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Light utilization and photosynthetic efficiency of *Nannochloris* sp. (Chlorophyceae) approached by spectral absorption characteristics and Fast Repetition Rate Fluorometry (FRRF)

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The photosynthetic performance of *Nannochloris* sp. (Chlorophyceae) batch cultures, grown in 16:8 h light:dark cycle, was evaluated with ^{14}C-incorporation, O_2-evolution, light absorption of PSII, and variable fluorescence. Cell pigmentation, elemental composition, and photosynthetic parameters were measured in the course of one light period from cultures acclimated to 314 and 39 μmol quanta m^{-2} s^{-1}. Both cultures had a biphasic trend during the light period. The productivity increased during the first half of the light period, and decreased afterwards. The growth rates in both of the cultures were mainly regulated by the internal cell cycle, rather than by light and nutrients. The nutrient metabolism obviously caused discrepancy between the ^{14}C-based and variable fluorescence-based primary productivity estimates. The relatively higher estimate of the rate of electron transport through PSII than of the ^{14}C-based biomass-specific primary productivity in the early phases of the experiment was mainly caused by the inability of the variable fluorescence method to take into account a partial loss of ATP, NADPH and ferredoxin pools caused by the active NO_3-N uptake and reduction. The temporal patterns of the fluorescence-based (ϕ_f) and the ^{14}C-based quantum yield for carbon fixation were much alike, since in the definition of ϕ_f processes other than carboxylation, consuming the reducing power and the chemical energy produced by the light reaction of photosynthesis, were taken into consideration. The problems associated with the inclusion of pre-set parameters in the electron-flow-based productivity models are also discussed.

Introduction

The bulk of aquatic primary productivity measurements in the last few decades have been carried out using the ^{14}C-labeled carbon assimilation technique (Steemann-Nielsen 1952). Although being rather sensitive and reproducible, the ^{14}C-technique has some uncertainties, like whether it measures gross or net primary production or something between them (Bender *et al.* 1987). Furthermore, sampling and incubation may affect the viability of algae (Eppley 1980). Demanding a lot of time in a laboratory, the number of samples measured *in situ* with this technique is limited. Alternative or, thus far, mainly supplementary techniques for the measurement of primary productivity have recently been introduced. Techniques based on variable chlorophyll fluorescence are instantaneous and measuring can be carried out continuously *in situ*, without preparation or incubation of the samples.

In the variable fluorescence methods the photosynthetic parameters of the phytoplankton assemblage are estimated from the changes in the fluorescence yield. The shortcoming of the widely-used Pulse-Amplitude-Modulated (PAM) technique (Schreiber 1986, Schreiber *et al.* 1993) is the inability to measure the functional absorption cross-section, and hence absolute production rates (*see* Hartig *et al.* 1998). This limitation was withdrawn when the Pump-and-Probe (PP) technique was introduced (Falkowski *et al.* 1986), in which the functional absorption cross-section is measured by gradually increasing the intensity of the pump flash (Kolber and Falkowski 1993). The Fast Repetition Rate (FRR) technique, developed from the PP-technique, utilizes a rapid series of excitation flashes and offers an efficient way to study the photosynthetic activity of phytoplankton (Kolber and Falkowski 1992). In the FRR technique, the prompt measurement of the functional absorption cross-section allows a reliable determination of the amount of light energy used in photosynthesis (Falkowski and Kolber 1995). The FRR technique has already been utilized in several field studies (Greene *et al.* 1994, Kolber *et al.* 1994, Vassiliev *et al.* 1994, Falkowski and Kolber 1995, Babin *et al.* 1996, Behrenfeld *et al.* 1996, Strutton *et al.* 1997), the interpretation of the results obtained has progressed (Kolber *et al.* 1998), and more specialized modifications of this technique have been introduced very recently (Gorbunov *et al.* 1999, Gorbunov *et al.* 2000).

Comparisons with the ^{14}C-technique were made with PAM-technique (Hartig *et al.* 1998), using PP-technique (Kolber and Falkowski 1993, Boyd *et al.* 1997), and also using FRR-technique (Babin *et al.* 1996). Complementing the productivity measurements with the spectral light harvesting properties of photosynthetic pigments is essential for the understanding of the effect of light variability on the photosynthetic parameters of algae. However, no studies of this kind have been published for the FRR-technique, as far as we know.

We studied the FRR, ^{14}C, and O_2-techniques in the evaluation of the algal light utilization and primary productivity during a light period of a diurnal cycle, and completed them with spectral measurements to derive the rate of light absorption by PSII. Furthermore, we supplemented bio-optical measurements with growth and nutrient uptake rates that gave an insight into the physiological status of the cells. For practical but also ecological reasons we selected the small unicellular Chlorophyte, *Nannochloris* sp., as a test organism for this study. The pigmentation, as well as the energy transfer processes in the photosystems of Chlorophytes are better documented than for other algal groups (Thornber 1986, Kirk 1994), and thus they serve better as test organisms for new techniques. The small (diameter two to three μm) coccoid shape of *Nannochloris* sp. allowed us to determine its biovolume, and consequently the interpretation of optical measurements was not disturbed by a complex cell shape. Later on, after this methodological study, more ecologically important species will be studied, including species forming harmful blooms.

Materials and methods

Experimental design

Nannochloris sp. (strain TV1b1) was isolated from the northern Gulf of Finland, Baltic Sea,

and maintained at the Tvärminne Zoological Station, University of Helsinki (Hällfors and Hällfors 1992). The strain was originally erroneously determined as *Nannochloropsis* sp. (Eustigmatophyceae), but recently re-classified, based on the pigmentation, as *Nannochloris* sp. (Chlorophyceae) (G. Hällfors, pers. comm.). Experimental cultures were grown at 20 °C in 16-l polycarbonate tubes in a modified Erd-Schreiber medium, containing 5 ml of soil extract, 2 mg NO_3-N, 0.1 mg NH_4-N, 0.3 mg PO_4-P, 0.2 mg vitamin B_1, and 0.1 mg vitamin B_{12} in one liter of filtered Baltic Sea water (salinity 6.0 PSU). To maintain a constant CO_2 level, cultures were continuously bubbled with air filtered through 0.2-μm filters (Sartorius Minisart). Cultures were illuminated from opposite sides with cool-white fluorescent tubes (Philips TLD 36W/95). Two subcultures were grown at irradiance levels (PAR, 400 to 700 nm) 314 (referred henceforth as HL), and 39 μmol quanta m^{-2} s^{-1} (LL). Spectral irradiance was measured with a spectroradiometer (LI-1800, Li-Cor Inc.) in the centre of the tubes (Fig. 1). The lower irradiance was obtained by coating the culture tube with spectrally neutral films (Rosco). Temperature in the culture room was maintained at a constant level with a fan directed between the two light panels. Cultures were adapted for 12 days to the prevailing irradiances and the 16:8 h light:dark cycle. After the adaptation period, cultures were diluted with fresh media to ensure that exponential growth would continue and to prevent nutrient limitation during the experiment. A range of dilutions (1:4 to 1:10) was selected to ensure that both subcultures had similar chlorophyll *a* (chl *a*) concentrations, and thus comparable optical thickness, in the initial phase of experiments. The resulting initial chl *a* concentrations during the first experimental sampling, the day after the dilutions, were from 57 to 58 μg l^{-1}.

