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Photoacclimation of *Dunaliella tertiolecta* (Chlorophyceae) under fluctuating irradiance

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Abstract

We investigated photoacclimation of *Dunaliella tertiolecta* (Butcher) in irradiance (*I*) regimes simulating mixed layer conditions of turbid estuarine waters or lakes. *D. tertiolecta* was exposed to a range of fixed *I* regimes to establish baseline physiology-*I* relationships that were compared with subsequent photoacclimation to a simulated mixed layer. Measured indices of photoacclimation included cellular pigmentation, chlorophyll variable fluorescence, and effective photosystem 2 antenna size. While *D. tertiolecta* grown under fluctuating *I* maintained division rates comparable to cells grown at high *I*, the cells exhibited characteristics of photoacclimation consistent with cells grown under a stable regimes at irradiances considerably lower than the average *I* of the simulated mixed layer.

Additional key words: absorption and fluorescence excitation spectra; carotenoids; chlorophylls; diurnal course; fluorescence induction; growth rate; photosystem 2 antenna size; phytoplankton; vertical mixing; xanthophyll cycle pigments.

Introduction

The aquatic environment presents a highly variable irradiance (*I*) field with changes occurring over a wide range of time scales (Denman and Gargett 1983, Falkowski 1984). For example, changes in *I* on short time scales can result from focusing and defocusing of radiation by waves at the surface (Dera and Gordon 1968, Walsh and Legendre 1983). Longer time scale changes can result from variable cloud cover or turbulent motion that transports phytoplankton across the exponential *I* gradient of the surface mixed layer. In a well-mixed surface layer, phytoplankton experiences long periods of low *I* interspersed by short periods of saturating or even supersaturating *I* (Falkowski and Wirick 1983, Lewis *et al.* 1984, Cullen and Lewis 1988, McIntyre *et al*. 2000). The diurnal solar cycle causes changes in *I* on even longer time scales (Prézelin and Sweeney 1977, Owens *et al.* 1980, Henley 1993).

To cope with the highly variable radiation environment, phytoplankton has developed numerous strategies to optimize photosynthesis, while minimizing susceptibility to photodamage (Richardson *et al.* 1983). Photosynthetic acclimation to *I* over time scales of hours to days proceeds through changes in cellular pigmentation or structural characteristics, *e.g*. size and number of photosynthetic units (Falkowski 1980, Wilhelm 1984, Herzig and Dubinsky 1992). On shorter time scales, cells adjust photon utilization efficiencies by changing the distribution of harvested energy between photosystems (state transitions) (Warwick 1979, Kroon 1994) or by dissipating excess energy through non-photochemical processes, *e.g*. xanthophyll cycle or photoinhibition (Demmig-Adams 1990).

Understanding the physiology of photoacclimation is central to aquatic primary production models and has been the focus of intensive investigations. Two experimental approaches have been (*1*) the determination of acclimation kinetics (Falkowski and Wirick 1981, Cullen and Lewis 1988, Lande and Lewis 1989, Geider *et al.* 1996), and (*2*) the direct measurements of photoacclimation states in simulated variable *I* regimes (*e.g*. Kromkamp and Limbeek 1993, Ibelings *et al.* 1994, Flameling and Kromkamp 1997, Jakob *et al*. 1999, Fietz and Nicklisch 2002). The basis of the former approach is to characterize rates of acclimation in experimental 'light shift' treatments and then apply these measured kinetics to a modelled mixed layer. Depending on model

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assumptions (the magnitude of asymmetry between rate constants for acclimation to an increase *vs*. decrease in *I*, the rate of mixing relative to the rate of acclimation), this approach has yielded a variety of conclusions with respect to *I* to which phytoplankton acclimate in a mixed layer. The 'direct measurement' approach has generally consisted of comparing photoacclimation states in cells grown under variable *I* with cells grown under a constant or sinusoidal regime of the same temporal average *I*. These studies have largely indicated that cells in variable *I* have characteristics significantly different than expected for their average *I*.

