

# Fast repetition rate and pulse amplitude modulation chlorophyll *a* fluorescence measurements for assessment of photosynthetic electron transport in marine phytoplankton

DAVID J. SUGGETT<sup>1</sup>, KEVIN OXBOROUGH<sup>1</sup>, NEIL R. BAKER<sup>1</sup>,  
HUGH L. MACINTYRE<sup>2</sup>, TODD M. KANA<sup>3</sup> AND RICHARD J. GEIDER<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, University of Essex, Colchester CO4 3SQ, UK

<sup>2</sup>Dauphin Island Sea Laboratory, 101 Bienville Blvd, Dauphin Island, Alabama 36528, USA

<sup>3</sup>University of Maryland Centre for Environmental Science, Horn Point Laboratory, P.O. Box 775, Cambridge, Maryland 21613-0775, USA

(Received 3 December 2002; accepted 1 July 2003)

Pulse amplitude modulation (PAM) and fast repetition rate (FRR) fluorescence are currently used to estimate photosynthetic quantum yields and photosynthetic rates in aquatic systems. Here we compare simultaneous measurements of the photochemical efficiency of photosystem II obtained from the two techniques and independent estimates of the rate of light absorption by photosystem II. We measured the light-dependencies of the photochemical efficiency of photosystem II ( $F_q'/F_m'$ ) in five phytoplankters using FRR and Xe-PAM approaches. The FRR and PAM estimates were related in a non-linear fashion. At low irradiances,  $F_q'/F_m'$  measured using PAM fluorescence exceeded  $F_q'/F_m'$  measured using FRR fluorescence by about 20%. At high irradiances, measurements of  $F_q'/F_m'$  from the two approaches converged. The differences in  $F_q'/F_m'$  reflect the distinct techniques by which FRR and PAM protocols excite PSII and are amplified when estimating electron transfer rates as a result of the irradiance term. We also found that measurements of the effective light absorption cross-section for photosystem II obtained by FRR fluorescence compared well with estimates obtained from measured light absorption and photosynthetic unit size. Finally, we compared the photon efficiency of gross oxygen evolution from measurements of gross oxygen evolution and light absorption ( $\Phi_{PO_2}^{18}$ ) with FRR measurements of  $F_q'/F_m'$ . We found that measurements of  $F_q'/F_m'$  were highly linearly correlated, but were lower by a factor of  $\sim 1.5$ , than  $\Phi_{PO_2}^{18}$ .

**Key words:** electron transport, FRRF, light absorption, PAM, phytoplankton, PSII, quantum yield

## Introduction

The development 20 years ago of fluorescence-based methods for measuring the photosynthetic quantum yield in actinic (ambient) light was a major advance in studies of plant photosynthesis under undisturbed conditions in nature. Development of this technique took two parallel, but distinct, paths. One path uses a pulse amplitude modulation (PAM) fluorometer, where the fluorescence from a 'weak' modulated light source is evaluated independently of the fluorescence that arises from actinic light (Schreiber *et al.*, 1986, 1993). The PAM approach uses light pulses of  $\sim 3000$  to  $10\,000\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$  for 300–1200 ms duration to modify fluorescence yields. This technique has been used in lakes (e.g. Gilbert *et al.*, 2000), algal cultures (e.g. Parkhill *et al.*, 2001) and microphytobenthos (e.g. Kromkamp *et al.*, 1998, Perkins *et al.*, 2002), but is not sensitive

enough for use in oligotrophic, open ocean, conditions. The second path measures changes in fluorescence that occur during a short (150–400  $\mu\text{s}$ ) but intense light flash of  $> 20\,000\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$  in the presence of actinic light (Kolber *et al.*, 1998). This fast repetition rate (FRR) approach can be used at chlorophyll *a* concentrations ranging from  $\sim 30$  to  $< 0.1\ \text{mg m}^{-3}$  for work in the open ocean (e.g. Falkowski & Kolber, 1995; Babin *et al.*, 1996; Aiken *et al.*, 2000) and nutrient impoverished lakes.

The PAM and FRR fluorometers are fundamentally distinct in the way they 'perturb' the redox state of photosystem II (PSII) with each flash (see Kromkamp & Forster, 2003). The FRR technique fully reduces the primary electron acceptor,  $Q_A$ , and allows a simultaneous, single closure event (single turnover, ST) of all PSII reaction centres. In contrast, the PAM technique generates multiple photochemical charge separations (multiple turnover, MT) and fully reduces  $Q_A$ , the secondary

Correspondence to: D. J. Suggett. e-mail: dsuggett@essex.ac.uk

acceptor, Q<sub>B</sub>, and plastoquinone (PQ). Other commercially available fluorometers, for example, the Photon Systems Instrument (PSI, Brno, Czech Republic) and the Algal Online Analyser (AOA, Moldaenke, GmbH, Kiel, Germany), also provide ST or MT protocols for assessment of PSII photochemistry (Koblížek *et al.*, 2001; Beutler *et al.*, 2002) but have been used less widely in algal research.

Both PAM and FRR fluorometers can be used to determine a range of fluorescence parameters, which are defined in Table 1. Fluorescence under actinic light ( $F'$ ) and maximum fluorescence when all photosystem II (PSII) reaction centres are closed ( $F_m'$ ) are the basic measurements that are made. The ratio  $(F_m' - F')/F_m'$  ( $= F_q'/F_m'$ ) is one of the most useful parameters that can be obtained from these measurements.  $F_q'/F_m'$  (otherwise known as  $\Delta F/F_m'$ , Kromkamp & Forster, 2003) equals the effective photochemical efficiency of PSII (Butler, 1978, also reviewed by Geider *et al.*, 1993a; Parkhill *et al.*, 2001) and provides an estimate of the quantum efficiency of photosynthetic electron transport (Genty *et al.*, 1989).

$F_q'/F_m'$  has been used to estimate the quantum yield of PSII photochemistry in algae ( $\Phi_P$ , e.g. Hofstraat *et al.*, 1994; Gilbert *et al.*, 2000; Table 2) following the approach established by Genty *et al.* (1989) for leaves of vascular plants,

$$\Phi_P = \frac{(F_q'/F_m')}{k} \quad [1]$$

where  $k$  is the number of stable charge separation events at PSII required to reduce 1 mol of O<sub>2</sub> and has a theoretical minimum value of 4 mol electrons (mol O<sub>2</sub>)<sup>-1</sup>.

In order to estimate the rate of non-cyclic electron transport, additional knowledge of the photosynthetically active irradiance (E) and proportion of light absorbed by PSII is required (Gilbert *et al.*, 2000). Light absorption by algae is typically expressed relative to the concentration of the predominant light harvesting pigment, chlorophyll *a* ( $a^{\text{chl}}$ , Table 2). Generally, it is assumed that half of the absorbed light is distributed to PSII (Hartig *et al.*, 1998; Gilbert *et al.*, 2000). Consequently, electron transfer rates, expressed as P<sup>chl</sup>, can be obtained from:

**Table 1.** Definition of parameters. Fluorescence parameters follow Baker & Oxborough (2003) and Kromkamp & Forster (2003). All parameters are measured or derived (in full in a separate column); units are in parentheses following each definition, except when dimensionless. Absorption by photosynthetically and non-photosynthetically active pigments is derived following Bidigare *et al.* (1990).

Parameter	Definition	Derivation
E	Irradiance ( $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ )	
E <sub>κ</sub>	Light saturation parameter ( $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ )	
$a^{\text{chl}}_{\text{PSII}}$	PSII chl <i>a</i> -specific absorption cross section ( $\text{m}^2 (\text{mg chl } a)^{-1}$ )	See Table 2
$\sigma_{\text{PSII}}$	Effective absorption cross section of PSII ( $\text{m}^2 (\text{mol RCII})^{-1}$ )	
$\sigma_{\text{PSII}}'$	$\sigma_{\text{PSII}}$ measured under actinic light ( $\text{m}^2 (\text{mol RCII})^{-1}$ )	
$n_{\text{PSII}}$	Photosynthetic unit size of PSII (mol RCII (mol chl <i>a</i> ) <sup>-1</sup> )	
$a^{\text{chl}}$	Chlorophyll <i>a</i> -specific absorption coefficient ( $\text{m}^2 (\text{mg chl } a)^{-1}$ )	
$a_{\text{PH}}(\lambda)$	Absorption by photosynthetically active pigments ( $\text{m}^{-1}$ )	$\Sigma a^{\text{chl}}_i(\lambda) \cdot C_i$
$a_{\text{NPH}}(\lambda)$	Absorption by non-photosynthetically active pigments ( $\text{m}^{-1}$ )	$\Sigma a^{\text{chl}}_i(\lambda) \cdot C_i$
$C_i$	Concentration of pigment <i>i</i> ( $\text{mg m}^{-3}$ )	
$F_o$	Minimum fluorescence yield—dark adapted state	
$F_m$	Maximum fluorescence yield—dark adapted state	
$F_v$	Variable fluorescence yield—dark adapted state	$F_m - F_o$
$F_v/F_m$	Potential photochemical efficiency of open RCII	$(F_m - F_o)/F_m$
$F'$	Fluorescence yield at any point between $F_o'$ and $F_m'$	
$F_o'$	Minimum fluorescence yield – actinic light	$F_o/[ (F_v/F_m) + (F_o/F_m') ]$
$F_m'$	Maximum fluorescence yield – actinic light	
$F_v'$	Variable fluorescence yield – actinic light	$F_m' - F_o'$
$F_q'$	Difference between fluorescence yields $F_m'$ and $F'$	$F_m' - F'$
$F_q'/F_m'$	Photochemical efficiency of PSII – actinic light	$(F_m' - F')/F_m'$
$F_q'/F_v'$	PSII efficiency factor – actinic light	$(F_m' - F')/(F_m' - F_o')$
$F_v'/F_m'$	Maximum efficiency of PSII – actinic light	$(F_m' - F_o')/F_m'$
$\Phi_{\text{P}}\text{O}_2^{18}$	Quantum yield of oxygen evolution (mol O <sub>2</sub> (mol photons) <sup>-1</sup> ) from the <sup>18</sup> O <sub>2</sub> isotope technique	Gross O <sub>2</sub> evolved/ $a^{\text{chl}}$
$\Phi_{\text{P}}$	Quantum yield of PSII photochemistry (mol O <sub>2</sub> (mol photons) <sup>-1</sup> )	See Table 2
1/ $k$	Ratio of PSII electron transport for O <sub>2</sub> evolution (0.25 mol O <sub>2</sub> (mol electrons) <sup>-1</sup> )	
P <sup>chl</sup>	Oxygenic photosynthetic rate ( $\mu\text{mol O}_2 (\text{mg chl } a)^{-1} \text{ h}^{-1}$ )	See Table 2
relative P <sup>chl</sup>	Relative rate of PSII electron transport ( $\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	$\Phi_{\text{P}} \cdot E$

**Table 2.** Equations and models used to construct and compare the FRRF and PAM algorithms of photosynthetic electron transport. All parameters are as noted in Table 1. The conversion factors:  $3.6 \times 10^{-3}$  (Eq. 2, 3) accounts for seconds to hours and  $\mu\text{mol}$  photons to  $\text{mol}$  photons;  $0.00675$  (Eq. 4) accounts for  $\text{\AA}^2$  to  $\text{m}^2$ , quanta to  $\text{mols}$  photons and  $\text{mols chl } a$  to  $\text{mg chl } a$ ;  $0.5$  (Eq. 8) represents the fraction of absorbed light transferred to PSII.

