

Light dependence of quantum yields for PSII charge separation and oxygen evolution in eucaryotic algae

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Abstract

Quantum yields of photosystem II (PSII) charge separation (Φ_p) and oxygen production (Φ_{O_2}) were determined by simultaneous measurements of oxygen production and variable fluorescence in four different aquatic microalgae representing three different taxonomic groups: the freshwater alga *Scenedesmus protuberans* (Chlorophyceae) and the marine algae *Phaeocystis globosa* (Prymnesiophyceae), *Emiliania huxleyi* (Prymnesiophyceae), and *Phaeodactylum tricornerutum* (Bacillariophyceae). In *S. protuberans*, *P. tricornerutum*, and *E. huxleyi*, light-dependent variability was observed in the ratio of Φ_{O_2} to Φ_p , i.e. in the number of oxygen molecules produced per electron generated by PSII. The ratio $\Phi_{O_2}:\Phi_p$ was highly variable at low light intensities ($E < 0.5E_k$), and at higher light intensities ($E > 0.5E_k$) $\Phi_{O_2}:\Phi_p$ showed a nonlinear decrease with increasing light intensity. In contrast, in *P. globosa*, a trend in $\Phi_{O_2}:\Phi_p$ could not be distinguished, and this species showed a decrease in $\Phi_{O_2}:\Phi_p$ during the day, indicating a dependency of $\Phi_{O_2}:\Phi_p$ on light history. Additionally, considerable interspecific quantitative differences in $\Phi_{O_2}:\Phi_p$ were observed. Two possible interpretations to explain the variability in $\Phi_{O_2}:\Phi_p$ are discussed. Assuming that Φ_p is a reliable measure of the quantum yield for charge separation at PSII, one interpretation is that net oxygen production is influenced by processes that consume oxygen or affect linear electron transport (e.g. cyclic electron transport around PSII, pseudocyclic electron transport in the Mehler reaction, Rubisco oxygenase activity, and light-dependent mitochondrial respiration). A second interpretation, however, suggests that at saturating light, changes in photosynthesis turnover time occur, such that Φ_p does not predict the steady-state O_2 yield.

Quantum yields of phytoplankton photosynthesis are usually defined as the quantum yields for O_2 production (Φ_{O_2}) or C fixation (Φ_{CO_2}) (Kok 1948; Myers 1980; Babin et al. 1996). Because measurements of Φ_{O_2} and Φ_{CO_2} are laborious and time-consuming, much attention has been focused lately on the use of variable chlorophyll fluorescence as a tool to measure the quantum yield of charge separation in photosystem II (PSII) reaction centers (Genty et al. 1989). This technique is rapid and noninvasive and may offer high temporal and spatial resolution when used in field measurements (Schreiber et al. 1986). Theoretically, the rate of noncyclic photosynthetic electron transport of a PSII (J) can be calculated from the quantum yield of PSII charge separation (Φ_p) according to

$$J = \Phi_p E \sigma_{PSII}, \quad (1)$$

where E is the incident light intensity and σ_{PSII} is the absorption cross section of PSII, which determines the fraction of the incident light intensity that is actually used by PSII (e.g. Kolber and Falkowski 1993; Kroon 1991; Hofstra et al. 1994; Biehler and Fock 1995).

In order to use J as a measure for photosynthesis, the relationships between Φ_p , Φ_{O_2} , and Φ_{CO_2} have to be well established. According to the stoichiometry of the Z-scheme, four stable charge separations at both PSII and PSI are need-

ed for the production of one oxygen molecule. Therefore, Φ_{O_2} has a theoretical maximum of $0.125 \times \Phi_p$ (Kok 1948). The ratio of the quantum yields for oxygen production and charge separation ($\Phi_{O_2}:\Phi_p$) is a useful parameter to describe the efficiency of oxygen production by linear electron transport generated by PSII. Because charge separation and oxygen production are closely coupled processes, both representing PSII activity, large variability in $\Phi_{O_2}:\Phi_p$ is not expected under physiological conditions. In contrast, the coupling between Φ_{O_2} and Φ_{CO_2} is supposed to be less strict because of the occurrence of cyclic electron transport around photosystem I (PSI) and because photosynthetically generated reducing power is used for processes other than CO_2 fixation, i.e. NO_3^- reduction and thiolation of chloroplast enzymes (Robinson 1988; Williams and Robertson 1991).

Experimental comparison of Φ_p , Φ_{O_2} , and Φ_{CO_2} has yielded contradictory results. Nonlinearity of Φ_p and Φ_{O_2} was observed in marine microalgae (Schreiber et al. 1995a), macroalgae (Hanelt and Nultsch 1995), and higher plants (Biehler and Fock 1995). In contrast, a linear relationship between Φ_p and Φ_{O_2} was observed in green alga (Kroon 1991; Holmes et al. 1989) and in some higher plants (Strand and Lundmark 1995; Genty et al. 1992). Indications for linearity between Φ_p and Φ_{CO_2} were obtained in higher plants (Genty et al. 1989; Edwards and Baker 1993). Close coupling between Φ_p and Φ_{O_2} , but not between Φ_{O_2} and Φ_{CO_2} , has been observed by Kroon et al. (1993) in the dinoflagellate *Heterocapsa pygmaea*. Clearly, further research is needed in order to use J as a reliable measure of photosynthesis.

We studied the light dependency and the occurrence of diurnal patterns in Φ_p and Φ_{O_2} in light-limited continuous cultures of four different aquatic microalgae representing different taxonomic groups: the freshwater alga *Scenedesmus*

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Table 1. Medium type and culture conditions of the different species. Maximum light intensity (E_{\max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) refers to the peak intensity imposed on the algae in a sinusoidal light regime during the light period of a 10:14 L/D cycle. D is dilution rate (d^{-1}); T is culture temperature ($^{\circ}\text{C}$).

	E_{\max}	D	T	Medium
<i>S. protuberans</i>	80	0.12	20	BG/11
<i>P. globosa</i>	100	0.20	17.5	MN + vit. B1 and B12
<i>E. huxleyi</i>	60	0.08	17.5	MN + vit. B1, B12, and H
<i>P. tricornutum</i>	90	0.11	15	MN + silicate + vit. B1, B12, and H

protuberans (Chlorophyceae) and the chlorophyll *c*-containing marine algae *Phaeodactylum tricornutum* (Bacillariophyceae), *Phaeocystis globosa* (Prymnesiophyceae) and *Emiliana huxleyi* (Prymnesiophyceae). The results show considerable light-dependent variability in Φ_{O_2} : Φ_{P} in all species.

Methods

The freshwater alga *S. protuberans* was cultivated in O_2 -saturated, filter-sterilized BG/11 freshwater medium enriched with trace metal mix. The marine species were grown in filter-sterilized MN medium enriched with trace metal mix and the vitamins thiamine (B, 74 nM), cyanocobalamin (B12, 18 nM), and biotine (H, 100 nM) (both media according to Rippka et al. 1979). B12 was not added to *P. globosa* medium because it was found to impair growth of this species. *P. globosa* did not form colonies. Na_2SiO_3 (150 μM) was added to the *P. tricornutum* medium. Continuous cultures were grown in sterilized flat culture vessels under a 10:14 L/D photocycle. The cultures were illuminated by 400-W high-pressure lamps (Philips HPIT E40). A water jacket, connected to a temperature-controlled water bath, was mounted between the cultures and the lamps to keep growth at constant temperature. The cultures were continuously bubbled with sterile water-saturated air to prevent CO_2 limitation or sedimentation of the cells. The diurnal course of the light intensity in the cultures was regulated by a computer-controlled system of Venetian blinds, as described by Kromkamp and Limbeek (1993). The cultures were exposed to a sinusoidal diurnal light regime, simulating diurnal light intensity in the absence of vertical mixing. Total daily light dose and maximum light intensity are given in Table 1, together with other culture variables. Chl *a* was measured spectrophotometrically after extraction in 95% boiling methanol for *S. protuberans* (Iwamura et al. 1970) or extraction in 95% acetone for the other species (Jeffrey and Humprey 1975). In vivo absorption was measured spectrophotometrically according to Shibata et al. (1954). Chlorophyll-specific absorption cross sections (α^* ; $\text{m}^2 (\text{mg Chl})^{-1}$) were calculated from the chlorophyll concentration and the in vivo absorption (Dubinsky et al. 1986). All absorption measurements were performed using a Uvikon 940 double-beam scanning spectrophotometer.

