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FLUORESCENCE-BASED MAXIMAL QUANTUM YIELD FOR PSII AS A DIAGNOSTIC OF NUTRIENT STRESS[1]

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In biological oceanography, it has been widely accepted that the maximum quantum yield of photosynthesis is influenced by nutrient stress. A closely related parameter, the maximum quantum yield for stable charge separation of PSII, $(\phi_{PSII})_m$, can be estimated by measuring the increase in fluorescence yield from dark-adapted minimal fluorescence (F_o) to maximal fluorescence (F_m) associated with the closing of photosynthetic reaction centers with saturating light or with a photosynthetic inhibitor such as 3′-(3,4-dichlorophenyl)-1′,1′-dimethyl urea (DCMU). The ratio F_v/F_m (= $(F_m - F_o)/F_m$) is thus used as a diagnostic of nutrient stress. Published results indicate that F_v/F_m is depressed for nutrient-stressed phytoplankton, both during nutrient starvation (unbalanced growth) and acclimated nutrient limitation (steady-state or balanced growth). In contrast to published results, fluorescence measurements from our laboratory indicate that F_v/F_m is high and insensitive to nutrient limitation for cultures in steady state under a wide range of relative growth rates and irradiance levels. This discrepancy between results could be attributed to differences in measurement systems or to differences in growth conditions. To resolve the uncertainty about F_v/F_m as a diagnostic of nutrient stress, we grew the neritic diatom *Thalassiosira pseudonana* (Hustedt) Hasle et Heimdal under nutrient-replete and nutrient-stressed conditions, using replicate semicontinuous, batch, and continuous cultures. F_v/F_m was determined using a conventional fluorometer and DCMU and with a pulse amplitude modulated (PAM) fluorometer. Reduction of excitation irradiance in the conventional fluorometer eliminated overestimation of F_o in the DCMU methodology for cultures grown at lower light levels, and for a large range of growth conditions there was a strong correlation between the measurements of F_v/F_m with DCMU and PAM ($r^2 = 0.77$, $n = 460$). Consistent with the literature, nutrient-replete cultures showed consistently high F_v/F_m (∼0.65), independent of growth irradiance. Under nutrient-starved (batch culture and perturbed steady state) conditions, F_v/F_m was significantly correlated to time without the limiting nutrient and to nutrient-limited growth rate before starvation. In contrast to published results, our continuous culture experiments showed that F_v/F_m was not a good measure of nutrient limitation under balanced growth conditions and remained constant (∼0.65) and independent of nutrient-limited growth rate under different irradiance levels. Because variable fluorescence can only be used as a diagnostic for nutrient-starved unbalanced growth conditions, a robust measure of nutrient stressed oceanic waters is still required.

Key index words: **DCMU; fluorescence; F_v/F_m; maximal quantum yield; nutrient limitation; nutrient starvation; nutrient stress; PAM; phytoplankton**

Abbreviations: **ANOVA, analysis of variance; DCMU, 3′-(3,4-dichlorophenyl)-1′,1′-dimethyl urea; E, irradiance; F_m, maximal fluorescence; F_o, initial fluorescence; F_v, variable fluorescence ($F_m - F_o$); F_v/F_m, fluorescence-based maximum quantum yield for PSII; NH_4^+, ammonia; NO_3^-, nitrate; PAM, pulse amplitude modulation; ϕ_C, quantum yield of carbon fixation; $(\phi_C)_m$, maximum quantum yield of carbon fixation; ϕ_{PSII}, quantum yield of PSII; $(\phi_{PSII})_m$, maximum quantum yield of PSII; μ, specific growth rate; μ_{max}, maximum growth rate**

The determination of phytoplankton biomass, photosynthetic capacity, and photosynthetic efficiency is essential for quantifying primary production in the oceans. Photosynthesis is dependent on light, temperature, and nutrients (Eppley 1972, Cullen et al. 1992, Falkowski and Kolber 1993, Kirk 1994). The relationship between photosynthesis and irradiance (Jassby and Platt 1976) and the influence of light on growth rates (Eppley 1980, Langdon 1988) and photosynthetic efficiency (Dubinsky 1992) have been well documented and quantified, as well as other light-dependent physiological effects such as acclimation (Ibelings et al. 1994, Geider et al. 1996) and inhibition of photosynthesis (Marra 1978, Neale and Richerson 1987). The effects of temperature on algal physiology and growth have also been reviewed extensively (Eppley 1972, Davison 1991). However, disparate results persist for the role of nutrients in the growth and physiology of phytoplankton (Cullen et al. 1992). Dugdale (1967), Eppley (1981), and Levasseur et al. (1993) suggested that nitrogen limits growth rates of microalgae in regions of the ocean, although Goldman (1980) suggested that phytoplankton in the field were growing at or near their maximum relative growth rate. Although nutrient stress is known to cause changes in cellular physiology of microalgae, a review by Cullen et al. (1992)

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suggested that photosynthetic parameters could not be used as robust indicators of nitrogen stress for both unbalanced (nutrient starved) and acclimated nitrogen-limited growth because published studies produced fundamentally different results for relationships between photosynthetic capacity and N-limited growth rates. This study called into question the use of measures of photosynthetic performance as diagnostics of nutrient limitation. A robust diagnostic of nutrient stress is required to resolve uncertainties about the relationships between photosynthesis, nutrition, and growth rates.

Diagnostic tools such as fluorescence metrics have shown promise for determination of photosynthetic efficiency (Schreiber 1986, Falkowski et al. 1992) and, in turn, for describing the effects of nutrition on photosynthetic performance of phytoplankton (Genty et al. 1989, Geider et al. 1993, Kolber and Falkowski 1993, Babin et al. 1996b). Fluorescence measurements have the advantage of being rapid, sensitive, and minimally invasive. A particularly useful measure is the fluorescence-based maximum quantum yield of charge separation for PSII (F_v/F_m; Table 1 summarizes significant symbols). This measure is calculated from the ratio of maximal fluorescence (photosynthetic pathways blocked by light or with an inhibitor) minus minimal fluorescence (determined using a non-actinic light source) over the maximal fluorescence, determined from a dark-adapted sample. However, the relationships between fluorescence patterns and nutrient limitation may not be as straightforward as some studies suggest. For example, Kolber et al. (1988) investigated five species of marine unicellular algae, representing three phylogenetic classes, and found that a fluorescence-based measure of photosynthetic quantum yield of PSII was high and constant for nutrient-replete cultures, regardless of irradiance, and was depressed for nitrogen-limited cultures grown in chemostats. Batch cultures starved of nitrogen similarly showed depression of fluorescence-based quantum yield (Cleveland and Perry 1987). If sensitivity of fluorescence-based quantum yield for PSII to nutrient stress holds for all growth conditions and light levels, fluorescence can be used as a diagnostic for nutrient-stressed growth of phytoplankton. However, Cullen et al. (1992) showed that F_v/F_m, based on fluorescence ± the photosynthetic inhibitor 3′-(3,4-dichlorophenyl)-1′,1′-dimethyl urea (DCMU), was insensitive to N-limitation of growth rate for cultures of a neritic diatom in balanced growth. Subsequently, MacIntyre et al. (1997) demonstrated that F_v/F_m of the toxic dinoflagellate *Alexandrium tamarense* (Lebour) Balech was not reduced when cultures were fully acclimated to N-limited growth.

In this article, we examine the role of nutrient stress on phytoplankton physiology and its effect on F_v/F_m of unialgal cultures in controlled laboratory experiments. Two methods for measuring F_v/F_m are compared, and we test the generalization that maximum quantum yield of PSII is reduced under nutrient stress in steady state (i.e. fully acclimated) cultures (Kolber et al. 1998). Starvation experiments with batch cultures were used to demonstrate the difference between nutrient-limited and nutrient-starved conditions and the effect of nutrient status on photosynthetic physiology.

BACKGROUND

F_v/F_m as a proxy for maximum quantum yield of PSII. The relationship between fluorescence and photosynthesis (Fig. 1 and assumptions below) is based on energy conversion theory and biophysical models (Butler 1978, Weis and Berry 1987, Genty et al. 1989, Owens 1991, Kolber and Falkowski 1993, Kroon et al. 1993, Lavergne and Trissl 1995). To use fluorescence parameters as a proxy for maximal quantum yield for PSII (F_v/F_m), three assumptions and several simplifications must be made (Schreiber et al. 1995). First, the sum of the three probabilities of photochemistry (Ψ_p), heat dissipation (Ψ_d), and fluorescence (Ψ_f) represents all possible energy fates:

$$\Psi_p + \Psi_d + \Psi_f = 1 \tag{1}$$

Second, just after a saturating light pulse or treatment with an electron transport inhibitor (i.e. DCMU), when all the reaction centers are closed (subscript m), the probability of photochemistry becomes zero:

$$(\Psi_d)_m + (\Psi_f)_m = 1 \tag{2}$$

Finally, the ratio between the quantum yield of fluorescence and the quantum yield of heat dissipation is constant, regardless of the physiological status of the cell:

$$\frac{\Psi_d}{\Psi_f} = \frac{(\Psi_d)_m}{(\Psi_f)_m} \tag{3}$$

Through algebraic manipulation of Equations 2 and 3, $(\Psi_d)_m$ can be replaced by $1 - (\Psi_f)_m$, and Ψ_d can be expressed as $\Psi_f/(\Psi_f)_m - \Psi_f$ so the probability of photochemistry can be expressed solely in terms of probability of fluorescence.

$$\begin{aligned}\Psi_p &= 1 - \Psi_f - \left[\frac{\Psi_f}{(\Psi_f)_m} - \Psi_f\right] \\ &= 1 - \frac{\Psi_f}{(\Psi_f)_m} = \frac{(\Psi_f)_m - \Psi_f}{(\Psi_f)_m}\end{aligned} \tag{4}$$

Knowing the relative increase in fluorescence allows quantification of an algal cell's ability to undergo photosynthetic processes, and changes in fluorescence yield can be attributed to the probability of photochemical energy conversion (Falkowski et al. 1986, Schreiber 1986, Kiefer and Reynolds 1992, Schreiber et al. 1995). Fluorescence-based measures of maximum quantum yield reflect the probability that PSII reaction centers will use the available excitation energy. Therefore, the quantum yield of fluorescence and the quantum yield of PSII (ϕ_{PSII}) should be inversely related (Butler and Kitajima 1975).