		Equation
1	$\Phi_P$ ( $\text{mol O}_2$ ( $\text{mol photons}$ ) $^{-1}$ )	$= F_q'/F_m' \cdot 1/k$
2, 3	$P^{\text{chl}}$ ( $\text{mol O}_2$ ( $\text{mg chl } a$ ) $^{-1}$ $\text{hr}^{-1}$ )	$= a^{\text{chl}}_{\text{PSII}} \cdot \Phi_P \cdot E \cdot 3.6 \times 10^{-3}$
4	$a^{\text{chl}}_{\text{PSII}}$ FRR method ( $\text{m}^2$ ( $\text{mg chl } a$ ) $^{-1}$ )	$= \sigma_{\text{PSII}} \cdot n_{\text{PSII}} \cdot 0.00675$
5	$F_q'/F_m'$	$= F_q'/F_v' \cdot F_v'/F_m'$
6	$a^{\text{chl}}_{\text{LED}}$ ( $\text{m}^2$ ( $\text{mg chl } a$ ) $^{-1}$ )	$= \sum (a^{\text{chl}}_{(\lambda)} \cdot E_{\text{LED}(\lambda)}) / \sum E_{\text{LED}(\lambda)}$ where $\lambda = 400-700$ nm
7	$a_{\text{LED}}$ ( $\text{m}^2$ ( $\text{mg pigment}$ ) $^{-1}$ )	$= (\sum (a^{\text{chl}}_{(\lambda)} \cdot E_{\text{LED}(\lambda)}) / \sum E_{\text{LED}(\lambda)}) \cdot C$
8	$a^{\text{chl}}_{\text{PSII}}$ $a^{\text{chl}}$ method ( $\text{m}^2$ ( $\text{mg chl } a$ ) $^{-1}$ )	$= a^{\text{chl}}_{\text{LED}} \cdot [a_{\text{LED}}]_{\text{PH}} / ([a_{\text{LED}}]_{\text{PH}} + [a_{\text{LED}}]_{\text{NPH}}) \cdot 0.5$
9	$\Phi_{\text{P(G)}}$ (Gorbunov <i>et al.</i> , 2000, 2001) where $F_q'/F'$ represents photochemical reduction to the maximum quantum yield, and $\sigma_{\text{PSII}'}/\sigma_{\text{PSII}}$ represents non-photochemical reduction to the maximum quantum yield	$= (F_q'/F')/k \cdot (\sigma_{\text{PSII}'}/\sigma_{\text{PSII}})$
10	Dependence of photon efficiency of photosynthesis on irradiance ( $F_q'/F_m'$ versus $E$ )— $F_q'/F_m' = A[1 - \exp(-E/E_k)]/E$ where $A = F_q'/F_m'_{\text{MAX}} \cdot E_k$ .	
11	Dependence of photosynthesis on irradiance (ETR versus $E$ )— $P^{\text{chl}} = P_m^{\text{chl}} (1 - \exp(-\alpha^{\text{chl}} E/P_m^{\text{chl}}))$ where $P_m^{\text{chl}}$ ( $\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) is the chl $a$ -specific photosynthetic rate at irradiance, $E$ ( $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ); $P_m^{\text{chl}}$ is the light-saturated rate of photosynthesis that would be observed in the absence of photoinhibition; $\alpha^{\text{chl}}$ ( $\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) ( $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) $^{-1}$ is the initial slope of the $P^{\text{chl}}-E$ curve (Jassby & Platt, 1976). $P^{\text{chl}} = P_m^{\text{chl}} (1 - \exp(-\alpha^{\text{chl}} E/P_m^{\text{chl}}))(\exp(-\beta^{\text{chl}} E/P_m^{\text{chl}}))$ as above except $\beta$ ( $\text{mmol O}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) ( $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) $^{-1}$ is the inhibition parameter (Platt <i>et al.</i> , 1980).	

$$P^{\text{chl}} = E \cdot \left( \frac{a^{\text{chl}}}{2} \right) \cdot \Phi_P \quad [2]$$

Since not all absorbed light is used for photochemistry and some pigments do not contribute to photosynthesis, it is more appropriate to express  $P^{\text{chl}}$  in terms of the optical absorption cross section of PSII,  $a^{\text{chl}}_{\text{PSII}}$  (Equation 3) rather than in terms of  $a^{\text{chl}}$  (Equation 2).

$$P^{\text{chl}} = E \cdot a^{\text{chl}}_{\text{PSII}} \cdot \Phi_P \quad [3]$$

$a^{\text{chl}}_{\text{PSII}}$  can be obtained from  $a^{\text{chl}}/2$  by correcting for the proportion of total absorption attributable to photosynthetically active pigments (Bidigare *et al.*, 1990; Tables 1, 2), the distribution of photons between PSI and PSII (Prášil *et al.*, 1996; Yamamoto & Bassi, 1996) and the transfer efficiency of photons between light harvesting pigments and PSII reaction centres (Mauzerall & Greenbaum, 1989).

In addition to providing a measurement of  $F_q'/F_m'$ , the FRR method provides a measurement of the effective absorption cross section of PSII,  $\sigma_{\text{PSII}'}$ . Under dark-adapted conditions ( $\sigma_{\text{PSII}}$ ), this can be used to estimate  $a^{\text{chl}}_{\text{PSII}}$  provided the ratio of functional PSII reaction centres to chlorophyll  $a$  (referred to as  $n_{\text{PSII}}$  in Tables 1 and 2) is known (Mauzerall & Greenbaum, 1989):

$$a^{\text{chl}}_{\text{PSII}} = \sigma_{\text{PSII}} \cdot n_{\text{PSII}} \quad [4]$$

Both the FRR and PAM methods can provide *in situ* estimates of  $P^{\text{chl}}$  at high spatial and

temporal resolution and, therefore, offer considerable advantages over conventional methods for measuring aquatic photosynthesis (Sakshaug *et al.*, 1997). Comparisons of  $P^{\text{chl}}$  values derived from fluorescence techniques with direct measurements of  $\text{O}_2$  evolution rates (e.g. Flaming & Kromkamp, 1998; Franklin & Badger, 2001; Masojídek *et al.*, 2001) or  $^{14}\text{C}$ -uptake rates (e.g. Kolber & Falkowski, 1993; Boyd *et al.*, 1997; Hartig *et al.*, 1998; Gilbert *et al.*, 2000; Suggett *et al.*, 2001) in algae have generated a range of relationships. These studies report that fluorescence-based estimates of  $P^{\text{chl}}$  can differ from conventional measurements of production by factors between 0.25 and 15. The variability between conventional and fluorescence-based estimates of algal photosynthesis has been largely assumed to be the result of differences between fluorescence and processes associated with carbon uptake or the relative rates of oxygen evolution and uptake. The comparisons are further complicated by (1) a lack of understanding of the relationship between FRR and PAM estimates of  $F_q'/F_m'$ , (2) the need to confirm the validity of the assumed relationship between  $F_q'/F_m'$  and  $\Phi_P$  (Equation 1) and (3) the need to estimate  $a^{\text{chl}}_{\text{PSII}}$  accurately. Several investigations have compared the different fluorescence yields that are obtained from ST and MT protocols (Schreiber *et al.*, 1995; Kolber *et al.*, 1998; Koblížek *et al.*, 2001); however, a direct comparison of FRR and PAM fluorescence-based measurements of  $\Phi_P$  has not been previously made.

In this study, we compared simultaneous FRR and PAM fluorescence measurements from five species of marine phytoplankton exposed to a wide range of irradiances. We show that values of  $F_q'/F_m'$  obtained from FRR and PAM techniques are related but in a non-linear fashion. We also report the results of a comparison of  $\Phi_P$  from FRR measurements of  $F_q'/F_m'$  with measurements of the quantum yield of gross oxygen evolution in three species of microalgae. These two factors are linearly related but require an additional factor to yield equivalency in two of the three species. Finally, we also demonstrate that estimates of  $a^{\text{chl}}_{\text{PSII}}$  from spectrophotometric absorption measurements agreed well with biophysical (FRR) estimates of  $a^{\text{chl}}_{\text{PSII}}$  from  $\sigma_{\text{PSII}}$  and  $n_{\text{PSII}}$ .

## Materials and methods

### Algal culture

Four species of eukaryotic microalgae were grown for comparison of FRR and PAM fluorescence parameters: the diatom *Chaetoceros muelleri* (CCAP/1010/3), the dinoflagellate *Prorocentrum minimum* (CCAP 1136/14), the chlorophyte *Dunaliella tertiolecta* (CCAP 19/6B), and the prymnesiophyte *Emiliania huxleyi* (CCAP 920/6). All were grown in 250 ml batch cultures in filtered seawater enriched with f/2 nutrients (Guillard & Ryther, 1962). *E. huxleyi* was grown at 18 °C under 375 ('HL') or 75 ('LL')  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . All other species were grown at 25 °C under 400 ('HL') or 50  $\mu\text{mol m}^{-2} \text{s}^{-1}$  ('LL'). All cultures were grown in temperature controlled growth chambers with a 14 h light:10 h dark daily cycle. Cultures were routinely sub-cultured during the exponential growth phase. Samples for each experiment were drawn at the end of the exponential growth phase and  $3 \pm 0.5$  h after the start of the light period. In addition, the prochlorophyte *Prochlorococcus marinus* (PCC 9511), supplied by D. Scanlan, (University of Warwick), was grown in PCR-S11 medium (Partensky et al., 1999) at 23 °C and under 5–10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  ('LL') continuous light. Chlorophyll *a* concentrations at the time of sampling were between 0.1 and 0.2  $\text{mg l}^{-1}$ .

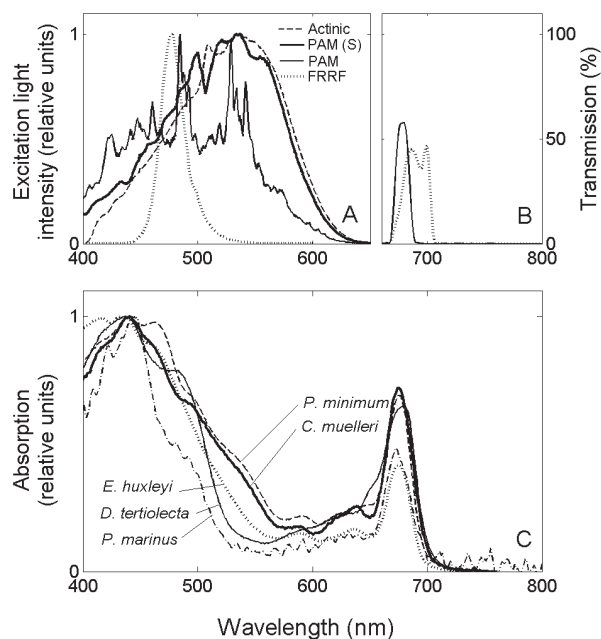
Three species of microalgae were grown for comparison of FRR estimates of  $F_q'/F_m'$  and measurements of the quantum yield of gross oxygen evolution: the diatom *Thalassiosira weissflogii* (CCMP 1047) and the chlorophytes *Dunaliella tertiolecta* (CCMP 1320) and *Nannochloris atomus* (CCMP509). These were maintained in exponential phase in 1500 ml semi-continuous cultures in filtered seawater enriched with f/2 nutrients (Guillard & Ryther 1962). All were grown under continuous light (c. 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) at 20 °C. Chlorophyll *a* concentrations at the time of sampling were between 0.2 and 0.4  $\text{mg l}^{-1}$ .