At 1, 5, and 7.5 h after the start of the light period, subsamples were drawn from the culture. Photosynthetic oxygen

evolution and PAM fluorescence of the subsamples were recorded simultaneously in a specially designed Dubinsky chamber (Dubinsky et al. 1987) at the growth temperature. The Chl *a* concentration in the chamber varied between 6.0 and 6.3 mg liter^{-1} for *S. protuberans*, 0.94 and 0.98 mg liter^{-1} for *P. globosa*, 1.19 and 1.45 mg liter^{-1} for *E. huxleyi*, and 1.36 and 1.57 mg liter^{-1} for *P. tricornutum*. The algae were dark-adapted for 15 min and subsequently exposed for 2–4 min to increasing irradiances of white light. Steady-state fluorescence (F_s) and maximal fluorescence (F_m') were measured using a PAM-Walz 101-103 fluorometer (H. Walz, Effeltrich; Schreiber et al. 1986). A light-emitting diode delivered a low-intensity modulated measuring light beam that was weak enough not to induce any significant variable fluorescence. The measuring light was guided to the Dubinsky chamber through a glass-fiber connection (101-F5, Walz), which also collected the fluorescence emitted by the sample. At intervals of 30 s a high-intensity saturating light pulse (Schott KL1500-E; $E > 10,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) with a duration of 0.5–0.7 s was applied to the culture sample in order to close all reaction centers. In this situation, photochemical fluorescence quenching is reduced to zero, and fluorescence is maximal. Increasing the pulse duration or decreasing the Chl concentration in the chamber had no effect on PSII efficiency, indicating that the pulse was indeed saturating. F_s and F_m' were recorded before and during a saturating light pulse, respectively. All measured fluorescence values were corrected for background signals. Oxygen production was measured using a polarographic oxygen electrode (YSI 5331).

Maximum quantum yield for charge separation (F_v/F_m ; mol charge separation (mol quanta) $^{-1}$) was measured at the end of the dark-adaptation period. F_v/F_m was calculated as

$$F_v/F_m = (F_m - F_o)/F_m, \quad (2)$$

in which F_m and F_o represent the maximum and minimum fluorescence of open reaction centers, i.e. after >15 min of dark adaptation. F_v/F_m was independent of cell density of the sample (data not shown).

Operational quantum yields for charge separation (Φ_{P}) were calculated as:

$$\Phi_{\text{P}} = (F_m' - F_s)/F_m', \quad (3)$$

where F_m' and F_s represent maximum and steady-state fluorescence under actinic illumination.

Gross oxygen production was calculated by adding the initial dark respiration to the oxygen evolution data. P - E curves were fitted according to the hyperbolic tangent function of Jassby and Platt (1976). Photosynthesis efficiency (α^B ; $\text{mg O}_2 (\text{mg Chl})^{-1} \text{h}^{-1} (\mu\text{mol m}^{-2} \text{s}^{-1})^{-1}$), maximal photosynthetic capacity (P^B_{\max} ; $\text{mg O}_2 (\text{mg Chl})^{-1} \text{h}^{-1}$), and saturating light intensity ($E_k = P^B_{\max}/\alpha^B$; $\mu\text{mol m}^{-2} \text{s}^{-1}$) were derived from the fit.

Maximum quantum yield for O_2 production ($\Phi_{\text{O}_2, \max}$; mol quanta (mol O_2) $^{-1}$) was calculated as

$$\Phi_{\text{O}_2, \max} = \alpha^B/(115 \times \alpha^*), \quad (4)$$

where the constant 115 is needed to provide uniform dimensions. The operational quantum yield for O_2 production (Φ_{O_2}) was calculated as

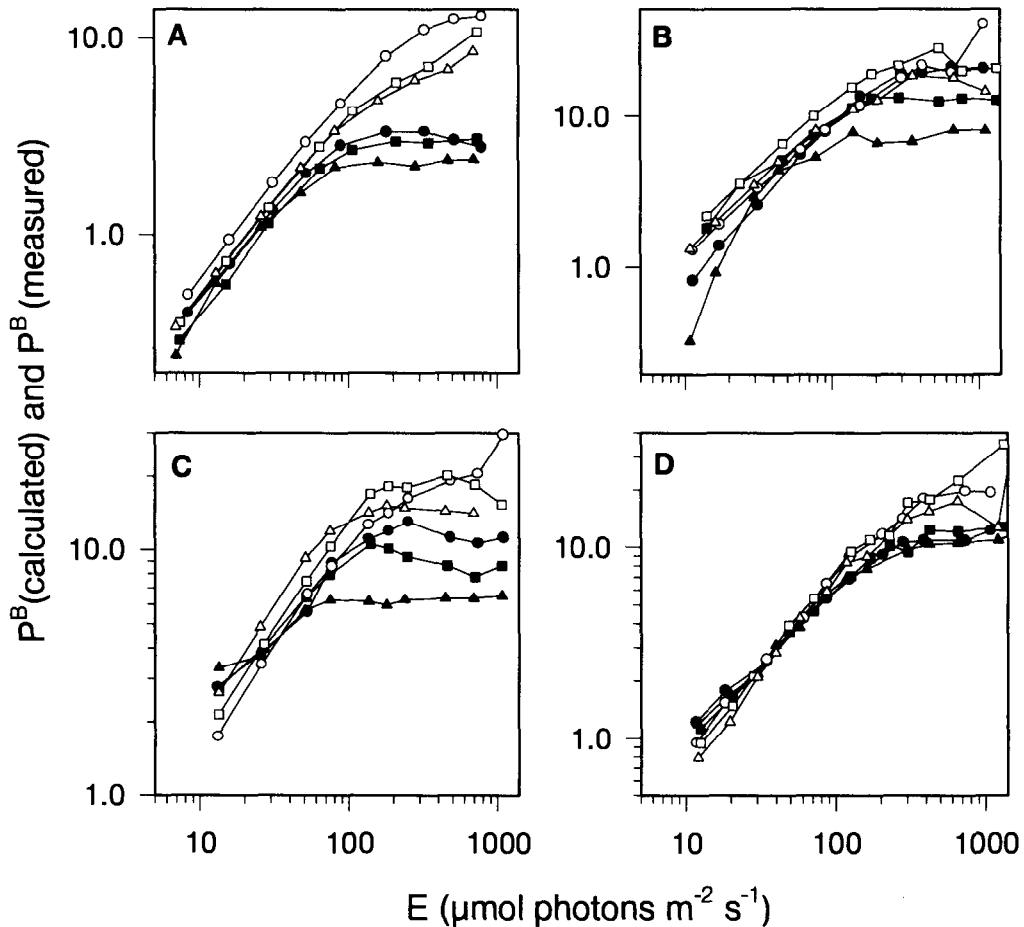


Fig. 1. Net photosynthesis (P^B ; $\text{mg O}_2 \text{ mg Chl}^{-1} \text{ h}^{-1}$) as a function of light intensity at 1 h (circles), 5 h (squares), and 7.5 h (triangles) after the start of the light period. Closed symbols indicate polarographically measured O_2 production; open symbols indicate O_2 production calculated according to Eq. 6. A. *Scenedesmus protuberans*. B. *Phaeocystis globosa*. C. *Emiliana huxleyi*. D. *Phaeodactylum tricornutum*.

$$\Phi_{\text{O}_2} = P^B / (115 \times a^* \times E). \quad (5)$$

Results

P-E data—All measurements were performed at least in duplicate. Because measurements performed on different days showed the same pattern, for reasons of clarity we chose to use a dataset obtained on a single day for each species. Fig. 1 and Table 2 present photosynthesis characteristics and chlorophyll-specific absorption cross sections of the four different species, measured 1, 5, and 7.5 h after the start of the light period.