The photosynthetic parameters were recorded during the light period. One day after HL and LL cultures were diluted with fresh media, they were sampled at 4-h intervals (signed as 0 h, 4 h, 8 h, 12 h, and 16 h) throughout the light period of 16 hours, with some exceptions specified below. After sampling, variable fluorescence measurements were carried out inside the experimental tubes.

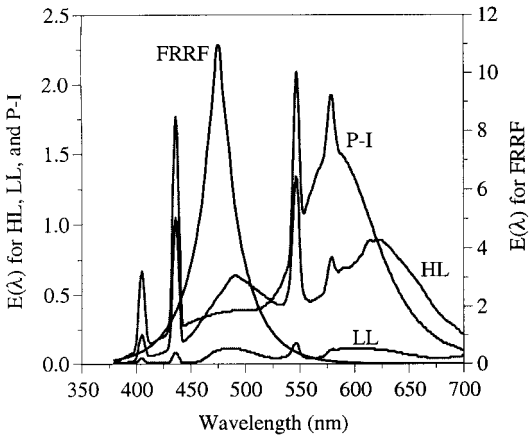

Fig. 1. *Nannochloris* sp. cultures HL and LL. The spectral irradiances $E(\lambda)$ (μmol quanta m^{-2} s^{-1} nm^{-1}) (380–700 nm) for the light source of the P-I incubator (P-I), for the light source of FRRF fluorometer (FRRF), and inside the cultures (HL and LL). Note: the 2nd y-axis refers to the scale for FRRF with units mmol quanta m^{-2} s^{-1} nm^{-1}.

Algal biomass, pigments and elemental composition

Samples for cell number counts were fixed with Lugol's solution. Number of cells, and their size distribution were measured with an Elzone particle counter (Particle Data Europe). Total algal biovolume in samples (bv, mm^3 l^{-1}) was obtained by integrating a product of cell number and cell volume between observed minimum and maximum diameter (1.8 to 5.0 μm) for *Nannochloris* sp. Mean diameter ($\overline{D}$) of cells was estimated as described in Stramski and Reynolds (1993).

For the determination of the algal pigments, from 10 to 20 ml of the subsamples were filtered through Whatman GF/F filters, and filters were stored in darkness at –20 °C for one month prior to analyses. Pigments were extracted with 96% ethanol, and the absorption was recorded from 380 to 750 nm with a Shimadzu UV-2101PC spectrophotometer. Spectra for the range from 590 to 700 nm was deconvoluted by the non-negative least-square regression technique in the components of chlorophylls *a* and *b* (chl *b*) using the spectra of pure pigments, and the specific absorption coefficients given by Wintermans and DeMots (1965). Carotenoids

do not absorb in this range, and only minor quantities of chlorophyll degradation products were assumed to be present as the cells were at the exponential growth phase. Obtained chl *a* concentrations were in perfect agreement with the values calculated with the equations of Wintermans and DeMots (1965). The spectra of total carotenoids were obtained by subtracting chl *a* and *b* spectra from the sample spectra. Concentration of total carotenoids was calculated with the specific absorption coefficient of 250 l g^{-1} cm^{-1} (Rowan 1989).

Subsamples from 10 to 15 ml for particulate C, N, and P (POC, PON, and POP) were filtered through acid-washed and precombusted Whatman GF/F filters. POC and PON were measured from the same filters with Roboprep/Tracermass mass spectrometer (Europa Scientific, UK), and POP was measured according to Solorzano and Sharp (1980). Using the linear relationship between all the measured values of the bv of algae and POC (r^2 = 0.98) or PON (r^2 = 0.97), we estimated the detrital pools of POC and PON caused by non-algal particles in the culture media. Detrital pools for POP were estimated accordingly, but different equations were used for each culture, as the detrital pools of POP were evidently culture-specific. These pools were subtracted from the measured values to obtain the elemental composition of algae.

Biovolume-, chl *a*-, and particulate nutrient-specific net growth rates μ (d^{-1}) were calculated as

$$\mu_x = \ln\left(X_{t_2} X_{t_1}^{-1}\right) \times \left[\left(t_2 - t_1\right) / 24\right]^{-1} \qquad (1)$$

where X is either bv, chl *a*, POC, PON or POP, and $(t_2 - t_1)$ presents time (hours) between subsequent samplings.

In vivo spectral measurements

In vivo absorption and fluorescence spectra of the whole cells were measured from the samples taken at 4 h. For absorption, cells were filtered on Whatman GF/F filter, and scanned from 380 to 800 nm with a Shimadzu UV-2101PC spectrophotometer. The pathlength amplification factor for *Nannochloris* sp. was determined as suggested by Cleveland and Weidemann (1993). The chl *a*-specific absorption at red maximum $a^*(679)$ (m^2 (mg chl $a)^{-1}$) was calculated for each sample using a statistical relationship between $a^*(679)$ and the ratio of cellular chl *a* to the projected area of a cell (r^2 = 0.83, n = 25) obtained for the same species (J. Seppälä, unpubl.).

$$a^*(679) = -0.4057 \times \text{chl } a \times \text{cell number}^{-1} \times (\pi(\overline{D} / 2)^2)^{-1} + 0.0266 \qquad (2)$$

Reasoning behind such a relationship is given by e.g. Morel and Bricaud (1986), and for the range of our data the relationship was evidently linear. Values measured at 4 h fitted the regression line.