### Comparative fluorescence measurements

A FRR fluorometer (Chelsea Instruments, West Molesey, UK) and Xe-PAM fluorometer (Walz, Effeltrich,

Germany; referred to as 'PAM' throughout) were used to measure fluorescence yields of all LL grown cultures. The FRR fluorometer was programmed to generate single turnover (ST) saturation from 100 flashlets of 1.1  $\mu\text{s}$  duration within the shortest possible period (280  $\mu\text{s}$ , i.e. a flashlet cycle of 2.8  $\mu\text{s}$  at 200KHz) and applied at 1 s intervals. When the excitation energy used for the ST protocol was increased using 65 and 46 flashlets of 2.2  $\mu\text{s}$  and 3.3  $\mu\text{s}$ , respectively, over 280  $\mu\text{s}$ , no change in  $F'$ ,  $F_m'$  and, therefore,  $F_q'/F_m'$  was observed (see also Kolber et al., 1998). Excitation light was emitted from a bank of blue LEDs with peak output at 478 nm (Fig. 1). The fluorescence emission was collimated, passed through band pass interference and RG665 filters with the spectral characteristics illustrated in Fig. 1, and finally detected by an R928 photomultiplier tube (PMT). All measurements were made at the highest gain setting and neutral density filters were placed over the detection window to avoid saturation of the photomultiplier tube from the chlorophyll *a* concentrations used for experimentation. Each ST saturation curve was used to calculate the values of minimal and maximal fluorescence, the effective absorption cross section and the connectivity parameter ( $\rho$ ) using software (v4) provided by Sam Laney (<http://picasso.oce.orst.edu/ORSOO/FRRF/>). This software fits an algorithm to the saturation curve generating residuals and a goodness of fit ( $\chi^2$ ).

The Xe-PAM fluorometer used throughout provided modulated measuring light (2 Hz) from a xenon flash



**Fig. 1.** (a) Spectral outputs of the actinic light and of the light sources used for the FRR and PAM fluorometers (where PAM and PAM(S) refer to the modulated and saturating light sources, respectively); (b) Transmission spectra of the FRR fluorometer (FRRF) and PAM filters prior to detection (legend as for a). FRRF excitation spectra and pre-detection filters were supplied by John Atkins, *Chelsea Instruments*; (c) Absorption spectra of LL grown species. All spectra, except transmission (b) are normalised against the maximum value and expressed as relative units.

lamp and pulses of saturating light from a halogen lamp to achieve  $F_m'$ . Both measuring and saturating light were passed via a BG39 band pass filter (Coherent, Watford, UK) to yield the excitation spectrum illustrated in Fig. 1. A combination of long pass (RG645) and dichroic (R65) filters (Schott, Mainz, Germany) were placed between sample and detector. The excitation and detection pathways were connected through a single fibreoptic arm. The instrument was routinely operated at a gain of 10–12 and damping of 2–5. A 700 ms saturation pulse of approximately  $4000 \mu\text{mol m}^{-2} \text{s}^{-1}$  was applied at 20 s intervals. Decreasing this pulse length reduced the fluorescence yield; however, increasing the pulse length did not increase the fluorescence yield in any of the species examined, demonstrating that the pulse was saturating. The inter-pulse interval was determined from the minimum time required for fluorescence to return to a consistent, pre-saturation, level throughout the range of irradiances used.

Cultures were sub-sampled (10 ml) into a  $45 \times 25 \times 25$  mm quartz glass cuvette and placed over the detection window of the FRR fluorometer's open 'light' chamber. The excitation window of the FRR fluorometer lies at a right angle to the detection window. The PAM fibreoptic arm was positioned to one side of the cuvette and aligned with the FRR fluorometer optical window in order to ensure that the PAM and FRR were sampling the same volume of the cuvette. A projector lamp, positioned opposite to the FRR fluorometer's LED window and therefore at  $90^\circ$  to the detectors of both fluorometers, provided actinic light via a BG39 band pass filter (Fig. 1). Cultures were maintained at growth temperature using a Peltier temperature control probe (US-T, Walz, Effeltrich, Germany). The contents of the cuvette were regularly mixed using a pipette to prevent the cells from settling.

Neutral density filters were used to provide actinic irradiance ( $E$ ) of 10 to  $650 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The irradiance was measured inside the cuvette using a Hansatech Quantitherm calibrated against a Biospherical Instruments  $4\pi$  PAR sensor. Attenuation of  $E$  from the front to the back of the cuvette was always  $< 10\%$  and the cultures were considered to be optically thin. FRR fluorescence ST flashes and the PAM fluorescence modulated light source were run continuously throughout each experiment. The PAM minimum fluorescence yield became constant 3–5 min after each increase of irradiance. PAM saturation pulses were initiated once the minimum fluorescence yield was observed to be constant for 1 min. FRR fluorescence yields collected over 1 min prior to the PAM saturation pulse period were compared with PAM fluorescence yields collected throughout the saturation pulse period. Additional dark-adapted fluorescence yields were measured from sub-samples kept in darkness and at constant temperature throughout the experiment. A blank consisting of growth media was measured by both fluorometers at the end of each experiment and was  $< 2\%$  of the fluorescence from the cultures.

DCMU ( $< 1\%$  in ethanol) was added to a final concentration of  $15 \mu\text{M}$  during some FRR and PAM modulated fluorescence measurements of cells exposed to  $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ . PAM saturation pulses were

applied after the DCMU addition when the fluorescence yield was observed to be constant for 1 min. Dark-adapted measurements were made in the absence of DCMU.

#### *O<sub>2</sub> flash yield measurements*

All LL and HL grown eukaryotic algae were used for estimations of photosynthetic unit size ( $n_{\text{PSII}}$ ). We followed the technique described by Falkowski *et al.* (1981), which requires algal concentrations between  $\sim 1$  and  $5 \text{ mg l}^{-1}$ . All algal suspensions were concentrated using gravity filtration. Algae were collected on 47 mm GF/C filters (Whatman, UK) and re-suspended in 4–5 ml of the filtrate. This procedure did not change  $F_v/F_m$ .

Oxygen concentrations were measured using a Clarke-type electrode housed within a DW1 liquid-phase oxygen electrode chamber (Hansatech Instruments, King's Lynn, UK). A ST saturation flash system consisting of three blocks of 100 blue LEDs (Bartington Associates, Essex, UK) was positioned around the reaction chamber. A Fresnel lens was placed in front of each block to focus the light. The flash system was controlled via a PC interface using a NI-DAQ (National Instruments, Texas, USA) high-speed timer card. Flash length was  $100 \mu\text{s}$  and the output from each block measured by the Hansatech Quantitherm within the electrode chamber was  $\sim 5000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Flash yields of  $\text{O}_2$  were constant for flash frequencies between 10 and 50 flashes  $\text{s}^{-1}$  and measurements were performed routinely at 20, 30 and 40 flashes  $\text{s}^{-1}$ . Measurements of photosynthetic unit size were obtained by expressing  $\text{O}_2$  flash yields relative to total chlorophyll  $a$  content.  $n_{\text{PSII}}$  is reported as the product of PSII photosynthetic unit size ( $\text{mol O}_2 (\text{mol chl } a)^{-1}$ ) and 4 mol RCII ( $\text{mol O}_2$ ) $^{-1}$ ; i.e.  $\text{mol RCII} (\text{mol chl } a)^{-1}$  throughout (Table 1). FRR fluorescence measurements were made before and after concentrating each dark-adapted sub-sample and following the  $\text{O}_2$  flash yield experiment.

#### *Absorption and pigment measurements*

The concentrated sub-sample and growth media were subsequently placed in matched 10 mm quartz cuvettes and optical density measured using a U-3000 spectrophotometer fitted with an integrating sphere ( $\phi 60$ , Hitachi High-Technologies, Berkshire, UK). Absorption by the microalgae was calculated from optical density after correction for pathlength and residual scattering at 750 nm, and subtraction of the growth-media blank. The chlorophyll  $a$ -specific absorption coefficient ( $a^{\text{chl}}$ ) was obtained by normalising absorption to the chlorophyll  $a$  concentration measured in a parallel subsample of culture by HPLC. The sub-sample was centrifuged and methanol added to the pellet to a final volume of 10 ml. Each sample was stored at  $4^\circ\text{C}$  for 12–16 h for pigment extraction. Pigment concentrations were determined using a Beckman HPLC with a gradient of 80% methanol:20% ammonium acetate (solvent A) and 70% methanol:30% ethyl acetate (Solvent B). Detection was at 436 and 450 nm. Pigments were identified by elution time and quantified from the diode-array spectra by comparison with pigment standards (chlorophyll  $a$ , chlorophyll  $c_1$ , fucoxanthin, peridinin, lutein, 19'hexa-

nonyloxyfucoxanthin, diadinoxanthin and zeaxanthin; DHI, Hørsholm, Denmark) and specific extinction coefficients (Jeffrey *et al.*, 1997). Pigments were classified as photosynthetic (PH; chlorophylls *a*, *b* and *c*, 19'-hexanoyloxyfucoxanthin, fucoxanthin and peridinin) and non-photosynthetic (NPH;  $\beta$ -carotene, diadinoxanthin, diatoxanthin, lutein, and zeaxanthin) following Bidigare *et al.* (1990) and Babin *et al.* (1996).

### <sup>18</sup>O<sub>2</sub> measurements

In separate experiments, gross oxygen evolution was determined using a modified <sup>18</sup>O<sub>2</sub> dilution technique on discrete samples (Kana, 1990) of the chlorophytes *Dunaliella tertiolecta* and *Nannochloris atomus*, and the diatom *Thalassiosira weissflogii*. Each sample was held within a closed glass cuvette placed over the FRR fluorometer optical window and within a water bath, and exposed to a continuous irradiance (as above) for 30–40 min. FRR measurements were made continuously whilst oxygen isotope concentrations were determined at the beginning and end of this period using a membrane inlet mass spectrometer (Kana *et al.*, 1994). The experiment was repeated under 50, 200 and 800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , as measured with a QSL-101 scalar quantum sensor (Biospherical Instruments, San Diego, CA, USA). Corresponding measurements of chlorophyll *a* and mean light absorption,  $a^{\text{chl}}$ , were made (as described above) to determine the quantum yield of O<sub>2</sub> evolution,  $\Phi_{\text{P}}\text{O}_2^{18}$ , for comparison with the FRR measurement of  $F_q'/F_m'$  from the experiment. The comparison was performed on three occasions with *N. atomus* and *T. weissflogii* and twice with *D. tertiolecta*.