The interspecific differences in α^B and P^B_{max} were considerable, covering respectively a half and almost one order of magnitude. The highest values of P^B_{max} were reached in *P. globosa*, whereas both α^B and P^B_{max} were lowest in *S. protuberans*, the only Chl *b*-containing species. P^B_{max} decreased during the light period in *S. protuberans*, *P. globosa*, and *E. huxleyi*. This diurnal pattern was especially pronounced in *P. globosa*. Diurnal patterns in α^B and in chlorophyll-specific absorption cross section (a^*) could not be observed (Table

2). Despite the large interspecific differences in photosynthetic characteristics, maximum quantum yields of charge separation and oxygen production (F_v/F_m and $\Phi_{\text{O}_2, \text{max}}$) of the different species had comparable values (Table 2). To a great extent, the observed interspecific differences in α^B can be explained by differences in a^* . Similar results were obtained by Welschmeyer and Lorenzen (1981) in the comparison of carbon-based efficiencies and quantum yields of a number of phytoplankton species. Apparently, interspecific variability in photosynthetic efficiencies of light-limited algae can only to a limited extent be explained by variability in quantum yields.

Fluorescence—The course of F_s/F_m' of the different species at a range of light intensities is shown in Fig. 2. When $E < E_k$, F_s remained constant in *S. protuberans*, whereas F_s increased by 15–30% in the Chl *c*-containing algae. Similar differences between light-dependent behavior of F_s of chlorophytes and Chl *c*-containing algae were observed by Falkowski et al. (1986b). As light became saturating ($E > E_k$), F_s was progressively quenched in all species. Quenching of

Table 2. α^B (mg O₂ (mg Chl)⁻¹ h⁻¹ (μmol m⁻² s⁻¹)⁻¹), P_{\max}^B (mg O₂ (mg Chl)⁻¹ h⁻¹), E_k (μmol m⁻² s⁻¹), R^B (mg O₂ (mg Chl)⁻¹ h⁻¹) ($R^B(\text{dac})$, R^B in dark-adapted cells; $R^B(\text{ai})$, R^B after illumination), $\Phi_{\text{O}_2, \max}$ (photons O₂⁻¹), F_v/F_m , and a^* (m₂ (mg Chl)⁻¹) of the four species measured at different times of the day (n.d., not done).

	Time (h)	α^B	P_{\max}^B	E_k	$R^B(\text{dac})$	$R^B(\text{ai})$	$\Phi_{\text{O}_2, \max}$	F_v/F_m	a^*
<i>S. protuberans</i>	0900	0.048	3.2	66.7	0.36	n.d.	0.059	0.69	0.0070
	1300	0.042	3.0	71.4	0.50	1.22	0.051	0.68	0.0072
	1530	0.044	2.4	54.6	0.67	1.11	0.051	0.66	0.0075
<i>P. globosa</i>	0900	0.10	21.9	219.0	1.83	n.d.	0.057	0.60	0.0154
	1300	0.13	13.7	105.4	1.49	4.73	0.065	0.57	0.0175
	1530	0.10	8.0	80.0	2.63	3.89	0.046	0.54	0.0187
<i>E. huxleyi</i>	0900	0.14	11.8	84.3	0.60	4.38	0.037	0.63	0.033
	1300	0.17	9.3	54.7	1.75	2.00	0.049	0.51	0.030
	1530	0.21	6.2	29.5	1.92	3.26	0.061	0.49	0.030
<i>P. tricornutum</i>	0900	0.072	10.1	140.3	1.23	4.38	0.048	0.35	0.0131
	1300	0.069	11.4	165.2	1.33	2.33	0.038	0.35	0.0160
	1530	0.069	10.9	158.0	1.49	3.33	0.043	0.34	0.0141

F_s to values below F_o was observed in *S. protuberans*, *E. huxleyi*, and *P. tricornutum*. This pattern was especially pronounced in *P. tricornutum*, where F_s was quenched to <20% of F_o at high light intensities. According to Falkowski et al. (1986b), a light-dependent increase of F_m' may be expected in Chl *c*-containing algae at $E < E_k$ owing to light-induced changes in σ_{PSII} . Indeed, increases in F_m' at low light intensities ($E < 0.2E_k$) were observed in *P. globosa* and *P. tricornutum* but not in *E. huxleyi*. Maximum fluorescence yield was reached at $I \approx 0.2I_k$ in both species and was slightly quenched at higher, subsaturating light intensities. In *S. protuberans* and *E. huxleyi*, variable fluorescence was maximal in dark-adapted cells, and F_m' was slightly quenched with increasing light intensities at subsaturating light intensities ($E > E_k$). At saturating light intensities ($E > E_k$), F_m' sharply decreased to values close to F_s in all species.

With the exception of *P. tricornutum*, it may be stated that quenching of both F_s and F_m' was more pronounced and started at lower light intensities in the samples that were taken at the end of the light period. This is not surprising, since P_{\max}^B (and subsequently E_k) decreased during the day in these species. Consistent with this observation, diurnal patterns in P_{\max}^B were absent in *P. tricornutum* (Table 2).

Maximum and operational quantum yields—Although maximum quantum yield of charge separation (F_v/F_m) has been reported to reach values of 0.7–0.8 in various phytoplankton species or assemblages growing under light-limited conditions (e.g. Gilmour et al. 1984; Greene et al. 1994; Hofstraat et al. 1994), in this study F_v/F_m had consistently lower values for all species. F_v/F_m ranged from 0.7 in *S. protuberans* to 0.4 in *P. tricornutum* (Table 2). However, for the latter species the low values recorded in this study may be due to specific culture conditions, since F_v/F_m values of ~0.65 were observed by Greene et al. (1991, 1992) and Geel et al. (1997). Longer dark adaptation did not result in higher values for F_v/F_m in any of the species. Apparently 15 min of dark adaptation were sufficient to achieve complete relaxation of photochemical quenching. One explanation for the relatively low F_v/F_m ratios might be that we used rela-

tively high measuring light intensities (settings 6–8 on PAM-101) in order to get a reliable signal. This could have caused some variable fluorescence, and hence an overestimation of F_o . Also, the algal species we used, with the exception of *Scenedesmus*, do not form grana in the chloroplast. This difference in chloroplast organization might be responsible for an apparently lower F_v/F_m .

A general pattern in operational quantum yields of charge separation (Φ_p) could be observed in all species (Fig. 3, closed symbols). At low light intensities ($E < 30 \mu\text{mol m}^{-2} \text{s}^{-1}$), Φ_p was more or less constant and close to F_v/F_m , indicating that linear electron transport proceeded with almost maximal efficiency. At higher light intensities, Φ_p was inversely related to light intensity. The light-dependent patterns in Φ_{O_2} resembled the patterns in Φ_p (Fig. 3, open symbols). At low light intensities ($E < 20 \mu\text{mol m}^{-2} \text{s}^{-1}$), Φ_{O_2} was highly variable.

Calculation of $\Phi_{\text{O}_2}:\Phi_p$ revealed that, in general, the efficiency of oxygen production due to linear electron transport generated by PSII was maximal in low light. In *S. protuberans*, *P. tricornutum*, and *E. huxleyi* $\Phi_{\text{O}_2}:\Phi_p$ decreased more or less logarithmically with increasing light intensity (Fig. 4). In *S. protuberans*, $\Phi_{\text{O}_2}:\Phi_p$ started to decrease at saturating light intensities (i.e. $E > E_k$), whereas in *P. tricornutum* and *E. huxleyi*, $\Phi_{\text{O}_2}:\Phi_p$ started to decrease before saturating light intensities were reached ($E < E_k$). *E. huxleyi* was the only species in which $\Phi_{\text{O}_2}:\Phi_p$ reached a constant, minimum value at light intensities $>100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (i.e. $>E_k$). In *P. globosa*, the patterns in $\Phi_{\text{O}_2}:\Phi_p$ were less clear. In this species, $\Phi_{\text{O}_2}:\Phi_p$ was highest at the start of the light period.

