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REGULAR PAPER

Fitting light saturation curves measured using modulated fluorometry

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Abstract A blue diode PAM (*P*ulse *A*mplitude *M*odulation) fluorometer was used to measure rapid Photosynthesis (P) versus Irradiance (E) curves (P vs. E curves) in *Synechococcus* (classical cyanobacteria), *Prochlorothrix* (prochlorophyta), *Chlorella* (chlorophyta), *Rhodomonas* (cryptophyta), *Phaeodactylum* (bacillariophyta) *Acaryochloris* (Chl d/a cyanobacteria) and Subterranean Clover (*Trifolium subterraneum*, Papilionaceae, Angiospermae). Effective quantum yield (Φ_{PSII}) versus irradiance curves could be described by a simple exponential decay function ($\Phi_{PSII} = \Phi_{PSII,\ max}e^{-kE}$) although Log/Log transformation was sometimes found to be necessary to obtain the best fits. Photosynthesis was measured as relative Electron Transport Rate (rETR) standardised on a chlorophyll basis. P versus E curves were fitted to the waiting-in-line function (an equation of the form $P = P_{max} \cdot k \cdot E \cdot e^{-kE}$) allowing half-saturating and optimal irradiances ($E_{optimum}$) to be estimated. The second differential of the equation shows that at twice optimal light intensities, there is a point of inflection in the P versus E curve. Photosynthesis is inhibited 26.4% at this point of inflection. The waiting-in-line model was found to be a very good descriptor of photosynthetic light saturation curves and superior to hyperbolic functions with an asymptotic saturation point (Michaelis–Menten, exponential saturation and hyperbolic tangent). The exponential constants (k) of the Φ_{PSII} versus E and P versus E curves should be equal because rETR is directly proportional to $\Phi_{PSII} \times E$. The conventionally calculated Non-Photochemical Quenching (NPQ) in *Synechococcus* was not significantly different to zero but NPQ versus E curves for the other algae could be fitted to an exponential saturation model. The kinetics of NPQ does not appear to be related to the kinetics of Φ_{PSII} or rETR.

Keywords *Acaryochloris* · *Chlorella* · Subterranean clover · *Phaeodactylum* · *Prochlorothrix* · *Rhodomonas* · *Synechococcus* · *Trifolium subterraneum* · Oxyphotobacteria · Photosynthesis · PAM fluorometry · Modulated fluorometry · Effective quantum yield · Electron transport rate · Non-photochemical quenching · Light saturation curves · P versus E · PE

Abbreviations

E	Irradiance (mol m^{-2} s^{-1}) PAR
Φ_{PSII}	Effective quantum yield (measured using standard settings)
rETR	Relative electron transport rate (measured using standard settings)
NPQ	Non-photochemical quenching

Introduction

This study investigated the measurement and modelling of light saturation curves determined by modulated fluorometry (pulse-amplitude modulated, PAM) on representative organisms of the currently known different types of oxygenic photosynthetic organisms (Gantt and Cunningham 2001; Larkum et al. 2003). These are:

(a) Conventional cyanobacteria (example *Synechococcus R-2* PCC 7942) containing only chlorophyll *a*, allophycocyanin, phycocyanin and zeaxanthin (Gantt and Cunningham 2001; Larkum et al. 2003).

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(b) The unusual oxyphotobacterium *Prochlorothrix hollandica* PCC 9006, which has been loosely grouped into the "prochlorophyta" and contains chlorophyll *a* & *b*, allophycocyanin, phycocyanin and zeaxanthin (Burger-Weirsma and Post 1989; Gantt and Cunningham 2001; Larkum et al. 2003).

(c) Eukaryotic organisms with chloroplasts containing chlorophyll *a* & *b* and lutein, neoxanthin, violaxanthin and zeaxanthin (Gantt and Cunningham 2001; Larkum et al. 2003). This group includes green algae (Chlorophyta), charophytes and archeogoniophytes, which include vascular and non vascular plants. The examples used here were the unicellular green alga *Chlorella* sp. and the dicot vascular plant, Subterranean Clover (*Trifolium subterraneum*).

(d) Eukaryotic organisms with chloroplasts containing chlorophyll $a + c_1$, phycoerythrin and alloxanthin (Gantt and Cunningham 2001; Larkum et al. 2003). The example used in the present study was *Rhodomonas* sp. (Cryptophyta).

(e) Eukaryotic organisms with chloroplasts containing chlorophyll $a + c_1$ & c_2 and the xanthophylls diatoxanthin and fucoxanthin (Gantt and Cunningham 2001; Larkum et al. 2003). The example used in the present study was the diatom (Bacillariophyta) *Phaeodactylum* sp.

(f) The unusual cyanobacterium *Acaryochloris marina*, which uses chlorophyll *d* as its primary photosynthetic chlorophyll and contains chlorophylls *a* & *d*, allophycocyanin, phycocyanin and the carotenoids alpha-carotene and zeaxanthin (Gantt and Cunningham 2001; Larkum et al. 2003). The novelty of this variant of oxygenic photosynthesis has aroused intense interest (Burger-Weirsma and Post 1989; Miyashita et al. 1996, 1997, 2003; Hu et al. 1998; Chen et al. 2002, 2005a, b; Larkum et al. 2003; Mimuro et al. 2004; Kühl et al. 2005; Miller et al. 2005; Gloag et al. 2007).

Modulated chlorophyll fluorometry, using the so-called PAM technique (Schreiber et al. 1995a, b), provides a means to make rapid and accurate measurements of key photosynthetic parameters (Genty et al. 1989; Krause and Weis 1991; Schreiber et al. 1995a, b; Hartig et al. 1998; Kühl et al. 2005; Gloag et al. 2007). In particular, it has made photosynthetic light response curves (P vs. Irradiance) very easy to obtain compared to a time-consuming routines required for such measurements using oxygen electrode, ^{14}C-fixation or infrared gas analyser (IRGA) methods. A rapid light curve (White and Critchley 1999) on a plant, such as pea (*Pisum sativum*) can be measured in about 2 min. Multiple turnover modulated fluorometry employs the florescence emission of chlorophyll that results from a brief but strong light pulse of known intensity. The technique measures variable fluorescence in response to brief pulses of light and with this information one can estimate the *E*lectron *T*ransport *R*ate (ETR) (Genty et al. 1989; van Kooten and Snel 1990; Krause and Weis 1991; Schreiber et al. 1995a, b; Franklin and Badger 2001; Gloag et al. 2007). PAM fluorometers, such as the Junior-PAM used in the present study, were primarily designed for use on vascular plants which have Chl a as the primary photosynthetic pigment and Chl b as the main auxiliary photosynthetic pigment but can be used on most photosynthetic organisms with Chl a as their primary photosynthetic pigment. Modulation fluorometry techniques also work well on *Acaryochloris*, which has a Chl d-based photosynthetic mechanism (Gloag et al. 2007).

In this investigation, I discuss the modelling effective quantum fluorescence yield (Φ_{PSII}) versus irradiance (E) and the use of the Waiting-in-Line Curve as a model for fitting light saturation curves (rETR vs. E) measured as the relative Electron Transport Rate (rETR). The relationship between Effective Quantum Yield (Φ_{PSII}) and rETR will be investigated as well as curves to describe the conventionally calculated Non-Photochemical Quenching (NPQ) (van Kooten and Snel 1990).

Methods

Culture conditions and experimental materials

Synechococcus R-2 (PCC 7942) originating from the Pasteur Culture Collection, *Prochlorothrix hollandica* (PCC 9006) (CCAP 1490/1, Dunstaffnage Marine laboratory, Scotland) and *Chlorella* sp. (Sydney University Algal Culture Collection) were grown in BG-11 medium (Allen 1973). *Acaryochloris marina* (MBIC11017, Marine Biology Institute Culture Collection, Kamaishi, Japan) and the cryptophyte *Rhodomonas* sp. and the marine diatom, *Phaeodactylum* sp. (Sydney University Teaching Collection) were cultured in enriched seawater C medium (MBIC medium No 8) (Ritchie 2006; Gloag et al. 2007). Subterranean Clover (*Trifolium subterraneum*) was growing as a pasture crop at Camden farms (Agronomy Building CO2, Sydney University Camden Farms, Camden, NSW in September 2005).

Cyanobacteria are notorious for giving erratic results in modulation fluorometry experiments. In the present study, it was found that the most consistent results for *Synechococcus* were obtained on cells grown on magnetic stirrer with constant aeration (pH about 7.5–8.5). The other algae were grown on an orbital shaker ($\approx$80 rpm) fitted with overhead fluorescent lights (Sylvania Gro-Lux) in continuous light at about 25°C. The light intensity was

approximately 100 μE m^{-2} s^{-1} (PAR 400−700 nm), using a Li-Cor photon flux meter Model LI-189 (Li-Cor Corp, USA). *Acaryochloris* and *Prochlorothrix* consistently grew better on the edge of the shaker where the light intensity was lower (≈40 μE m^{-2} s^{-1} PAR).