TABLE 1. Symbols and units, from a compilation of fluorescence research papers (van Kooten and Snel 1990, Falkowski and Kolber 1993, Kolber and Falkowski 1993, Schreiber et al. 1995).

Symbols	Definitions	Units
F_o	Initial fluorescence	Relative
F_m	Maximal fluorescence	Relative
F_v	Variable fluorescence ($F_m - F_o$)	Relative
F_v/F_m	Fluorescence-based maximum quantum yield for PSII	Dimensionless
Ψ_f	Probability of fluorescence	Dimensionless
Ψ_d	Probability of heat dissipation	Dimensionless
Ψ_p	Probability of photochemistry	Dimensionless
ϕ_C	Quantum yield of carbon fixation	mol C fixed·(mol photons absorbed)$^{-1}$
$(\phi_C)_m$	Maximum quantum yield of carbon fixation	mol C fixed·(mol photons absorbed)$^{-1}$
ϕ_{PSII}	Quantum yield of PSII	mol electron·(mol photons absorbed)$^{-1}$
$(\phi_{PSII})_m$	Maximum quantum yield of PSII	mol electron·(mol photons absorbed)$^{-1}$
E	Irradiance	μmol photons·m^{-2}·s^{-1}
μ	Specific growth rate	d^{-1}
μ_{max}	Maximum growth rate at a growth E and temperature	d^{-1}

A problem with using fluorescence-based maximum quantum yield as an indicator of nutrient stress is the assumption that a constant relationship exists between Ψ_d and Ψ_f (Eq. 3), the ratio between heat dissipation and fluorescence, regardless of conditions. Olaizola and Yamamoto (1994) showed changes in these ratios under light-saturated conditions and concluded a breakdown of the fundamental assumptions relating Ψ_d, Ψ_f, and Ψ_p (Eqs. 3 and 4). They attributed the departure from the linear relationship to nonphotochemical quenching. Dark acclimation of samples (>30 min) minimizes the effects of nonphotochemical quenching, but the assumption of a constant ratio between heat dissipation and fluorescence (Eq. 3) may be an oversimplification of a complex relationship.

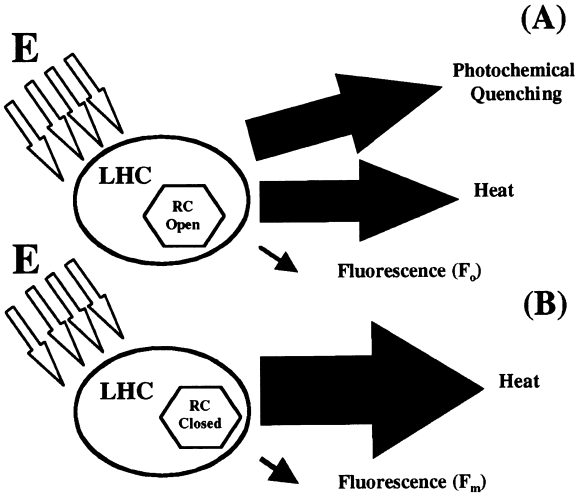

FIG. 1. A schematic showing the interaction between fluorescence, heat, and photochemical quenching. (A) If the reaction centers (RC) are open, the incident irradiance (E) is absorbed by the light harvesting complex (LHC) where it is dissipated by fluorescence, heat and photochemical reactions. (B) If the reaction centers are closed, photochemical reactions cannot occur and the dissipation of the energy absorbed by the LHC is directed to heat and fluorescence exclusively. Area of the arrows represent the probability of energy transfer by the respective pathways for energy absorbed by the photosynthetic unit through to charge stabilization in PSII.

Nutrition and growth. Precise definitions help to focus discussions of the nutrition and growth of phytoplankton. When growth and photosynthesis of phytoplankton are not restricted by the supply of nutrients, growth conditions are considered nutrient replete and growth rate is limited by irradiance and temperature (μ_{max}). Nutrient-replete conditions can be achieved using semicontinuous cultures, replacing media as fast as the algae can grow, and maintaining low biomass and high nutrient concentration.

Nutrient stress refers to both nutrient limitation and nutrient starvation. Nutrient limitation refers to balanced growth, where growth rate is determined by the rate of nutrient supply and the cells are fully acclimated to this restriction (Bannister and Laws 1980, Cullen et al. 1992). During acclimated growth on light:dark cycles, growth is balanced over a photoperiod (Shuter 1979). Nutrient starvation refers to unbalanced growth during which the availability of a limiting nutrient decreases relative to the cellular demand so that the rates of photosynthesis and growth decline (Shuter 1979, Eppley 1981, Cullen et al. 1992). The terms steady-state and acclimated growth are appropriate for conditions of nutrient limitation, whereas unbalanced and unacclimated growth refer to nutrient starvation. Although there are fundamental physiological differences between acclimated and unbalanced growth, the distinction between nutrient limitation and starvation is not always recognized. All conditions except for nutrient-replete growth can be considered nutrient stress.

Under batch culture experiments, an essential nutrient can be provided in short supply so it is depleted from the medium and becomes limiting, resulting in unbalanced growth, altered physiological status, and eventual cessation of growth. Limiting nutrients, in this case nitrogen, influence the physiological status of the cell, including its photosynthetic efficiency and ability to react to environmental stresses. Falkowski (1992) showed that nutrient starvation is correlated to the de-

cline of key reaction center proteins, leading to the inactivation of PSII reaction centers and changes in chemical composition.

Balanced growth can be achieved in continuous cultures where cells grow in an invariable environment with respect to nutrients and light. In these conditions, cells exhibit constant cellular compositions because carbon and nutrients are assimilated at identical cell-specific rates (Shuter 1979, Eppley 1981, Cullen et al. 1992). Cyclostat cultures, allowing for light:dark cycles, will never truly be in steady state because variations in growth rate and cellular constituents will occur over a photoperiod, but these cultures can be in acclimated growth, balanced over a 24-h period. Nutrient-limited growth rate is determined by the rate of dilution of the culture with fresh media. Biomass is determined by the concentration of the limiting nutrient in the media. Sampling the cultures at the same time each day can minimize confounding signals from diel variations. Continuous culture systems allow for the investigation of nutrient effects on phytoplankton physiology by minimizing the uncertainties of changing growth rates and growth conditions associated with sampling batch cultures. Comparisons between cyclostat, chemostat, and batch culture can reveal different aspects of nutrient stress.

Conventional versus active fluorometry. Conventional fluorometers can be used to estimate F_v/F_m. These measurements can play an important role in probing physiological state, but they have the disadvantage of requiring an electron transport inhibitor (i.e. DCMU) for determination of maximal fluorescence yield and thereby lose the ability to resolve important physiological parameters *in situ* under ambient light (Owens 1991). The body of knowledge on phytoplankton fluorescence using inhibitors (e.g. Prézelin et al. 1977, Samuelsson and Öquist 1977, Cullen and Renger 1979, Roy and Legendre 1979, Neale et al. 1989) and the increasing number of experiments that use active fluorescence techniques (Falkowski et al. 1986, Schreiber 1986, Genty et al. 1989, Öquist and Chow 1992, Kolber and Falkowski 1993, Babin et al. 1996a, Flameling and Kromkamp 1998) to determine different physiological parameters depends on the validity of the methods used to resolve fluorescence metrics. Disagreement between studies are difficult to resolve. Our experiments allow a direct intercomparison between conventional and active fluorometers.

MATERIALS AND METHODS

General culture conditions. Semicontinuous, batch, and chemostat/cyclostat culture experiments were performed to determine the variability of F_v/F_m in cultures of a neritic diatom, *Thalassiosira pseudonana*, Clone 3H, provided by the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP 1015). Triplicate cultures for each experimental treatment were grown under 40-W Vita-lite full-spectrum fluorescent bulbs (Duro-test Canada Inc., Rexdale, Ontario, Canada) under a 12:12 light:dark cycle, except for chemostat cultures and a set of replete cultures grown under continuous light. Desired light levels were achieved using neutral density screening (cellulose acetate; Lee Filters, Dartmouth, Nova Scotia, Canada) to reduce ambient light conditions. PAR (μmol photons$\cdot m^{-2}\cdot s^{-1}$) was measured with a Biospherical Instruments Inc. (San Diego, CA) QSL-100 4π sensor. The temperature for all cultures was controlled at $20 \pm 0.5^\circ$ C, and the chemostat/cyclostat samples were continuously mixed with magnetic stirrers and aerated with sterile air. Batch and replete cultures were manually agitated daily. The medium was f/2 (Guillard and Ryther 1962), made from artificial seawater (Keller et al. 1987), modified by omission of all nitrogen sources. Nitrogen was added to the medium aseptically in desired concentrations (Table 2). Chemostat and semicontinuous cultures were set up to repeat the experiment of Kolber et al. (1988) using the same clone (3H) and growth conditions.

Growth conditions. Cultures were preconditioned to their respective light regimes for a minimum of 2 weeks before each experiment. Continuous cultures were grown under reduced nitrogen f/2 medium (50 μmol$\cdot L^{-1}$) before the experiments so that the physiological stress on the cultures investigated would be minimized.

Semicontinuous cultures were maintained in f/2 medium through many generations to study acclimated growth, replete in all nutrients. Cultures (2.5 L) were grown in triplicate in 4-L Erlenmeyer flasks for each treatment. They were diluted daily to maintain a constant cell density and, after acclimation, a constant maximal specific growth rate (μ_{max}). Chl concentrations did not exceed 150 mg chl$\cdot m^{-3}$. Data for other semicontinuous experiments grown at different irradiance levels (Table 2) are from previously described experiments (Cullen et al. 1992, Zhu et al. 1992).