Discussion

From the results, it can be concluded that the efficiency of oxygen production by linear electron transport generated by PSII ($\Phi_{\text{O}_2}:\Phi_p$) is generally maximal in low light. By using the dark-adapted maximum values of $\Phi_{\text{O}_2, \max}/(F_v/F_m)$ (Fig. 3), the theoretical gross photosynthetic O₂ production

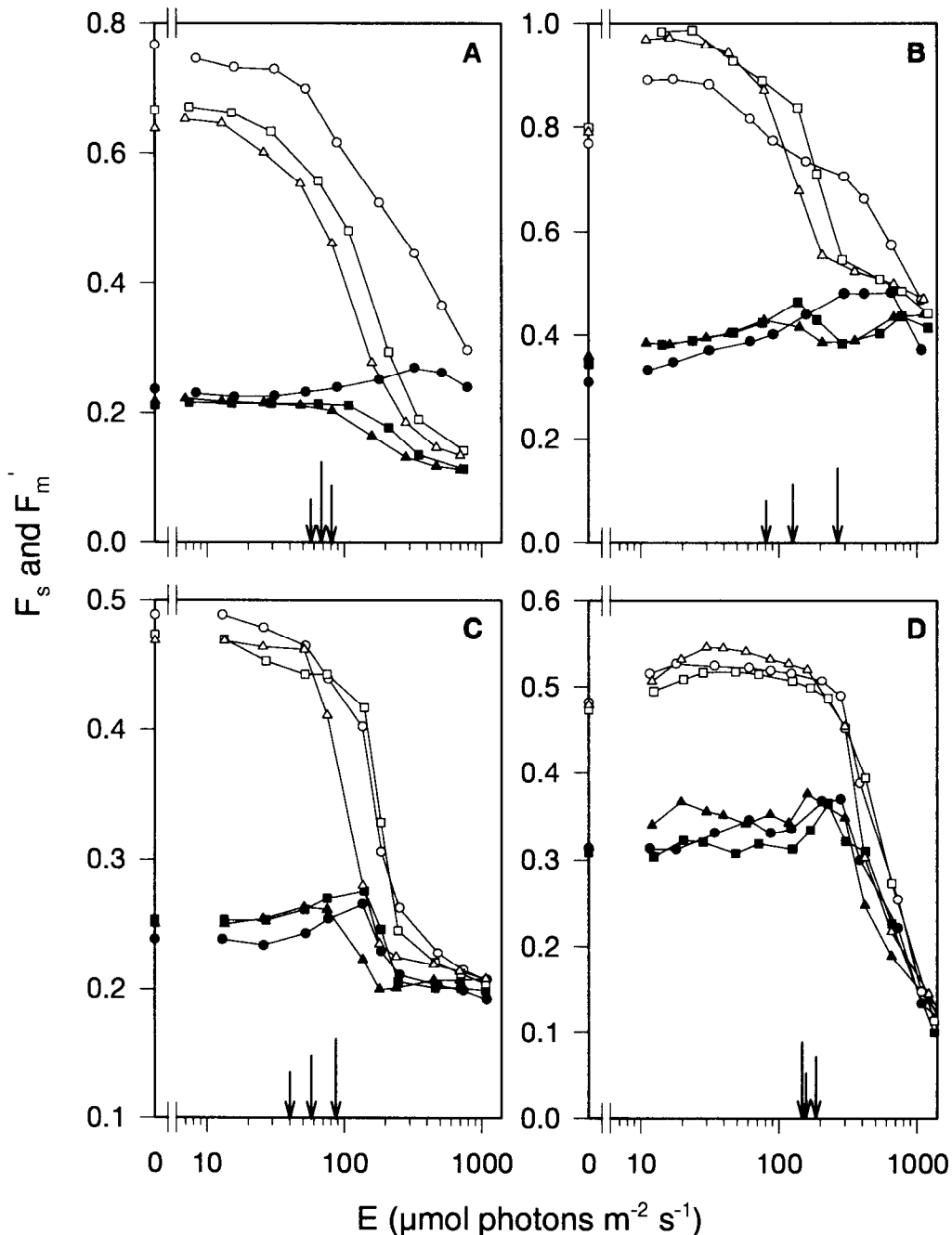


Fig. 2. Steady-state fluorescence (F_s ; closed symbols) and maximal fluorescence (F'_m ; open symbols) as a function of light intensity at 1 h (circles), 5 h (squares), and 7.5 h (triangles) after the start of the light period. Minimum and maximum fluorescence (F_o and F_m) are shown on the y-axis. A. *Scenedesmus protuberans*. B. *Phaeocystis globosa*. C. *Emiliania huxleyi*. D. *Phaeodactylum tricornutum*. The arrows indicate E_k at 1 h (long arrow), 5 h (intermediate arrow), and 7.5 h (short arrow).

can be calculated from fluorescence measurements (assuming a constant σ_{PSII}):

$$P^B = Ea * [\Phi_{O_2, \max} / (F_v / F_m)] \Phi_p. \quad (6)$$

Comparison of calculated and measured O_2 production shows a consistent overestimation of O_2 production with increasing light intensity in all species (Fig. 1, open symbols)

owing to the decline in Φ_{O_2} / Φ_p . The difference between calculated and measured values may be up to 300%, as in *S. protuberans*.

Explanations for the variability in Φ_{O_2} / Φ_p —Basically, there are two possible interpretations of the observed non-linearity of Φ_{O_2} and Φ_p , yet these interpretations are not mu-

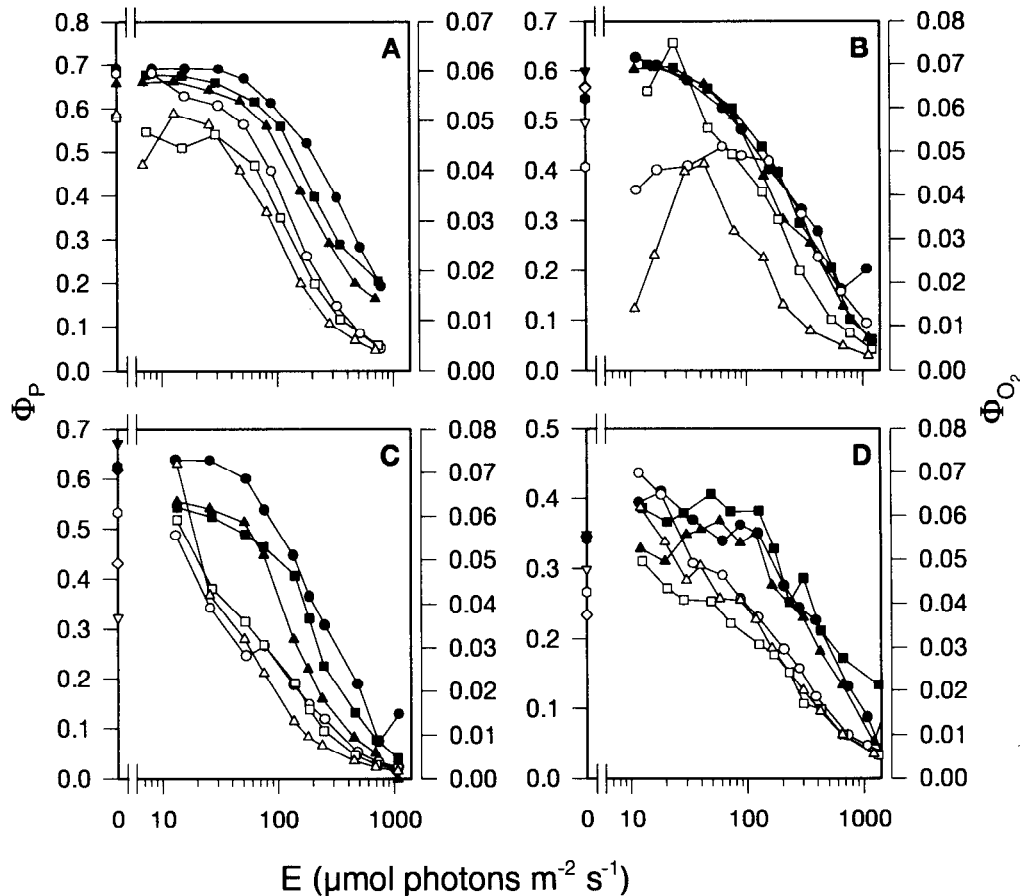


Fig. 3. Operational quantum yields for charge separation (Φ_p ; closed symbols) and oxygen production (Φ_{O_2} ; open symbols) as a function of light intensity at 1 h (circles), 5 h (squares), and 7.5 h (triangles) after the start of the light period. F_v/F_m and $\Phi_{O_2, \max}$ are shown on the y-axis. A. *Scenedesmus protuberans*. B. *Phaeocystis globosa*. C. *Emiliania huxleyi*. D. *Phaeodactylum tricornutum*.

tually exclusive. The processes that they assume may occur simultaneously and have a cumulative effect.