Modulation fluorometry

Light saturation curve measurements were made using a Junior PAM portable chlorophyll fluorometer (Gademann Instruments GmbH, Wurzburg, Germany) fitted with a 1.5-mm diametre optic fibre and a blue diode (485 ± 40 nm) light source. The Junior PAM uses a magnetic clamp to hold specimens about 1 mm from the end of the light pipe. PAM parameters (effective quantum yield, rETR, NPQ) were calculated using the WINCONTROL software (2.133/03.00) using standard settings for rapid light curves (Heinz Walz Gmbh, Effeltrich, Germany) (Genty et al. 1989). The default adsorptance factor of 0.84 and the default value of 0.5 for estimated absorption of light by PSI & PSII were used on the Junior PAM to calculate the relative *E*lectron *T*ransport *R*ate or rETR (see, Schreiber et al. 1995a, b; Kühl et al. 2005). On the standard settings for a rapid light curve (White and Critchley 1999), sets of PAM light curve measurements took about 88s to complete with 10 s between actinic flashes of light and each flash of light was 0.8 s duration. The flashes were in order of increasing intensity but the steady-state fluorescence yield (F_s) did not change by more than 10% over the course of a P versus E run. At least nine rapid light curve experiments were run on independent samples of each species used.

Replicate samples of algal cells (usually 5 or 10 ml cells suspensions) were filtered onto Whatman GF-C glass fibre filters (Whatman International, Maidstone, England, UK) in a Millipore apparatus for 25-mm filters then dark treated in a dish of seawater or BG-11 medium, as appropriate, for at least 10 min. Only one light saturation experiment was run on each filter to avoid confounding effects of multiple experimental treatments. The inside diameter of the Millipore filtration apparatus was 15.9 mm and so the disks of algae adhering to the glass-fibre filter had a surface area of 198.6×10^{-6} m^2. The algal-impregnated disks provided highly reproducible material for experiments. Care was taken to avoid the algae-impregnated disks from drying out. I kept the disks in Petri dishes with wet filter paper (seawater or BG-11 medium as appropriate) in the dark for at least 30 min before use in an experiment. Sub-clover leaves were selected at random and placed in Petri dishes with wet filter paper in the dark to dark-adapt for at least 30 min before use in modulation fluorometry experiments. The measurements were made in the bright green central parts of the leaflets. Leaf disks (6.3 mm diameter) were taken for chlorophyll determination.

Chlorophyll determinations

After photosynthetic electron transport determinations, chlorophyll was extracted from the glass fibre disks using ethanol and chlorophylls determined using the algorithms of Ritchie (2006). Chlorophylls were extracted in 100% (99.5%) ethanol neutralised with magnesium carbonate. It was difficult to extract chlorophyll from *Chlorella*, clover and *Prochlorothrix* in ethanol unless cells were heated in alcohol in a water bath at about 80°C for about 3 min. After dissolving the chlorophylls, the glass or leaf disks were removed, the alcohol extracts made up to 5 ml were then cleared by centrifugation and stored at −20°C as described previously (Ritchie 2006). Soaking algae in ethanol overnight to extract chlorophyll was not employed because it provides an opportunity for chlorophyllase to convert chlorophylls to chlorophyllides. Extracts were stored in the dark in a freezer at −20°C before spectrophotometric assay for as short a time as practicable.

Chlorophylls were determined from spectrophotometric readings made using a Shimadzu UV-2550 UV-visible spectrophotometer using quartz cuvettes as described previously (Ritchie 2006). Generally chlorophyll assays were made within a few hours of extraction or the next day. Replicate disks from the same batch of cells varied by less than ±2% in chlorophyll content.

Calculation of rETR on a chlorophyll basis

ETR is usually calculated on a surface area basis (the surface area of the object illuminated by the beam of light) as mol m^{-2} s^{-1}. This makes it difficult to make interspecific and intraspecific comparisons between different batches of algal cells or different leaves of vascular plants. The diametre of the glass fibre disks of algae or leaf disks and their chlorophyll content were both known and so mg of chlorophyll per square metre could be calculated. rETR in mol m^{-2} s^{-1} was converted to mol mg Chl^{-1} h^{-1} using the chlorophyll assays (as mg chlorophyll m^{-2}). rETR was calculated as mol mg Chl a^{-1} h^{-1} except for *Acaryochloris* where photosynthesis was calculated on a Chl d basis (mol mg Chl d^{-1} h^{-1}).

Statistics

All errors quoted are ±95% confidence limits. The number of replicates are quoted in brackets (*n*), where the brackets contain two numbers (*a* and *b*) the first refers to the number of independent experiments and the second refers to the total number of data points. Curves were fitted by non-linear least squares fitting and the asymptotic errors calculated by matrix inversion (Ritchie 2006; Gloag et al. 2007). EXCEL files to fit the functions used in the present article are available in the Supplementary Material.

Theory

The fluorescence yield was calculated using the WinControl software as the Effective Quantum Yield (Φ_{PSII}) as defined by Genty et al. (1989), van Kooten and Snel (1990) and Franklin and Badger (2001).

$$\Phi_{PSII} = \frac{F'_m - F_s}{F'_m} \quad (1)$$

where, Φ_{PSII} is the Effective Quantum Yield of photosynthesis of a plant given an irradiance E, F'_m is the maximum fluorescence after a flash of saturating light (E), F_s is the background steady-state fluorescence (in the presence of the measuring light ≈ 1 μmol m^{-2} s^{-1}) measured just prior to the pulse of saturating light.

The range of light intensities was adjusted so that, if possible, the optimum light was near the mode of the range of light intensities used. Thus, if photosynthesis saturated at about 200 μmol m^{-2} s^{-1} PAR a range of light intensities up to 351 μmol m^{-2} s^{-1} was appropriate but for plants grown in full sunlight, a range setting was chosen where the maximum light was 1,950 μmol m^{-2} s^{-1} PAR.

Effective Quantum Yield ranges from 0 to 1. It is found experimentally that if Φ_{PSII} is plotted against irradiance (E) it follows a simple exponential decay function of the form $Y = e^{-kx}$ (Poisson-type distribution).

$$\Phi_{PSII} = \Phi_{PSII,max} \cdot e^{-k_w E} \quad (2)$$

where Φ_{PSII} is the effective quantum yield, $\Phi_{PSII,max}$ is the effective quantum yield at theoretical zero irradiance, k_w is a constant and E is the irradiance.

The Electron Transport Rate (ETR) is an estimate of gross photosynthesis is defined as,

$$\begin{aligned} ETR = {} & \Phi_{PSII} \times E \times \text{PSI/PSII allocation factor (0.5)} \\ & \times \text{leaf absorptance factor (0.84)} \end{aligned} \quad (3)$$

where Φ_{PSII} is the effective quantum yield, E is the irradiance (mol m^{-2} s^{-1} PAR), the PSI/PSII allocation factor (0.5) allows for about 50% of quanta being absorbed by PSII and the leaf absorptance constant (0.84) is the mean absorptance factor for plants determined by Bjorkman and Demmig (1987), Knapp and Carter 1998) and Runcie and Durako (2004). Both these default values are approximations but are used routinely.

In Eq. 3, the PSI/PSII allocation factor of 0.5 allows for about 50% of the light being absorbed by PSII. The actual ratio of photosynthetic photosystems vary considerably in different phototrophs and under different growth conditions although considerations of the relative proportions of PSII and PSI and their relative efficiencies in absorbing light result in a more or less equal absorption of the light by the two photosystems (Melis 1989). However, the optimum irradiance and the shape of light curves are not affected by the values of these constants. They affect the calibration of ETR with oxygen evolution or carbon fixation. Since in the present study the default values for the PSII absorption factor (0.5) and the ETR absorption facto (0.84) were used, the ETR quoted in the present study will be designated the relative Electron Transport Rate (rETR). Four electrons are moved through PSII for each O_2 produced in photosynthesis and so an ETR of 4 μmol m^{-2} s^{-1} is equivalent to an approximate gross photosynthetic rate of 1 μmol m^{-2} s^{-1} in terms of oxygen (O_2) evolution.

The rETR values were plotted as light response curves (photosynthetic rate (P) vs. light intensity or irradiance (E); P vs. E). It follows from the finding that plots of Φ_{PSII} versus E obey a simple exponential decay function that plots of rETR versus irradiance should obey an exponential function of the form $Y = x \cdot e^{-x}$. This equation is known as the Waiting-in-Line model (probability density function or exponential waiting time distribution). The Waiting-in-Line equation can be used to model a variety of systems where a rate is initially directly proportional to the amount substrate but eventually saturates at a given substrate density and then is inhibited if the substrate is further increased. The model has been used an empirical model for P (as carbon fixation) versus E curves by Steele (1962) and Jassby and Platt (1976) but it is not used routinely. It does not appear to have been used for ETR versus E curves until recently (Gloag et al. 2007).