Triplicate N-deficient batch cultures (2.5 L each) were grown in 4-L Erlenmeyer flasks and were maintained until the culture reached late senescence (26 days) to observe changes in physiological and cellular parameters. The cultures had an initial concentration of 150 μmol$\cdot L^{-1}$ $NaNO_3$. Nutrient concentrations were monitored to ensure that nitrogen was the limiting resource and to show when nitrate was depleted.

Nitrogen starvation was also imposed by stopping flow of the medium in N-limited continuous cultures acclimated to different growth rates. Therefore, N-starvation experiments were conducted on both nutrient-replete cultures and N-limited acclimated cultures (Table 2).

Triplicate chemostat or cyclostat cultures were grown for a minimum of 10 generations, allowing cells to acclimate to the nutrient-limited conditions. It is important to stress the importance of preconditioning (light levels, nutrient concentration) of the cultures to facilitate physiological acclimation with minimal disruption. Chemostat cultures were grown under continuous light of 150 μmol photons$\cdot m^{-2}\cdot s^{-1}$, whereas cyclostat cultures were grown at 55 and 350 μmol photons$\cdot m^{-2}\cdot s^{-1}$ (PAR) on a 12:12 light:dark cycle (Table 2). Cultures (2.1 L) were grown in 2.5-L polycarbonate bottles. The growth rates of the chemostat cultures were predetermined by controlling the dilution rates (inflow/outflow of medium). The nitrogen source for the chemostat cultures was 75 μmol $NH_4^+\cdot L^{-1}$, whereas the cyclostat cultures were diluted with fresh medium containing 50 μmol $NO_3^-\cdot L^{-1}$. Data from other cyclostat experiments (Table 2) are from previously described experiments (Cullen et al. 1992, Zhu et al. 1992) using essentially the same culturing methods.

Measurements. Routine sampling was conducted 3 h into the light period (10:00 h) and included measures of chl concentration, cell size and cell density, nutrient concentration, dilution and overflow volumes, and fluorescence as determined by pulse amplitude modulation (PAM) (Schreiber et al. 1995) and DCMU methodology (Samuelsson and Öquist 1977). Cultures were sampled at the same time ($\pm$30 min) to minimize effects of diel periodicity in algal physiological factors (Prézelin et al. 1977). Chl *a*, corrected for phaeopigments (Strickland and Parsons 1972), was measured using a Turner Designs (Sunnyvale, CA) fluorometer (10-005 R) calibrated with pure chl *a* (Sigma Chemical Co., St. Louis, MO). Duplicate volumes of 1 mL each were filtered on Whatman GF/F filters (Whatman Inc., Clifton, NJ) and extracted in 10 mL of 90% acetone in the dark at -15° C for at least 24 h. Cell size and density were determined on triplicate samples after dilution on a Coulter Multisizer II Particle Analyzer (Coulter Electronics of Canada, LTD., Burlington,

TABLE 2. Summary of experiments performed, with the initial nutrient concentrations, light levels, growth rates, maximal growth rates, and duration of the individual experiments.

Type of experiment	Initial N levels ($\mu mol \cdot L^{-1}$)	Irradiance ($\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$)	μ (d^{-1})	μ_{max}[a] (d^{-1})	Duration (d)
Unbalanced growth					
Batch N starvation	150 (NO_3^-)	350	Variable	1.5	26
N starvation of	<0.2 (NO_3^-)	350	Variable (initial = 0.8)	1.5	7
N-limited	<0.2 (NO_3^-)	350	Variable (initial = 0.4)	1.5	7
Continuous cultures	<0.2 (NH_4^+)[b]	150	Variable (initial = 0.3)	1.78	5
	<0.2 (NO_3^-)	55	Variable (initial = 0.4)	0.7	7
	<0.2 (NO_3^-)	55	Variable (initial = 0.2)	0.7	7
Balanced growth					
Semicontinuous (N-replete)	880(NH_4^+)[b]	150	1.78	1.78	15
	880(NO_3^-)[c]	9	0.05	0.05	36
	880(NO_3^-)[c]	25	0.38	0.38	20
	880(NO_3^-)[c]	50	0.67	0.67	12
	880(NO_3^-)[c]	75	0.85	0.85	8
	880(NO_3^-)[c]	100	0.97	0.97	12
	880(NO_3^-)[c]	200	1.33	1.33	9
	880(NO_3^-)[c]	410	1.56	1.56	6
	880(NO_3^-)[c]	912	1.82	1.82	6
Chemostat (N limited)	75(NH_4^+)[b]	150	0.30	1.78	26
Cyclostat	50(NO_3^-)	350	1.20	1.50	34
N limited	50(NO_3^-)	350	0.80	1.50	35
PAM and DCMU	50(NO_3^-)	350	0.40	1.50	35
	50(NO_3^-)	350	0.40	1.50	35
	50(NO_3^-)	55	0.43	0.71	37
	50(NO_3^-)	55	0.21	0.71	35
Cyclostat[c]	50(NO_3^-)[b]	75	0.13	0.85	15
N limited	50(NO_3^-)[c]	75	0.35	0.85	11
DCMU only	50(NO_3^-)[c]	75	0.56	0.85	15
	50(NO_3^-)[c]	75	0.61	0.85	25
	50(NO_3^-)[c]	200	0.20	1.33	32
	50(NO_3^-)[c]	200	0.43	1.33	26
	50(NO_3^-)[c]	200	0.59	1.33	13
	50(NO_3^-)[c]	200	0.84	1.33	25
	50(NO_3^-)[c]	200	1.11	1.33	17

Cultures were grown under 12:12 light:dark cycles, unless otherwise indicated.
[a] Growth rate in nutrient-replete semicontinuous culture at the same irradiance.
[b] 24-hour light cycle.
[c] Data from previously described experiments of Zhu et al. (1992) and Cullen et al. (1992).

Ontario, Canada) calibrated with latex beads. Concentrations of NO_3^- and NH_4^+ were determined from filtered (0.22 μm) samples of culture medium by a Technicon II Autoanalyzer (Technicon Co., Tarrytown, NY) (Grasshoff et al. 1976). Ammonium concentration was determined for chemostat cultures only. The overflow volumes and dilution rate for chemostat and cyclostat cultures were measured on a daily basis. For semicontinuous cultures, growth rate (μ, d^{-1}) was determined using the exponential growth equation to describe changes in cell density (N, $cells \cdot mL^{-1}$), accounting for dilution (D) (volume fresh medium/total volume) over a discrete period of time (ΔT, d):

$$\mu = \frac{1}{\Delta T}\left(1n\frac{[N_{T+\Delta T}]/(1-D)}{[N_T]}\right) \quad (5)$$

For fluorescence measurements, samples from each culture were dark adapted for 30 min. For the DCMU methodology, fluorescence was measured, on triplicate 10-mL samples, before and 30 s after the addition of 50 μL of 3 mM DCMU in ethanol. To test if the light level within the fluorometer was high enough to close reaction centers, thereby violating the assumption for measurement of F_o, neutral screening (cellulose acetate, 60% transmission) was placed around the cuvettes and fluorescence was measured on a parallel set of subsamples for comparison. PAM fluorescence measurements using the PAM101/102/103 system (Walz, Effeltrich, Germany) with a photomultiplier tube accessory and the emitter-detector-cuvette assembly (ED101) were done on duplicate samples. The digital signal was recorded using Labview 4.0 (National Instruments, Austin, TX). Minimal fluorescence (F_o) was measured using a light-emitting diode delivering a modulated measuring light beam (λ = 650 nm), too weak to induce reaction center closure. For the measurement of maximal fluorescence (F_m), a minimum of 16 saturating pulses (Schott KL1500-E; E > 5000 $\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$) each of 600-ms duration were delivered at 30-s intervals. The interval between saturation pulses allows for reoxidation of the reaction centers. Investigation into the duration, intensity, and time between saturation pulse was performed to ensure the proper settings on the PAM fluorometer (data not shown).

RESULTS

Comparison of measurement systems. Two independent fluorescence measurement systems were compared for describing F_v/F_m of cultures under nutrient-replete, nutrient-starved, and nutrient-limited growth conditions. Comparison between PAM fluorometry (active) and DCMU methodology (conventional) showed a strong positive linear correlation (r^2 = 0.63, $P < 0.001$, n = 460), although departure from a one-to-one relationship was apparent (slope = 0.79 ± 0.03 [95% CI]) and deviations for the individual experiments grown under vary-

ing light levels were observed (Fig. 2A). F_v/F_m measured by conventional fluorometry using DCMU was lower than when measured with PAM for cultures grown in low irradiance (<150 μmol photons·m^{-2}·s^{-1}) (Fig. 2B).

Neutral density screen was used to reduce excitation irradiance from the Turner Designs fluorometer for cultures grown at 55 μmol photons·m^{-2}·s^{-1}. This eliminated the artifactually low F_v/F_m, due to actinic light closing reaction centers, thereby giving elevated values of F_o for low light cultures. With this modification of the method, a much better relationship between DCMU and PAM methodologies was observed (Fig. 2C). The regression line from this comparison explains 77% of the variance and shows a strong one-to-one agreement (slope = 1.03 ± 0.02 [95% CI]). A paired Student's *t* test identified no significant difference when neutral density screening was used in the measurement of F_v/F_m for acclimated and subsequently starved cultures grown under 150 μmol photons·m^{-2}·s^{-1} and 350 μmol photons·m^{-2}·s^{-1} ($P > 0.10$, $n = 144$, F_v/F_m range = 0.11–0.73). Therefore, neutral density screen was used only for cultures grown at the lower irradiance.

F_v/F_m for nutrient-replete cultures. Triplicate semicontinuous cultures, replete in all nutrients and grown under continuous light (150 μmol photons·m^{-2}·s^{-1}), showed nearly identical growth rates (average of daily determinations; 1.78 d^{-1} ± 0.04 d^{-1} [± SE, $n = 3$], Fig. 3). Measurements of F_v/F_m (PAM) during this time course were relatively high and changed little (0.60 ± 0.02 [± SE, $n = 45$]) (Fig. 3A), consistent with previous studies of F_v/F_m in nutrient-replete cultures. Estimates of F_v/F_m with DCMU (0.61 ± 0.03 [± SE, $n = 45$]) were nearly the same as with PAM, confirming the strong agreement between instruments.