First, it can be assumed that Φ_p is a reliable measure of the operational quantum yield for charge separation at PSII (Genty et al. 1989), and hence that the product of Φ_p and E is a good measure for the rate of PSII electron transport. However, several processes that consume O_2 or influence photosynthetic O_2 evolution, without affecting the number of electrons generated at PSII, may be responsible for the light-dependent decrease in $\Phi_{O_2} : \Phi_p$. This explanation is supported by Genty et al. (1992), who demonstrated a linear relationship between Φ_p and Φ_{O_2} in barley and beans using different stable isotopes of oxygen.

Among the processes influencing net PSII electron production or net oxygen evolution are cyclic electron flow around PSII, light-dependent mitochondrial respiration, pseudocyclic electron transport (or Mehler reaction), Rubisco oxygenase activity, and PSII heterogeneity. We briefly discuss each of these processes here.

Falkowski et al. (1986a,b) and Prasil et al. (1996) found deviation of linearity of oxygen-flash yield and variable fluorescence yield in a number of microalgae. The deviation started when photosynthetic rates reached light saturation; in

other words, there was a residual amount of variable fluorescence yield left when oxygen-flash yield approached zero. These results were attributed to the occurrence of a cyclic electron flow around PSII at saturating light intensities. The authors suggested a model in which electrons are transported from PQH₂ via Cyt *b* 559 to Z. This process is only likely to occur at a high redox state of the plastoquinone pool, i.e. at or near saturating light intensities.

A different, simpler explanation for the deviation of Φ_p and Φ_{O_2} may be that mitochondrial respiration rates are controlled by light intensity. Not including this probability in the calculation of gross oxygen production, as was the case in this study, may result in consistent underestimation of gross oxygen production rates and consequently of Φ_{O_2} . Experimental evidence for increases in mitochondrial respiration rate in the light has been found for a number of Chl *c*-containing algae (Grande et al. 1989; Weger et al. 1989; Daneri et al. 1992; Beardall et al. 1994). The higher mitochondrial respiration rates in the light are probably due to increased substrate supply by photosynthesis. This would imply a quantitative relationship between photosynthesis and respiration. Consequently, light-induced increases in mitochondrial respiration probably occur at subsaturating light

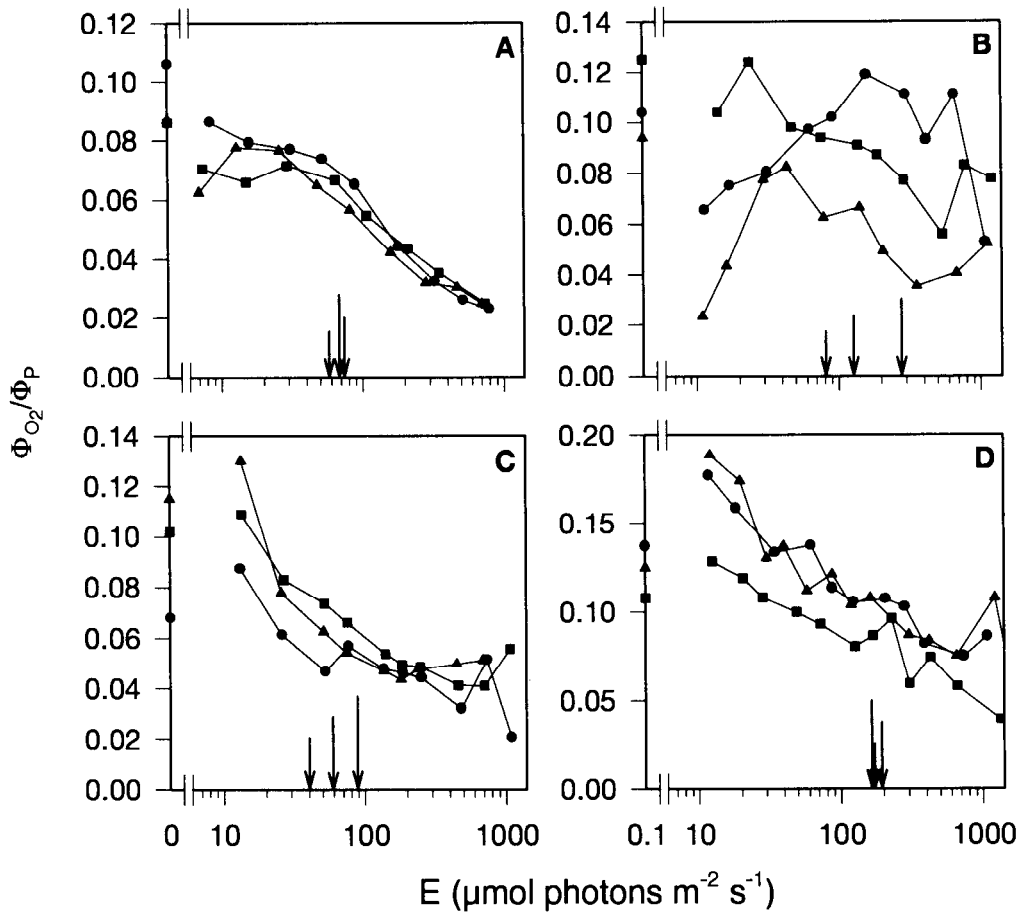


Fig. 4. $\Phi_{O_2}:\Phi_P$ as a function of light intensity at 1 h (circles), 5 h (squares), and 7.5 h (triangles) after the start of the light period. $\Phi_{O_2,max}/(F_v/F_m)$ is shown on the y-axis. A. *Scenedesmus protuberans*. B. *Phaeocystis globosa*. C. *Emiliana huxleyi*. D. *Phaeodactylum tricornutum*. The arrows indicate E_k at 1 h (long arrow), 5 h (intermediate arrow), and 7.5 h (short arrow).

intensities, and stabilize at $E > E_k$. Although mitochondrial respiration was not directly measured in the light in this study, increased dark respiration rates were observed in the first 15 min of darkness following a $P-E$ measurement. The increases were highly variable (Table 2). Although not direct evidence, these enhanced post-illumination respiration (EPIR) rates are indicative of increased mitochondrial respiration in the light. We have recalculated Φ_{O_2} , with the assumption that respiration linearly increased from the dark-adapted value to EPIR at $E < E_k$, and respiration was constant at $E > E_k$. This removes most of the nonlinearity of $\Phi_{O_2}:\Phi_P$ in *E. huxleyi*, because this species showed a more or less constant ratio of $\Phi_{O_2}:\Phi_P$ at higher irradiances. However, increased respiration cannot explain the decrease in the $\Phi_{O_2}:\Phi_P$ ratio above E_k for the other species.

A third process that has potential impact on the ratio of Φ_P and Φ_{O_2} is the Mehler peroxidase reaction, which is also referred to as pseudocyclic electron transport (Robinson 1988). In the Mehler reaction, reduced ferredoxin at the donor site of PSI donates electrons to O_2 instead of to $NADP^+$, which is the usual terminal electron acceptor in the photosynthetic electron transport chain. Reduction of oxygen results in the formation of free superoxide radicals, which are

rapidly converted in the chloroplast in a series of enzymatic reactions to oxygen and water. Mehler reaction activity has been observed in a wide range of organisms, including cyanobacteria (Kana 1992, 1993), *Scenedesmus* spp. (Radmer and Kok 1976; Radmer and Ollinger 1980; Radmer et al. 1978), and higher plants (e.g. Canvin et al. 1980; Neubauer and Yamamoto 1994). Rees et al. (1992) found evidence for strong Mehler reaction activity in the green alga *Dunaliella*. The net oxygen yield of the Mehler reaction is zero. Mehler reaction activity will preferably occur when the oxygen concentration is high and the supply of reductant is in excess of the demands of reductant by the carbon-fixation cycle, i.e. when light intensity is saturating and the rate of photosynthetic carbon fixation is maximal (Kana 1992). However, such activity was also observed at subsaturating irradiances in a cyanobacterium (Kana 1993). In higher plants, Mehler activity was shown to be a function of light intensity at maximal carbon-fixation rates (Canvin et al. 1980). This underlines the possible role of the Mehler reaction as a mechanism to support additional linear electron transport and ATP production in conditions where the rate of linear electron transport is limited by the rate of $NADP^+$ removal by the carbon-fixation cycle. The maximum quantitative contribution of

Mehler-type electron transport to the total linear electron transport may be considerable, ranging from 10 to 50% (Furbank et al. 1982; Kana 1993). Schreiber et al. (1995b) hypothesized that the trans-thylakoid ΔpH gradient that is induced by the Mehler reaction plays a role in the dissipation of excess energy at high light intensity. Thus, the Mehler reaction may function as a protective mechanism against the harmful effects of high light intensity.