The waiting-in-line equation is,

$$Y = x \cdot e^{-x} \quad (4)$$

Equation 4 has a maxima ($dy/dx = 0$) at $x = 1$, the slope of the line at $x = 0$ is 1 and there is a point of inflection ($d^2y/dx^2 = 0$) at $x = 2$. A form suitable for modelling photosynthesis (Gloag et al. 2007) is,

$$P = A \cdot k_w \cdot E \cdot e^{-k_w E} \quad (5)$$

where, P is photosynthesis measured as rETR, O_2 evolution or CO_2 uptake, A is a scaling constant for the height of the curve, k_w is a scaling constant for the X-axis, E is the Irradiance (mol (quanta) m^{-2} s^{-1} 400–700 nm PAR).

In Eq. 5, it can be shown that P_{max} (the maximum velocity) is equivalent to A/e and so an equivalent form of Eq. 5 is $P = P_{max} \cdot k_w \cdot E \cdot e^{1-k_w \cdot E}$. It can also be shown that the exponential constant (k_w) in the exponential yield function (Eq. 2) should equal the k_w value determined by fitting Eq. 5. The P_{max} occurs at an irradiance value of $1/k_w$ ($E_{optimum} = 1/k_w$). The maximum photosynthetic efficiency (Alpha,α) is the initial slope of the curve at $E = 0$ ($\alpha = A \cdot k_w$ or $\alpha = P_{max} \cdot e \cdot k_w$). At very low light intensities photosynthesis is directly proportional to irradiance. It can be shown by analysis of this function that the half-maximum photosynthesis ($P_{half\text{-}max}$) occurs at 0.231961 times $E_{optimum}$ and photosynthesis is inhibited by 50% at 2.67341 times $E_{optimum}$.

From a mechanistic view it is useful to have the differentiated equation for the waiting-in-line model,

$$\frac{dP}{dE} = A \cdot k_w \cdot e^{-k_w E} \cdot (1 - k_w E)$$
$$\text{or} \quad (6)$$
$$\frac{dP}{dE} = P_{max} \cdot k_w \cdot e^{-k_w E} \cdot (1 - k_w E)$$

The differentiated equation has a maximum value at zero light intensity, reflecting the theoretical maximum photosynthetic efficiency (α), a value of zero at the optimum light intensity ($E_{optimum}$) and at infinite light intensity, and a minimum at the point of inflection of Equation 5 (at $E = 2/k_w$ or $2\times E_{optimum}$) (see Figs. 3 or 4). Photosynthesis is inhibited by 26.4% at this point of inflection which lies at twice that of the optimum irradiance. Photosynthesis will decrease almost exponentially at light intensities higher than the point of inflection.

In performing non-linear least squares fits it is usual to assume that the error in the data is a constant independent of the magnitude of the independent variable. In many biological situations the error is often proportional to the magnitude of the independent variable (Johnson and Faunt 1992; Ritchie and Prvan 1996a, b; Ritchie 2006; Gloag et al. 2007). Effective Quantum Yield is calculated as a proportion and so it is reasonable to expect that it would have a constant relative error type of error structure. Where there is a constant relative error it is appropriate to Log/Log transform the data before least squares fitting. The Log/Log transforms of Eqs. 2 and 5 are,

$$\text{Ln}(\Phi_{PSII}) = \text{Ln}(\Phi_{PSII,max}) - k_w E \quad (2a)$$

$$\text{Ln}(P) = \text{Ln}(A) + \text{Ln}(k_w) + \text{Ln}(E) - k_w E \quad (5a)$$

Analysis of Log/Log transformed data requires the elimination of some data points because Log (0) is undefined. In the present study, it was expected that k_w calculated from Φ_{PSII} versus irradiance and rETR versus irradiance curves would be very similar. Where they were found to be different, estimates of k_w from fits of Log/Log transformed Φ_{PSII} versus irradiance data (Eq. 2a) much more closely corresponded to those determined from the rETR versus irradiance data (Eq. 5). In the present study fitted values for constants (A) and (k_w) determined using Eqs. 5 and 5a were found to be similar and so there was no justification in using Eq. 5a over Eq. 5.

Asymptotic alternative fitting models

Light saturation curves are typically fitted to asymptotic curves that reach saturation at infinity. Such models cannot take account of photoinhibition effects. Fitting such models also involves a loss of information about photoinhibition effects at high irradiances. The most common models are the Michaelis–Menten, exponential saturation and hyperbolic tangent models (Jassby and Platt 1976; Ritchie and Prvan 1996a, b; Thornley 1976; Chalker 1981; Harrison and Platt 1986; Strzepek and Harrison 2004; Falkowski and Raven 2007). All of these models have the limitation that data points for irradiances substantially beyond saturation of photosynthesis, where photoinhibition becomes apparent, need to be excluded from the fitting procedure. It is often overlooked that such models are valid for only restricted ranges of irradiances. The equations for these three models are shown in Table 1 with the parameters for the Waiting-in-Line model included for comparison. The asymptotic errors of the k values and P_{max} in each case can be calculated by matrix inversion. The errors of the estimates of saturating irradiance and 1/2 saturating irradiance in the case of the waiting-in-line model and the 1/2 saturating irradiance in the case of the other three equations can then be calculated.

Non Photochemical quenching (NPQ) is a measure of the quenching of the photochemistry of photosynthesis and so is a measure of energy absorbed by the photosynthetic apparatus that is not lost as fluorescence or used in photosynthetic electron transport. NPQ as defined by Genty et al. (1989) and calculated by the WINCONTROL software is,

$$\text{NPQ} = \frac{F_m - F'_m}{F'_m} \quad (7)$$

where F_m is the maximum fluorescence measured in the dark (or more accurately in the presence of the dim background measuring light) and F'_m is the maximum florescence measured in the saturating light pulse.

NPQ represents a loss of potential energy by the system, probably involving a number of factors including energy losses involved in setting up the pH gradient across thylacoid membranes, thermodynamic losses as waste heat and losses in the xanthophyll cycle of photosynthesis in some phototrophs (Genty et al. 1989; Krause and Weis 1991; Ting and Owens 1993; Schreiber et al. 1995a, b; Campbell et al. 1998; Beer and Axelsson 2004; Holt et al. 2004). NPQ typically exhibits a simple exponential saturation curve with a value of zero at the origin and an asymptotic maxima ($\text{NPQ} = \text{NPQ}_{max}(1 - e^{-kE})$, see Table 1 in Holt et al. (2004).

Results

Modulation fluorometry

Figures 1 and 2 show plots of effective quantum yield (Φ_{PSII}) plotted against irradiance for *Synechococcus* and *Chlorella*. Both datasets could be best fitted to an

Table 1 Mathematical models used for light saturation curves

	Waiting-in-Line model	Michaelis–Menten	Exponential saturation	Hyperbolic tangent
Equation	$P = P_{max} \cdot k_w \cdot E \cdot e^{1-k \cdot E}$	$P = \frac{P_{max} \cdot E}{k_m + E}$	$P = P_{max} \cdot (1 - e^{-k_e \cdot E})$	$P = P_{max} \cdot \mathrm{Tanh}(k_h \cdot E)$
Photosynthetic efficiency (α) (dP/dE at E = 0)	$P_{max} \cdot e \cdot k_w$	P_{max}/k_m	$k_e \cdot P_{max}$	$k_h \cdot P_{max}$
1/2 Saturation irradiance	$0.231961/k_w$	k_m	$0.6931/k_e$	$0.5493/k_h$
Saturating light	$1/k_w$	∞	∞	∞

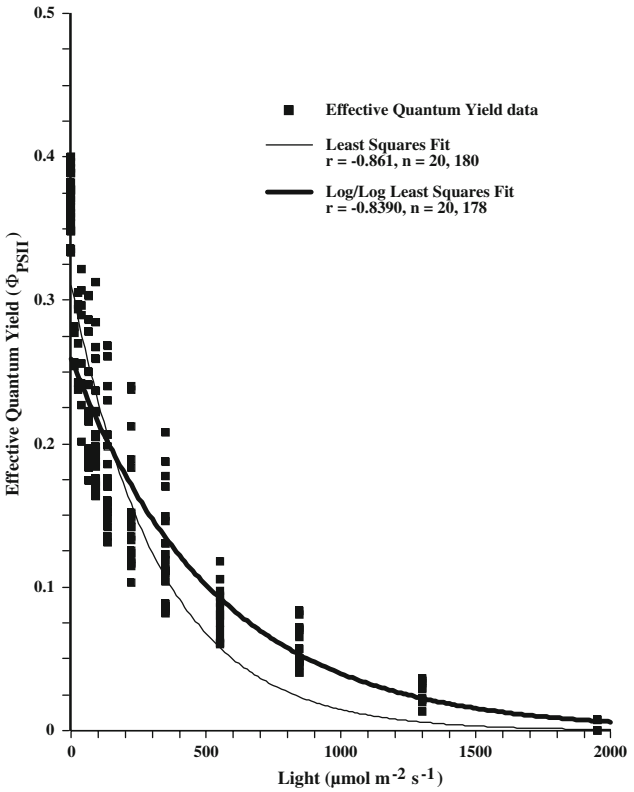

Fig. 1 Effective Quantum Yield (Φ_{PSII}) of *Synechococcus* versus irradiance fitted to an exponential model. Light is irradiance 400–700 nm PAR. Least squares fits to untransformed and Log/Log transformed data sets are shown. Five ml samples of cell suspensions were filtered onto glass fibre disks. The fit is notably poor compared to other classes of photosynthetic organisms

