ_{O_2} relative to Φ_{PSII} in *Ulva* and *Porphyra* as PPFD rose (higher ratio of Φ_{PSII} to Φ_{O_2}). 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 Φ_{PSII} to Φ_{O_2} 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 reduced plastoquinone pool at high PPFD, especially at low C_i concentration in *Ulva* and *Zonaria* (cf. Fig. 2 and Fig. 5). Surprisingly, the plastoquinone pools in *Porphyra* appeared to be only transiently reduced in low C_i (Fig. 2). ETRs as calculated from light response curves (Fig. 5C) in *Zonaria* were always higher than expected but similar to gross O_2 evolution during a C_i response curve at low PPFD (Fig. 8C). The higher phi PSII at a given phi O_2 (Fig. 3C) supports the hypothesis of stimulated cyclic electron flow around PSII, but the low reduction status of the Q pool at low PPFD is not in keeping with this interpretation.

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 absorptance, 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 PSII and PSI. *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 absorptance 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 absorptance value obtained for *Zonaria* was only slightly less than that typically measured for equally thick higher plant leaves (Evans 1996). Our absorptance 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 absorptance 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 absorptance 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 is 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 Φ_{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 Φ_{PSII} to derive estimates of algal productivity should be treated with great caution.

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Asada, K. 1999. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 50:601–40.

Badger, M. R. 1985. Photosynthetic oxygen-exchange. *Annu. Rev. Plant Physiol.* 36:27–53.

Badger, M. R., Andrews, T. J., Whitney, S. M., Ludwig, M., Yellowlees, D. C., Leggat, W. & Price, G. D. 1998. The diversity and

coevolution of Rubisco, plastids, pyrenoids, and chloroplast-based CO_2-concentrating mechanisms in algae. *Can. J. Bot.* 76:1052–71.

Badger, M. R., von Caemmerer, S., Ruuska, S. & Nakano, H. 2000. Electron flow to O_2 in higher plants and algae: rates and control of direct photoreduction (Mehler reaction) and Rubisco oxygenase. *Phil. Trans. R. Soc. Lond. B* 335:1433–46.

Beer, S. & Björk, M. 2000. Measuring rates of photosynthesis of two tropical seagrasses by pulse amplitude modulated (PAM) fluorometry. *Aquat. Bot.* 66:69–76.

Beer, S., Larsson, C., Poryan, O. & Axelsson, L. 2000. Photosynthetic rates of *Ulva* (Chlorophyta) measured by pulse amplitude modulated (PAM) fluorometry. *Eur. J. Phycol.* 35:69–74.

Beer, S., Vilenkin, B., Weil, A., Veste, M., Susel, L. & Eshel, A. 1998. Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry. *Mar. Ecol. Prog. Ser.* 174:293–300.

Bennoun, P. 1982. Evidence for a respiratory chain in the chloroplast. *Proc. Natl. Acad. Sci. USA* 79:4352–6.

Biehler, K. & Fock, H. 1995. Estimation of non-cyclic electron transport *in vivo* of *Triticum* using chlorophyll fluorescence and mass spectrometric O_2 evolution. *J. Plant Physiol.* 145:422–6.

Bilger, W. & Björkman, O. 1990. Role of the xanthophyll cycle in photoprotection elucidated by measurements of light induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynth. Res.* 25:173–85.

Canvin, D. T., Berry, J. A., Badger, M. R., Fock, H. & Osmond, C. B. 1980. Oxygen exchange in leaves in the light. *Plant Physiol.* 66:117–23.

Evans, J. R. 1996. Developmental constraints on photosynthesis: effects of light and nutrition. *In* Baker, N. R. [Ed.] *Photosynthesis and the Environment*, Vol. 5. Kluwer Academic Publishers, Dordrecht, pp. 281–304.

Falkowski, P. G., Fugita, Y., Ley, A. & Mauzerall, D. 1986. Evidence for cyclic electron flow around photosystem II in *Chlorella pyrenoidosa*. *Plant Physiol.* 81:310–2.