Photorespiration, initiated by the oxygenase activity of ribulosebisphosphate carboxylase/oxygenase (Rubisco), may be a fourth mechanism decreasing $\Phi_{\text{O}_2}:\Phi_{\text{P}}$. However, it is not likely that photorespiration is quantitatively important in most microalgae, since photorespiratory activity in these organisms is effectively suppressed by the presence of CO_2 -concentrating mechanisms (Ogren 1984; Glover 1989; Raven and Johnston 1991). *E. huxleyi* may be an exception to this (Raven and Johnston 1991). We have tested the presence of photorespiration in batch cultures of *E. huxleyi* and *P. tricornutum*. Nonphotorespiratory (NPR) conditions were applied by addition of $0.2 \text{ g liter}^{-1} \text{ NaHCO}_3^-$, in combination with flushing with N_2 and CO_2 gas in order to lower the pH to 7 and the oxygen concentration to 40% saturation. Φ_{P} and Φ_{O_2} were measured as usual. NPR conditions had no influence on patterns in $\Phi_{\text{O}_2}:\Phi_{\text{P}}$ in both species (Fig. 5). Note that the application of NPR conditions is more complicated in aquatic systems than in air, since CO_2 concentration in the water is mainly a function of pH. This makes it difficult to separate the effects of increased CO_2 concentration from direct pH effects.

PSII heterogeneity might also add to the nonlinearity between Φ_{P} and Φ_{O_2} (see Melis 1991 for a review). Hormann et al. (1994) and Schreiber et al. (1995a) demonstrated a linear relationship between Φ_{P} and Φ_{O_2} at high irradiances but a deviation from nonlinearity at high quantum efficiencies, i.e. at low irradiances. By using artificial electron acceptors these authors were able to demonstrate that this nonlinearity was related to the presence of two different populations of PSII. This type of PSII heterogeneity has been observed in *E. huxleyi* (Kromkamp et al. unpubl.).

In the second interpretation, steady-state photosynthesis in saturating light is limited by Calvin cycle reactions rather than by electron transport (Heber et al. 1988; Leverenz et al. 1990). The residual capacity for oxygen production of cells with light-saturated steady-state photosynthesis can be revealed by the administration of saturating flashes (Falkowski et al. 1988). It can be assumed that the relationship between Φ_{P} and steady-state oxygen production is not constant over a range of light intensities, because turnover time (τ) decreases as a result of a decrease in the number of PSII per Rubisco at high light intensity (Sukenik et al. 1987).

This explanation seems to be contradicted by the fact that linearity of Φ_{O_2} and Φ_{P} over a range of light intensities has actually been observed in many higher plants in nonphotorespiratory conditions (see Table 3 and the section on comparison with other studies below). Deviations from linearity of Φ_{O_2} and Φ_{P} in higher plants are mainly found at low light intensities (i.e. high quantum yields) and are ascribed to the presence of PSII heterogeneity (e.g. Schreiber et al. 1995a; Hormann et al. 1994). However, microalgae have a more flexible photosynthetic machinery and can more easily adjust

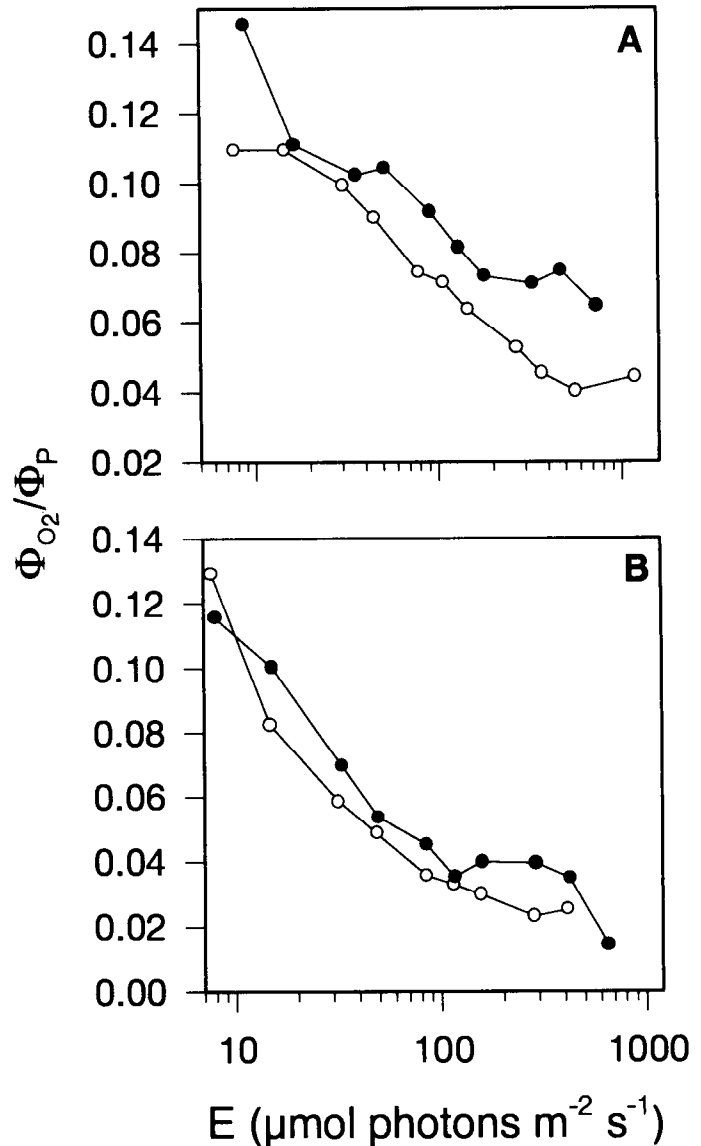


Fig. 5. $\Phi_{\text{O}_2}:\Phi_{\text{P}}$ as a function of light intensity in batch cultures. Open circles indicate nonphotorespiratory (NPR) conditions; solid circles indicate control. A. *Emiliana huxleyi*. B. *Phaeodactylum tricornutum*.

their optical properties. Also, unlike in microalgae, it is very difficult to truly saturate photosynthesis in higher plants. Therefore possible nonlinearity between Φ_{O_2} and Φ_{P} because of limitation by Calvin cycle processes might be difficult to reveal in higher plants.

If this interpretation is correct, it means that models relating fluorescence to steady-state oxygen production, like the ones used for higher plants (Genty et al. 1989), cannot work for microalgae for the full range of light intensities, without taking changes in τ into account (Kolber and Falkowski 1993).

Comparison with other studies—Table 3 presents the results of a number of studies (including this study) on the coupling of Φ_{P} and Φ_{O_2} or Φ_{CO_2} . Note that this survey does

Table 3. Comparison of the relationship between Φ_p , Φ_{O_2} , and Φ_{CO_2} in several species.