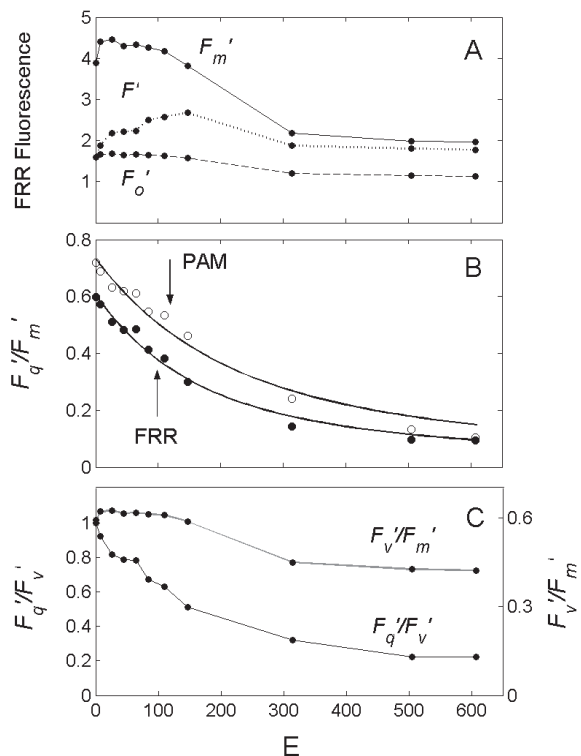
## Results and discussion

### Light dependence of fluorescence

Light-response experiments were performed for all LL grown cultures. The response of fluorescence parameters  $F'$  and  $F_m'$  from the FRR fluorometer to increasing actinic light ( $E$ ) is shown in Fig. 2a for *Prorocentrum minimum*.  $F'$  increased whilst  $F_m'$  remained relatively constant as  $E$  was increased from 0 to 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . These fluorescence yields declined once  $E$  exceeded 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . The effective photochemical efficiency of PSII photochemistry ( $F_q'/F_m'$ ) decreased with the increase in  $E$  (Fig. 2b). To assess the causes of this decline, we separated  $F_q'/F_m'$  into contributions from changes to the maximum efficiency of photochemistry,  $F_v'/F_m'$ , and changes to the PSII efficiency factor,  $F_q'/F_v'$  (Table 1).

$$\frac{F_q'}{F_m'} = \frac{F_v'}{F_m'} \cdot \frac{F_q'}{F_v'} \quad [5]$$

$F_q'/F_v'$  is equivalent to the factor qP, commonly defined as the fraction of open reaction



**Fig. 2.** (a) Fluorescence from cells of *Prorocentrum minimum* under actinic light ( $F'$ , dotted line) and the minimum ( $F_o'$ , broken line) and maximum ( $F_m'$ , solid line) fluorescence, measured with the FRR fluorometer with increasing irradiance ( $E$ ,  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ); (b) Photochemical efficiency of PSII,  $F_q'/F_m'$ , estimated using the FRR fluorescence measurements in (a) with increasing irradiance (●). Measurements of  $F_q'/F_m'$  using the PAM technique are also shown (○). Curve fits to determine  $E_k$ , the point of light saturation, are indicated by the arrows for both methods. Curves were fitted by least-squares non-linear regression according to equation 10, Table 2. FRR  $E_k = 95.6$ ,  $r^2 = 0.986$  and PAM  $E_k = 125.0$ ,  $r^2 = 0.975$ ; (c) Maximum efficiency of PSII,  $F_v'/F_m'$ , and the PSII efficiency factor,  $F_q'/F_v'$ , estimated using the FRR fluorescence measurements in (a) with increasing irradiance.

centres (qP, Kolber & Falkowski, 1993; Kromkamp & Forster, 2003). However, we refer to  $F_q'/F_v'$  as the PSII efficiency factor since the linear relationship assumed between  $F_q'/F_v'$  and the fraction of open PSII reaction centres is modified by the degree of connectivity between reaction centres (Baker & Oxborough, 2003). Calculation of both  $F_v'/F_m'$  and  $F_q'/F_v'$  requires knowledge of the minimum fluorescence yield under actinic light,  $F_o'$  (Table 1).  $F_o'$  is a difficult parameter to measure accurately (see Kromkamp & Forster, 2003); therefore, we estimated  $F_o'$  indirectly from  $F_m'$  and corresponding dark-adapted fluorescence measurements (Table 1, following Oxborough & Baker, 1997).

Between 0 and 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $F_q'/F_v'$  decreased whilst  $F_v'/F_m'$  remained constant (Fig. 2c). At higher irradiances,  $F_q'/F_v'$  continued to

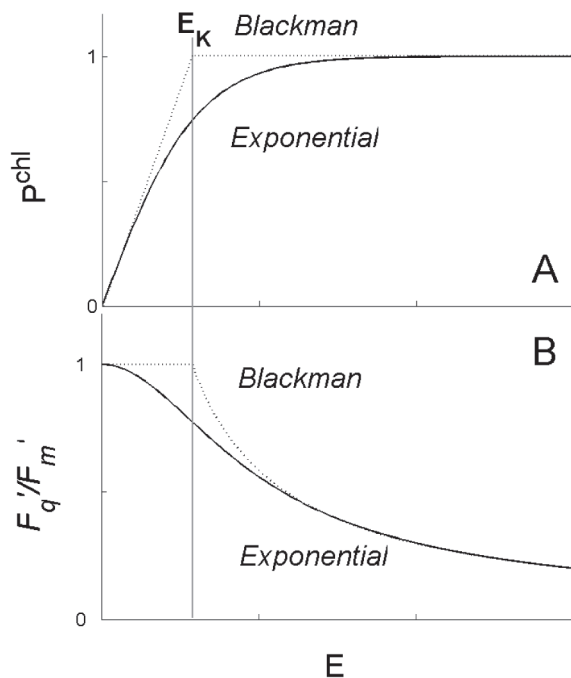
decline whilst  $F_v'/F_m'$  declined to a lower, but reasonably stable, value. Changes to  $F_q'/F_m'$  with irradiance result from changes in photochemical and non-photochemical quenching processes and are characteristic of photosynthetic activity. The reduction of  $F_q'/F_m'$  between 0 and  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  is due to a reduction of  $F_q'/F_v'$  and indicates that the decrease in photochemistry is almost entirely from the utilisation of electron transport products downstream of PSII (Baker & Oxborough, 2003). The decrease of  $F_v'/F_m'$  above  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  reflects the activation of additional non-photo-chemical quenching processes.

#### Light dependence of photosynthesis and quantum yield

The light-response of photosynthesis can be described by a variety of mathematical functions (Jassby & Platt, 1976; Laws *et al.*, 2002). The most basic is a Blackman-type function (Jassby & Platt, 1976; Fig. 3a), however, an exponential function is more commonly used (Equation 11, Table 2). These functions describe the transition from light-dependent photosynthesis, where changes in photosynthesis are directly related to changes in E, to light-independent photosynthesis, where photosynthesis and PSII electron turnover are maximal and maintained by a variety of physiological

mechanisms (e.g. Sakshaug *et al.*, 1997). The light-saturation parameter,  $E_k$ , marks the transition point between light-dependent and light-independent photosynthesis in the Blackman function and is used to quantify the transition point when other functions are used.

Light-response relationships are generated directly from gas-exchange measurements. In contrast, fluorescence techniques provide a direct measure of the quantum yield. The quantum yield can be converted into an estimate of productivity given knowledge of the light absorption coefficient (equations 2 and 3). However, the derived light-response may result in a change to the distribution of error and the values for the light-response parameters, such as  $E_k$  (Laws *et al.*, 2002). Light-response functions can also be applied to the quantum yield of photochemistry (Fig. 3b). The relationship of  $F_q'/F_m'$  with E can, therefore, be described relative to the point of light saturation as in equation 10, Table 2.  $E_k$  was estimated by fitting  $F_q'/F_m'$  to this function for all light response experiments (see Fig. 2b) to aid the interpretation of the light response observed from measures of  $F_q'/F_m'$ . This exponential model for  $F_q'/F_m'$  versus E routinely produced better fits for the FRRF than the PAM (e.g. Fig. 2b legend).

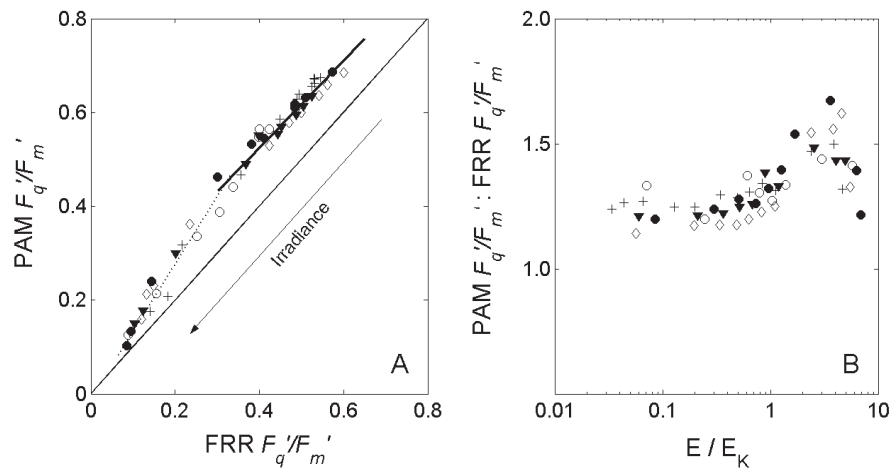


**Fig. 3.** Models of the response of photosynthesis as (a)  $P_{chl}$  and (b) the quantum yield of PSII photochemistry,  $F_q'/F_m'$ , to changes in irradiance (E). A Blackman-type or exponential function is used to describe the response (Jassby & Platt, 1976) and determine the point of light saturation,  $E_k$ . The exponential equation (a) follows equation 11 and (b) follows equation 10 (Table 2). All units are relative.

#### Light-response of $F_q'/F_m'$

All five species showed a similar non-linear relationship between values of  $F_q'/F_m'$  and E (Fig. 4). Whether measured by PAM or FRR fluorometers, values of  $F_q'/F_m'$  decreased as E increased from 0 to  $650 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Fig. 2c, Fig. 4a). The decrease was greater for FRR than PAM measurements (Fig. 4A, and is emphasized by plotting the ratio of  $F_q'/F_m'$  obtained from the two instruments versus irradiance (Fig. 4b). Dark-adapted values of photochemical efficiency ( $F_v'/F_m'$ ) were a factor of  $1.23 \pm 0.072$  higher from the PAM than the FRR fluorometer (data not shown). At low irradiances,  $F_q'/F_m'$  values from the PAM fluorometer exceeded those from FRR fluorescence by a factor of 1.17 and were comparable to the ratios measured in darkness. The ratio of corresponding values of  $F_q'/F_m'$  increased with irradiance and was highest at an  $E/E_k$  of 2.5–3, but subsequently converged (Fig. 4b).

The differences in  $F_q'/F_m'$  between PAM and FRR measurements over a wide range of irradiances are likely to be the result of the different effects of single and multiple turnover flashes upon the photosynthetic apparatus. The ST method typically provides lower values of  $F_m'$  than the MT method (Kolber *et al.*, 1998; Vasilév



**Fig. 4.** (a) Simultaneous measures of  $F_q'/F_m'$  (dimensionless) from the PAM and FRR fluorometers throughout the light gradient. Solid line indicates regression,  $y = 1.05x + 0.09$  ( $r^2 = 0.961$ ,  $n = 35$ ) for data where  $E < E_k$  ( $E_k$  determined by fitting equation 10 to the light response of  $F_q'/F_m'$  from the FRRF for each taxa). (b) Ratio of simultaneous measures of  $F_q'/F_m'$  from PAM and FRR fluorescence throughout the light gradient.  $E$  is plotted relative to values of  $E_k$ , determined as above, to reduce variability from differences in the growth irradiance. X-axis is plotted as a log scale. Symbols are ( $\diamond$ ) *Chaetoceros muelleri* ( $\blacktriangledown$ ) *Dunaliella tertiolecta* ( $\circ$ ) *Emiliania huxleyi* ( $+$ ) *Prochlorococcus marinus* ( $\bullet$ ) *Prorocentrum minimum*.