The objective of this study was to analyze the differences between physiological adjustments of cells *D. tertiolecta* exposed to different degrees in *I* variability and to assess the light level to which *D. tertiolecta* photoacclimate in a mixed layer.

Materials and methods

The green alga *Dunaliella tertiolecta* was cultured under four different *I*: L1 consisted of six constant *I*, L2 contained six time courses of sinusoidal *I*, each with a different maximum, and L3 and L4 were two light regimes that simulated conditions in hypothetical mixed layers with different average *I* (Fig. 1). The treatment L2 was representative of expected depth-dependent changes in daily *I* for a perfectly stratified water column. The central objective of this experimental design was to develop physiological baselines of photoacclimation for two relatively stable I regimes (L1 and L2) and then to compare photoacclimation characteristics in the two simulated mixing regimes (L3, L4) with these baselines.

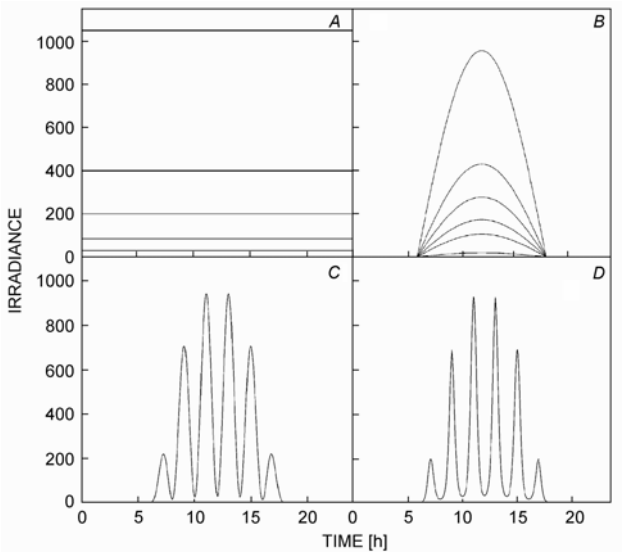

Fig. 1. Diurnal courses of irradiance (*I*) [μmol(photon) m^{-2} s^{-1}] for the four acclimation *I* regimes compared during this study. The constant *I* regime, L1 (*A*) and the sinusoidal *I* regime L2 (*B*) provided acclimation data for the 'baseline' relationships, against which acclimation in the simulated vertical mixing conditions [L3 (*C*) and L4 (*D*)] were compared.

Culture conditions*: D. tertiolecta* (CCMP 1320) was grown in f/2 medium (Guillard and Ryther 1962) in semi-continuous turbidostats. Optically thin cultures (~5×10^5 cells cm^{-3}) were grown in 1 000 cm^3 flat Rouxe's culture flasks, bubbled with air to ensure CO_2 supply and mixing, and placed in temperature-controlled water baths (18±0.5 °C). Irradiation was provided by a bank of *Osram DULUX L55W/12-950 "Lumilux daylight"* compact fluorescent lamps that allowed computer control of "white light" between 0 and 1 100 μmol(photon) m^{-2} s^{-1} (Bruyant *et al.* 2001). Cultures were allowed to acclimate to their given light regime for one week prior to each experiment; the steady state of cultures was confirmed by <5 % variations in chlorophyll (Chl) content in three consecutive days. The six constant *I* levels in treatment L1 were 8, 16, 45, 130, 500, and 1 100 μmol(photon) m^{-2} s^{-1}, corresponding to total daily radiation dose (TDLD) of 0.7, 1.4, 3.9, 11.2, 43.2, and 95 mol(photon) m^{-2} (Fig. 1*A*). The sinusoidal radiation regimes consisted of 12/12 h light/dark periods of TDLD: 0.5, 3.0, 4.8, 7.8, 13.0, and 25.9 mol(photon) m^{-2} (Fig. 1*B*). The two variable radiation regimes (L3 and L4) simulated *I* in a simplified mixed layer (*i.e.* random trajectories of individual cells were not considered and water motion was assumed to follow a defined period and mixing depth). The L3 and L4 regimes differed in the width of individual peaks of *I* maxima and thus by the average *I*—otherwise both the maxima and minima of individual cycles were identical.