Flameling, I. A. & Kromkamp, J. 1998. Light dependence of quantum yields for PSII charge separation and oxygen evolution in eucaryotic algae. *Limnol. Oceanogr.* 43:284–97.

Fork, D. C., Herbert, S. K. & Malkin, S. 1991. Light energy distribution in the brown alga *Macrocystis pyrifera* (giant kelp). *Plant Physiol.* 95:731–9.

Franklin, L. A., Levavasseur, G., Osmond, C. B., Henley, W. J. & Ramus, J. 1992. Two components of onset and recovery during photoinhibition of *Ulva rotundata*. *Planta* 186:399–408.

Franklin, L. A. & Larkum, A. W. D. 1996. Photoinhibition of photosynthesis on a coral reef. *Plant Cell Environ.* 19:825–36.

Frost-Christensen, H. & Sand-Jensen, K. 1992. The quantum efficiency of photosynthesis in macroalgae and submerged angiosperms. *Oecologia* 91:377–84.

Geel, C., Versluis, W. & Snel, J. F. H. 1997. Estimation of oxygen evolution by marine phytoplankton from measurement of the efficiency of photosystem II electron flow. *Photosynth. Res.* 51:61–70.

Genty, B., Briantais, J.-M. & Baker, N. R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* 990:87–92.

Genty, B., Goulas, Y., Dimon, B., Peltier, G., Briantais, J. M. & Moya, I. 1992. Modulation of efficiency of primary conversion in leaves, mechanisms involved at PS2. *In* Murata, N. [Ed.] *Research in Photosynthesis*, Vol. IV. Kluwer Academic Publishers, Netherlands, pp. 603–10.

Genty, B., Harbinson, J., Briantais, J.-M. & Baker, N. R. 1990. The relationship between non-photochemical quenching of chlorophyll fluorescence and the rate of photosystem II photochemistry in leaves. *Photosynth. Res.* 25:249–57.

Gerbaud, A. & Andre, M. 1980. Effect of CO_2, O_2, and light on photosynthesis and photo-respiration in wheat. *Plant Physiol.* 66:1032–6.

Green, T. G. A., Schroeter, B., Kappen, L., Seppelt, R. D. & Maseyk, K. 1998. An assessment of the relationship between chlorophyll a fluorescence and CO_2 gas exchange from field measurements on a moss and lichen. *Planta* 206:611–8.

Guillard, R. R. L. & Ryther, J. H. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *J. Microbiol.* 8:229–39.

Hager, A. 1980. The reversible, light-induced conversions of xanthophylls in the chloroplast. *In* Czygan, R. D., [Ed.] *Pigments in Plants*, Fisher, Stuttgart, pp. 57–79.

Hanelt, D. & Nultsch, W. 1995. Field studies of photoinhibition show non-correlations between oxygen and fluorescence measurements in the arctic red alga *Palmaria palmata*. *J. Plant Physiol.* 145:31–8.

Harbinson, J., Genty, B. & Baker, N. R. 1990. The relationship between CO_2 assimilation and electron transport in leaves. *Photosynth. Res.* 25:213–24.

Hartig, P., Wolfstein, K., Lippemeier, S. & Colijn, F. 1998. Photosynthetic activity of natural microphytobenthos populations measured by fluorescence (PAM) and ^{14}C-tracer methods: a comparison. *Mar. Ecol. Progr. Ser.* 166:53–62.

Henley, W. J. 1992. Growth and photosynthesis of *Ulva rotundata* (Chlorophyta) as a function of temperature and square wave irradiance in indoor culture. *J. Phycol.* 28:625–34.

Henley, W. J., Levavasseur, G., Franklin, L. A., Lindley, S. T., Ramus, J. & Osmond, C. B. 1991. Diurnal responses of photosynthesis and fluorescence in *Ulva rotundata* acclimated to sun and shade in outdoor culture. *Mar. Ecol. Prog. Ser.* 75:19–28.

Henley, W. J. & Ramus, J. 1989. Optimization of pigment content and the limits of photoacclimation for *Ulva rotundata* (Chlorophyta). *Mar. Biol.* 103:267–74.