Fluorescence was measured from DCMU treated samples (20 μM final concentration of DCMU) using far-red emission of chl *a* at 730 nm, and the excitation from 380 to 700 nm (Neori *et al.* 1988) with a Shimadzu RF-5001 spectrofluorometer. Fluorescence spectra were corrected for instrument optics with the commercial dye Basic Blue 3 (Kopf and Heinze 1984), and further adjusted using spectra of pure chl *a* (*see* Lutz *et al.* 1998). We assumed no remarkable changes in the shape of spectral fluorescence during the study, and the culture-specific shapes obtained at the 4 h samples were also used for other sampling times. Fluorescence spectra were scaled 1:1 at the red peak with a value of $a^*(679)$. Thus obtained scaled fluorescence spectra $F^*(\lambda)$ with same units as $a^*(679)$, is used here as an approximation of light absorption by photosynthetic pigments (*see* Johnsen and Sakshaug 1996, Johnsen *et al.* 1997).

Photosynthetic rates and P-I curves

An apparent net carbon fixation was measured as ^{14}C-CO_2 uptake according to Steemann-Nielsen (1952), and as modified by Niemi *et al.* (1983). To estimate algal gross productivity, fixed carbon both in the particulate and dissolved matter was measured. The activity of the ^{14}C-labelled $NaHCO_3$ (VKI, Denmark) aqueous solution was 20 μCi ml^{-1}. Samples were incubated in *in situ*

light in Greiner transparent 50-ml tissue culture flasks. The incubation time was limited to 100 min in order to obtain primary productivity estimates as close to the gross productivity as possible. The ^{14}C-incorporation rates were corrected for the dark uptake of ^{14}C. Radioactivity was measured with 1217 Rackbeta liquid scintillation counter (LKB Wallac Co, Finland). The amount of total inorganic carbon was analysed using a Unicarbo carbon analyser (Elektro Dynamo Oy, Laitila, Finland). The ^{14}C-based biomass-specific primary productivity P_c (mol *C* (mol chl *a*)$^{-1}$ s^{-1}) was defined as:

$$P_c = P \times [\text{chl } a]^{-1} \times 2.068 \times 10^{-2} \qquad (3)$$

where P is the ^{14}C-based primary productivity (mg C m^{-3} h^{-1}). The unit for chl *a* concentration is mg m^{-3}, and 2.068×10^{-2} is the factor for a conversion to molar ratios and a per second rate.

Oxygen evolution and dark respiration rates were estimated by the changes in O_2 concentrations during the incubation periods lasting from 75 to 100 min. Samples were incubated in 120-ml glass bottles in ambient light. O_2 concentrations at the start and at the end of the incubations were measured using the Winkler technique and an automatic titrator (Toledo DL53, Mettler).

O_2 and ^{14}C incubations were made as duplicate and triplicate samples in HL, and as single and duplicate samples in LL, respectively. ^{14}C-CO_2 uptake and O_2 production was not measured from samples taken at 12 h (LL) or at 16 h (both subcultures). The photosynthetic quotient (PQ) was defined as mole O_2 produced per mole C fixed.

A relation between ^{14}C-CO_2 uptake and irradiance was measured with a P-I incubator (Hydrobios) equipped with 10 Osram L8W/20 cool white fluorescent tubes (for spectra, *see* Fig. 1). Photosynthetic responses were measured at 12 irradiance levels ranging from 0 to 400 μmol quanta m^{-2} s^{-1}. Incubation time varied from 80 to 100 min. Light saturation was not achieved in any P-I measurement. Since a deviation from the initial slope section of the P-I curve was observed only at light levels of 400 μmol quanta m^{-2} s^{-1} at 4 h and 16 h in HL, and at 16 h in LL, no non-linear model could be fitted to the data. Consequently, the maximum light utilization coefficient α (mg C (mg chl a)$^{-1}$ h^{-1} (μmol quanta m^{-2} s^{-1})$^{-1}$) was determined from the samples situated along the initial slope with a linear regression model. The r^2- and p-values of the resulting fits were > 0.92 and < 0.001 in all the samples, respectively (data not shown).

The ^{14}C-based maximum quantum yield for carbon fixation ϕ_{max} (mol C (mol quanta)$^{-1}$), i.e. photosynthetic efficiency, was calculated by dividing α by the light absorption of photosynthetic pigments $\overline{F}^*$ (m^2 (mg chl *a*)$^{-1}$).

$$\phi_{max} = \alpha \overline{F}^{*-1} \times 2.315 \times 10^{-2} \qquad (4)$$

where 2.315×10^{-2} is a conversion factor to molar ratios. $\overline{F}^*$ is the average chl *a*-specific *in vivo* absorption by photosynthetic pigments weighted by the irradiance spectrum of the P-I incubator $E(\lambda)$.

$$\overline{F}^* = \int_{380}^{700} F^*(\lambda) E(\lambda)\, d(\lambda) \left(\int_{380}^{700} E(\lambda)\, d(\lambda) \right)^{-1} \qquad (5)$$

Fluorescence measurements

In this paper, we follow the nomenclature of van Kooten and Snel (1990) (*see* Abbreviations). We measured the increase in fluorescence yield from minimal to maximal level at the ambient irradiance (from F to F_m') with a FRR-fluorometer Fasttracka (Chelsea Instruments Ltd.). To create this increase, we used an excitation protocol where the FRR-fluorometer emitted a flash sequence of 100 flashlets of 1.1 μs duration, and 2.8 μs intervals**,** within a single turnover of photosystem II (PSII). The time between flashlets was so small that cumulative excitation energy reduced the electron transport chain downstream from the reaction centre II (RCII) (Falkowski and Kolber 1995). The energy of a single flashlet was 6.6×10^{-5} quanta Å^{-2}, and the energy flux of the saturation protocol was calculated to be from 1.7 to 2.2 quanta RCII^{-1}, depending on the size of the functional absorption cross-section of PSII. Kolber *et al.* (1998) recently noted that the lower limit of excitation energy to neglect Q_a^- reoxidation is 1.6 quanta RCII^{-1}, and therefore we assumed Q_a^- reoxidation to be

negligible in our experiment. The fluorescence parameters F, F_m', ϕ', and σ_{PSII}' (for explanations, *see* abbreviations) were calculated from the raw fluorescence data with the Fasttracka post-processing software FRS v. 1.6, which is based on equations presented in Kolber *et al.* (1998). For the overall theory behind the FRR-technique and the related fluorescence parameters, *see* Kolber and Falkowski (1993) and Kolber *et al.* (1998).