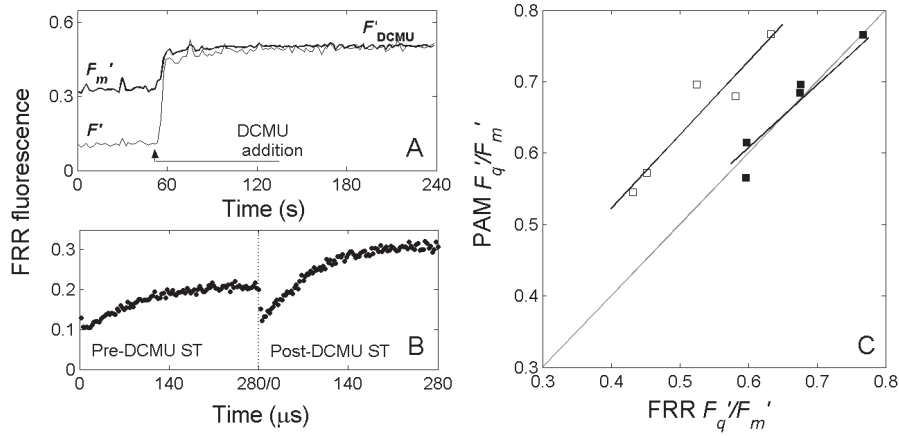
& Bruce, 1998; Samson *et al.*, 1999; Koblizek *et al.*, 2001). Single turnover flashes fully reduce the pool of the primary electron acceptor,  $Q_A$ , but have minimal effect on the redox state of the secondary electron acceptor,  $Q_B$ , or the plastoquinone pool (PQ) (e.g. Falkowski & Raven, 1997; Kolber *et al.*, 1998), and induce the photochemical phase of chlorophyll *a* fluorescence induction (Samson *et al.*, 1999). Conversely, multiple turnover flashes almost completely reduce  $Q_B$  to  $Q_B^-$  and PQ to plastoquinol (e.g. Koblížek *et al.*, 2001) and induce a secondary, thermal, phase of chlorophyll *a* fluorescence induction (Samson *et al.*, 1999). PQ is a quencher of chlorophyll fluorescence while plastoquinol is not. Consequently, the maximum fluorescence yield measured using MT flashes can increase by an estimated 20% when PQ is fully reduced to plastoquinol (Vernotte *et al.*, 1979; Kramer *et al.*, 1995). The extent of quenching by PQ may be modified by the interaction of PQ bound to  $Q_B$  (Samson *et al.*, 1999; Yaakoubd *et al.*, 2002, see below); however, the difference between  $F_m'$  from ST and MT excitation protocols is not well understood (Samson *et al.*, 1999). Neither PAM nor FRR method affects the redox state of the PQ pool during measurement of  $F'$ . As such, values of  $F_q'/F_m'$  are lower, and changes in  $F'$  relative to  $F_m'$  are greater, for the FRR than PAM fluorometer under relatively low irradiances.

$F_q'/F_m'$  values converge for the two methods once  $E$  exceeds  $E_k$  presumably since PQ becomes reduced by excess excitation energy and the amount of quenching by PQ decreases. However, the irradiance at which convergence begins to occur does not correspond with  $E_k$ , but at approximately

three times  $E_k$ . This offset reflects the ability with which  $E_k$  predicts the onset of light-saturated photosynthesis, the difference between the rate of production at  $E_k$  and the maximum rate of photosynthesis (e.g. Fig. 3).

#### Effect of DCMU on $F_q'/F_m'$

In microalgae,  $F_v/F_m$  measured in the presence and absence of DCMU using a Turner Fluorometer has been shown to equal  $F_v/F_m$  measured using PAM fluorescence (Hormann *et al.* 1994; Parkhill *et al.*, 2001) and exceed  $F_v/F_m$  using single turnover pump and probe fluorescence (Geider *et al.*, 1993a), which is analogous to FRR fluorescence. We observed an increase in values of  $F_m'$  and  $F_q'/F_m'$  measured by FRR fluorescence upon addition of DCMU, when LL grown cells were illuminated with low levels of light ( $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $E < E_k$ ; Figs. 5a,b). Values of  $F_m'$  measured by PAM fluorescence were not significantly affected by addition of DCMU (data not shown). A comparison of values of  $F_q'/F_m'$  measured by the two techniques in the presence of DCMU produced a 1:1 relationship (Fig. 5c). These results are consistent with recent observations by Yaakoubd *et al.* (2002) that demonstrate that the contribution of PQ to the thermal phase of fluorescence is largely the result of PQ that is bound to  $Q_B$ . DCMU preferentially binds to  $Q_B$  and thus prevents quenching by bound PQ. 'Free' PQ accounts for a much smaller proportion of the thermal phase and some quenching by 'free' PQ may still occur in the presence of DCMU. Similar processes could explain (1) the rise in  $F_o$  ( $F'$ ),  $F_m$  ( $F_m'$ ) and  $F_v/F_m$  ( $F_q'/F_m'$ ) using FRR fluorescence



**Fig. 5.** (a) Response of  $F'$  and  $F_m'$  measured with the FRRF upon addition of DCMU to cells of *Procoentrum minimum* (see main text for details) at  $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; (b) Kinetics of fluorescence induction in *P. minimum* during a single turnover saturating flash from the FRRF in the presence and absence of  $15 \mu\text{M}$  DCMU in the dark. DCMU was added 2 min prior to the first post-DCMU FRR measurement to allow penetration of the herbicide into the cells. Values of  $F_o$  before (0.114) and after (0.120) DCMU addition were not significantly different ( $t$ -test), whilst values of  $F_m$  before (0.207) and after (0.307) DCMU addition were significantly different ( $t$ -test,  $p < 0.001$ ); (c) Relationship between  $F_q'/F_m'$  measured using FRR and PAM fluorimeters before ( $\square$ ) and after ( $\blacksquare$ ) addition of DCMU for each LL grown species at  $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ . PAM  $F_q'/F_m' = 1.03 \cdot \text{FRR } F_q'/F_m' + 0.11$  ( $r^2 = 0.908$ ). PAM  $(F'_{\text{DCMU}} - F')/F'_{\text{DCMU}} = 0.88 \cdot \text{FRR } (F'_{\text{DCMU}} - F')/F'_{\text{DCMU}} + 0.08$  ( $r^2 = 0.932$ ).

and (2) the close agreement between  $F_q'/F_m'$  from FRR and PAM fluorescence, in the presence of DCMU.

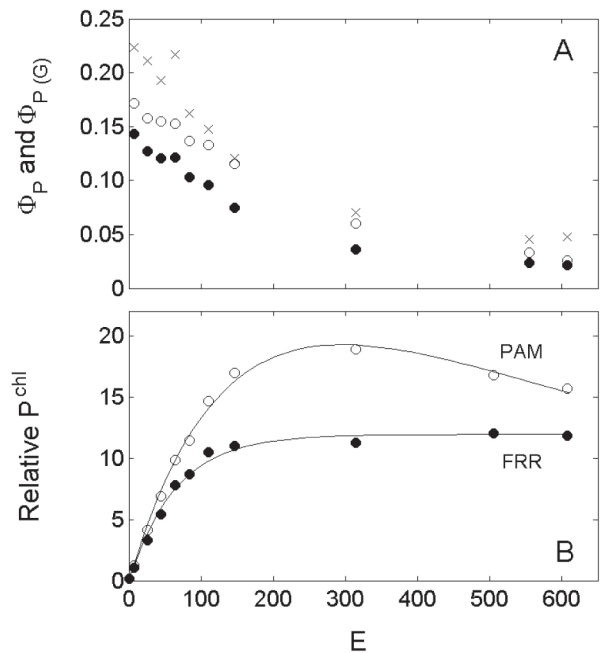
#### Estimation of the quantum yield of PSII photochemistry ( $\Phi_P$ )

The quantum efficiency of photosynthesis,  $\Phi_P$  (Genty *et al.*, 1989, equation 1) is obtained from the product of  $F_q'/F_m'$  and  $1/k$ :

$$\Phi_P = (F_q'/F_m')/k, \quad [6]$$

where  $1/k$  is the maximum yield of  $\text{O}_2$  from each electron transfer step through PSII, and typically assumed to be a constant  $0.25 \text{ mol O}_2$  (mol electrons) $^{-1}$  (Kolber & Falkowski, 1993; Gilbert *et al.*, 2000). However, estimates of  $\Phi_P$  from PAM and FRR measurements differ, reflecting the differences in  $F_q'/F_m'$  obtained from the two techniques (Fig. 4).

Gorbunov *et al.* (2000, 2001) have modified this approach and set  $\Phi_P$  equal to  $(F_q'/F_v')/k$ , designated  $\Phi_{P(G)}$ . Estimation of  $F_q'/F_m'$  and the maximum efficiency of PSII,  $F_v'/F_m'$ , assumes that non-photochemical dissipation of excitation occurs solely in the light-harvesting antennae (Butler, 1978); however, it is possible that processes may quench fluorescence in the PSII reaction centres (Falkowski & Raven, 1997; Bernhardt & Trissl, 1999). Consequently,  $\Phi_{P(G)}$  employs the ratio of the effective absorption cross section of PSII under actinic light,  $\sigma_{\text{PSII}}$ , to that under dark-adaptation,



**Fig. 6.** Light response of (a) the quantum yield of PSII photochemistry ( $\Phi_P = (F_q'/F_m')/k$ , Table 2) from PAM ( $\circ$ ) and FRR ( $\bullet$ ) fluorescence measurements of *Procoentrum minimum*. Also shown is the quantum yield of PSII photochemistry estimated according to Gorbunov *et al.* (2000, 2001) ( $\Phi_{P(G)} = (F_q'/F_v')/k \cdot (\sigma_{\text{PSII}}'/\sigma_{\text{PSII}})$ , Table 2) using FRR fluorescence measurements ( $\times$ ); (b) estimates of relative electron transfer rates (relative  $P^{\text{chl}} = F_q'/F_m' \cdot 0.25 \cdot E$ , Table 1) from FRR and PAM fluorescence measurements of  $F_q'/F_m'$  for *P. minimum*. Curves were fitted by least-squares non-linear regression according to the single exponential photosynthesis-irradiance model (equation 11, Jassby & Platt, 1976). PAM data were better fit, in particular at high light, with a double exponential model (equation 11, Platt *et al.*, 1980);  $0.90 < r^2 < 0.99$  for all curve fits.

$\sigma_{\text{PSII}}$ , to estimate the amount of non-photochemical quenching in the antennae. Use of  $(F_q'/F_v')/k$  ( $\sigma_{\text{PSII}'}/\sigma_{\text{PSII}}$ ) results in an exponential response of  $\Phi_{\text{P(G)}}$  with E (Fig. 6). A highly significant linear relationship was observed between  $(F_q'/F_v')/k \cdot (\sigma_{\text{PSII}'}/\sigma_{\text{PSII}})$  and  $F_q'/F_m'/k$  from all light-response experiments (data not shown):  $[(F_q'/F_v')/k \cdot (\sigma_{\text{PSII}'}/\sigma_{\text{PSII}})] = F_q'/F_m'/k \cdot 1.64$  ( $r^2 = 0.983$ ). This difference between approaches reflects the degree of quenching in the PSII reaction centres and of the thermal phase of chlorophyll fluorescence.