For semicontinuous replete cultures, grown on a 12:12 light:dark regime, growth rate (μ_{max}) was a saturation function of irradiance (Fig. 3B). Using DCMU methodology, F_v/F_m remained consistently high (~0.65) for all growth irradiances tested except for decreases in F_v/F_m at low irradiances (<150 μmol photons·m^{-2}·s^{-1}), which can be attributed to underestimation due to the artifact for low-light adapted that was subsequently identified (Fig. 2).

F_v/F_m during N starvation. For N-starved cultures, reduced F_v/F_m and growth rate as determined from cell density and fluorescence measurements were apparent after nitrogen was depleted (Fig. 4). For the N-starved

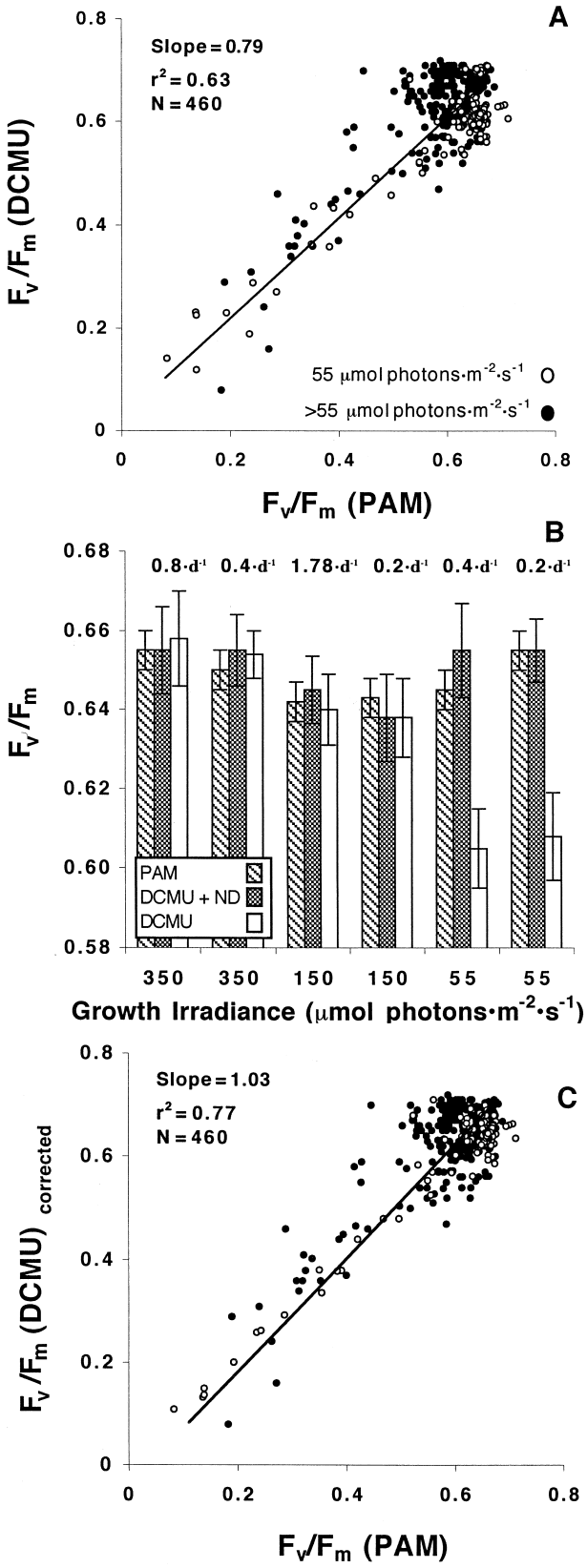

FIG. 2. Comparison between active fluorometry (PAM) and fluorometry determined by DCMU methodology. (A) F_v/F_m determined with PAM vs. DCMU (no neutral density screen) for different growth conditions and light levels from Table 2 ($n = 460$). Although the relationship shows agreement, clusters are apparent for low light cultures (55 μmol photons·m^{-2}·s^{-1} = ○). (B) F_v/F_m for acclimated cyclostat cultures of *Thalassiosira pseudonana* at different growth irradiance and different N-limited growth rates for the PAM (▨) and DCMU methodology with 60% neutral density screening (■) and without 60% neutral density screening (□). Means ± SD for three replicates. Correction of the method through the use of neutral density screening around the cuvettes was necessary for cultures grown under low light to prevent closing of reaction centers and overestimation of F_o. (C) Comparison of the two fluorescence measurement systems when the corrected DCMU methodology is used (see text).

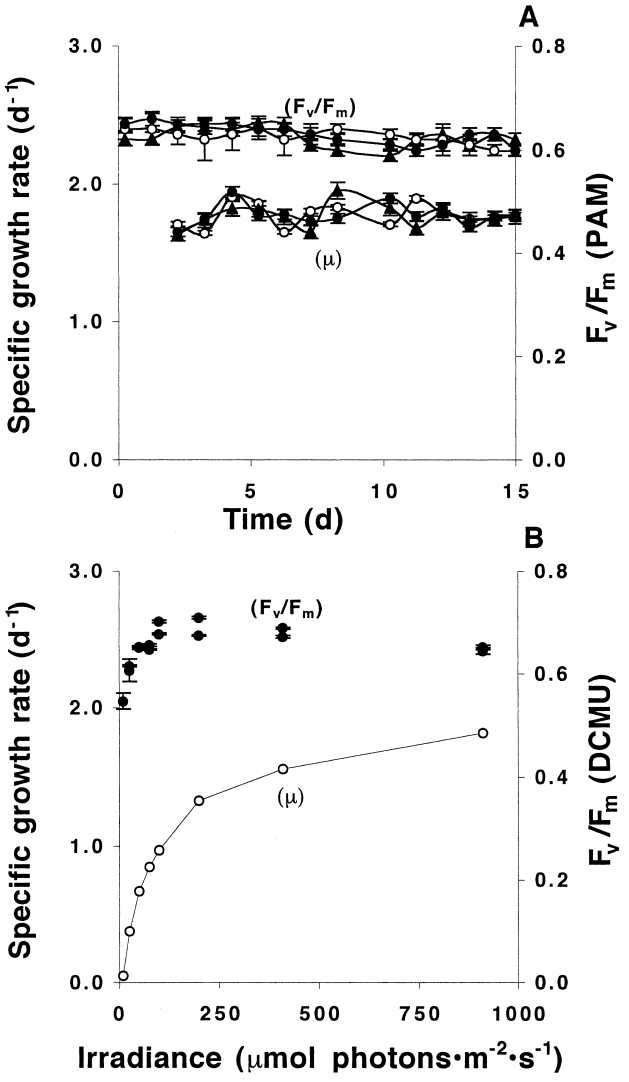

FIG. 3. (A) Specific growth rate as a function of time (lower points) for NH_4^+ replete cultures of *Thalassiosira pseudonana* grown under 150 μmol photons·m^{-2}·s^{-1} continuous light. Growth rate was determined at daily intervals (ΔT = 1d) using equation 5. The upper points show F_v/F_m determined from active fluorometry (PAM) over the same time-course. The symbols represent triplicate cultures and error bars represent standard error. (B) μ_{max} and F_v/F_m, determined from a conventional fluorometer (DCMU methodology), as a function of irradiance for nutrient-replete cultures grown under a 12:12 light regime. Actinic light from the fluorometer was not reduced, so the slight decline of F_v/F_m for low-light cultures may be an artifact as described in Figure 2. Means ± SE for three replicate cultures.

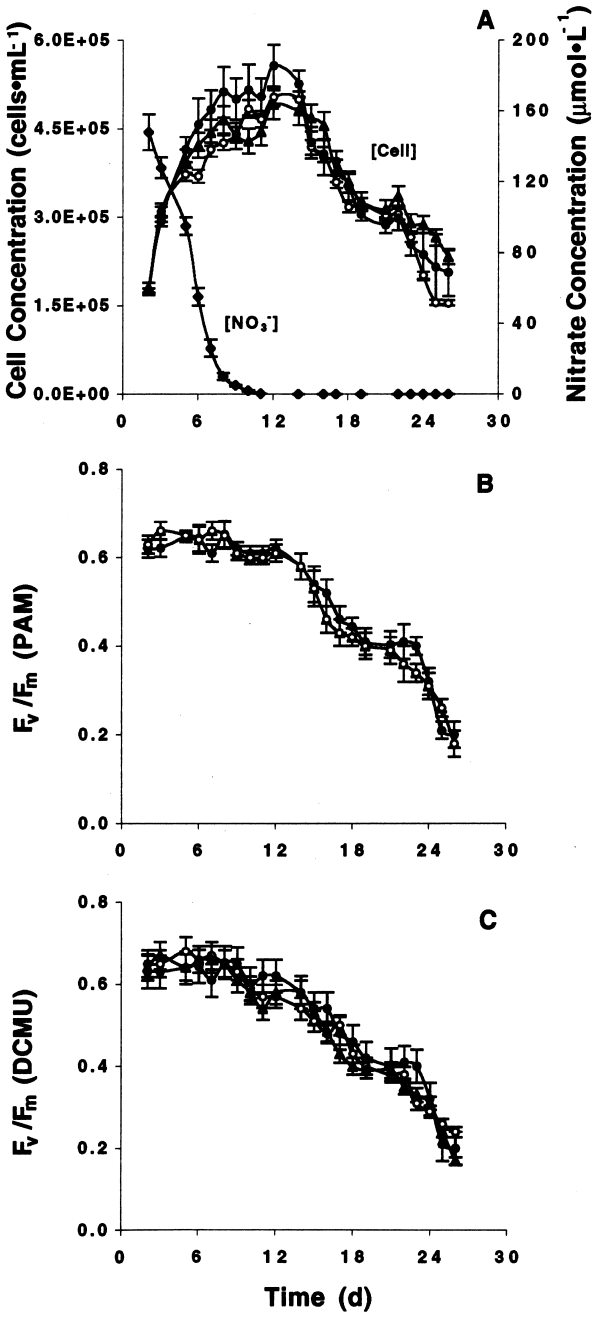

FIG. 4. (A) Cell concentration and ambient nitrate concentration (symbol = ◆) as a function of time for *Thalassiosira pseudonana* batch cultures (350 μmol photons·m^{-2}·s^{-1} PAR), while F_v/F_m for the same triplicate cultures is shown as a function of time as measured with active fluorometry (PAM) (B), and DCMU methodology using a conventional fluorometer (C). Means ± SE for three replicates for all three graphs. Nitrate concentrations with values below detection limit (<0.1 μM) are represented with a zero value. Nitrate data represent means of triplicate cultures ± SD.

batch experiment, routine measures showed cultures under unbalanced growth conditions and showed a reduced F_v/F_m as a function of nutrient starvation (Fig. 4). F_v/F_m determined by DCMU (Fig. 4C) agreed well with F_v/F_m determined by PAM for batch cultures (r^2 = 0.91, n = 150).