The rate of electron transport through PSII P_f (mol e^- (mol RC)$^{-1}$ s^{-1}) was modified from Kolber and Falkowski (1993), and Gorbunov *et al.* (2000), and is calculated as a product of ambient irradiance E (μmol quanta m^{-2} s^{-1}), a functional absorption cross-section under ambient light σ_{PSII}' (Å^2 quanta^{-1}), a proportion of the functional reaction centres at ambient irradiance f', and the quantum yield of photochemistry within PSII ϕ_{RC} (mol e^- (mol quanta)$^{-1}$):

$$P_f = E\sigma_{PSII}' f' \phi_{RC} 6.022 \times 10^{-3} \quad (6)$$

where 6.022×10^{-3} is a conversion factor to molar ratios. f' is calculated as $\phi'/0.65$ (Kolber and Falkowski 1993), and ϕ_{RC} is assumed to equal one (Falkowski and Raven 1997).

The fluorescence-based quantum yield for carbon fixation ϕ_f (mol C (mol quanta)$^{-1}$) is modified from Babin *et al.* (1996):

$$\phi_f = (\sigma_{PSII}' \phi_c f' \phi_{RC} n_{PSII}) \times (\overline{F} * PQ)^{-1} \times 6.740 \times 10^{-3} \quad (7)$$

where 6.740×10^{-3} is a conversion factor to molar ratios, ϕ_c is the quantum yield of electron transport in PSII, and assumed to be 0.25 mol O_2 (mol e^-)$^{-1}$ (Dubinsky *et al.* 1986), n_{PSII} is a ratio of RCII to total PSII chl *a* pigments, and assumed to be 0.002 mol e^- (mol chl *a*)$^{-1}$ (Kolber and Falkowski 1993), and PQ is the photosynthetic quotient (mol O_2 (mol C)$^{-1}$). For this equation $\overline{F}$ * was calculated using the spectral irradiance of the LEDs of the FRR-fluorometer (*see* Fig. 1).

Results

Pigmentation, elemental composition and growth rates of cultures

The cell size distribution measurements (data not shown) indicated the increase of mean cell size during the day; from diameter 2.4 in HL, or 2.7 μm in LL at 0 h to 3.0 μm at 16 h in both HL and LL. Apparently, for this species, the majority of cell divisions in HL takes place during the dark period (*see* DuRand and Olson 1998), but in LL a fraction of cells divides during the light period. Due to changes in cell size, we present cellular variables as intracellular concentrations rather than normalized to cell numbers. The number of cells remained practically constant in HL culture, and increased about 50% in LL culture.

Table 1. *Nannochloris* sp. cultures HL and LL. Pigment and chemical content. Car = total carotenoids. Ratios are calculated for weights, except those noted. Values present averages of the 16-h light period and the observed ranges (minimum–maximum) are given in parenthesis.

	HL	LL
chl *a* bv^{-1} (mg mm^{-3})	0.0030 (0.0019–0.0039)	0.0101 (0.0092–0.0108)
chl *b* chl *a*$^{-1}$	0.054 (0.023–0.067)	0.080 (0.051–0.134)
Car chl *a*$^{-1}$	0.37 (0.30–0.45)	0.16 (0.14–0.19)
C bv^{-1} (mg mm^{-3})	0.170 (0.150–0.187)	0.206 (0.192–0.231)
N bv^{-1} (mg mm^{-3})	0.027 (0.018–0.030)	0.036 (0.034–0.040)
P bv^{-1} (mg mm^{-3})	0.005 (0.003–0.006)	0.007 (0.006–0.007)
chl *a* C^{-1}	0.017 (0.013–0.021)	0.049 (0.040–0.056)
N P^{-1}	6.1 (4.7–7.1)	5.5 (5.3–5.6)
C P^{-1}	39.4 (28.1–55.2)	31.3 (28.3–35.6)
C N^{-1}	6.5 (5.4–8.4)	5.7 (5.2–6.7)

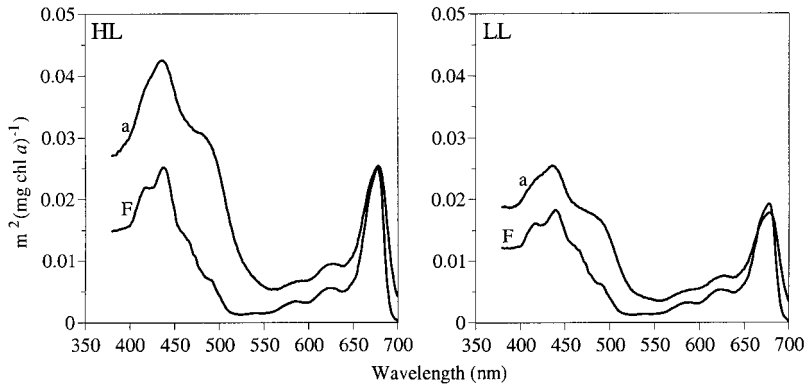

Fig. 2. *Nannochloris* sp. cultures HL and LL. Total absorption (a) and scaled fluorescence excitation spectra (*F*).

As a response to the lower light level, LL cells had three times higher ratio of chl *a* to biovolume, and accordingly higher ratio of chl *a* to C than HL cells (Table 1). A similar pattern was also noted for chl *b*, while to a lesser extent for the total amount of carotenoids. The ratio of chl *b* to chl *a* for this species was very low and similar to that previously estimated by HPLC measurements (J. Seppälä and G. Johnsen, unpubl.), indicating that light harvesting through chl *b* was not very important. Relative importance of chl *b*, as indicated by the ratio of chl *b* to chl *a* and by spectral measurements (*see* below), was higher in the LL cells. High amounts of carotenoids relative to chl *a* in HL cells emphasize their photoprotective role. The difference between the absorption spectra of total cells and that of PSII, estimated from the corrected fluorescence spectra, actually quantify the proportion of photoprotective carotenoids from total light absorption (Fig. 2). The spectral shape of fluorescence also indicates the minor role of photosynthetic carotenoids, like lutein, in light harvesting. We are, however, aware that the reasoning above ignores the qualitative differences in pigmentation of PSI and PSII possibly leading to slightly erroneous spectra of total photosynthetic pigments. Especially for the LL cells the quantity of photoprotective carotenoids should be minimized.

LL cells contained more macronutrients than HL cells (Table 1). Development of cellular elemental ratios indicated rather balanced uptake for LL cells. HL cells, in turn, showed luxury P, and to a lesser extent, N uptake during the first four experimental hours (Fig. 3). In HL, after 4 h the P uptake ceased and the N uptake also decreased by more than 60%. After that, pigment build-up rate reached its maximum between 4 and 8 h. Changes in POC or fresh weight accumulation rates were moderate during the study. For LL, maximum nutrient accumulation occurred later, between 4 and 8 h, and actually occurred at the same time as a major chl *a* build-up. Growth rates for different cellular variables were in good agreement when calculated for the whole experimental period (Fig. 3), and averaged 1.60 and 1.12 d^{-1} for HL and LL, respectively.