#### Estimation of photosynthetic electron transfer rates

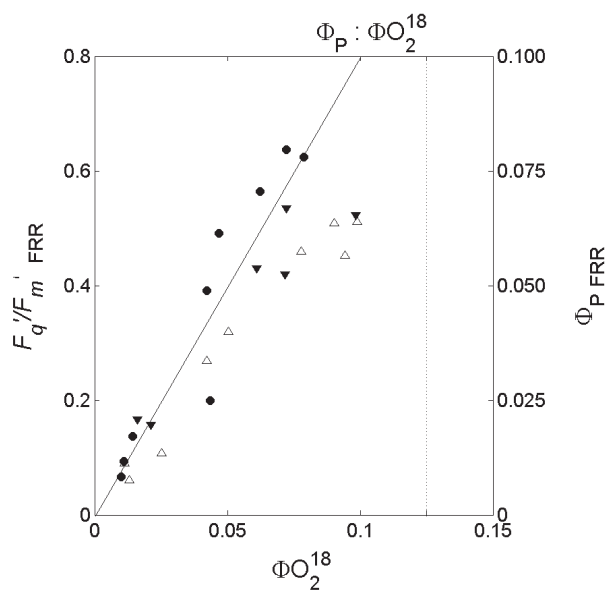
An estimate of relative electron transport rates can be obtained from the product of  $\Phi_{\text{P}}$  and E (relative  $\text{P}^{\text{chl}}$ , Hartig *et al.*, 1998). Calculations of relative  $\text{P}^{\text{chl}}$  from PAM and FRR measurements of  $F_q'/F_m'$  further emphasise the differences between the two approaches (Fig. 6). Values of relative  $\text{P}^{\text{chl}}$  are similar under low irradiances but diverge as E increases. This divergence follows that of  $F_q'/F_m'$  (Fig. 4) but E is an important determinant of relative  $\text{P}^{\text{chl}}$  and consequently amplifies the difference. The difference between relative  $\text{P}^{\text{chl}}$  from the two approaches is greatest where E exceeds  $E_k$  but is subsequently reduced. Consequently, the FRR and PAM fluorescence protocols tested here give rise to different light-response curves.

The photosynthesis-light response from the FRRF can be described by a model with a single exponential function (Table 2). However, the photosynthesis-light response from the PAM routinely displayed inhibition at high light (Fig. 6) and was better described using a model containing a second exponential function (Fig. 6 legend). This is consistent with the fits determined from the exponential model used to describe the light response of  $F_q'/F_m'$  from the two techniques (see Fig. 3). The different light response curves, in particular, the degree of photoinhibition, may partly reflect the MT and ST protocols used in this experiment. Other investigators report a range of light response functions using the PAM approach (Hofstraat *et al.*, 1994; Flameling & Kromkamp, 1998).

Parameters obtained from light response curves are used to characterize the photosynthetic response at scales of individual cells (MacIntyre *et al.*, 2002) to entire aquatic systems (Sathyendranath *et al.*, 1995). Hence, it is important to verify ST and MT fluorescence based estimates of  $\text{P}^{\text{chl}}$  with independent measures of oxygen evolution. However, what is the most appropriate dimensionless fluorescence-based measure to use with  $1/k$  to estimate the quantum yield of oxygen evolution? We addressed this question through simultaneous measurements of PSII quantum efficiency,  $F_q'/F_m'$ ,

from FRR measurements and the quantum yield of oxygen evolution using the  $^{18}\text{O}_2$  isotope technique ( $\Phi_{\text{P}}\text{O}_2^{18} = \text{O}_2 \text{ production}/a^{\text{chl}}$ ), which gives a true rate of gross oxygen evolution (Fig. 7).

We found that  $F_q'/F_m'$  was linearly related to  $\Phi_{\text{P}}\text{O}_2^{18}$  for each of the three species studied (Fig. 7). In two of the three species (*Dunaliella tertiolecta* and *Thalassiosira weissflogii*), the slope of the relationship between  $(F_q'/F_m')/k$  and  $\Phi_{\text{P}}\text{O}_2^{18}$  was significantly less than predicted (Table 3). This difference between  $(F_q'/F_m')/k$  and  $\Phi_{\text{P}}\text{O}_2^{18}$  is similar to that observed between  $(F_q'/F_m')/k$  and  $(F_q'/F_v')/k \cdot (\sigma_{\text{PSII}'}/\sigma_{\text{PSII}})$  from the



**Fig. 7.** Relationship between quantum yield of oxygen evolution, measured using the oxygen-18 isotope technique ( $\Phi_{\text{O}_2^{18}}$ , mol  $\text{O}_2$  (mol photons) $^{-1}$ ) and both the quantum efficiency of PSII,  $F_q'/F_m'$  and  $\Phi_{\text{P}}$  (mol  $\text{O}_2$  (mol photons) $^{-1}$ , =  $(F_q'/F_m')/k \cdot E$ ) from FRR fluorescence measurements.  $\Phi_{\text{P}}$  was estimated from equation 1 (Table 2) but multiplied by an additional factor of 0.5 to account for absorption by both PSII and PSI included in  $a^{\text{chl}}$  for  $\Phi_{\text{O}_2^{18}}$ . The solid line represents exact equivalence between  $\Phi_{\text{P}}$  and  $\Phi_{\text{O}_2^{18}}$ . The dotted line represents the theoretical maximum for  $\Phi_{\text{O}_2^{18}}$  according to the z-scheme. Data are from *Dunaliella tertiolecta* ( $\blacktriangledown$ ) *Thalassiosira weissflogii* ( $\Delta$ ) and *Nannochloris atomus* ( $\bullet$ ).

**Table 3.** Regression parameters from comparison (Bartlett's Type II regression) of estimates of the quantum yield of gross photosynthesis by the FRR (as  $[F_q'/F_m']/k$ ) and estimates by  $^{16}\text{O}/^{18}\text{O}$  mass spectrometry (see Fig. 7). Data are presented by species as mean  $\pm$  standard error.

	Slope	Intercept	$r^2$
<i>Dunaliella tertiolecta</i>	$0.69 \pm 0.10$	$0.007 \pm 0.007$	0.902
<i>Nannochloris atomus</i>	$1.06 \pm 0.13$	$-0.001 \pm 0.006$	0.903
<i>Thalassiosira weissflogii</i>	$0.65 \pm 0.05$	$0.003 \pm 0.003$	0.962

light-response experiments and may partly reflect the underestimation of  $\Phi_P$  from  $F_q'/F_m'$  given non-photochemical quenching processes in the reaction centres (see above). However, it is not possible to assess fairly the utility of FRR for estimating quantum yields of photosynthesis from such a limited sample size (three species grown under nutrient-replete conditions at a single irradiance).

While the data are in good agreement for *Nannochloris atomus*, the FRR underestimated  $\Phi_{PO_2}^{18}$  across all irradiances in the other two species. If these observations prove to be repeatable, they cannot be ascribed to taxonomically-based differences in pigment complement or photosynthetic physiology as both *N. atomus* and *D. tertiolecta* are chlorophytes. The most obvious differences between *N. atomus* and the other two species are size and shape: *N. atomus* is a coccoid cell with a diameter of c. 2  $\mu\text{m}$  and the others are ovoid/cylindrical with axial lengths of c. 10–15  $\mu\text{m}$ . As scattering by *N. atomus* is therefore closer to being anisotropic than for the other two species, the likelihood of overestimating its absorption, even when using a cuvette at the port of an integrating sphere, is higher. It is quite possible that such an overestimate would cause a corresponding underestimate in  $\Phi_{PO_2}^{18}$  and that the relationship between  $(F_q'/F_m')/k$  and  $\Phi_{PO_2}^{18}$  for *N. atomus* should be closer to that of the other two species. While the FRR estimates of quantum yield are highly correlated with observed values, it appears that another factor may be needed to reconcile them.

Other comparisons between measurements of the quantum yield of oxygen evolution and  $F_q'/F_m'$  using the PAM report both linear and curvilinear relationships (Flameling & Kromkamp, 1998; Franklin & Badger, 2001; Masojidek *et al.*, 2001). The loss of linearity may occur under conditions of low or high actinic light and varies according to both taxa and growth treatment (see also Flameling & Kromkamp, 1998; Franklin & Badger, 2001). These comparisons were performed over a wider range of irradiances than were ours. However, the high degree of linearity between  $(F_q'/F_m')/k$  and  $\Phi_{PO_2}^{18}$  and the lack of significant non-zero intercepts observed here, suggest that the discrepancy is not dependent on irradiance. As the relationship between FRR and PAM estimates of  $F_q'/F_m'$  is non-linear and irradiance-dependent, the non-linearity reported between measured quantum yields of oxygen evolution and  $(F_q'/F_m')/k$  determined by PAM fluorescence (Flameling & Kromkamp, 1998; Franklin & Badger, 2001; Masojidek *et al.*, 2001) may partly be attributable to the differences in the ST versus MT protocols.

### PSII light absorption

In order to calculate photosynthetic electron transfer rates from  $F_q'/F_m'$ , a knowledge of the rate of light absorption by PSII is required. Typically, the chlorophyll *a* specific absorption cross section for PSII, designated  $a_{\text{PSII}}^{\text{chl}}$ , is calculated from equation 8 (Table 2; see also equation 2), which accounts for the effect of non-photosynthetic pigments and the assumption that excitation energy is distributed equally between PSII and PSI. We tested the validity of equation 8 by measuring the functional cross-section for PSII ( $\sigma_{\text{PSII}}$ ), the ratio of PSII to chlorophyll *a* ( $n_{\text{PSII}}$ ), the chlorophyll *a* specific absorption coefficient ( $a^{\text{chl}}$ ) and the pigment composition. The value of  $a^{\text{chl}}$  was weighted according to the spectral composition of LED excitation from the FRRF (Fig. 1),

$$a_{\text{LED}}^{\text{chl}} = \frac{\sum a_{(\lambda)}^{\text{chl}} \cdot E_{\text{LED}}(\lambda)}{\sum E_{\text{LED}}(\lambda)} \quad [7]$$

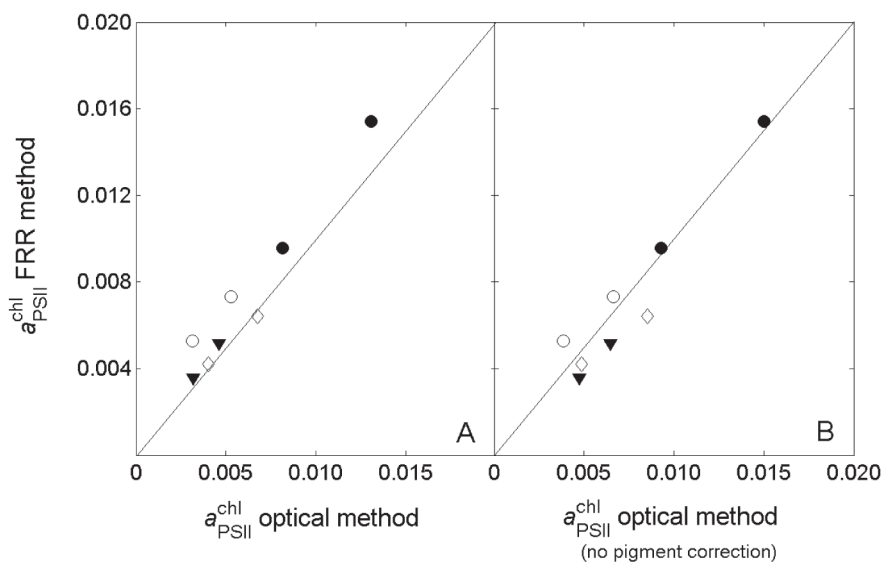
where  $E_{\text{LED}}$  is the photon flux of the FRRF LEDs and  $\lambda$  is from 400 to 700 nm. A similar weighting was applied when estimating the contribution of light absorption from photosynthetic pigments (PH) and non-photosynthetic pigments (NPH),

$$[a_{\text{LED}}]_{\text{PH,NPH}} = \left[ \frac{\sum a_{(\lambda)}^* \cdot E_{\text{LED}}(\lambda)}{\sum E_{\text{LED}}(\lambda)} \cdot C \right]_{\text{PH,NPH}} \quad [8]$$

where  $a^*$  is the light absorption coefficient (Bidigare *et al.*, 1990) and  $C$  is the concentration of pigment.