	Taxonomic group; C3/C4 metabolism	Measured quantum yields	Methods	Reported conditions during measurement	Observed relationship	References
<i>Palmaria palmata</i>	Rhodophyceae	Φ_p, Φ_{O_2}	PAM* 2000, oxygen electrode	—†	Biphasic linear relationship; pivoting point at $\Phi_p = 0.4F_p/F_r$	Hanelt and Nultsch 1995
<i>Chlorella pyrenoidosa</i>	Chlorophyceae	$\Delta\Phi_p, \ddagger \Phi_{O_2}$	Pump and probe; oxygen electrode	1% CO ₂	Linear relationship at $\Delta\Phi > 0.2 \Delta\Phi_{max, \ddagger}$ deviation from linearity at high light intensities ($\Delta\Phi < 0.2 \Delta\Phi_{max}$)	Falkowski et al. 1986a
<i>Chlorella vulgaris</i>	Chlorophyceae	$\Delta\Phi, \Phi_{O_2}$	Pump and probe; oxygen electrode	—	Linear relationship at intermediate light intensities; deviations at low and high light intensities	Falkowski et al. 1986b
<i>Dunaliella tertiolecta</i>	Chlorophyceae	$q_p \times \Phi_p, \Phi_{CO_2}$	Pump and probe; ¹⁴ C incorporation	—	Linear relationship	Falkowski et al. 1991
<i>Isochrysis galbana</i>	Prymnesiophyceae	$q_p \times \Phi_p, \Phi_{CO_2}$	Pump and probe; ¹⁴ C incorporation	—	Linear relationship	Kolber and Falkowski 1993
<i>Bacillariophyceae</i>	Bacillariophyceae	$\Phi_p, \Phi_{O_2}, \Phi_{CO_2}$	PAM 101; oxygen electrode; ¹⁴ C incorporation	Well below O ₂ saturation	Close coupling (<10% variation) between Φ_p and Φ_{O_2} ; deviations from linearity at very low light intensities. Additional variability between Φ_{O_2} and Φ_{CO_2} throughout the range in light intensities	Kroon et al. 1993
<i>Phytoplankton community</i>	—	Φ_p, Φ_{O_2}	PAM 101; oxygen electrode	—	Linear relationship	Hanelt et al. 1995
<i>Heterocapsa pygmaea</i>	Dinophyceae	$\Phi_p, \Phi_{O_2}, \Phi_{CO_2}$	PAM; oxygen electrode PAM 2000; CO ₂ porometer	—	Nonlinear relationship No clear relationship	Rees et al. 1992 Schroeter et al. 1992
<i>Dictyota dichotoma</i>	Phaeophyceae	Φ_p, Φ_{O_2}	PAM 101; oxygen electrode	—	<i>E. huxleyi</i> : linear relationship at $\Phi_p < 0.40$; $\Phi_{O_2} = 0.055\Phi_p$; curvilinear relationship at lower light intensities ($\Phi_p > 0.40$). Other species: curvilinear relationships throughout the range in light intensities; high variability in low light	This study
<i>Dunaliella</i> sp.	Chlorophyceae	Φ_p, Φ_{O_2}	PAM; oxygen electrode	—	Nonlinear relationships for Φ_p and Φ_{O_2} ; and Φ_p and Φ_{CO_2}	Biehler and Fock 1995
<i>Trebouxia</i> sp. (photobiont) in <i>Buella frigida</i> (lichen)	Chlorophyceae	Φ_p, Φ_{CO_2}	PAM 2000; CO ₂ porometer	—		
<i>Emiliania huxleyi</i>	Prymnesiophyceae	Φ_p, Φ_{O_2}	PAM 101; oxygen electrode	—		
<i>Scenedesmus protuberans</i>	Chlorophyceae					
<i>Phaeocystis globosa</i>	Prymnesiophyceae					
<i>Phaeodactylum tricoratum</i>	Bacillariophyceae					
<i>Triticum aestivum</i>	Monocotyledonae	$\Phi_p, \Phi_{O_2}, \Phi_{CO_2}$	PAM; ¹⁶ O ₂ / ¹⁸ O ₂ mass spectrometry	21% O ₂ , variable CO ₂		

Table 3. Continued.

	Taxonomic group; C3/C4 metabolism	Measured quantum yields	Methods	Reported conditions during measurement	Observed relationship	References
<i>Hordeum vulgare</i> (wild type and Chl <i>b</i> -less mutant)	Monocotyledonae	Φ_p , Φ_{O_2}	PAM 101; oxygen elec- trode	21% O ₂ , 1% CO ₂	Linear relationship at Φ_p < 0.60; Φ_{O_2} = 0.063 Φ_p (<i>H. vulgare</i>); Φ_{O_2} = 0.056 Φ_p (<i>H.</i> <i>vulgare</i> , Chl <i>b</i> -less mutant); Φ_{O_2} = 0.093 Φ_p (other spe- cies); curvilinear rela- tionship at low light intensities ($\Phi_p >$ 0.60), except for the Chl <i>b</i> -less mutant of <i>H. vulgare</i>	Öquist and Chow 1992
<i>Pisum sativum</i>	Dicotyledonae					
<i>Spinacia oleracea</i>	Dicotyledonae					
<i>Tradescantia albiflora</i>	Dicotyledonae					
<i>Zea mays</i>	Monocotyledonae					
<i>Hordeum vulgare</i> (wild type and Chl <i>b</i> -less mutant)	Monocotyledonae	Φ_p , Φ_{CO_2}	PAM 101; IRGA [†]	1% O ₂ , 2.5% CO ₂	Linear relationship at 0.1 < Φ_p < 0.7; Φ_{CO_2} = 0.133 Φ_p (wild type); α_{CO_2} = 0.077 Φ_p (Chl <i>b</i> -less mutant)	Genty et al. 1989
<i>Hordeum vulgare</i>	Monocotyledonae	Φ_p , Φ_{CO_2}	PAM 101; IRGA	2% O ₂ /20% O ₂	Linear relationship at 0.1 < Φ_p < 0.6 in 2% O ₂ ; Φ_{CO_2} = 0.092 Φ_p ; linear relationship at 0.1 < Φ_p < 0.5 in 20% O ₂ ; Φ_{CO_2} = 0.060 Φ_p	Genty et al. 1990
<i>Phaseolus vulgaris</i>	Dicotyledonae	Φ_p , Φ_{O_2}	PAM 101; ¹⁶ O ₂ / ¹⁸ O ₂ mass spectrometry	0.3% CO ₂ /5% CO ₂	Linear relationship at 0.15 < Φ_p < 0.8; Φ_{CO_2} = 0.098 Φ_p + 0.0045	Genty et al. 1992
<i>Atriplex spongiosa</i>	Dicotyledonae	Φ_p , Φ_{O_2}	PAM 101; oxygen elec- trode	CO ₂ saturation, NPR condi- tions	Linear relationship at Φ_p < 0.60; Φ_{O_2} = 0.075 Φ_p ; curvilinear relationship at low light intensities ($\Phi_p >$ 0.60)	Seaton and Walker 1990
<i>Cucumis sativus</i>	Dicotyledonae					
<i>Echinum pininana</i>	Dicotyledonae					
<i>Ginkgo biloba</i>	Ginkgophyta					
<i>Glycine max</i>	Dicotyledonae					
<i>Hordeum vulgare</i>	Monocotyledonae					
<i>Kalanchoë crenata</i>	Dicotyledonae					
<i>Liriodendron tulipifera</i>	Dicotyledonae					
<i>Nicotiana tabacum</i>	Dicotyledonae					
<i>Ocimum basilicum</i>	Dicotyledonae					
<i>Osmonda regalis</i>	Pterophyta					
<i>Panicum miliaceum</i>	Monocotyledonae					
<i>Polygonum cuspidatum</i>	Dicotyledonae					
<i>Spinach oleracea</i>	Dicotyledonae					
<i>Viburnum lantana</i>	Dicotyledonae					
<i>Zea mays</i>	Monocotyledonae					

Table 3. Continued.