Light utilization

Estimated chl *a*-specific light absorption at red maximum (679 nm) ranged from 0.0173 for LL to 0.0253 m^2 (mg chl *a*)$^{-1}$ for HL (Table 2). By scaling the corrected spectral fluorescence to this peak, we obtained the estimate of light harvesting by the photosynthetic pigments (Fig. 2). Taking into account the differences in $E(\lambda)$, $F^*(\lambda)$ and chl *a* levels, the actual amount of light harvested by the photosynthetic pigments per unit of chl *a* in LL was on average 9.8% of that in HL. Difference spectra (not shown) of $F^*(\lambda)$ for HL and LL indicate that the slight mismatch in the shapes of these two spectra is due to chl *b*.

A distinct periodicity in the initial slopes of the P-I curve (α) was observed during the light period. Values of α increased, similarly for both cultures, until the maximum at 8 h (Table 2 and Fig. 4). After this, α decreased, and in the last samples at 16 h they were lower than the initial values at 0 h. The level of α was similar for HL and LL; it varied between 0.0068 and 0.0198, and between 0.0085

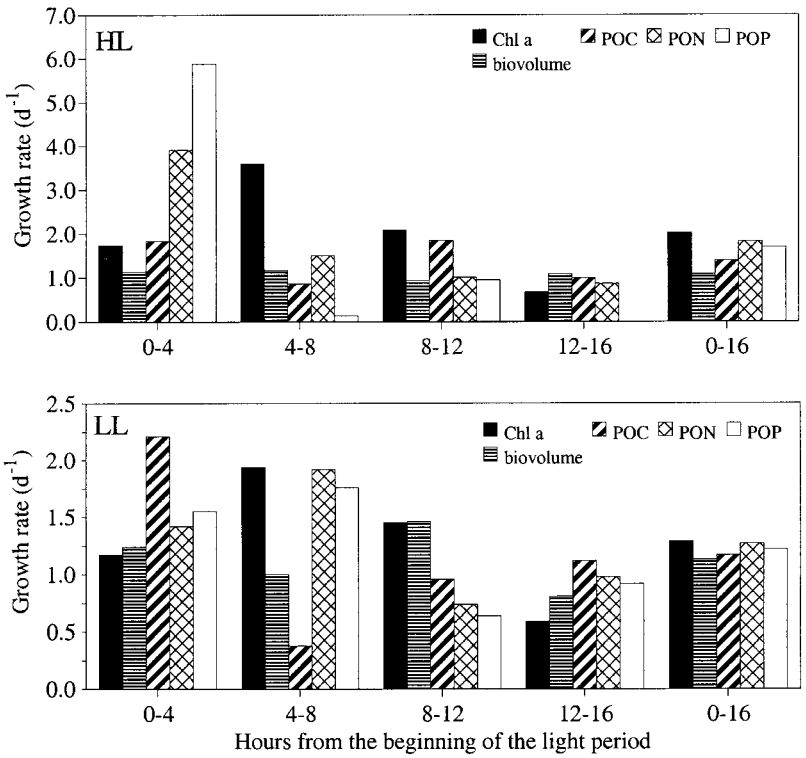

Fig. 3. *Nannochloris* sp. cultures HL and LL. Growth rates expressed as chl *a*, biovolume, POC, PON and POP at four-hour intervals and for the whole light period (time 0 to 16 h).

and 0.0206 mg C (mg chl *a*)$^{-1}$ h^{-1} (μmol quanta m^{-2} s^{-1})$^{-1}$ in HL and LL, respectively. As there were no remarkable changes in the spectrally weighted chl *a*-specific absorption coefficient for photosynthetic pigments ($\overline{F}$ *) in the course of the study, the variability in the ^{14}C-based quantum yield for carbon fixation (ϕ_{max}) was similar to α (Table 2). ϕ_{max} varied between 0.0260 and 0.0746, and between 0.0404 and 0.0996 mol C (mol quanta)$^{-1}$ in HL and LL, respectively. The level of $\overline{F}$ * in LL was, on average, 81% of that of HL. Consequently, ϕ_{max} was higher for LL (paired *t*-test: $n = 4$, $p < 0.001$). Thus, LL was able to utilize the absorbed quanta more efficiently.

Table 2. *Nannochloris* sp. cultures HL and LL. Light utilization and photosynthetic parameters. Time = hours from the beginning of the light period. For units, *see* abbreviations. nd = no data.

	Time	$a^*(679)$	$\overline{F}$ *	ϕ_{max}	α	PQ	P_c	ϕ'	σ_{PSII}'	P_f	ϕ_f
HL	0 h	0.0253	0.0066	0.0407	0.0113	2.57	0.061	0.40	358	421	0.0265
	4 h	0.0251	0.0065	0.0602	0.0166	1.21	0.125	0.39	351	403	0.0544
	8 h	0.0242	0.0063	0.0746	0.0198	1.47	0.100	0.42	320	392	0.0456
	12 h	0.0235	0.0061	0.0572	0.0148	1.60	0.071	0.36	341	358	0.0394
	16 h	0.0235	0.0061	0.0260	0.0068	nd	nd	0.33	373	354	nd
LL	0 h	0.0192	0.0054	0.0611	0.0136	2.26	0.019	0.54	281	54.4	0.0367
	4 h	0.0192	0.0055	0.0756	0.0169	1.38	0.025	0.53	285	54.3	0.0591
	8 h	0.0175	0.0050	0.0996	0.0206	1.59	0.027	0.50	294	52.9	0.0555
	12 h	0.0173	0.0049	nd	nd	nd	nd	0.44	289	46.0	nd
	16 h	0.0173	0.0049	0.0404	0.0085	nd	nd	0.38	281	38.9	nd

Fluorescence and productivity measurements

Functional absorption cross section under ambient irradiance (σ_{PSII}'), which represents the effective target size of the antenna serving PSII, was significantly higher in HL than in LL (two-group *t*-test: $n = 5$, $p < 0.01$). Whereas σ_{PSII}' had a minimum at 8 h in HL, in LL the σ_{PSII}' level remained markedly stable throughout the study (Table 2). The functional photosynthetic energy conversion efficiency (ϕ'), which represents the upper limit for all subsequent photosynthetic events under ambient irradiance (Schofield *et al.* 1998), was, in turn, significantly higher in LL than in HL (two-group *t*-test: $n = 5$, $p < 0.005$). The overall trend in ϕ' for both of the cultures was to decrease towards the end of the study, except that there was a maximum at 8 h in HL (Table 2 and Fig. 4). This pattern of ϕ' between 0 and 8 h was clearly different from the pattern of α, especially in LL (Fig. 4).