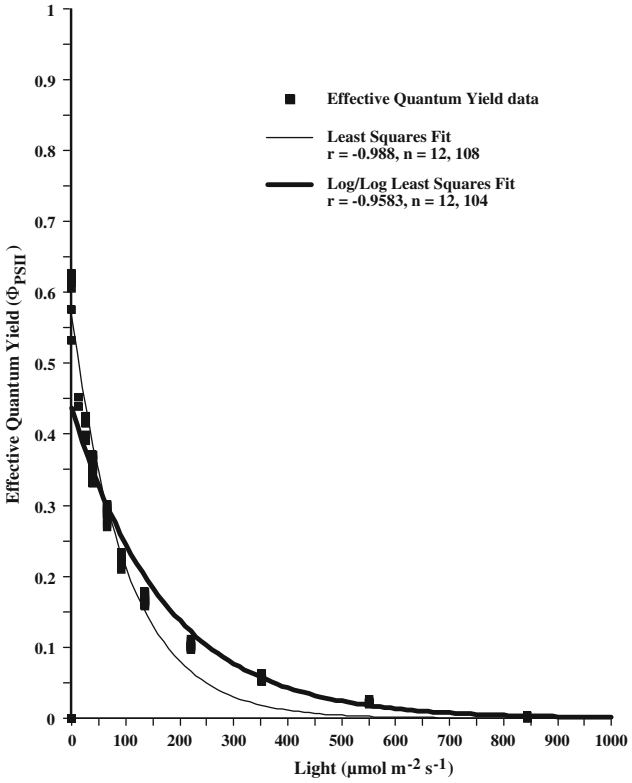

Fig. 2 Effective Quantum Yield (Φ_{PSII}) of *Chlorella* versus irradiance fitted to an exponential model. Light is irradiance 400–700 nm PAR. Least squares fits to untransformed and Log/Log transformed data are shown. Five ml samples of cell suspensions were filtered onto glass fibre disks

exponential decay function after Log/Log transformation of the data but transformation of the other data sets was not judged to be necessary because the estimates of k_w in Table 2 on untransformed data were close to those in Table 3. The statistics for the fitted curves are shown in Table 2 for *Synechococcus, Prochlorothrix, Chlorella*, Subterranean Clover, *Rhodomonas, Phaeodactylum* and *Acaryochloris*. Where the algae had more than one type of chlorophyll, the chlorophyll ratios found in the present study (Table 2) are similar to those found for the algae in previous studies (Ritchie 2006). All fits were highly significant ($P \ll 0.001$) but the fit was notably poorer for *Synechococcus* and *Rhodomonas* compared to *Chlorella* and the other representatives of the classes of photosynthetic organisms used in the present study.

Waiting-in-Line curves were fitted to plots of rETR versus irradiance. Figures 3 and 4 show light saturation curves for *Synechococcus* and *Chlorella*. Plots for the other species are available in the Supplementary material. The fitted parameters for all seven species used in the present study are shown in Table 3. P_{max} is quoted on both an rETR and O_2 basis ($P_{max,\ rETR}$ is four times higher than $P_{max,Oxygen\ basis}$). Photosynthetic efficiencies are also quoted on rETR and oxygen (O_2) bases. *Synechococcus* had the highest saturating photosynthetic rate on a chlorophyll basis ($P_{max,\ rETR} = 1252 \pm 89$ µmol mg Chl a^{-1} h^{-1}) and *Rhodomonas* had the lowest ($P_{max,\ rETR} = 72 \pm 9$ µmol

Table 2 Exponential decay parameters fitted to plots of effective quantum yield (Φ_{PSII}) versus irradiance

	Synechococcus *R*-2	*Prochlorothrix hollandica*	*Chlorella* sp.	*Trifolium subterraneum*	*Rhodomonas* sp.	*Phaeodactylum* sp.	*Acaryochloris marina*
Exponential (k_w)	0.001877 ± 0.0009993	0.01208 ± 0.0004264	0.005800 ± 0.0001874	0.003432 ± 0.0007194	0.02417 ± 0.002554	0.01931 ± 0.001398	0.006549 ± 0.0001929
Maximum yield ($F_{v,o}$)	0.2589 ± 0.01278	0.5250 ± 0.007976	0.4375 ± 0.0200	0.5602 ± 0.0554	0.2257 ± 0.01163	0.4263 ± 0.01458	0.6116 ± 0.007790
Correlation *r* (number of samples, data points)	−0.8390 (20,178)	−0.9959 (9,81)	−0.9583 (12,104)	−0.8492(11,99)	−0.9428(16,144)	−0.9706 (16,144)	−0.9954 (16,144)
Transform	Log/Log transformed	No Log/Log transform	Log/Log transformed	No Log/Log transform	No Log/Log transform	No Log/Log transform	No Log/Log transform

All except the *Trifolium* experiments were done on cell suspensions impregnated onto glass fibre disks (*Synechococcus*, 59.4 ± 0.383 mg Chl a m^{-2}; *Prochlorothrix*, 67.4 ± 0.803 mg Chl a m^{-2}, Chl b/a = 0.290 ± 0.0045; *Chlorella*, 90.8 ± 0.255 mg Chl a m^{-2}, Chl b/a = 0.489 ± 0.00632; Trifolium *subterraneum* leaf disks, 346 ± 18.4 mg Chl a m^{-2}, Chl b/a = 0.482 ± 0.0103; *Rhodomonas*, 68.08 ± 0.74 mg Chl a m^{-2}, Chl c_1/a = 0.182 ± 0.0013; *Phaeodactylum*, 71.9 ± 0.657 mg Chl a m^{-2}, Chl c_1c_2/a = 0.237 ± 0.011; *Acaryochloris*, 106 ± 0.80 mg Chl d m^{-2}, Chl a/d = 0.0296 ± 0.00031). The number of separate disks of cells and the total number of data points used are shown in brackets. All decay curves fitted an exponential decay model but some required Log/Log transformation of the data

mg Chl a^{-1} h^{-1}). However, the saturating light intensity was very high for *Synechococcus* (598 ± 41 μmol m^{-2} s^{-1}) but much lower for the other algae (≈40–200 μmol m^{-2} s^{-1}). *Prochlorothrix*, *Chlorella* and subterranean clover (all Chl a + b photoautotrophs) shared the highest photosynthetic efficiencies compared to the other autotrophs. As would be expected, the subterranean clover grown in full spring sunlight had the highest saturating light intensity ($E_{optimum}$ = 908 ± 53 μmol m^{-2} s^{-1} PAR). All statistical fits to the Waiting-in-Line model were highly significant ($P \ll 0.001$).

Tables 4 and 5 compare the curve fits for the Waiting-in-line, Michael–Menten, exponential saturation and hyperbolic tangent models for the same sets of data for *Synechococcus* and *Chlorella*. (For similar analyses for *Prochlorothrix*, Subterranean Clover, *Rhodomonas*, *Phaeodactylum* and *Acaryochloris* please refer to the Supplementary Material). The Waiting-in-Line models in Table 3 for the algae utilised the full data sets. The analyses shown in Tables 4 & 5 (and the Tables in the Supplementary Material) used the photosynthesis versus irradiance data only up to the first light intensity above the calculated saturating light intensity shown in Table 3. All four models gave quite good statistical fits ($P \ll 0.001$) but the correlations were considerably worse for *Synechococcus* ($r \approx 0.91$) and *Rhodomonas* ($r \approx 0.86$) than for the other algae ($r \approx 0.95$ or higher). The Michaelis–Menten model consistently overestimated P_{max} and photosynthetic efficiency (α). In all cases the hyperbolic tangent model gave estimates of P_{max}, 1/2-saturating light intensity and photosynthetic efficiency closest to those obtained using the Waiting-in-Line model. The exponential saturation model also generally gave estimates of P_{max} and 1/2 saturating light intensity very close to the Waiting-in-Line model but tended to overestimate the photosynthetic efficiency (α).