Independent estimates of  $a_{\text{PSII}}^{\text{chl}}$  from FRR measurements of  $\sigma_{\text{PSII}}$  and spectrophotometric measurements of light absorption,  $a^{\text{chl}}$  (equation 8, Table 2) were closely correlated (Fig. 8a), although the biophysical measurements gave values of  $a_{\text{PSII}}^{\text{chl}}$  that were about 20% higher than the optical measurements. This difference was reduced when the proportion of total light absorption by non-photosynthetic pigments was not considered for the estimation of  $a_{\text{PSII}}^{\text{chl}}$  from  $a^{\text{chl}}$  (Fig. 8b).

The higher values of  $a_{\text{PSII}}^{\text{chl}}$  from the FRRF approach suggest that photons absorbed at 480 nm are preferentially delivered to PSII, consistent with the greater contribution of 480 nm excitation to PSII than to PSI photochemistry (see Baker & Webber, 1987). The  $a^{\text{chl}}$  approach assumes 50% of absorbed light is transferred to PSII. The data in Fig. 8 suggest that 49% of the excitation energy for *Chaetoceros mulleri* is delivered to PSII and this increases to 70% for *Emiliania huxleyi*. Therefore, estimates of



**Fig. 8.** (a) Relationship between the chlorophyll *a*-specific absorption cross-section for PSII,  $a_{\text{PSII}}^{\text{chl}}$  ( $\text{m}^2 (\text{mg chl } a)^{-1}$ ), estimated from the optical absorption by photosynthetic pigments ( $= a_{\text{LED}}^{\text{chl}} \cdot [a_{\text{LED}}]_{\text{PH}} / ([a_{\text{LED}}]_{\text{PH}} + [a_{\text{LED}}]_{\text{NPH}}) \cdot 0.5$ ) and from the FRR ( $= \sigma_{\text{PSII}} \cdot n_{\text{PSII}} \cdot 0.00675$ ).  $a_{\text{PSII}}^{\text{chl}} \text{ FRR} = 1.17 \cdot a_{\text{PSII}}^{\text{chl}} \text{ optical}$  ( $r^2 = 0.944$ ,  $n = 8$ ,  $p < 0.001$ ). The relationship remains significant ( $y = 1.19x$ ,  $r^2 = 0.773$ ,  $0.005 > p > 0.002$ ) upon the removal of the highest value, indicating that the relationship is not based on the range of estimates. (b) Relationship between  $a_{\text{PSII}}^{\text{chl}}$  from optical and FRR methods as in (a); however, the optical absorption approach is not corrected for the proportion of total absorption by photosynthetic pigments ( $= a_{\text{LED}}^{\text{chl}} \cdot 0.5$ ):  $a_{\text{PSII}}^{\text{chl}} \text{ FRR} = 0.97 \cdot a_{\text{PSII}}^{\text{chl}} \text{ optical}$ ,  $r^2 = 0.932$ ,  $n = 8$ ,  $p < 0.001$ . Symbols for both panels as Fig. 4. Error bars were within the area of the symbols.

$P^{\text{chl}}$  (equation 3) are likely to be higher when using the biophysical approach than the  $a^{\text{chl}}$  approach for determining  $a_{\text{PSII}}^{\text{chl}}$  and may complicate the interpretation of absolute electron transfer rates from FRR and PAM protocols. This may also help to explain why measurements of  $\Phi_{\text{p}}\text{O}_2^{18}$  using  $a^{\text{chl}}$  are higher than corresponding estimates using  $F_q'/F_m'$  from the FRR fluorometer in some species (Fig. 7). However, this comparison does not consider the unequal distribution of photosynthetic and non-photosynthetic pigments between PSII and PSI (Yamamoto & Bassi, 1996), differences in pigment-specific transfer efficiencies between light harvesting pigments and PSII reaction centres (Mauzerall & Greenbaum, 1989) and inclusion of systematic measurement errors, such as those for light absorption (scattering) and/or  $\sigma_{\text{PSII}}$  (Sam Laney, personal communication).

The biophysical estimate of  $a_{\text{PSII}}^{\text{chl}}$  (equation 4, Table 2) varies between taxa at each growth irradiance. Within a taxon, the biophysical  $a_{\text{PSII}}^{\text{chl}}$  is consistently higher for the HL than LL treatment ( $t$ -test,  $p < 0.05$  for each taxa) reflecting increases in  $n_{\text{PSII}}$  and  $\sigma_{\text{PSII}}$  (data not shown) as well as  $a^{\text{chl}}$  for algae grown under the HL conditions. The results presented here are consistent with observations of co-variation of  $a^{\text{chl}}$  and  $\sigma_{\text{PSII}}$  from pigment package effects (Geider *et al.*, 1993b). Similarly, values of  $n_{\text{PSII}}$  increase at high growth irradiances and are indicative of a photo-acclimative response (Falkowski *et al.*, 1981).

## Conclusions

Bio-optical approaches are being used increasingly in aquatic research to estimate primary productivity with high temporal and spatial resolution (Bidigare *et al.*, 1992; Sakshaug *et al.*, 1997). These approaches often employ a sophisticated treatment of the rate of light absorption, but rely on an independent and often poorly constrained specification of the relationship between quantum efficiency and irradiance. PAM and FRR fluorescence approaches can make a major contribution to understanding variability of photosynthesis in aquatic systems by providing accurate estimates of quantum yield, *in situ*; however, whether these techniques can improve estimates of primary productivity in aquatic systems remains an open question.

Here we show that (1) optical and biophysical techniques can provide similar estimates of light absorption given a sophisticated detailed treatment of how light is delivered to PSII reaction centres and (2) that care must be taken when applying the FRR and PAM techniques to estimate quantum yields. Estimates of  $F_m'$  and, therefore,  $F_q'/F_m'$ , from FRR and PAM measurements differed as a result of the different methods that are used to close PSII reaction centres. In contrast to the ST method, MT measures of  $F_m'$  prevent non-photochemical quenching mechanisms that are inherent to measures of  $F'$ . Consequently, the MT method is not consistent with the rate constants required to

generate  $F_q'/F_m'$  (following Butler, 1978) and may be inappropriate, in particular under low actinic irradiances, where PQ remains highly oxidised (see also Fryer *et al.*, 1998). Furthermore, the differences emphasise that a clearer distinction should be made between ST and MT measures of fluorescence and estimates of PSII photochemistry (Kromkamp & Forster, 2003). ST measurements of  $F_q'/F_m'$  using FRR fluorescence and  $\Phi_P$  co-varied with simultaneous, but independent, measures of the quantum yield of oxygen evolution,  $\Phi_{PO_2}^{18}$ . If the linearity of the relationship holds under other growth conditions and in other species and if the magnitude of the slope varies little, the FRR will be a valuable tool for estimating productivity free from the constraints of inappropriate light regimes imposed by bottle incubations (MacIntyre *et al.*, 2000).

### Acknowledgements

We thank David Scanlon (University of Warwick) for cultures of *Prochlorococcus*, John Bartington (University of Essex) for constructing the flash yield system and Paul Beckwith (University of Essex), David Weiss (University of Maryland) and Laurie van Heukelem (University of Maryland) for technical support. We are indebted to Sam Laney (Oregon State University) for advice and provision of processing software for the FRR Fluorometer. Comments by two anonymous reviewers and by Jacco Kromkamp helped to improve the paper. This work was supported by the National Environment Research Council, UK (NER/A/S/2000/01237 to RJG), with additional support from the US National Science Foundation (grant OCE-9907702 to TMK and HLM). This is Contribution 3667 of the University of Maryland Centre for Environmental Science.