A similar discrepancy was observed with the productivity estimates (Table 2). The rate of electron transport through PSII (P_f) decreased through the entire experiment, whereas the ^{14}C-based biomass-specific primary productivity (P_c) had a maximum at 4 h and 8 h in HL and LL, respectively. Consequently, the highest ratios of P_f to P_c in both of the cultures were observed at 0 h (Fig. 5). Generally, the ratio of P_f to P_c was lower in LL than in HL.

The ratio of the fluorescence-based quantum yield for carbon fixation (ϕ_f) to ϕ_{max} ranged from 0.61 to 0.90, and from 0.56 to 0.78 in HL and LL, respectively (Fig. 5). The pattern of this ratio was much alike in both of the cultures, contrary to the ratio of P_f to P_c, and the ratio had maximum values at 4 h.

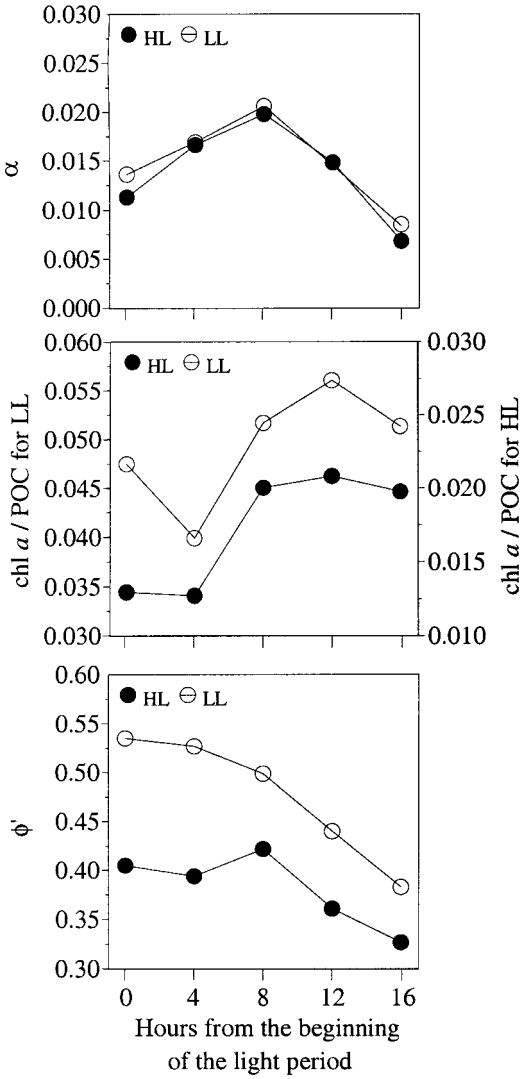

Fig. 4. *Nannochloris* sp. cultures HL and LL. The initial slope of the P-I curve α (mg C (mg chl *a*)$^{-1}$ h^{-1} (μmol quanta m^{-2} s^{-1})$^{-1}$), chl/POC ratio, and the functional photosynthetic energy conversion efficiency ϕ' (relative) for the whole light period.

Discussion

Cell cycle and nutrient uptake of *Nannochloris* sp.

The growth of *Nannochloris* sp. was obviously regulated by the diurnal physiological cycle. Decline in photosynthetic efficiency after 8 h was probably caused by the cell division pattern. The need for an additional biomass build-up, and thus nutrient uptake and light utilization, was down-regulated by the cell division. Indeed, the algal biovolume in both cultures doubled during the 16 h light period. We assume that growth, considering the whole period, was more

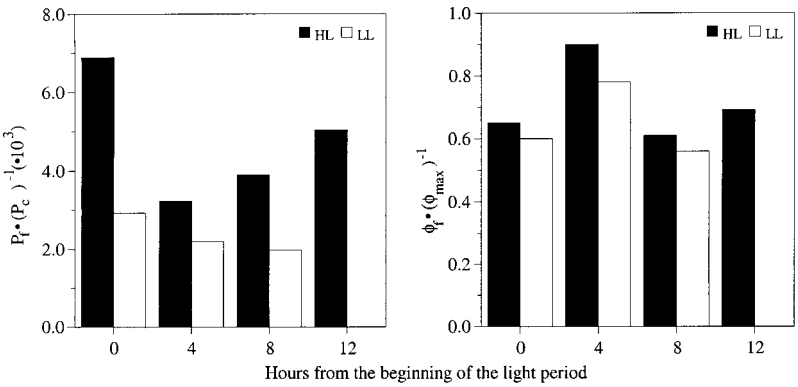

Fig. 5. *Nannochloris* sp. cultures HL and LL. Left: the ratio of the rate of electron transport through PSII to the ^{14}C-based biomass-specific primary productivity ($P_f \times P_c^{-1}$) for the whole light period. Note: values must be multiplied with 1000. Right: the ratio of the fluorescence-based quantum yield for carbon fixation to the ^{14}C-based maximum quantum yield for carbon fixation ($\phi_f \times \phi_{max}^{-1}$) for the whole light period.

regulated by the internal diel cell cycle than by light and nutrients, although we have no measurements for the dark period (*see also* DuRand and Olson 1998).

HL cells had apparently a very high affinity for nutrients in the early hours of the experiment (Fig. 3), but they were not likely to be nutrient limited at that time. Rather the high amount of available light allowed HL cells the luxury uptake of nutrients for later use. The high nutrient uptake during early hours is supported by studies, where nitrate reductase activity peaked during the first three hours of the light period within 14:10 h (Gao *et al.* 1992, Berges *et al.* 1995) and 12:12 h light:dark cycle (Vergara *et al.* 1998). According to Vergara *et al.* (1998) nitrate reductase activity increased already prior to the onset of the light period thus enabling the quick increase in cellular N quota right after the dawn. In our study both the N and P uptake were fastest from 0 to 4 h, and from 4 to 8 h in HL and LL, respectively (data not shown).

The photosynthetic apparatus of LL culture received roughly 10 times less light quanta per chl *a* than HL did. The cells in LL were light limited as they showed high cellular concentrations of pigments and nutrients, constant elemental ratios, and relatively stable nutrient uptake rates over the whole study period (Zevenboom 1986). LL cells were able to compensate light limitation partly by increasing their cellular pigmentation, and partly by having higher quantum yield for carbon fixation. The resulting carbon-specific C uptake rates were comparable between cultures, indicating the flexibility of *Nannochloris* sp. pigmentation and energy transfer systems to adapt to a wide range of irradiances and retain high growth.