Table 6 shows the results of non-linear least squares fits of a simple exponential saturation model to plots of the conventionally calculated NPQ versus irradiance in the six types of photoautotrophs used in the present study fitted to (see Table 1). This seems a logical consequence of the observation that Effective Quantum Yield versus irradiance curves follow an exponential decay model (Table 2). Attempts were made to fit Michaelis-Menten and Tanh curves to the NPQ data (not shown) but in each case the exponential saturation curves produced a better fit (correlation closer to 1). The exponential saturation model fitted the NPQ versus irradiance curves very well ($P \ll 0.001$) for *Prochlorothrix*, *Chlorella*, Subterranean Clover, *Rhodomonas*, *Phaeodactylum* and *Acaryochloris* but except at very low irradiances all the NPQ values for *Synechococcus* were zero and so there was no significant fit of the exponential saturation model for the *Synechococcus* data. The

Table 3 Light saturation curve parameters fitted to rETR versus irradiance using the Waiting-in-Line model using the same data sets as those used to obtain the steady state fluorescence decay measurements shown in Table 1

	Synechococcus R-2	*Prochlorothrix hollandica*	*Chlorella* sp.	*Trifolium subterraneum*	*Rhodomonas* sp.	*Phaeodactylum* sp.	*Acaryochloris marina*
Exponential (*k*)	0.00167 ± 0.000115	0.01240 ± 0.000579	0.00558 ± 0.000177	0.001101 ± 0.0000648	0.0224 ± 0.00218	0.0141 ± 0.000649	0.00515 ± 0.000103
Maximum photosynthesis (P_{max})	1252 ± 88.7 (ETR μmol mg Chl a^{-1} h^{-1}) 313 ± 22.2 (μmol O_2 mg Chl a^{-1} h^{-1})	348 ± 20.1(ETR μmol mg Chl a^{-1} h^{-1}) 87.0 ± 5.03 (μmol O_2 mg Chl a^{-1} h^{-1})	812 ± 30.6 (ETR μmol mg Chl a^{-1} h^{-1}) 203 ± 7.65 (μmol O_2 mg Chl a^{-1} h^{-1})	490 ± 32.4 (ETR μmol mg Chl a^{-1} h^{-1}) 127 ± 8.10 (μmol O_2 mg Chl a^{-1} h^{-1})	71.5 ± 9.44 (ETR μmol mg Chl a^{-1} h^{-1}) 17.9 ± 2.36 (μmol O_2 mg Chl a^{-1} h^{-1})	178 ± 10.3 (ETR μmol mg Chl a^{-1} h^{-1}) 44.5 ± 2.58 (μmol O_2 mg Chl a^{-1} h^{-1})	528 ± 12.8(ETR μmol mg Chl d^{-1} h^{-1})132 ± 3.20 (μmol O_2 mg Chl a^{-1} h^{-1})
Optimum light (μmol m^{-2} s^{-1})	598 ± 41.2	80.7 ± 3.77	179 ± 5.66	908 ± 53.45	44.7 ± 4.37	70.8 ± 3.25	194 ± 3.90
1/2 Optimum light (μmol m^{-2} s^{-1})	139 ± 9.55	18.7 ± 0.875	41.5 ± 1.31	211 ± 12.4	10.4 ± 1.01	16.4 ± 0.754	45.1 ± 0.904
Inflection Irradiance (μmol m^{-2} s^{-1})	1194 ± 82.4	161 ± 7.34	358 ± 11.3	1816 ± 107	89.4 ± 8.72	141 ± 6.50	388 ± 7.80
Photosynthetic Efficiency (Alpha, α),	$\alpha_{ETR} = 158 \pm 15.6$ ($\times 10^{-6}$ m^2 mg Chl a^{-1}) $\alpha_{O2} = 39.5 \pm 3.90$ ($\times 10^{-6}$ m^2 mg Chl a^{-1})	$\alpha_{ETR} = 325 \pm 24.2$ ($\times 10^{-6}$ m^2 mg Chl a^{-1}) $\alpha_{O2} = 81.4 \pm 6.05$ ($\times 10^{-6}$ m^2 mg Chl a^{-1})	$\alpha_{ETR} = 342 \pm 16.8$ ($\times 10^{-6}$ m^2 mg Chl a^{-1}) $\alpha_{O2} = 85.6 \pm 4.20$ ($\times 10^{-6}$ m^2 mg Chl a^{-1})	$\alpha_{ETR} = 407 \pm 36.0$ ($\times 10^{-6}$ m^2 mg Chl a^{-1}) $\alpha_{O2} = 101.8 \pm 9.00$ ($\times 10^{-6}$ m^2 mg Chl a^{-1})	$\alpha_{ETR} = 121 \pm 19.8$ ($\times 10^{-6}$ m^2 mg Chl a^{-1}) $\alpha_{O2} = 30.2 \pm 4.95$ ($\times 10^{-6}$ m^2 mg Chl a^{-1})	$\alpha_{ETR} = 190 \pm 14.0$ ($\times 10^{-6}$ m^2 mg Chl a^{-1}) $\alpha_{O2} = 47.5 \pm 3.50$ ($\times 10^{-6}$ m^2 mg Chl a^{-1})	$\alpha_{ETR} = 205 \pm 6.45$ ($\times 10^{-6}$ m^2 mg Chl d^{-1}) $\alpha_{O2} = 51.3 \pm 1.61$ ($\times 10^{-6}$ m^2 mg Chl a^{-1})
Correlation *r* (number of samples, data points)	0.9139 (20,180)	0.9654 (9,81)	0.9702 (12,108)	0.9503 (9,99)	0.8304(16,144)	0.9424 (16,144)	0.9851 (16,144)

rETR has been standardised onto chlorophyll *a* as μmol mg Chl a^{-1} h^{-1} except in the case of *Acaryochloris* where it has been calculated on a chlorophyll *d* basis. All the light saturation curves fitted the waiting-in-line model without Log/Log transformation

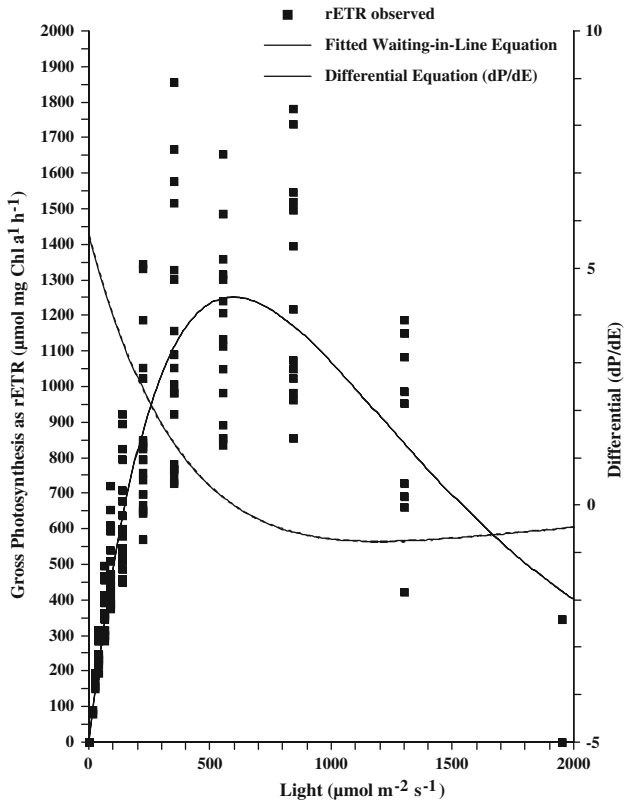

Fig. 3 Light saturation data on *Synechococcus* fitted to the Waiting-in-Line model. Relative Electron Transport Rates (rETR) have been standardised on the chlorophyll *a* content of cell suspensions impregnating glass fibre disks. Light is irradiance 400–700 nm PAR. The differential with respect to light (dP/dE) is also shown on the right Y-axis. The fit is notably poor compared to other classes of photosynthetic organisms

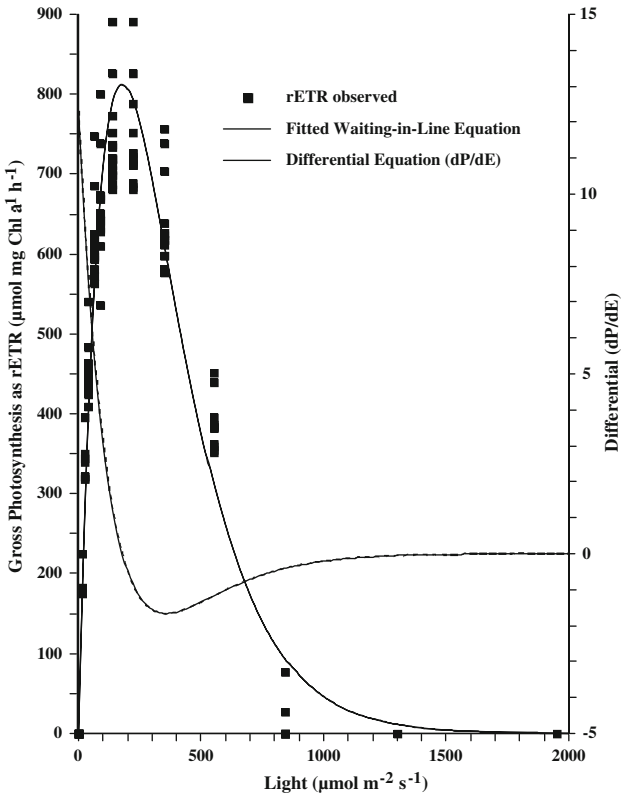

Fig. 4 Light saturation data on *Chlorella* fitted to the Waiting-in-Line model. Relative Electron Transport Rates (rETR) have been standardised on the chlorophyll *a* content of cell suspensions impregnating glass fibre disks. Light is irradiance 400–700 nm PAR. The differential with respect to light (dP/dE) is also shown on the right Y-axis

exponential constants of Effective Quantum Yield versus irradiance (Table 2) and for the rETR versus irradiance fits (Table 3) do not seem to be related to those found for the NPQ versus irradiance curves (Table 6). Log/Log transformation of the NPQ versus irradiance data did not improve the correspondence of the k values estimated with those shown in Tables 2 and 3.