Starvation was also imposed on the N-limited continuous cultures by stopping the inflow of nutrients into the system. When the cultures were perturbed by turning off the pump, F_v/F_m of the previously acclimated algal cultures declined as a function of both time without nutrient supply and preconditioned N-limited growth rate (Fig. 5). The rate of decrease of F_v/F_m was determined from linear regression for the

final 4 days of each nitrate-starvation experiment (triplicate cultures at four growth rates). The slopes varied little (mean = -0.09 ± 0.02 d^{-1} [± SE, n = 12]), indicating that the decline of F_v/F_m was independent of N-limited growth rate preconditioning and irradiance level.

Cultures that were previously acclimated to lower N-limited growth rates had a shorter time interval before the decline of F_v/F_m during N starvation compared with higher N-limited growth rates (Fig. 5). For each culture, changes in F_v/F_m for the 5 days before the termination of nutrient flow and 3 days after were analyzed by repeated-measures analysis of variance (ANOVA). No significant difference in F_v/F_m through time was observed before imposed nutrient starvation, although significant declines were detected after termination of nutrient supply. The time of onset of the decline in F_v/F_m was determined post hoc using a least-squares means comparison to find the first sample with significantly lower F_v/F_m. Significant declines in F_v/F_m were evident at 0.5 ± 0.2 days (mean ± SE, n = 3) after imposed N starvation for both the μ/μ_{max} = 0.26 and μ/μ_{max} = 0.29 cultures, whereas cultures previously acclimated to μ/μ_{max} = 0.53 and 0.60 showed an onset of F_v/F_m decline at 1.0 ± 0.5 and 2.0 ± 0.5 days, respectively (Fig. 5, A and B). Cultures grown replete for nutrients (μ/μ_{max} = 1.0; initial nitrate concentration 880 μmol·L^{-1}) showed no decline in F_v/F_m when replenishment of the medium was stopped for 4 days. The 24-h, continuous light, NH_4^+-limited 0.17 μ/μ_{max} cultures showed the most rapid onset of the decline of F_v/F_m (0.4 ± 0.2 days, n = 3) (Fig. 5C). In other words, the start of the decline of F_v/F_m was related to the nutrient-limited preconditioning, although the rate of decline was independent of preconditioning. Consequently, F_v/F_m 4 days after interruption of the nutrient supply is a strong function of N-limited growth rate at the time of the interruption (Fig. 5C). The assumption of normality underlying ANOVA was tested in each analysis using a G-test at α = 0.10.

F_v/F_m under acclimated N limitation. When chemostat and cyclostat cultures were acclimated to N limitation, F_v/F_m was maximal (~0.65) for the range of light intensities investigated (Fig. 6). Both the fluorescence measurement systems (PAM and DCMU) provided similar evidence for F_v/F_m being independent of both irradiance and acclimated nutrient-limited growth rate (Fig. 6) for all balanced growth experiments con-

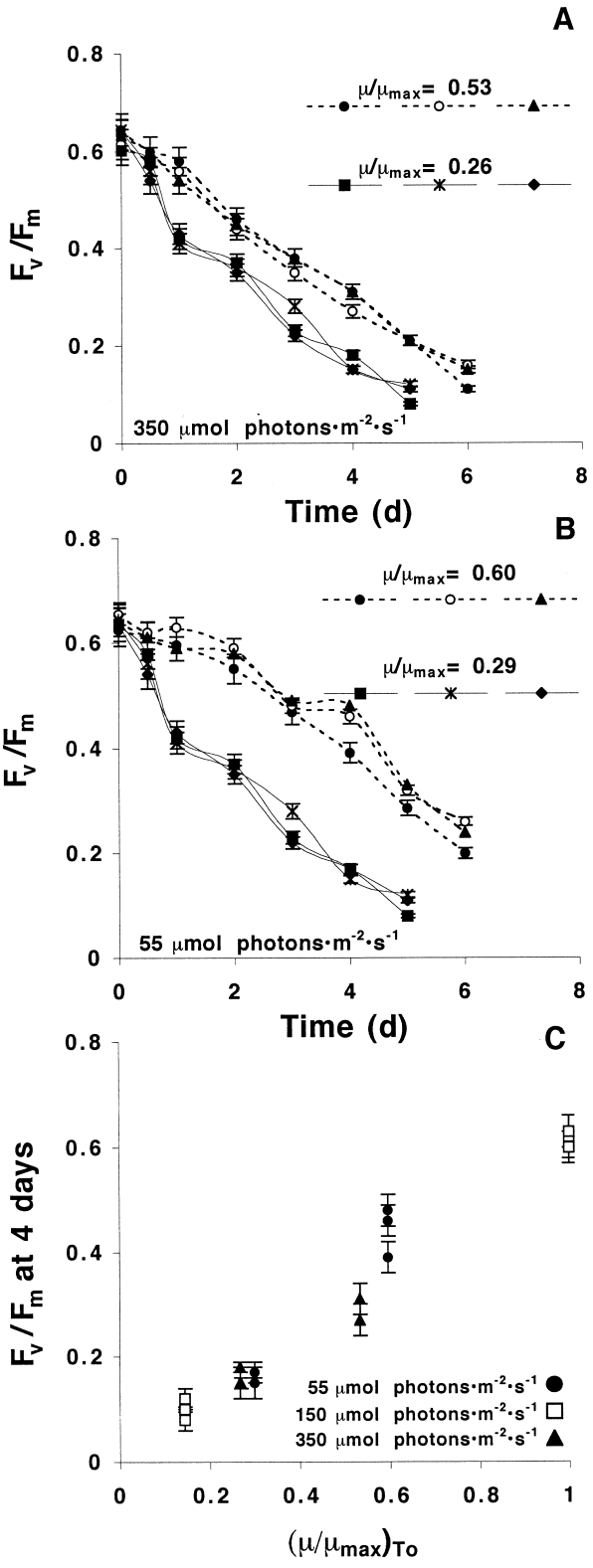

FIG. 5. Nutrient starvation for cultures previously acclimated to nitrate-limited growth. F_v/F_m as a function of the duration of nutrient stress defined as the time after the flow of nutrients is stopped (time = 0) for four different pre-conditioned nutrient-limited relative growth rates at two different growth irradiances (A) 350 μmol photons·m^{-2}·s^{-1} (μ_{max} = 1.50 d^{-1}) and (B) 55 μmol photons·m^{-2}·s^{-1} (μ_{max} = 0.71 d^{-1}). Error bars show SD of triplicate cultures. (C) F_v/F_m, determined from PAM fluorometry, as a function of nutrient-dependent relative growth rate for perturbed continuous cultures, 4 days after the flow of nutrients was stopped for three different irradiance (● = 55 μmol photons·m^{-2}·s^{-1}, ▲= 350 μmol photons·m^{-2}·s^{-1}, ■ = 150 μmol photons·m^{-2}·s^{-1}). The points for μ/μmax = 1.0 represent nutrient replete semi-continuous cultures for which replacement of medium was stopped at time zero. Means ± SE for three replicate cultures.

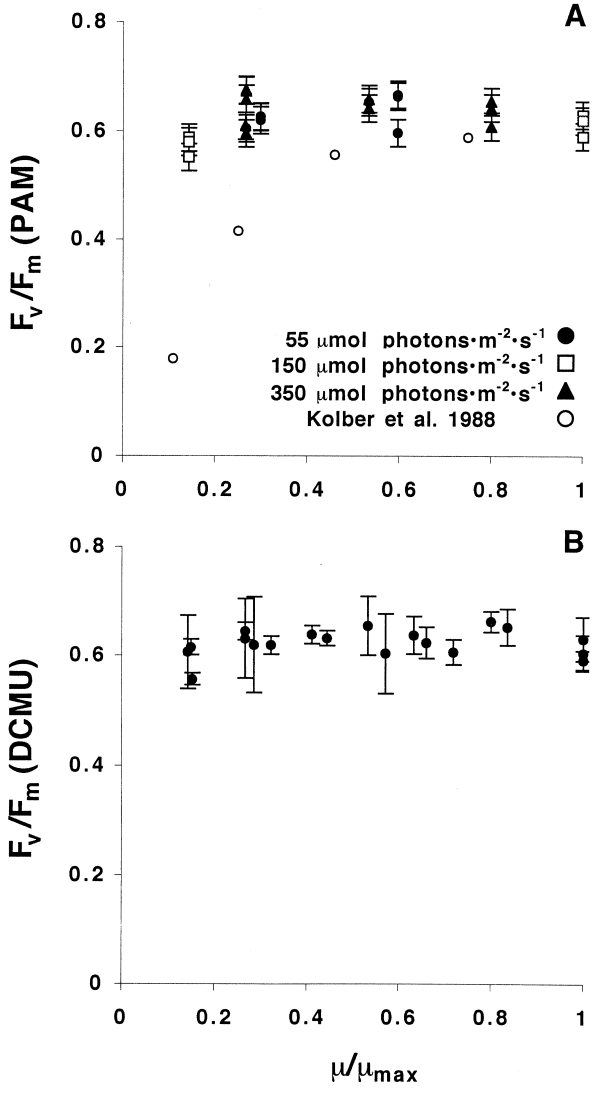

FIG. 6. (A) F_v/F_m determined from active fluorometry (PAM) under balanced growth conditions for a range of nutrient-dependent growth rates and growth irradiance (● = 55 μmol photons·m^{-2}·s^{-1}, ▲= 350 μmol photons·m^{-2}·s^{-1}, ■ = 150 μmol photons·m^{-2}·s^{-1}). Estimates of F_v/F_m from the findings of Kolber et al. (1988), who reported F_v/F_o measured with a Pump and Probe fluorometer on *Thalassiosira pseudonana* (3H), are shown for comparison (symbol = ○). (B) F_v/F_m determined by conventional fluorometry (DCMU methodology, no neutral density screen) for continuous cultures grown at different irradiances and N-limited growth rates. Data from previous experiments (Cullen et al. 1992 and Zhu et al. 1992). Means ± SE for three replicates.

ducted in our laboratory and previous experiments (Cullen et al. 1992, Zhu et al. 1992) (Fig. 6B). This contradicts the findings of Kolber et al. (1988), who showed that F_v/F_m was dependent on the degree of nutrient limitation in chemostats (Fig. 6A).