Light harvesting and utilization

As a sign of light-shade adaptation, low growth irradiance induced an increase in cellular photosynthetic pigments (*see* e.g. Kirk 1994). However, the rate of light harvesting for photochemistry is not determined solely by the amount of pigments per cell. The extent of light absorption per unit of pigment is affected by pigment density in cell suspension, inside the cell and chloroplast. In highly pigmented LL cells intracellular shading (i.e. package effect) led to a lower chl *a* absorption (a^*) than in HL.

A further complication in light harvesting is that cells grown in high light will increase the amount of light protecting carotenoids which do not contribute to the harvesting of light energy directed to photosynthesis. To eliminate the effect of photoprotective carotenoids, we utilized corrected and scaled far-red fluorescence as a proxy for light absorption by PSII (Johnsen and Sakshaug 1996). If this is not done, as in many earlier studies, the amount of light harvested for photosynthesis will be overestimated,

and consequently quantum yield for carbon fixation will be underestimated. In this study, the use of chl *a*-specific absorption by total pigments, instead of photosynthetic pigments, would have caused from 46 to 54% lower estimates of ϕ_{max}.

Comparison of fluorescence spectra of HL and LL cells indicate only slight changes in the composition of the light harvesting complexes of PSII due to light acclimation. Our further assumption that the spectra, and thus pigmentation, of PSII to be stable throughout the light period is not tested for the *Nannochloris* sp., as far as we know. However, the possible changes are certainly minor, and they should not introduce any major flaws for our calculations. The further assumption that pigmentation of PSI is similar to PSII is seldom true, but an approximation often made. Direct quantification for PSI and PSII absorption is hard to obtain and it requires the isolation of chromoproteins. Once this is done, the relative chl *a* content in PSI and PSII can be used in the scaling of the far-red fluorescence spectra and the total absorption spectra, yielding a true absorption by PSII (Johnsen *et al.* 1997).

Nannochloris sp. showed a high light utilization potential. The light-saturated photosynthetic rate was not reached in the P-I incubator even in LL. Consequently, high irradiance hardly was the main factor to decrease σ_{PSII}' between 0 and 8 h in HL, although the decrease in σ_{PSII} is usually associated with the down-regulation of PSII under excessive ambient light (Kolber *et al.* 1988, Mauzerall and Greenbaum 1989, Genty *et al.* 1990, Falkowski and Kolber 1995). The apparent momentary decrease in σ_{PSII}' could be seen as a result of the efficient nutrient uptake in the cultures in the early stages of the study. As cells in HL became more nutrient replete, the photosynthetic efficiency (ϕ') increased slightly between 4 and 8 h (Table 2). This increase was partly made by increasing the number of active RCs. As σ_{PSII} reflects only active RCs (Robinson *et al.* 1998), the measured σ_{PSII}' decreased, as more functional RCs shared a common pigment antenna.

Seen as a whole, the observed periodicity in HL — high nutrient uptake between 0 and 4 h, increase of chl *a* between 4 and 8 h, the highest productivity at 8 h, and subsequent decrease of photosynthetic efficiency — was probably endogenous. The changes in the ratio of chl *a* to C, as one indication of photosynthetic light utilization efficiency, showed a similar rhythm (Fig. 4). A circadian rhythm for several algal species was observed by Harding *et al.* (1981, 1983). They noted α to have a daily maximum in the morning or near midday in 12:12 h light:dark cycle. Despite the algal cells had enough resources to maintain high light utilization efficiency, they turned their activity down due to closing cell division.

The two different productivity approaches

The advantage of the PP and FRR techniques over other variable fluorescence techniques is the ability to evaluate the size of σ_{PSII} that permits the calculation of the absolute rate of quanta absorbed per RCII (Kolber *et al.* 1994, Falkowski and Kolber 1995). Thus, σ_{PSII} is essential in obtaining quantitatively good estimations of the fluorescence-based photosynthetic rates. The average level of P_f in this study was 50 and 390 mol e^- (mol RC)$^{-1}$ s^{-1} in LL and HL, respectively. The observed field results obtained using either PP or FRR-techniques either fall between these levels [up to 160 mol e^- (mol RC)$^{-1}$ s^{-1}, Boyd *et al.* (1997); up to 40 mol C (mol RC)$^{-1}$ s^{-1}, Kolber and Falkowski (1993), note the different units], or are at the same level as in HL [up to 80 mol C (mol RC)$^{-1}$ s^{-1}, (Falkowski and Kolber 1995)]. It must be stated that the equations these estimates are based on include the photochemical quenching (q_p, ranging from 0 to 1), which lowers these estimates, compared to the ones in this study.

The fluorescence-based quantum yield for carbon fixation (ϕ_f), taking into account (i) a pre-set ratio of 500 PSII chl *a* pigments per RCII (Myers and Graham 1983), (ii) the photosynthetic quotient (PQ, Table 2), and (iii) a theoretical relation of four e^- photoactivated to one O_2 evolved (Dubinsky *et al.* 1986, Babin *et al.* 1996), can be quantitatively compared to the ^{14}C-based ϕ_{max}. The range of ϕ_f in this study, from 0.027 to 0.059 mol C (mol quanta)$^{-1}$, was in accord with the field results observed by Babin *et al.* (1996). They reported levels from

0.02 to 0.03, and from 0.05 to 0.06 mol C (mol quanta)$^{-1}$ in the mesotrophic and eutrophic sites in the tropical Atlantic, respectively. However, ϕ_f was in our study, on average, 68% of ϕ_{max}. This discrepancy could be, at least partly, explained by the use of a pre-set parameters in the Eq. 7, namely n_{PSII} and ϕ_c, of which n_{PSII} is discussed more thoroughly. It is notable that both ϕ_f and ϕ_{max} are based on factors that take into account the absorption of only the light harvesting pigments serving PSII: $\overline{F}$ * and σ_{PSII}´ (Johnsen and Sakshaug 1993, Falkowski and Kolber 1995).