Discussion

Table 3 shows that optimum irradiance varied from a high of 908 µmol m^{-2} s^{-1} PAR for Subterranean Clover grown in full daylight, and 600 µmol m^{-2} s^{-1} PAR in *Synechococcus* to a low of only 45 µmol m^{-2} s^{-1} PAR in the case of *Rhodomonas* even though all the algae were grown under 50–100 µmol m^{-2} s^{-1} PAR. Thus, with the notable exception of *Synechococcus*, the optimum light intensity found using the modulation fluorometry data was approximately similar to the conditions under which the clover or algae had been grown. All the other phototrophs showed classical photoadaptation to the conditions under which they were grown with severe photoinhibition at supraoptimimal irradiances (Larkum et al. 2003). The classic cyanobacterium, *Synechococcus* seems to be exceptional in having a constitutive capacity to tolerate much higher light intensities than those it had previously encountered while growing in aerated culture.

Models for P versus irradiance curves are typically treated in the literature as empirical models rather than necessarily having an underlying theoretical basis (Steele 1962; Jassby and Platt 1976; Thornley 1976; Chalker 1981; Falkowski and Raven 2007). The Waiting-in-Line model discussed in the present article was introduced as an empirical model for P versus irradiance curves (measured as ^{14}C-fixation) by Steele (1962) and Jassby and Platt (1976) and for modulation fluorometry light saturation curves by Gloag et al. (2007). A model with a physiological basis has more value than an empirical model because of its much greater predictive value and hence testability. I have shown in the Theory that if the effective quantum yield versus irradiance relation obeys a simple exponential decay model then it follows that rETR would fit an equation of the form $Y = x \cdot e^{-x}$ simply because

Table 4 Comparison of the Light saturation curve parameters fitted to rETR versus irradiance using the Waiting-in-Line, Michaelis–Menten, Exponential saturation and hyperbolic tangent models for *Synechococcus*

	Waiting-in-Line model	Michaelis–Menten model	Exponential saturation model	Hyperbolic tangent
K	0.00164 ± 0.000160	204 ± 29.1	0.00521 ± 0.000842	0.00427 ± 0.000564
Maximum photosynthesis (P_{max})	1258 ± 103 (ETR µmol mg Chl a^{-1} h^{-1}) 315 ± 25.8 (µmol O_2 mg Chl a^{-1} h^{-1})	1615 ± 68 (ETR µmol mg Chl a^{-1} h^{-1}) 404 ± 17 (µmol O_2 mg Chl a^{-1} h^{-1})	1264 ± 84 (ETR µmol mg Chl a^{-1} h^{-1}) 316 ± 21 (µmol O_2 mg Chl a^{-1} h^{-1})	1213 ± 69.6 (ETR µmol mg Chl a^{-1} h^{-1}) 303 ± 17.4 (µmol O_2 mg Chl a^{-1} h^{-1})
Optimum light (µmol m^{-2} s^{-1})	610 ± 59.7	Not applicable	Not applicable	Not applicable
1/2 Optimum light (µmol m^{-2} s^{-1})	141 ± 13.9	204 ± 29.1	133 ± 21.5	129 ± 17.0
Inflection irradiance (µmol m^{-2} s^{-1})	1220 ± 119	Not applicable	Not applicable	Not applicable
Photosynthetic efficiency (Alpha, α),	α_{ETR} = 155 ± 19.8 ($\times 10^{-6}$ m^2 mg Chl a^{-1}) α_{O2} = 38.9 ± 4.95 ($\times 10^{-6}$ m^2 mg Chl a^{-1})	α_{ETR} = 220 ± 32.8 ($\times 10^{-6}$ m^2 mg Chl a^{-1}) α_{O2} = 55.1 ± 8.2 ($\times 10^{-6}$ m^2 mg Chl a^{-1})	α_{ETR} = 183 ± 72.6 ($\times 10^{-6}$ m^2 mg Chl a^{-1}) α_{O2} = 45.8 ± 18.2 ($\times 10^{-6}$ m^2 mg Chl a^{-1})	α_{ETR} = 144 ± 20.7 ($\times 10^{-6}$ m^2 mg Chl a^{-1}) α_{O2} = 36.0 ± 5.18 ($\times 10^{-6}$ m^2 mg Chl a^{-1})
Correlation r (number of samples, data points)	0.9137 (20,166)	0.9131 (20,166)	0.9140 (20,166)	0.9126 (20,166)

The Waiting-in-Line model in Table 3 utilised the full data set up to 1950 µmol m^{-2} s^{-1} PAR but since the optimum irradiance was found to be at about 600 µmol m^{-2} s^{-1} PAR, the rETR measurements at irradiances above 851 µmol m^{-2} s^{-1} PAR were omitted from use in fitting the curves

rETR is directly proportional to irradiance multiplied by the effective quantum yield. The waiting-in-line equation is used to model a very wide range of phenomena involved in entities filing in queues where a rate of entities passing through a system is at first directly proportional to numbers in the queue but eventually the finite time required to deal with individual items in the queue result in a saturated rate; if more individuals join the queue this results in congestion resulting in a decrease in the rate of processing items in the queue. It is therefore not surprising that the light reactions of photosynthesis can be fitted to a waiting-in-line equation because electrons are removed from water by the oxygen-evolving complex in a sequential manner and electrons flow through PSII, the electron transport chain and PSI in a file.

Modulation fluorometry methods are often difficult to use with cyanobacteria such as *Synechococcus R-2* (PCC 7942) (Schreiber et al. 1995a, b; Campbell et al. 1998). In my own experience PAM machines using blue-diode actinic light seem to give more consistent results than machines using red diode light sources. However, cyanobacterial material often gives fluorescence yields of zero at all irradiances even though oxygen electrode determinations show that the cyanobacterial cells evolved oxygen in the light. Even if a measurable effective quantum yield is detected, it is often difficult to obtain consistent results. It can be difficult to meaningfully compare results from different laboratories that used different experimental protocols and material grown under different culture conditions.

In the present study, I found that *Synechococcus* gave a measurable effective quantum yield or rETR only if the cells were grown with aeration and stirring. Even so, no significant NPQ was detectable. As pointed out previously, *Synechococcus* can be very unpredictable in PAM fluorescence studies; some cultures giving measurable effective quantum yields and rETR and others no detectable effective quantum yield at all (Gloag et al. 2007). In contrast, in other unpublished work I have found that useful PAM results can be consistently obtained on *Arthrospira* sp. cultures isolated from cyanobacterial crusts of marine beach rock similar to those studied in the field by Schreiber et al. (2002). Another classical cyanobacterium where I have been able to use a PAM fluorometer to obtain useful results is *Nodularia spumigena* (NSG L02-A1) (unpublished). As found in *Synechococcus*, no significant NPQ was detectable in *Arthrospira* or *Nodularia*. Recently Wilson et al. (2006) were able to successfully measure Effective Quantum Yield, rETR and NPQ in *Synechocystis* (PCC 6803) and compare wild-type responses to various mutants.