Herbert, S. K. & Waaland, J. R. 1988. Photoinhibition of photosynthesis in a sun and a shade species of the red algal genus *Porphyra*. *Mar. Biol.* 97:1–7.

Holmes, J. J., Weger, H. G. & Turpin, D. H. 1989. Chlorophyll a fluorescence predicts total photosynthetic electron flow to CO_2 or NO_3^-/NO_2^- under transient conditions. *Plant Physiol.* 91:331–7.

Husic, D. W., Husic, H. D. & Tolbert, N. E. 1987. The oxidative photosynthetic carbon cycle or C_2 cycle. *CRC Crit. Rev. Plant Sci.* 5:45–100.

Krall, J. P. & Edwards, G. E. 1990. Quantum yields of photosystem II electron transport and carbon dioxide fixation in C_4 plants. *Aust. J. Plant Physiol.* 17:579–88.

Krause, G. H. & Weis, E. 1991. Chlorophyll fluorescence and photosynthesis: the basics. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 42: 13–49.

Leisner, J. M. R., Green, T. G. A. & Lange, O. L. 1997. Photobiont activity of a temperate crustose lichen—long-term chlorophyll fluorescence and CO_2 exchange measurements in the field. *Symbiosis* 23:165–82.

Longstaff, B. J., Kildea, T., Runcie, J. W., Cheshire, A., Dennison, W. C., Hurd, C., Kana, T., Raven, J. A., & Larkum, W. D. 2001. *In situ* measurements of marine plant photosynthesis: a comparison of O2 exchange and electron transport rate methods using the marine macroalga *Ulva lactuca* (Chlorophyta). *Plant Cell Environ.* In press.

Lüning, K. & Dring, M. J. 1985. Action spectra and spectral quantum yield of photosynthesis in marine macroalgae with thin and thick thalli. *Mar. Biol.* 87:119–29.

Markager, S. 1993. Light absorption and quantum yield for growth in five species of marine macroalgae. *J. Phycol.* 29:54–63.

Osmond, C. B., Ramus, J., Levavasseur, G., Franklin, L. A. & Henley, W. J. 1993. Fluorescence quenching during photosynthesis and photoinhibition of *Ulva rotundata* Blid. *Planta* 190:97–106.

Prasil, O., Kolber, Z., Berry, J. A. & Falkowski, P. G. 1996. Cyclic electron flow around photosystem II *in vivo*. *Photosynth. Res.* 48:395–410.

Schreiber, U., Hormann, H., Neubauer, C. & Klughammer, C. 1995. Assessment of photosystem II photochemical quantum yield by chlorophyll fluorescence quenching analysis. *Aust. J. Plant Physiol.* 22:209–20.

Schreiber, U., Schliwa, U. & Bilger, W. 1986. Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res.* 10:51–62.

Seaton, G. G. R. & Walker, D. A. 1990. Chlorophyll fluorescence as a measure of photosynthetic carbon assimilation. *Proc. R. Soc. Lond. B* 242:29–35.

Sharkey, T. D., Berry, J. A. & Sage, R. F. 1988. Regulation of photosynthetic electron-transport in *Phaseolus vulgaris* L., as determined by room-temperature chlorophyll a fluorescence. *Planta* 176:415–24.

Stewart, D. H. & Brudvig, G. W. 1998. Cytochrome b_{559} of photosystem II. *Biochim. Biophys. Acta* 1367:63–87.

Taylor, M. W., Taylor, R. B. & Rees, T. A. V. 1999. Allometric evidence for the dominant role of surface cells in ammonium metabolism and photosynthesis in northeastern New Zealand seaweeds. *Mar. Ecol. Prog. Ser.* 184:73–81.

van Kooten, O. & Snel, J. F. H. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynth. Res.* 25:147–50.

Weger, H. G., Herzig, R., Palkowski, P. G. & Turpin, D. H. 1989. Respiratory losses in the light in a marine diatom: measurements by short-term mass spectrometry. *Limnol. Oceanogr.* 34:1153–61.

Weis, E. & Berry, J. A. 1987. Quantum efficiency of photosystem II in relation to energy-dependent quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* 894:198–208.