For the regime L3, *I* was calculated according to the equations:

$$I = I_{\max} \sin\left(\frac{t\,\pi}{D}\right)\frac{x}{2} \tag{1}$$

$$x = \left(1 - e^{-k\,h}\right)\left[\sin\left(\frac{2\,\pi\,t}{P} + \frac{\pi}{2}\right) + 1\right] + 2e^{-k\,h} \tag{2}$$

For regime 4, *I* was calculated according to the equations:

$$I = I_{\max} \sin\left(\frac{t\,\pi}{D}\right) e^{x} \tag{3}$$

$$x = -k\,h\,\frac{\sin\left(\frac{t\,2\pi}{P} + \frac{\pi}{2}\right) + 1}{2} \tag{4}$$

where I_{max} = the maximum *I* at the water surface [970 μmol(photon) m^{-2} s^{-1}], *P* = the circulation period (2 h), *t* = time [h], *k* = the mean attenuation coefficient for photosynthetic active *I* (0.75 m^{-1}), *h* = the mixing depth (5 m), and *D* = photoperiod duration (12 h). Both simulations L3 and L4 had the same diurnal change in surface *I*

with noon maximum I_{max} = 970 μmol(photon) m^{-2} s^{-1}, the same depth of mixing (5 m), but different TDLD of 13.4 and 8.2 mol(photon) m^{-2} (Fig. 1*D*).

Growth rates: Specific growth rates (μ) were determined after one week of acclimation to the respective *I* regime. Samples were counted with a Coulter Counter (*Beckman Multisizer III*) equipped with a 100 μm aperture and μ was calculated according to equation:

$$\mu = \frac{\ln(N_{t2} / N_{t1})}{t_2 - t_1} \quad (5)$$

where N_{t1} is the cell density at time t_1 and N_{t2} is the cell density at time t_2.

Pigment analysis: 5 cm^3 of cell suspensions were collected on glass fibre filters (*GF/F, Whatman*) and ground in 90 % acetone in a tissue homogenizer. Glass fibre debris was removed by centrifugation and absorption of the clarified acetone extracts was measured against 90 % acetone in a dual beam spectrometer (*Shimadzu UV 3000*). Chl amount was calculated according to Jeffrey and Humphrey (1975). To account for variability in cellular Chl resulting from (*1*) cell synchronization in the light : dark treatments and (*2*) differences in cell sizes under different *I*, Chl amount was normalized to total cell volume rather than to cell numbers. This yielded relatively constant values of Chl amount during the diel cycle, in contrast to Chl per cell which varied by ~50 %. Cell volumes were determined with the Coulter Counter.

Changes in the ratio of photosynthetically active (Chl *a*, Chl *b*, lutein, neoxanthin) to photoprotective pigments (antheraxanthin, violaxanthin, zeaxanthin, β-carotene) as a secondary index of photoacclimation were also followed. Pigment composition was determined by high performance liquid chromatography using acetone extracts collected in the same manner as those for Chl analysis, but using 100 % acetone. Separation was performed using a polymeric C_{18} reversed-phase column (*VYDAC 201TP*) with a linear gradient from eluent A (methanol : 0.5 M ammonium acetate, 80 : 20) to eluent B (methanol : acetone, 70 : 30) (Van Heukelem *et al.* 1992). Pigments were quantified by integration of the respective peak areas. The de-epoxidation state of xanthophyll cycle (DPS) was calculated as [Z] + 0.5 [A]/ [V] + [A] + [Z] (Casper-Lindley and Björkman 1998).