PAM fluorometers also work consistently well on the prochlorophyte *Prochloron* both in vivo and in situ and show light response curves that would fit a waiting-in-line

Table 5 Comparison of the Light saturation curve parameters fitted to rETR versus irradiance using the Waiting-in-Line, Michaelis–Menten, Exponential saturation and hyperbolic tangent models for *Chlorella*

	Waiting-in-Line model	Michaelis–Menten model	Exponential saturation model	Hyperbolic tangent
K	0.00665 ± 0.000297	36.7 ± 2.88	0.02407 ± 0.001911	0.01838 ± 0.001203
Maximum photosynthesis (P_{max})	773 ± 31.9(ETR μmol mg Chl a^{-1} h^{-1})193 ± 7.98 (μmol O_2 mg Chl a^{-1} h^{-1})	903 ± 25.6 (ETR μmol mg Chl a^{-1} h^{-1}) 226 ± 6.40 (μmol O_2 mg Chl a^{-1} h^{-1})	751 ± 18.8(ETR μmol mg Chl a^{-1} h^{-1}) 188 ± 4.70 (μmol O_2 mg Chl a^{-1} h^{-1})	733 ± 16.2 (ETR μmol mg Chl a^{-1} h^{-1}) 183 ± 4.05 (μmol O_2 mg Chl a^{-1} h^{-1})
Optimum light (μmol m^{-2} s^{-1})	150 ± 6.71	Not applicable	Not applicable	Not applicable
1/2 Optimum light (μmol m^{-2} s^{-1})	34.9 ± 1.56	36.7 ± 2.88	28.8 ± 2.29	29.9 ± 1.96
Inflection irradiance (μmol m^{-2} s^{-1})	300 ± 13.4	Not applicable	Not applicable	Not applicable
Photosynthetic efficiency (Alpha, α),	α_{ETR} = 388 ± 23.6 ($\times 10^{-6}$ m^2 mg Chl a^{-1}) α_{O2} = 97.0 ± 5.90 ($\times 10^{-6}$ m^2 mg Chl a^{-1})	α_{ETR} = 684 ± 57.2 ($\times 10^{-6}$ m^2 mg Chl a^{-1}) α_{O2} = 171 ± 14.3 ($\times 10^{-6}$ m^2 mg Chl a^{-1})	α_{ETR} = 502 ± 41.8 ($\times 10^{-6}$ m^2 mg Chl a^{-1}) α_{O2} = 126 ± 10.5 ($\times 10^{-6}$ m^2 mg Chl a^{-1})	α_{ETR} = 374 ± 25.8 ($\times 10^{-6}$ m^2 mg Chl a^{-1}) α_{O2} = 93.5 ± 6.45 ($\times 10^{-6}$ m^2 mg Chl a^{-1})
Correlation *r* (number of samples, data points)	0.9806 (12,99)	0.9806 (12,99)	0.9849 (12,99)	0.9847 (20,99)

The Waiting-in-Line model utilised in Table 3 the full data set up to an irradiance of 351 μmol m^{-2} s^{-1} PAR but since the optimum irradiance was found to be at about 180 μmol m^{-2} s^{-1} PAR the rETR measurements at irradiances above 221 μmol m^{-2} s^{-1} PAR were omitted from use in fitting the curves shown in this table

Table 6 Exponential saturation parameters fitted to plots of Non-Photochemical Quenching (NPQ) versus irradiance

	Synechococcus R-2	*Prochlorothrix hollandica*	*Chlorella* sp.	*Trifolium subterraneum*	*Rhodomonas* sp.	*Phaeodactylum* sp.	*Acaryochloris marina*
Exponential (k_w)	0.00000399± 0.0255	0.01178 ± 0.000764	0.00783 ± 0.000877	0.001971± 0.000384	0.009133 ± 0.00161	0.007056 ± 0.00165	0.004683 ± 0.000672
Maximum NPQ (E_∞)	0.0954± 609	0.1233 ± 0 .00340	0.6068 ± 0.0263	1.774 ± 0.146	0.3525 ± 0.0287	0.4856 ± 0.0636	0.1147 ± 0.00741
Correlation *r* (number of samples, data points)	0.229 n.s.(20,178)	0.9926 (9,81)	0.9628 (12,104)	0.9307(9,93)	0.9305 (16,144)	0.9215 (16,144)	0.9525(16,144)

The number of separate disks of cells and the total number of data points used are shown in brackets. All curves fitted an exponential saturation model except for the classic cyanobacterium *Synechocccus R-2* which showed nearly all zero NPQ values with no consistent pattern. Fits to the Michaelis–Menten and Tanh models were inferior to those obtained using the exponential saturation model and so are not shown

model very well (Schreiber et al. 1997; Chen et al. 2005a). PAM machines have also been used successfully on the prochlorophyte *Prochlorococcus* (Partensky et al. 1993, Moore et al. 1995; Bailey et al. 2005) and marine microbenthos communities apparently dominated by various cyanobacteria (Hartig et al. 1998).

It seems that oxyphotobacteria allied to cyanobacteria, but with photosynthetic pigment compositions different to classical cyanobacteria (*Acaryochloris*, *Prochlorothrix* and *Prochloron*) often have fluorescence properties more similar to eukaryotic photoautotrophs than the classical cyanobacteria, perhaps reflecting differences in light-harvesting complexes or electron transport mechanisms (Holt et al. 2004; Bailey et al. 2005). All these oxygenic photobacteria exhibit classical photoinhibition at high irradiances above the conditions under which they were grown.

In contrast, all the classical cyanobacteria in which I have been able to determine a significant effective fluorescent yield (Effective Quantum Yield or Φ_{PSII}) at a range of irradiances and plot rETR versus irradiance curves have very high apparent optimum light intensities (500–1,000 μmol m^{-2} s^{-1} PAR) which are far higher than the conditions under which they had been grown in culture (50–100 μmol m^{-2} s^{-1} PAR). Light saturation curves on *Synechocystis* (PCC6803) performed using oxygen electrode methods also have a high light saturation point and little or no photoinhibition in full sunlight (Wilson et al. 2006). Perhaps classical cyanobacteria have a constitutive ability to minimize photoinhibition at high irradiances.

PAM effective quantum yield (Φ_{PSII}) and light saturation curves carried out on *Prochlorothrix* and *Acaryochloris* appear to yield results similar to those for most other oxygenic photosynthetic organisms (Genty et al. 1989; Krause and Weis 1991; Schreiber et al. 1995a, b; Gloag et al. 2007) in contrast to those of typical cyanobacteria (Campbell et al. 1998). The photosynthetic efficiency (α) expressed in terms of oxygen (O_2) evolution (Tables 3 and 6) was found to be higher in *Acaryochloris* than data reported previously that was measured using a chamber-type oxygen electrode (about 438×10^{-6} m^2 mg Chl d^{-1} for red light and about 298×10^{-6} m^2 mg Chl d^{-1}) for white light (Gloag et al. 2007). This probably is a result of the PAM experiments described here measuring rETR using a beam of blue light on cells held stationary in a glass fibre matrix, whereas in an oxygen electrode the cells are in a stirred suspension and experience a scattered light source. In order to investigate the correlation between photosynthesis using PAM methods and either oxygen electrode or IRGA methods for measuring photosynthesis, a setup with equivalent light sources on photosynthetic preparations with similar geometry would be needed: for example an IRGA designed for PAM and CO_2-fixation measurements on leaves (Beer et al. 2000; Franklin and Badger 2001; Longstaff et al. 2002). In principle, using such setups it is possible to calibrate ETR onto O_2 or carbon-fixation based measures of photosynthesis and so replace rETR with an absolute ETR value. Unicellular algae suspended on glass fibres disks as used in the present study would be excellent material to systematically investigate the problem. However, taking advantage of the geometry of the flat-sheeted green alga *Ulva* it has been shown that the correlation between CO_2 fluxes and ETR is poor at high irradiances because ETR overestimated photosynthesis (Beer et al. 2000; Longstaff et al. 2002; Beer and Axelsson 2004). Thus the allocation of quanta to PSI and PSII (assumed here to be 0.5 to calculate rETR, see Theory) is not necessarily a constant.

Tables 3–5 show that the Waiting-in-Line curve is a very good model for P versus irradiance curves even if values at supraoptimum irradiances are not included in analyses. The curve gives estimates of maximum photosynthesis and 1/2 saturating light that are comparable to the Michaelis–Menten, exponential saturation and hyperbolic tangent models which are all asymptotic at infinite light and take no account of photoinhibition. In any set of P versus irradiance data from anything but the most unrealistic conditions there will always be some photoinhibition observed at high light conditions. A model capable of predicting photosynthesis at suboptimal, optimal and supraoptimal light is obviously a superior model to those that do not. The Michaelis–Menten model (Table 1) from enzymology has some justification as a potentially useful model for the light reactions of photosynthesis; however the light reactions involve many enzymes arranged in a complex series. For that reason it is not a good model for photosynthesis because it saturates too slowly and overestimates photosynthetic efficiency (Tables 3–6) (Jassby and Platt 1976; Thornley 1976; Chalker 1981; Harrison and Platt 1986; Beer et al. 2000; Longstaff et al. 2002; Falkowski and Raven 2007).

The exponential saturation model (Table 1) is also a good fit for P versus irradiance data sets restricted to rETR versus irradiance data below where substantial photoinhibition occurs (Tables 4 & 5 and Supplementary Material). The equation can be treated as an empirical relation (Thornley 1976; Chalker 1981; Harrison and Platt 1986; Falkowski and Raven 2007) or justified as a sharply saturating hyperbolic curve which can be used to represent the behaviour of a metabolic pathway of many enzymes arranged in series.