### References

- AIKEN, J., REES, N., HOOKER, S., HOLLIGAN, P., BALE, A., ROBINS, D., MOORE, G., HARRIS, R. & PILGRIM, D. (2000). The Atlantic Meridional Transect: Overview and synthesis of data. *Prog. Oceanography*, **45**: 257–312.
- BABIN, M., MOREL, A., CLAUSTRE, H., BRICAUD, C., KOLBER, Z. & FALKOWSKI, P.G. (1996). Nitrogen- and irradiance-dependent variations of the maximum quantum yield of carbon fixation in eutrophic, mesotrophic and oligotrophic marine systems. *Deep Sea Res.*, **43**(8): 1241–1272.
- BAKER, N.R. & OXBOROUGH, K. (2003). Chlorophyll fluorescence as probe of plant photosynthetic productivity. In *Chlorophyll fluorescence: A signature of photosynthesis* (Papageorgiou, C.G. & Govindjee, A.D., editors). Kluwer Academic Press, Dordrecht, in press.
- BAKER, N.R. & WEBBER, A.N. (1987). Interactions between photosystems. *Adv. Bot. Res.*, **13**: 1–66.
- BERNHARDT, K. & TRISSL, H.W. (1999). Theories for kinetics and yields of fluorescence and photochemistry: how, if at all, can different models of antenna organization be distinguished experimentally? *Biochim Biophys Acta*, **1409**(3): 125–142.
- BEUTLER, M., WILTSHIRE, K.H., MEYER, B., MOLDAENKE, C., LÜRING, C., MEYERHÖFER, M., HANSEN, U.P. & DAU, H. (2002). A fluorometric method for the differentiation of algal populations *in vivo* and *in situ*. *Photosynth. Res.*, **72**: 39–53.
- BIDIGARE, R.R., ONDRUSEK, M.E., MORROW, J.H. & KIEFER, D.A. (1990). *In vivo* absorption properties of algal pigments. *SPIE*, **1302** (Ocean Optics X): 289–302.
- BIDIGARE, R.R., PRÉZELIN, B.B. & SMITH, C.R. (1992). Bio-optical models and the problem of scaling. In *Primary productivity and biogeochemical cycles in the sea* (Falkowski, P.G. & Woodhead, A.D., editors), 175–212. Plenum Press, New York.
- BOYD, P.W., AIKEN, J.A. & KOLBER, Z.S. (1997). Comparison of radiocarbon and fluorescence based (pump and probe) measurements of phytoplankton photosynthetic characteristics in the northeast Atlantic Ocean. *Mar. Ecol. Prog. Ser.*, **149**: 215–226.
- BUTLER, W.L. (1978). Energy distribution in the photochemical apparatus of photosynthesis. *Ann. Rev. Plant Physiol.*, **29**: 345–378.
- FALKOWSKI, P.G. & KOLBER, Z.S. (1995). Variations in chlorophyll fluorescence yields in phytoplankton in the world's oceans. *Aust. J. Plant Physiol.*, **22**: 341–355.
- FALKOWSKI, P.G. & RAVEN, J.A. (1997). *Aquatic Photosynthesis*. Blackwell, USA.
- FALKOWSKI, P.G., OWENS, T.G., LEY, A.C. & MAUZERALL, D.C. (1981). Effects of growth irradiance levels on the ratio of reaction centres in two species of marine phytoplankton. *Plant Physiol.*, **68**: 969–973.
- FLAMELING, I.A. & KROMKAMP, J. (1998). Light dependence of quantum yields for PSII charge separation and oxygen evolution in eukaryotic algae. *Limnol. Oceanogr.*, **43**(2): 284–297.
- FRANKLIN, L.A. & BADGER, M.R. (2001). A comparison of photosynthetic electron transport rates in macroalgae measured by pulse amplitude modulated chlorophyll fluorometry and mass spectrometry. *J. Phycol.*, **37**: 756–767.
- FRYER, M.J., ANDREWS, J.R., OXBOROUGH, K., BLOWERS, D.A. & BAKER, N.R. (1998). Relationship between CO<sub>2</sub> assimilation, photosynthetic electron transport, and active O<sub>2</sub> metabolism in leaves of maize in the field during periods of low temperature. *Plant Physiol.*, **116**: 571–580.
- GEIDER, R.J., GREENE, R.M., KOLBER, Z., MACINTYRE, H.L. & FALKOWSKI, P.G. (1993a). Fluorescence assessment of the maximum quantum efficiency of photosynthesis in the western North Atlantic. *Deep Sea Res.*, **40**(6): 1205–1224.
- GEIDER, R.J., LA ROCHE, J., GREENE, R.M. & OLAIZOLA, M. (1993b). Response of the photosynthetic apparatus of *Phaeodactylum tricornerutum* (Bacillariophyceae) to nitrate, phosphate or iron starvation. *J. Phycol.*, **29**: 755–766.
- 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.
- GILBERT, M., DOMIN, A., BECKER, A. & WILHELM, C. (2000). Estimation of primary productivity by chlorophyll *a* *in vivo* fluorescence in freshwater phytoplankton. *Photosynthetica*, **38**(1): 111–126.
- GORBUNOV, M.Y., FALKOWSKI, P.G. & KOLBER, Z.S. (2000). Measurement of photosynthetic parameters in benthic organisms *in situ* using a SCUBA-based fast repetition rate fluorometer. *Limnol. Oceanogr.*, **45**(1): 242–245.
- GORBUNOV, M.Y., KOLBER, Z.S., LESSER, M.P. & FALKOWSKI, P.G. (2001). Photosynthesis and photoprotection in symbiotic corals. *Limnol. Oceanogr.*, **46**(1): 75–85.
- GUILLARD, R.R.L. & RYTHER, J.H. (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve). *Can. J. Microbiol.*, **8**: 229–239.

- HARTIG, P., WOLFSTEIN, K., LIPPEMEIER, S. & COLIJN, F. (1998). Photosynthetic activity of natural microphytobenthos populations measured by fluorescence (PAM) and  $^{14}\text{C}$ -tracer methods: a comparison. *Mar. Ecol. Prog. Ser.*, **166**: 53–62.
- HOFSTRAAT, J.W., PEETERS, J.C.H., SNEL, J.F.H. & GEEL, C. (1994). Simple determination of photosynthetic efficiency and photo-inhibition of *Dunaliella tertiolecta* by saturating pulse fluorescence measurements. *Mar. Ecol. Prog. Ser.*, **103**: 187–196.
- HORMANN, H., NEUBAUER, C. & SCHREIBER, U. (1994). On the relationship between chlorophyll fluorescence quenching and the quantum yield of electron transport in isolated thylakoids. *Photosynth. Res.*, **40**: 93–106.
- JASSBY, A.T. & PLATT, T. (1976). Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr.*, **21**: 540–547.
- JEFFREY, J.W., MANTOURA, R.F.C. & WRIGHT, S.W. (1997). *Phytoplankton pigments in oceanography: guidelines and modern methods*. UNESCO, France.
- KANA, T.M. (1990). Light-dependent oxygen cycling measured by an O-18 isotope dilution technique. *Mar. Ecol. Prog. Ser.*, **64**: 293–300.
- KANA, T.M., DARKANGELO, C., HUNT, M.D., OLDHAM, J.B., BENNETT, G.E. & CORNWELL, J.C. (1994). A membrane inlet mass spectrometer for rapid high precision determination of  $\text{N}_2$ ,  $\text{O}_2$  and Ar in environmental water samples. *Anal. Chem.*, **66**: 4166–4170.
- KOBLÍZEK, M., KAFTAN, D. & NEDBAL, L. (2001). On the relationship between non-photochemical quenching of chlorophyll fluorescence and the photosystem II harvesting efficiency. A repetitive flash fluorescence induction study. *Photosynth. Res.*, **68**: 141–152.
- KOLBER, Z. & FALKOWSKI, P.G. (1993). Use of active fluorescence to estimate phytoplankton photosynthesis *in situ*. *Limnol. Oceanogr.*, **38**(8): 1646–1665.
- KOLBER, Z.S., PRÁŠIL, O. & FALKOWSKI, P.G. (1998). Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. *Biochim. Biophys. Acta*, **1367**: 88–106.
- KRAMER, D.M., DIMARCO, G. & LORETO, F. (1995). Contribution of plastoquinone quenching to saturation pulse-induced rise of chlorophyll fluorescence in leaves. In *Photosynthesis from light to the biosphere* (Mathis, P., editor), vol 1: 147–150. Kluwer Academic Publishers, Dordrecht.
- KROMKAMP, J.C. & FORSTER, R.M. (2003). The use of variable fluorescence measurements in aquatic ecosystems: differences between multiple and single turnover measuring protocols and suggested terminology. *Eur. J. Phycol.*, **38**: 103–112.
- KROMKAMP, J.C., BARRANGUET, C. & PEENE, J. (1998). Determination of microphytobenthos PSII quantum efficiency and photosynthetic activity by means of variable chlorophyll fluorescence. *Mar. Ecol. Prog. Ser.*, **162**: 45–55.
- LAWS, E., SAKSHAUG, E., BABIN, M., DANDONNEAU, Y., FALKOWSKI, P., GEIDER, R., LEGENDRE, L., MOREL, A., SONDERGAARD, M., TAKAHASHI, M. & WILLIAMS, P.J. LE B. (2002). *Photosynthesis and primary productivity in marine ecosystems: Practical aspects and application of techniques*. JGOFS report 36.
- MACINTYRE, H.L., KANA, T.M. & GEIDER, R. J. (2000). The effect of water motion on short-term rates of photosynthesis by marine phytoplankton. *Trends Plant Sci.*, **5**: 12–17.
- MACINTYRE, H.L., KANA, T.M., ANNING, T. & GEIDER, R.J. (2002). Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. *J. Phycol.*, **38**: 17–38.
- MASOJÍDEK, J., GROBBELAAR, J.U., PECHAR, L. & KOBLÍZEK, M. (2001). Photosystem II electron transport rates and oxygen production in natural waterblooms of freshwater cyanobacteria during a diel cycle. *J. Plankton Res.*, **23**(1): 57–66.
- MAUZERALL, D. & GREENBAUM, N.L. (1989). The absolute size of a photosynthetic unit. *Biochim. Biophys. Acta*, **974**: 119–140.
- OXBOROUGH, K. & BAKER, N.R. (1997). Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components—calculation of  $qP$  and  $F_v'/F_m'$  without measuring  $F_o'$ . *Photosynth. Res.*, **54**: 135–142.
- PARKHILL, J.-P., MAILLET, G. & CULLEN, J.J. (2001). Fluorescence-based maximal quantum yield for PSII as a diagnostic of nutrient stress. *J. Phycol.*, **37**: 517–529.
- PARTENSKY, F., HESS, W.R. & VAULOT, D. (1999). *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. *Microbiol. Mol. Biol. Rev.*, **63**: 106–127.
- PERKINS, R.G., OXBOROUGH, K., HANLON, A.R.M., UNDERWOOD, G.J.C. & BAKER, N.R. (2002). Can chlorophyll fluorescence be used to estimate the rate of electron transport within microphytobenthic biofilms. *Mar. Ecol. Prog. Ser.*, **228**: 47–56.
- PLATT, T., GALLEGOS, C.L. & HARRISON, W.G. (1980). Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *J. Mar. Res.*, **38**: 687–701.
- PRÁŠIL, O., KOLBER, Z., BERRY, J.A. & FALKOWSKI, P.G. (1996). Cyclic electron flow around photosystem II *in vivo*. *Photosynth. Res.*, **48**(3): 395–410.
- SAKSHAUG, E., BRICAUD, A., DANDONNEAU, Y., FALKOWSKI, P.G., KIEFER, D.A., LEGENDRE, L., MOREL, A., PARSLAW, J. & TAKAHASHI, M. (1997). Parameters of photosynthesis: definitions, theory and interpretation of results. *J. Plankton Res.*, **19**(11): 1637–1670.
- SAMSON, G., PRÁŠIL, O. & YAAKOUBD, B. (1999). Photochemical and thermal phases of chlorophyll *a* fluorescence. *Photosynthetica*, **37**: 163–182.
- SATHYENDRANATH, S., LONGHURST, A., CAVERHILL, C.M. & PLATT, T. (1995). Regionally and seasonally differentiated primary production in the North Atlantic. *Deep Sea Res.*, **42**(10): 1773–1802.
- SCHREIBER, U., SCHLIWA, U. & BILGER, W. (1986). Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res.*, **10**: 51–62.
- SCHREIBER, U., NEUBAUER, C. & SCHLIWA, U. (1993). PAM fluorometer based on medium-frequency pulsed Xe-flash measuring light: A highly sensitive new tool in basic and applied photosynthesis research. *Photosynth. Res.*, **36**: 65–72.
- 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**: 209–220.
- SUGGETT, D., KRAAY, G., HOLLIGAN, P., DAVEY, M., AIKEN, J. & GEIDER, R. (2001). Assessment of photosynthesis in a spring cyanobacterial bloom by use of a fast repetition rate fluorometer. *Limnol. Oceanogr.*, **46**(4): 802–810.
- VASILÉV, S. & BRUCE, D. (1998). Non-photochemical quenching of excitation energy in Photosystem II. A picosecond time-resolved study of the low yield of chlorophyll *a* fluorescence induced by a single-turnover flash in isolated spinach thylakoids. *Biochemistry*, **37**: 11046–11054.
- VERNOTTE, C., ETIENNE, A.L. & BRIANTAIS, J.-M. (1979). Quenching of the system II chlorophyll fluorescence by the plastoquinone pool. *Biochim. Biophys. Acta*, **545**: 519–527.
- YAAKOUBD, B., ANDERSEN, R., DESJARDINS, Y. & SAMSON, G. (2002). Contributions of the free oxidised and  $\text{Q}_B$ -bound plastoquinone molecules to the thermal phase of chlorophyll *a* fluorescence. *Photosynth. Res.*, **74**: 251–257.
- YAMAMOTO, H.Y. & BASSI, R. (1996). Carotenoids: localization and function. In *Oxygenic Photosynthesis: the light reactions* (Ort, D.R. & Yocum, C.F., editors), 539–563. Kluwer Academic Press, The Netherlands.