Acclimation of our cultures was determined by constancy from day to day of cell density, cell size, chl, ambient nutrient concentrations, and fluorescence. Steady state was assumed when several parameters (i.e. chl, fluorescence, cell, and nutrient concentration) remained constant (±10%) over a 3-day period (Fig. 7). Figure 7 shows only the chl and fluorescence parameters over the duration of three representative experiments at the different growth irradiances, whereas cell and nutrient concentrations also played a role in determining status of the algal growth conditions being investigated (data not shown). It is clear that physiological changes can proceed for many days before acclimation is achieved.

DISCUSSION

Our results from experiments on N-starved batch and N-replete semicontinuous cultures support the established body of evidence that F_v/F_m can be used as a diagnostic of nutrient stress. However, we also show that this fluorescence metric is insensitive to N limitation when cultures are fully acclimated to N-stressed conditions. This result conflicts with previously published work on cultures grown in continuous cultures identified as being in steady state (Kolber et al. 1988). Possible reasons for this discrepancy may be due to instrumentation or growth conditions.

PAM versus DCMU methodology. The possibility exists that differences in methodology could explain the fundamental contrast between our results using PAM and DCMU and the results of Kolber et al. (1988), who used a Pump and Probe measurement system (Falkowski et al. 1986). Although our results show a strong positive correlation between the PAM and the DCMU methodology, no direct comparison between the PAM fluorometer and the Pump and Probe system was done. Geider et al. (1993) showed a significant correlation between measurements of F_v/F_m from the Pump and Probe fluorometer and from a Turner Designs fluorometer using DCMU, both in the laboratory (r^2 = 0.613, n = 28) and during a cross-shelf transect in the western North Atlantic (r^2 = 0.637, n = 24). Because we show a strong correlation between the Turner Designs fluorometer (DCMU methodology) and the PAM system, and Geider et al. (1993) show agreement between the Turner Designs and the Pump and Probe system, we conclude that the fundamental differences in results (Figs. 6A and 8) are not due to instrumentation.

We have not conducted a detailed comparison of methods for measuring F_v/F_m and we do not assert that any one method is best. This study shows that fundamental patterns in F_v/F_m as functions of nutrition can be reproduced by two methods (PAM and DCMU) and that differences between these and other studies is not likely due to differences in methodology.

Measurement of F_v/F_m with a conventional fluorometer and DCMU is convenient, relatively inexpensive, and easy, with a wide range of past and future ecophysiological applications (Cullen and Renger 1979, Neale et al. 1989, Krause and Weis 1991, Geider et al. 1993). We show that the method compares well with the more sophisticated PAM approach when an artifact for cultures grown under low light is avoided

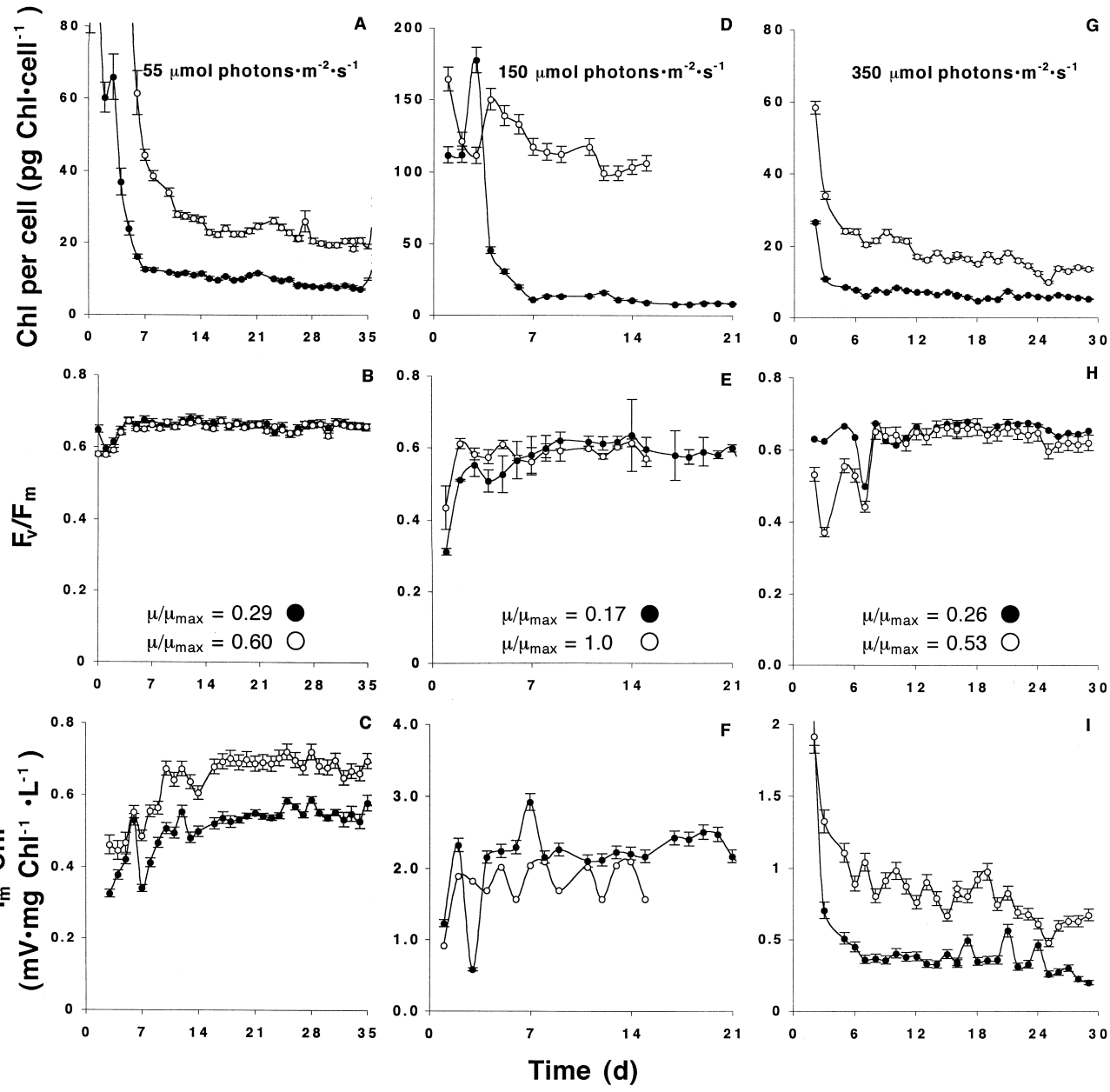

FIG. 7. Physiological parameters of *Thalassiosira pseudonana* as a function of time for determining balanced growth conditions. Representative cultures grown under low nutrient-limited growth rates (●) and high nutrient-limited growth rates (○) for different irradiance levels: 55 μmol photons·m^{-2}·s^{-1}, with growth rates of 0.43 and 0.21 d^{-1} (A-C), 150 μmol photons·m^{-2}·s^{-1}, with growth rates of 1.0 and 0.17 d^{-1} (D-F), 350 μmol photons·m^{-2}·s^{-1}, with growth rates of 0.80 and 0.40 d^{-1} (G-I)). Parameters shown as a function of time include changes in chl per cell, F_v/F_m and F_m per unit chl. Means ± SE for three replicate cultures.

through reduction of excitation irradiance (Fig. 2). This artifact is created by increased light sensitivity of the algae due to a physiological adaptation of increased chl concentration per cell, and likely increased chl per photosystem, for cultures grown under low light. Harris (1978) states that the low energy source from the Turner Designs fluorescence measurement system would not create the artifact of elevated initial fluorescence, although the studies did not include low light-adapted cultures. Further supporting evidence for an actinic light artifact for conventional fluorometers comes from our semicontinuous experiments, which showed a reduction in F_v/F_m for cultures grown under low light conditions using the conventional measurement system (Fig. 3). Once the artifact is eliminated, the regression line explains 77% of the variance (Fig. 2). We conclude that conventional and active fluorometry can be used interchangeably to assess fluorescence-based measurements of maximal quantum yield, if excitation energy in the fluorometer is reduced to en-

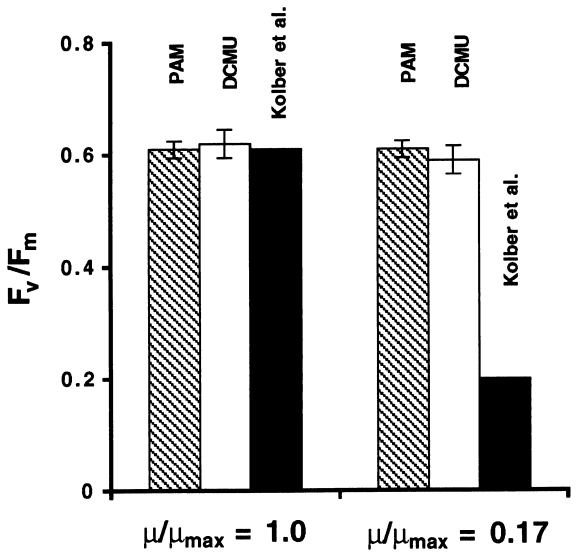

FIG. 8. Comparison of F_v/F_m determined during this study and in the study of Kolber et al. (1988) for both nutrient-replete (μ/μ_{max} = 1.0) and ammonium-limited (μ/μ_{max} = 0.17) steady-state cultures. Two independent measurement systems, PAM and DCMU methodology using a conventional fluorometer (means ± SE for three replicates), were compared to F_v/F_m determined using a Pump and Probe fluorometer by Kolber et al. (1988). Those values are interpreted from graphical representation and may not be exact.

sure that reaction centers are not significantly closed by the measurement system.