The hyperbolic tangent model (Table 1) was found in this study to be the best asymptotically saturating model for photosynthesis and has been a popular empirical model for P versus irradiance curves (Jassby and Platt 1976; Thornley 1976; Chalker 1980, 1981; Harrison and Platt 1986; Frenette et al. 1993; Beer et al. 2000; Longstaff et al.

2002; Strzepek and Harrison 2004; Falkowski and Raven 2007). The curve has some theoretical justification because it has been found to model systems with entities, which form up in a file, but do not congest and so the curve saturates asymptotically (Chalker 1980, 1981; Frenette et al. 1993). The Tanh(x) function increases almost linearly at low values of x, then rapidly flattens as it reaches its asymptote. It closely approximates a Blackman-type response curve (Thornley 1976).

The Platt (Exponential Difference) equation has frequently been used to model P versus irradiance curves that show photoinhibition in oxyphotobacteria and eukaryotic photosynthetic organisms including corals (Jassby and Platt 1976; Harrison and Platt 1986; Frenette et al. 1993; Longstaff et al. 2002; MacIntyre et al. 2002; Ulstrup et al. 2006). It does not seem to be widely known that its origin is empirical (Jassby and Platt 1976) rather than having theoretical justification. The physiological significance of the fitted parameters is not clear. The mathematics of fitting the Platt equation is set out by Jassby and Platt (1976) and Harrison and Platt (1986). A simple form of the Platt equation is,

$$\mathrm{P} = X \cdot (\mathrm{e}^{-k_1\mathrm{E}} - \mathrm{e}^{-k_2\mathrm{E}}) \tag{8}$$

Equation 8 is often expressed in a more cumbersome form as,

$$\mathrm{P} = X \cdot (1 - \mathrm{e}^{(k_1-k_2)\mathrm{E}}) \cdot \mathrm{e}^{-k_1\mathrm{E}} \tag{8a}$$

where, $K_2 > K_1 > 0$ and E is the irradiance.

Optimum light at dP/dE = 0

$$\mathrm{dP/dE} = -k_1 \cdot X \cdot \mathrm{e}^{-k_1\mathrm{E}} + k_2 \cdot X \cdot \mathrm{e}^{-k_2\mathrm{E}}$$

$$0 = -k_1 \cdot X \cdot \mathrm{e}^{-k_1\mathrm{E}} + k_2 \cdot X \cdot \mathrm{e}^{-k_2\mathrm{E}}$$

$$\therefore \mathrm{E_{optimum}} = \frac{\mathrm{Ln}(k_2) - \mathrm{Ln}(k_1)}{k_2 - k_1} \text{ and } \mathrm{P_{max}} = X \cdot (\mathrm{e}^{-k_1\mathrm{E_{opt}}} - \mathrm{e}^{-k_2\mathrm{E_{opt}}})$$

Photosynthetic Efficiency (α) = $X \cdot (k_2 - k_1)$.

The Platt equation is sometimes expressed in forms that make it difficult to recognise that they are forms of Eq. 8. The Platt equation is not recommended by the author for two reasons. One reason is that three, rather than two parameters need to be fitted and so it is fundamentally more difficult to fit Eqs. 8 or 8a to experimental data than using Eq. 5. The errors of X, k_1 and k_2 will be large and hence predicted irradiance parameters such as $\mathrm{E_{optimum}}$ and 1/2 $\mathrm{E_{optimum}}$ and $\mathrm{P_{max}}$ values will have large inherent errors. Most authors who use the model do not attempt to calculate the errors of the fitted parameters: if they calculated the asymptotic errors by matrix inversion (Johnson and Faunt 1992) they would be discouraged from using the model. For example, if we take the *Chlorella* data shown in Fig. 4 and fit it to Eq. 8, the correlation is very high ($r = 0.9758$, $P \ll 0.001$) but the estimate of the Optimum light is 166 ± 132 μmol $\mathrm{m^{-2}}$ $\mathrm{s^{-1}}$ PAR and the Pmax is 788 ± 336 μmol mg Chl $\mathrm{a^{-1}}$ $\mathrm{h^{-1}}$. These estimates are so imprecise (relative errors, ±80% and ±43% respectively) that the model has limited predictive value compared to those made using the waiting-in-line equation shown in Table 3.

The other reason is that Eq. 8 is actually redundant: different values for k_1 and k_2 in Eq. 8 generate a family of curves, all of which can be fitted to the simpler Eq. 5. It therefore follows from Eq. 5 that the 1/2 optimum irradiance is at 0.231961 × $\mathrm{E_{optimum}}$ and the point of inflection of the P versus irradiance curve is at 2 × $\mathrm{E_{optimum}}$. Algebraically $k_w = (k_2 - k_1)/\mathrm{Ln}(k_2/k_1)$ and so $\mathrm{P_{max}} = X \cdot (k_2 - k_1)/(\mathrm{e} \cdot k_w)$.

The NPQ measured in the present study (Eq. 7) was the conventionally calculated expression for NPQ. A measurable NPQ was found in light curves of all the phototrophs used in the present study except *Synechococcus* and some preliminary work on *Nodularia* and *Arthrospira* (data not shown).

Classic cyanobacteria such as *Synechococcus* (*Nodularia* and *Arthrospira*, unpublished) typically show no measurable NPQ as traditionally defined by Eq. 7 (Schreiber et al. 1995a, b; Campbell et al. 1998; Holt et al. 2004). No consistently measurable NPQ was found in *Synechococcus* (or *Nodularia* or *Arthrospira*) in the present study. Some other studies where a NPQ has been reported in *Synechococcus* and some other cyanobacteria have used alternative equations for calculating NPQ and did not use rapid light curves (see Campbell et al. 1998; Holt et al. 2004; Wilson et al. 2006).

If the pigment composition of classic cyanobacteria is compared to other oxygenic photosynthetic organisms, this lack of NPQ is not related to the presence of phycobilins because there are both eukaryotic and prokaryotic organisms with phycobilins that exhibit a readily measurable NPQ (Table 6) nor can it be related to the types of carotenoids possessed by different photoautotrophs (Gantt and Cunningham 2001; Larkum et al. 2003). *Synechocystis* (PCC6803) is apparently exceptional in this regard but the conditions of culture are critical (compare the results of the present study and those of Cadorat et al. 2004 with Wilson et al. 2006). The atypical cyanobacteria, *Prochlorothrix* and *Acaryochloris* have NPQ versus irradiance curves similar to eukaryotic photoautotrophs (Table 6) whether they have zeaxanthin or xanthophyll types of carotenoids (Ting and Owen 1993). In unpublished work I have also found that NPQ curves for the prochlorophyte *Prochloron* (Holt et al. 2004; Chen et al. 2005a) are those of a typical photoautotroph. *Prochlorococcus* (Prochlorophyta) is another example of an oxyphotobacterium which reportedly exhibits

a measurable NPQ versus irradiance curve (Partensky et al. 1993; Moore et al. 1995; Bailey et al. 2005) but only under conditions of iron stress where a light-harvesting gene called IsiA (or CP43′) is expressed. Bailey et al. (2005) however points out that the kinetics of NPQ in *Prochlorococcus* were different to that found in eukaryotic algae and vascular plants and so it might differ in important respects to NPQ as traditionally defined. Bailey et al. (2005) also points out that cyanobacteria are thought to regulate their light harvesting mechanisms in a different way to most oxygenic photoautotrophs (Mullineaux 1999).

Apparently such cyanobacterial photosynthetic mechanisms do not necessarily involve processes generating a measurable NPQ as traditionally defined (Holt et al. 2004). However, Cadorat et al. (2004) reported that *Synechocystis* (PCC6803) (a classical cyanobacterium generally similar physiologically to *Synechococcus*) exhibits NPQ under iron stress induced expression of IsiA but not under normal culturing conditions. The contrast in the results of Wilson et al. (2006) and Cadorat et al. (2004) thus might have a consistent physiological basis in the effects of iron-deficiency. An IsiA (CP43')-like protein also occurs in *Acaryochloris* (Chen et al. 2005a) and *Prochlorothrix* (van der Staay et al. 1998). Possibly expression of IsiA has some relationship to manifestation of NPQ phenomena in oxyphotobacteria (Campbell et al. 1998; van der Staay et al. 1998; Cadoret et al. 2004; Bailey et al. 2005; Chen et al. 2005a). It is not yet fully clear why a measurable NPQ is found in some cyanobacteria and not others nor why some cyanobacteria only exhibit a measurable NPQ when grown under certain conditions and using some experimental protocols. The role of nutrient status and carefully defined growth conditions on the fluorescence behaviour of cyanobacteria needs a systematic analysis.

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