	Taxonomic group; C3/C4 metabolism	Measured quantum yields	Methods	Reported conditions during measurement	Observed relationship	References
<i>Amaranthus cruentus</i>	Dicotyledonae (C4)	Φ_p, Φ_{CO_2}	PAM 101; IRGA	C3 plants: 2% O ₂ , 0.85% CO ₂	Linear relationship at Φ_p < 0.65; $\Phi_{CO_2} = 0.100\Phi_p$ (C3 plants)	Oberhuber et al. 1993
<i>Flaveria pringlei</i>	Dicotyledonae (C3)					
<i>Flaveria trinervia</i>	Dicotyledonae (C4)					
<i>Sorghum bicolor</i>	Monocotyledonae (C4)					
<i>Triticum aestivum</i>	Monocotyledonae (C3)				$\Phi_{CO_2} = 0.0735\Phi_p$ (C4 plants); deviations from linearity at low light intensities ($\Phi_p >$ 0.65)	
<i>Zea mays</i>	Monocotyledonae (C4)					
<i>Hedera canariensis</i>	Dicotyledonae	Φ_p, Φ_{O_2}	Custom-built fluorometer; oxygen electrode	20% O ₂ , 5% CO ₂	Linear relationship at 0.1 < Φ_p < 0.85; $\Phi_{O_2} =$ 0.1333 $\Phi_p - 0.0094$	Björkman and Demmig 1987
<i>Gossypium hirsutum</i>	Dicotyledonae	Φ_p, Φ_{O_2}	Custom-built fluorometer; oxygen electrode	—	Linear relationship at 0.1 < Φ_p < 0.9; $\Phi_{O_2} =$ 0.1367 $\Phi_p - 0.0106$	Demmig and Björkman 1987
<i>Monstera deliciosa</i>	Dicotyledonae	Φ_p, Φ_{O_2}				
<i>Rhizophora stylosa</i>	Dicotyledonae	Φ_p, Φ_{O_2}	PAM 101; oxygen electrode	5% CO ₂	Linear relationship at 0.1 < Φ_p < 0.65; $\Phi_{O_2} =$ 0.0893 $\Phi_p - 0.0017$	Strand and Lundmark 1995
<i>Picea abies</i>	Gymnospermae	Φ_p, Φ_{O_2}				
<i>Quercus cerris</i>	Dicotyledonae	Φ_p, Φ_{CO_2}	PAM 101, PAM 2000; open gas exchange system	1% O ₂ , 0.34% CO ₂	Linear relationship at $\Phi_{CO_2} = 0.0737\Phi_p +$ 0.0004	Valentini et al. 1995
<i>Phaseolus vulgaris</i>	Dicotyledonae	Φ_p, Φ_{CO_2}	PAM; IRGA	1%/21% O ₂ , 0.37%/1 1.50% CO ₂	Linear relationship at 0.1 < Φ_p < 0.7; $\Phi_{CO_2} =$ 0.1136 Φ_p	Cornic and Briantais 1991

* Pulse amplitude-modulated fluorimeter.

† No special treatment of the gas composition during Φ measurements reported; we assume atmospheric conditions.‡ $\Delta\Phi = (F'_m - F_m)/F_s$; $\Delta\phi_{max} = (F'_m - F_m)/F'_o$.§ $q_p = (F'_m - F_m)/(F'_m - F_m)$.|| We disagree with the authors, who conclude that there is a good relationship between Φ_p and Φ_{CO_2} .

¶ Infrared gas analyzer.

not pretend to be complete. Most of the studies have been performed on macroalgae and vascular plants, whereas there are only limited studies on microalgae (i.e. Falkowski 1986a,b, 1991; Kroon et al. 1993). Quantitative relationships between Φ_p and Φ_{O_2} or Φ_{CO_2} are only presented in the studies on higher plants. In these studies, $\Phi_p:\Phi_{O_2}$ or $\Phi_p:\Phi_{CO_2}$ is generally constant at intermediate to high light intensities. If Φ_p is plotted as a function of Φ_{O_2} or Φ_{CO_2} , deviations from linearity are usually found at high quantum yields ($\Phi_p > 0.6-0.7$), i.e. at low light intensities. The observed quantitative relationship in the linear range varies between $\Phi_{O_2(CO_2)} = 0.056 \Phi_p$ and $\Phi_{O_2(CO_2)} = 0.137 \Phi_p$. This variability was much larger between studies than within studies (i.e. Öquist and Chow 1992; Seaton and Walker 1990; Oberhuber et al. 1993; Demmig and Björkman 1987), suggesting that part of it can be explained by methodological errors or differences in growing conditions. The "true" range of variability in $\Phi_{O_2}:\Phi_p$ or $\Phi_{CO_2}:\Phi_p$ is probably rather small for higher plants.

In contrast to the situation in aquatic studies, most studies on higher plants mentioned in Table 3 were carried out in NPR conditions, i.e. in an O_2 -depleted or CO_2 -enriched atmosphere. The aim of these studies was to assess the validity of Φ_p as a tool for the prediction of Φ_{O_2} . NPR conditions had to be applied because in normal air, photorespiration is a very efficient sink of photosynthetic electron transport. As a result, $\Phi_p:\Phi_{O_2}$ or $\Phi_p:\Phi_{CO_2}$ in higher plants (especially C3 plants) may vary by a large extent in field conditions. It might be questioned whether a direct comparison between this study and higher plant studies, performed in NPR conditions, is correct. However, NPR conditions did not affect the patterns in $\Phi_p:\Phi_{O_2}$ observed in this study (Fig. 5), and according to literature, photorespiration is not likely to be quantitatively important in microalgae (Ogren 1984; Glover 1989; Raven and Johnston 1991).

Comparison of the results of studies on microalgae (including the present study) and studies on vascular plants suggests two major differences. First, there seems to be more light-dependent variability in the efficiency of oxygen production or carbon fixation by linear photosynthetic electron transport in microalgae. Whereas in vascular plants, deviations from linearity are usually restricted to low light intensities, in microalgae, nonlinearity is observed both at low and high light intensities (Falkowski et al. 1986a,b) or linearity is not observed at all (Rees et al. 1992; this study). As stated before, this might be related to the more flexible photosynthetic machinery of microalgae.

Second, the interspecific variability in the efficiency of oxygen production by linear photosynthetic electron transport is apparently larger in microalgae than in vascular plants. This statement is illustrated in Fig. 6, in which Φ_{O_2} is plotted against Φ_p for all species. The apparent larger interspecific variability in $\Phi_{O_2}:\Phi_p$ in microalgae, as compared to vascular plants, may be partly explained by the bigger diversity in pigment composition and, consequently, in spectral absorption characteristics of microalgae. To test the contribution of interspecific differences in spectral absorption characteristics to the interspecific variability in $\Phi_{O_2}:\Phi_p$ shown in Fig. 6, the chlorophyll-specific absorption cross sections used to calculate Φ_{O_2} were corrected for the spectral

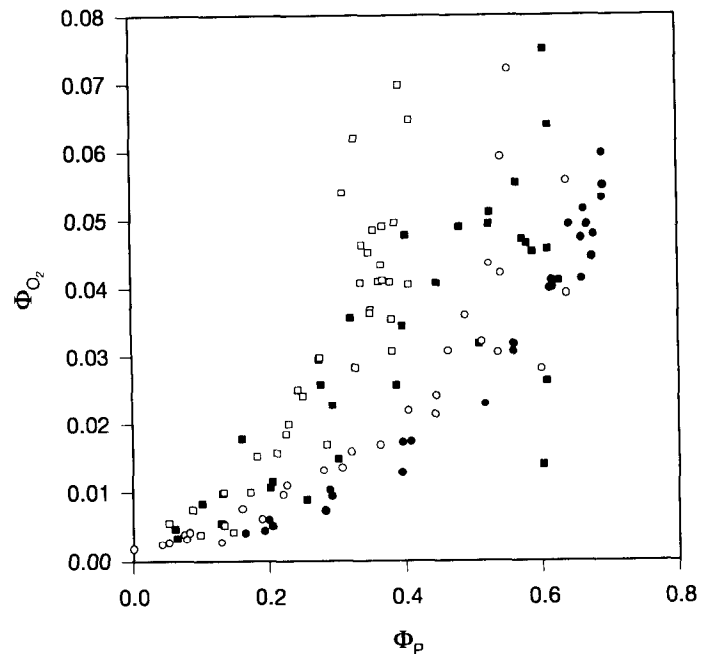


Fig. 6. Φ_{O_2} as a function of Φ_p . ●, *Scenedesmus protuberans*; ■, *Phaeocystis globosa*; ○, *Emiliania huxleyi*; □, *Phaeodactylum tricornutum*. Measurements at 1, 5, and 7.5 h after the start of the light period were pooled.

composition of the light source (Dubinsky et al. 1986). However, we found that differences in spectral absorption characteristics accounted for only <5% of the variability in Φ_{O_2} (data not shown).

Conclusions

The relationship between estimates of linear photosynthetic electron transport rates based on variable fluorescence measurements and oxygen-based rates of photosynthesis in microalgae is not linear. This may be explained by assuming that (1) the relationship between electrons generated in PSII and net oxygen production is disrupted by cyclic electron transport around PSII, mitochondrial respiration, pseudo-cyclic electron transport, or Rubisco oxygenase activity, and (2) light-dependent changes in τ disrupt the coupling between Φ_p and Φ_{O_2} .

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