F_v/F_m for nutrient-replete cultures. Our findings on replete cultures support the current literature (Falkowski and Kolber 1995, Kolber et al. 1988) that under semicontinuous exponential growth conditions the fluorescence-based measure of maximum quantum yield for PSII (F_v/F_m) remains maximal and constant and is insensitive to irradiance levels (Fig. 3). This result is not novel and only serves to increase the growing paradigm that F_v/F_m is not sensitive to growth irradiance for cultures grown under nutrient-replete conditions.

F_v/F_m during N starvation. Our batch culture experiment showed that under nutrient-starved conditions, F_v/F_m declined, reflecting the degree of nutrient stress (Fig. 4). After the cell has used its stores and is in a state of nutrient starvation, the culture will show adverse physiological effects (Cleveland and Perry 1987, Falkowski and Raven 1997, Berman-Frank and Dubinsky 1999). Once essential cellular components cannot be synthesized and balanced growth conditions have been perturbed for days, fluorescence-based maximal quantum yield for PSII is significantly reduced. The results from our batch culture experiments contribute to the established body of evidence that maximal quantum yield is a good indicator of nutrient starvation (Cleveland and Perry 1987, Geider et al. 1993, Falkowski and Raven 1997).

We also find that the reduction in fluorescence-based maximal quantum yield as a consequence of starvation is also a function of the preconditioned nutrient-dependent growth rates (Fig. 5). The onset of the decrease in F_v/F_m occurred more rapidly in the cultures previously acclimated to lower nutrient-limited growth rates. These findings show that phytoplankton experiencing lower nutrient-limited growth rates would be more susceptible to interruptions in nutrient supply in the laboratory or the field, consistent with the decline of F_v/F_m being related to depletion of cellular stores.

F_v/F_m under acclimated N limitation. Our results for chemostat and cyclostat cultures at multiple light intensities and growth rates show that under fully acclimated growth a constant fluorescence-based maximum quantum yield for PSII is obtained (Fig. 6). This is contradictory to the study of Kolber et al. (1988), which showed reduced fluorescence-based maximal quantum yield as a function of nutrient limitation (Fig. 8). We tried to replicate the experiments of Kolber et al. (1988), but we did not have the same measurement system. Nevertheless, we minimized this discrepancy by using two independent measurement systems and giving strict adherence to steady-state criteria. We hypothesize that disparate results may be attributed to the attention given to ensure that balanced growth conditions for all nutrient-limited growth rates were achieved (Fig. 7). Kolber et al. (1988) sampled cultures 7 days into the chemostat experiment, which may not provide sufficient time for the cultures to acclimate to their nutrient-dependent growth rate. Preconditioning the algal cultures to their growth irradiance and nutrient concentrations and waiting a minimum of 10 generations for the cultures to acclimate were done in our experiments. Our nutrient-interruption experiments and daily monitoring showed that slower growing N-stressed cultures are more susceptible to perturbations and slower to reach and maintain acclimated growth. Therefore, scrutiny of different physiological indicators of acclimated balanced growth, especially at low growth rates, is important in studies of cultures in steady-state growth.

Zhu et al. (1992) reported a constant fluorescence ratio for most N-limited relative growth rates, which supports our findings. The ratio broke down in their batch cultures similar to our experiments, which is shown in their article as a relative growth rate of zero. Zhu et al. (1992) show a decreased ratio of enhanced fluorescence to fluorescence (F_m/F_o) for the lowest relative growth rate (~0.15) in the continuous culture for the low light cultures (75 μmol photons·m^{-2}·s^{-1}). This result might be due to the overestimate of initial fluorescence by the Turner Designs measurement system in low light-adapted cultures. Further evidence of a high and constant F_v/F_m under nutrient limitation was reported by MacIntyre et al. (1997) for a dinoflagellate, *Alexandrium tamarense*, grown in semicontinuous culture. This support for our findings also emphasizes a limitation of our study. Our results are limited to one coastal species, and the investigation of F_v/F_m as a function of nutrient stress for other species, including oceanic isolates, is still required.

F_v/F_m as a diagnostic of N stress. Cleveland and Perry (1987) and Kolber et al. (1988) provided evidence that F_v/F_m can be used as an indicator of nutrient stress,

showing reductions under nitrogen starvation and limitation respectively. Graziano et al. (1996) and Geider et al. (1993) support this hypothesis of reduced F_v/F_m being an indicator of nutrient stress in the field. However, the conclusions of Graziano et al. (1996) may relate primarily to growth conditions of a eutrophic coastal environment at the final sampling station (station 8), because no other oligotrophic stations sampled (stations 1–7) showed significant correlation between nutrients, determined by nutrient addition experiments, and the fluorescence-based measure of maximum quantum yield (F_v/F_m). Babin et al. (1996b) provided further support for the role of nitrogen stress in reducing maximum quantum yield of carbon fixation $(\phi_C)_m$, but also identified covarying factors such as the contribution of nonphotosynthetic pigments to reduce maximum quantum yield. Kolber et al. (1990) and Babin et al. (1996b) showed patterns of fluorescence suggestive of nutrient stress in nature. However, our results suggest that, using F_v/F_m as a diagnostic, it is difficult to assess whether some natural populations are in acclimated nutrient limitation. The literature supports a dynamic environment hypothesis in which true balanced growth is never really achieved (Richerson et al. 1970, Harris 1978, Harris et al. 1980, Falkowski and Raven 1997), although a biological system will tend toward a steady-state condition and may be expected in some conditions to be nearly balanced (Shuter 1979, Eppley 1981). Thus, if sustained N limitation persists in nature, a degree of balanced growth is probable, causing uncertainty in F_v/F_m as an indicator of the degree of nutrient stress.

In summary, two independent fluorescence measurement systems, PAM fluorometry (active) and DCMU methodology (conventional), showed a strong 1:1 correlation when the DCMU method was corrected for overexcitation of minimal fluorescence for cultures grown under low light. These independent systems provide evidence that under nutrient-replete growth conditions, F_v/F_m for the neritic diatom *Thalassiosira pseudonana* (3H) is high and independent of growth irradiance, whereas during nutrient starvation F_v/F_m declines and is correlated with time without nutrients. These findings are consistent with the current literature. When nutrient starvation was imposed on acclimated N-limited cultures, the onset of the decline of F_v/F_m was a function of preconditioned N-limited growth rate, although the subsequent rate of decline of F_v/F_m was independent of preconditioned N-limited growth rates. This would suggest that F_v/F_m is more susceptible to perturbations in nutrient supply for phytoplankton with lower N-limited growth rates. In contrast to published results, F_v/F_m remained high and constant ($\sim$0.65) for our acclimated steady-state cultures at different nitrogen-limited growth rates, independent of growth irradiance. This result should be verified for a range of species isolated from different environments.

We conclude that fluorescence-based maximal quantum yield for PSII is not a robust diagnostic for all nutrient-stressed conditions. It is a sensitive indicator of nutrient stress during unbalanced growth, but when phytoplankton are acclimated to nutrient limitation, the relationship between F_v/F_m and nutrient stress breaks down. This limits the utility of F_v/F_m as a measure of phytoplankton physiological status in the laboratory and the field.

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References

Babin, M., Morel, A. & Gentili, B. 1996a. Remote sensing of sea surface sun-induced chlorophyll fluorescence: consequences of natural variations in the optical characteristics of phytoplankton and the quantum yield of chlorophyll a fluorescence. *Int. J. Remote Sensing* 17:2417–48.

Babin, M., Morel, A., Claustre, H., Bricaud, A., Kolber, Z. & Falkowski, P. G. 1996b. Nitrogen- and irradiance-dependent variations of maximum quantum yield of carbon fixation in eutrophic, mesotrophic, and oligotrophic marine systems. *Deep Sea Res.* 43:1241–72.

Bannister, T. T. & Laws, E. A. 1980. Modeling phytoplankton carbon metabolism. *In* Falkowski, P. G. [Ed.] *Primary Productivity in the Sea.* Plenum Press, New York, pp. 243–8.

Berman-Frank, I. & Dubinsky, Z. 1999. Balanced growth in aquatic plants: myth or reality? *Bioscience* 49(1):29–36.

Butler, W. L. 1978. Energy distribution in the photochemical apparatus of photosynthesis. *Ann. Rev. Plant Physiol.* 29:345–78.

Butler, W. L. & Kitajima, M. 1975. Fluorescence quenching of photosystem II in chloroplasts. *Biochim. Biophys. Acta* 376:116–25.

Cleveland, J. S. & Perry, M. J. 1987. Quantum yield, relative specific absorption and fluorescence in nitrogen-limited *Chaetoceros gracilis*. *Mar Biol.* 94:489–97.

Cullen, J. J. & Renger, E. H. 1979. Continuous measurement of the DCMU-induced fluorescence response of natural phytoplankton populations. *Mar Biol.* 53:13–20.

Cullen, J. J., Yang, X. & MacIntyre, H. L. 1992. Nutrient limitation of marine photosynthesis. *In* Falkowski, P. G. & Woodhead, A. D. [Eds.] *Primary Productivity and Biogeochemical Cycles in the Sea.* Plenum Press, New York, pp. 69–88.

Davison, I. R. 1991. Environmental effects on algal photosynthesis: temperature. *J. Phycol.* 27:2–8.