The pre-set n_{PSII} in the electron-flow-based productivity models

A pre-set n_{PSII}, the inverse of the size of the photosynthetic unit (PSU) with units mol e$^-$ (mol chl *a*)$^{-1}$, is one of the major problems to solve in the electron-flow-based productivity models. The algal cells tend to change their PSU size as a response to different light level (Myers and Graham 1971, Falkowski and Owens 1980, Falkowski *et al.* 1981, Perry *et al.* 1981, Raven 1984, Dubinsky *et al.* 1986). n_{PSII} also varies according to the phytoplankton species composition; Falkowski and Kolber (1993) noted σ_{PSII} to vary about five-fold in natural phytoplankton communities. The adaptation of the algae to excessive light levels with the non-photochemical quenching by the xanthophyll cycle (Demmig-Adams 1990) may lead to a 50% change in σ_{PSII} (Olaizola *et al.* 1994). As there were no estimates of PSU sizes for *Nannochloris* sp. available for us, n_{PSII} was determined to be 0.002, measured by Myers and Graham (1983) from *Chlorella pyredoinosa*. Raven (1984), however, noted that the reported PSU sizes varied two-fold in chlorophytes alone. Although PSU size has been observed to be very variable by nature, the current measurement protocols of PSU size — calculation of the oxygen evolution (chl *a* O_2^{-1}) obtained with single saturating flashes (Emerson and Arnold 1932, Joliot 1968) and the estimation of the ratio of chl *a* to RCI spectrophotometrically (Shiozawa *et al.* 1974) — are very time-consuming, and practically impossible to carry out in the field. Therefore, more representative field estimates of this parameter are needed e.g. for the Baltic Sea.

The temporal variability in the ratio of ϕ_f to ϕ_{max} — the role of PQ

Generally, the first half of the experiment was described with the decreasing values of P_f and ϕ´, representing the fluorescence-based technique, and increasing P_c and α, representing the ^{14}C-based technique (Table 2 and Figs. 4 and 5). The distinctively high ratio of P_f to P_c at 0 h is perhaps the best indication of the methological discrepancy between these two techniques. However, this discrepancy did not concern the ratio of ϕ_f to ϕ_{max}; the similar kind of a peak value at 0 h was not observed (Fig. 5). This was largely due to the inclusion of PQ in the electron-flow-based model (Eq. 7).

The fluorescence techniques are based on the variables that can be measured optically, and they do not provide any direct information about the physiological state of algal cells. However, they can provide some indirect information about the effects of nutrient limitation (e.g. Kolber *et al.* 1988, Greene *et al.* 1994), and light (e.g. Vassiliev *et al.* 1994, Babin *et al.* 1996) on the algal physiology. In our experiment, N uptake was most efficient between 0 and 4 h in HL, and between 4 and 8 h in LL (Fig. 3), which is related to high PQ values in the early stages of the study (Table 2). A high PQ value means that part of the reducing power and the chemical energy that the light phase of photosynthesis produces is transferred to serve reactions other than photosynthesis. The FRR-technique does not take into account that a part of the ATP, NADPH and ferredoxin molecules is used in active uptake and reduction of NO_3-N (Raven 1976, Eppley 1978). The carboxylation process can not use this part of the ATP and NADPH pool. Hence the fluorescence-based technique achieved a relatively higher productivity level than the ^{14}C-technique in the early part of the light period (Fig. 5). The inability to take into account this partial loss of ATP, NADPH and ferredoxin pools for serving the photochemistry seems to be a clear deficiency in the variable fluorescence technique. As PQ was included in

the Eq. 7, both the active N uptake, and the reduction of NO_3-N and SO_4-S as alternative sinks for electrons were included in the electron-flow-based productivity model.

Conclusions

The light utilization, and hence primary productivity of *Nannochloris* sp. was probably regulated by the internal diel cycle. *Nannochloris* sp. could adapt its photosynthetic apparatus to a wide range of irradiances. Thus, both of the cultures retained high growth rate. During high nutrient uptake rates the FRR-technique provided relatively higher estimates of photosynthetic rates than did the ^{14}C-technique. However, the FRR-technique provided somewhat lower estimates of the quantum yield for carbon fixation than the ^{14}C-technique did.

Despite the above-mentioned discrepancies, techniques based on variable fluorescence are promising tools for measuring algal photosynthetic parameters. However, there still are some problems to be solved, e.g. the use of constant values describing parameters in the electron-flow-based productivity models. The effect of active NO_3-N uptake was observed to lessen the ability of the fluorescence-based technique to describe algal productivity. Consequently, we will study the relationship of these two primary productivity methods more profoundly under different N-stress levels.

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Abbreviations

Q_a	quinone-a molecule
bv	algal biovolume ($mm^3\ l^{-1}$)
$\overline{D}$	mean diameter of algal cells
I	excitation energy (quanta $Å^{-2}$)
$E(\lambda)$	spectral irradiance (μmol quanta $m^{-2}\ s^{-1}\ nm^{-1}$).
E	integrated irradiance (380–700 nm) (μmol quanta $m^{-2}\ s^{-1}$).
a^*	chl *a*-specific absorption coefficient [m^2 (mg chl *a*)$^{-1}$]
$F^*(\lambda)$	scaled *in vivo* DCMU-enhanced fluorescence spectra [m^2 (mg chl *a*)$^{-1}$]
$\overline{F}^*$	chl *a*-specific, spectrally weighted *in vivo* absorption by PSII [m^2 (mg chl *a*)$^{-1}$]
α	the initial slope of the P-I curve [mg C (mg chl *a*)$^{-1}$ h^{-1} (μmol quanta $m^{-2}\ s^{-1}$)$^{-1}$]
$F,\ F_m'$	initial and maximal fluorescence at ambient irradiance (relative)
ϕ'	functional photosynthetic energy conversion efficiency at ambient irradiance (dimensionless number between 0 and 1). Defined as $\left(F_m' - F\right)F_m'^{-1}$.
f'	proportion of functional reaction centres at ambient irradiance (dimensionless number between 0 and 1)
σ_{PSII}'	the functional absorption cross-section of PSII at ambient irradiance ($Å^2$ quanta^{-1})
n_{PSII}	the ratio of PSII reaction centres to total PSII chl *a* pigments [mol e^- (mol chl *a*)$^{-1}$]
PQ	photosynthetic quotient [mol O_2 (mol C)$^{-1}$]
ϕ_{RC}	quantum yield of photochemistry within PSII [mol e^- (mol quanta)$^{-1}$].
ϕ_c	quantum yield of electron transport in PSII [mol O_2 (mol e^-)$^{-1}$]
P	^{14}C-based primary productivity (mg C $m^{-3}\ s^{-1}$)
P_c	^{14}C-based biomass-specific primary productivity [mol C (mol chl *a*)$^{-1}$ s^{-1}]
ϕ_{max}	^{14}C-based maximum quantum yield for carbon fixation [mol C (mol quanta)$^{-1}$]
P_f	the rate of electron transport through PSII [mol e^- (mol RC)$^{-1}$ s^{-1}]
ϕ_f	fluorescence-based quantum yield for carbon fixation [mol C (mol quanta)$^{-1}$]