Dubinsky, Z. 1992. The functional and optical absorption cross-sections of photosynthesis *In* Falkowski, P. G. & Woodhead, A. D. [Eds.] *Primary Productivity and Biogeochemical Cycles in the Sea.* Plenum Press, New York, pp. 31–45.

Dugdale, R. C. 1967. Nutrient limitation in the sea: Dynamics, identification and significance. *Limnol. Oceanogr.* 12:685–95.

Eppley, R. W. 1972. Temperature and phytoplankton growth in the sea. *Fish. Bull.* 70:1063–85.

Eppley, R. W. 1980. Estimating phytoplankton growth rates in the central oligotrophic oceans. *In* Falkowski, P. G. [Ed.] *Primary Productivity in the Sea.* Plenum Press, New York, pp. 231–42.

Eppley, R. W. 1981. Relationship between nutrient assimilation and growth rate in phytoplankton with a brief view of estimates growth rates in the ocean. *In* Platt T. [Ed.] *Physiological Bases of Phytoplankton Ecology.* Can. Bull. Fish. Aquat. Sci., Ottawa. pp. 251–63.

Falkowski, P. G. 1992. Molecular ecology of phytoplankton photosynthesis. *In* Falkowski, P. G. & Woodhead, A. D. [Eds.] *Primary Productivity and Biogeochemical Cycles in the Sea.* Plenum Press, New York, pp. 47–67.

Falkowski, P. G. & Raven, J. A. 1997. *Aquatic Photosynthesis.* Blackwell Science, Oxford, UK, 375 pp.

Falkowski, P. G. & Kolber, Z. 1995. Variations in chlorophyll fluorescence yields in phytoplankton in the world oceans. *Aust. J. Plant Phys.* 22:341–55.

Falkowski, P. G. & Kolber, Z. 1993. Estimation of phytoplankton photosynthesis by active fluorescence. *ICES Mar. Sci. Symp.* 197:92–103.

Falkowski, P. G., Greene, R. M. & Geider, R. J. 1992. Physiological limitations on phytoplankton productivity in the ocean. *Oceanogr. Mag.* 5:84–91.

Falkowski, P. G, Wyman, K., Ley, A. C. & Mauzerall, D. 1986. Relationship of steady state photosynthesis to fluorescence in eukaryotic algae. *Biochim. Biophys. Acta* 849:183–92.

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

Geider, R. J., Greene, R. M., Kolber, Z., MacIntyre, H. L. & Falkowski, P. G. 1993. Fluorescence assessment of the maximum quantum efficiency of photosynthesis in the western North Atlantic. *Deep Sea Res.* 40:1205–24.

Geider, R. J., MacIntyre, H. L. & Kana, T. M. 1996. A dynamic model of photoadaptation in phytoplankton. *Limnol. Oceanogr.* 41:1–15.

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

Goldman, J. C. 1980. Physiological processes, nutrient availability, and the concept of relative growth rate in marine phytoplankton ecology. *In* Falkowski, P. G. [Ed.] *Primary Productivity in the Sea.* Plenum Press, New York, pp. 179–94.

Grasshoff, K., Ehrhardt, M. & Kremling, K. 1976. Methods of Seawater Analysis. Verlag Chemie, Weinheim, pp. 125–57.

Graziano, L. M., Geider, R. J., Li, W. K. W. & Olaizola, M. 1996. Nitrogen limitation of North Atlantic phytoplankton: analysis of physiological condition in nutrient enrichment experiments. *Aquat. Microb. Ecol.* 11:53–64.

Guillard, R. R. L. & Ryther J. H. 1962. Studies of marine plankton diatoms. 1. *Cyclotella nana* Hustedt, and *Detonula* (Cleve) Gran. *Can. J. Microbiol.* 8:229–39.

Harris, G. P. 1978. Photosynthesis, productivity and growth: the physiological ecology of phytoplankton. *Arch. Hydrobiol. Beih. Ergeb. Limnol.* 10:1–171.

Harris, G. P., Haffner, G. D. & Piccinin, B. B. 1980. Physical variability and phytoplankton communities II. Primary productivity by phytoplankton in a physically variable environment. *Arch. Hydrobiol.* 88:393–425.

Ibelings, B. W., Kroon, B. M. A. & Mur, L. R. 1994. Acclimation of photosystem II in a cyanobacterium and a eukaryotic green alga to high and fluctuating photosynthetic photon flux densities, simulating light regimes induced by mixing in lakes. *New Phytol.* 128:407–24.

Jassby, A. D. & Platt, T. 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr.* 21:540–7.

Keller, M. D., Selvin, R. C., Claus, W. & Guillard, R. R. L. 1987. Media for the culture of oceanic ultraphytoplankton. *J. Phycol.* 23:633–8.

Kiefer, D. A. & Reynolds, R. A. 1992. Advances in understanding phytoplankton fluorescence and photosynthesis. *In* Falkowski, P.G. & Woodhead, A.D. [Eds.] *Primary Productivity and Biogeochemical Cycles in the Sea.* Plenum Press, New York, pp. 155–74.

Kirk, J. T. O. 1994. *Light &Photosynthesis in Aquatic Ecosystems.* 2nd edition. Cambridge University Press, Cambridge, UK, 509 pp.

Kolber, Z. S. & Falkowski, P. G. 1993. Use of active fluorescence to estimate phytoplankton photosynthesis *in situ*. *Limnol. Oceanogr.* 38:1646–65.

Kolber, Z. S., Zehr, J. & Falkowski, P. G. 1988. Effects of growth irradiance and nitrogen limitation on photosynthetic energy conversion in photosystem II. *Plant Physiol.* 88:72–9.

Kolber, Z. S., Wyman, K. D. & Falkowski, P. G. 1990. Natural variability in photosynthetic energy conversion efficiency: a field study in the Gulf of Maine. *Limnol. Oceanogr.* 35:72–9.

Krause, G. H. & Weis, E. 1991. Chlorophyll fluorescence and photosynthesis: the basics. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 42:313–49.

Kroon, B., Prézelin, B. B. & Schofield, O. 1993. Chromatic regulation of quantum yields for photosystem II charge separation, oxygen evolution, and carbon fixation in *Heterocapsa pygmaea* (Pyrrophyta). *J. Phycol.* 29:453–62.

Langdon, C. 1988. On the causes of interspecific differences in the growth-irradiance relationship for phytoplankton. II. A general review. *J. Plank. Res.* 10:1291–1312.

Levasseur, M., Thompson, P. A. & Harrison, P. J. 1993. Physiological acclimation of marine phytoplankton to different nitrogen sources. *J. Phycol.* 29:587–95.

Lavergne, J. & Trissl, H. W. 1995. Theory of fluorescence induction in photosystem II- derivation of analytical expressions in a model including exciton-radical-pair equilibrium and restricted energy transfer between photosynthetic units. *Biophys. J.* 68:2474–92.

MacIntyre, J. G., Cullen, J. J. & Cembella, A. D. 1997. Vertical migration, nutrition and toxicity in the dinoflagellate *Alexandrium tamarense*. *Mar. Ecol. Prog. Ser.* 148:201–16.

Marra, J. 1978. Effects of short-term variations in light intensity on photosynthesis of a marine phytoplankton: a laboratory simulation study. *Mar. Biol.* 46:191–202.

Neale, P. J. & Richerson, P. J. 1987. Photoinhibition and the diurnal variation of phytoplankton photosynthesis- I. Development of a photosynthesis-irradiance model from studies of *in situ* responses. *J. Plankton Res.* 9:167–93.

Neale, P. J., Cullen, J. J. & Yentsch, C. M. 1989. Bio-optical inferences from chlorophyll a fluorescence: What kind of fluorescence is measured in flow cytometry? *Limnol. Oceanogr.* 34: 1739–48.

Olaizola, M. & Yamamoto, H. Y. 1994. Short-term response of the diadinoxanthin cycle and fluorescence yield to high irradiance in *Chaetoceros muelleri* (Bacillariophyceae). *J. Phycol.* 30:606–12.

Öquist, G. & Chow W. S. 1992. On the relationship between the quantum yield of photosystem II electron transport, as determined by chlorophyll fluorescence and the quantum yield of CO_2-dependent O_2 evolution. *Photosyn. Res.* 33:51–62.

Owens, T. G. 1991. Energy transformation and fluorescence in photosynthesis. *In* Demers, S. [Ed.] *Particle Analysis of Oceanography.* Springer-Verlag, Berlin. pp. 101–36.

Prézelin, B. B., Meeson, B. W. & Sweeney, B. M. 1977. Characterization of photosynthetic rhythms in marine dinoflagellates. II. Photosynthesis-irradiance curves and *in vivo* chlorophyll *a* fluorescence. *Pl. Physiol. Lancaster* 60:388–92.

Richerson, P. J., Armstrong, R. & Goldman, C. R. 1970. Contemporaneous disequilibrium. A new hypothesis to explain the "paradox of the plankton". *Proc. Natl. Acad. Sci.* 67:1710–14.

Roy, S. & Legendre, L. 1979. DCMU-enhanced fluorescence as an index of photosynthetic activity in phytoplankton. *Mar Biol.* 55:93–101.

Samuelsson, G. & Öquist, G. 1977. A method for studying photosynthetic capacities of unicellular algae based on in vivo chlorophyll fluorescence. *Plant Physiol.* 40:315–19.

Schreiber, U. 1986. Detection of rapid induction kinetics with a new type of high frequency modulated chlorophyll fluorometer. *Photosyn. Res.* 9:261–72.

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

Shuter, B. 1979. A model of physiological adaptation in unicellular algae. *J. Theor. Biol.* 78:519–52.

Strickland, J. D. H. & Parsons, T. R. 1972. *A Practical Handbook of Seawater Analysis. Can. Bull. Fish. Res.* Canada.

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

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

Zhu, M., Yang, X. & Cullen, J. J. 1992. The study of fluorescence characteristics and biochemical composition of a marine diatom *Thalassiosira pseudonana* 3H in light and dark cycles. *Acta Oceanol. Sinica* 12:457–64.