Absorption and fluorescence spectroscopy: Absorption spectra of whole cells were recorded in 1 nm steps from 400 to 750 nm using the *Shimadzu UV3000* spectrophotometer set to 1 nm slit width. In order to minimize scattering, the 1 cm cuvette with cell suspension (A_{750nm} ~ 0.01) was placed in a holder in front of the photomultiplier window. Chl fluorescence excitation spectra of whole cells were measured at room temperature using the *Aminco-Bowman* series *II* spectrophotometer. Cell suspensions were placed into standard 3 cm^3 fluorescence cuvettes (*Hellma*). The emission monochromator was set at 683 nm and 4 nm slit width. Excitation spectra were recorded in 1 nm steps from 400 to 675 nm, with 2 nm slit width. The instrument function was corrected by dividing raw emission spectra by simultaneously recorded signal from the reference diode.

Variable fluorescence measurements: Chl variable fluorescence was measured using the *FL-3000* fluorometer (*Photon System Instruments*, Brno, Czech Republic) after the cells were kept for 3 min in darkness. The difference between F_v/F_m values of cells kept for 2 and 20 min in darkness was less than 25 %. Chl fluorescence yield was determined by weak blue (460 nm) measuring pulses. The intrinsic yield F_0 was measured in the dark, while the maximal fluorescence yield F_m – ST was determined following short (<30 μs) single turnover saturating flashes from actinic red LEDs. The multiple turnover maximal fluorescence yield F_m – MT was induced by a series of 100 sub-saturating actinic flashes of 15 μs duration, 500 μs apart. Effective photosystem 2 (PS2) antenna sizes were estimated from changes in fluorescence induction curves following addition of DCMU (final concentration 10^{-5} M) (Malkin and Kok 1966). During the L3 and L4 experiments, Chl variable fluorescence measurements were conducted every hour when *I* was at local maximum or minimum.

Results

Chl content decreased exponentially as growth *I* increased and varied from 2.9 to 16.5 pg μm^{-3} (mg m^{-3}) for the six constant *I* treatments (L1) (Table 1) and from 3.9 to 13.1 pg μm^{-3} for the six sinusoidal *I* treatments (L2) (Fig. 2*A*). These two exponential curves thus provided baseline Chl-*I* relationships against which results from the fluctuating *I* could be compared. For fluctuating *I* regime L3, the average Chl content was 6.9±0.35 pg μm^{-3}. When compared to the baseline curves (Fig. 2*A*), this Chl content corresponded to cells grown in sinusoidal *I* with TDLD of 4.8 mol(photon) m^{-2}, which was significantly lower than TDLD of the L3 treatment [13.4 mol(photon) m^{-2}]. Likewise, fluctuating *I* regime L4 yielded a Chl content of ~8.7±0.5 pg μm^{-3}, which corresponded to an acclimation to TDLD of 2.5 mol(photon) m^{-2} according to the baseline relationships, rather than acclimation to the treatment's TDLD of 8.2 mol(photon) m^{-2} (Fig. 2*A*).

In higher plant and chlorophyte algae, the Chl *a/b* ratio is often used as an indicator of acclimation of the photosynthetic apparatus to *I* (Lichtenthaler *et al.* 1981). Under constant *I*, Chl *a/b* varied with the growth *I* between 4.9 and 7.6. Under the fluctuating *I* regimes L3 and L4 this ratio reached values 7.5 and 7.0, respectively (Table 1).

Table 1. Pigment characteristics of *D. tertiolecta* cultures grown under different irradiances (*I*) [mol(photon) m^{-2}]: L1 = constant *I*, L3 and L4 = fluctuating *I* regime of different average *I*. Chlorophyll *a*+*b* per cell volume [pg μm^{-3}] are average values of three consecutive measurements. The other parameters were calculated based on data from HPLC analyses that were not repeated. T_{car} is a sum of all carotenoids, DPS is de-epoxidation state of xanthophyll cycle pigments.

I regime/TDLD	Chl *a*+*b*	Chl *a*/*b*	Chl *a*+*b*/T_{car}	DPS
L1/0.7	15.3±1.43	5.7	1.6	0.27
L1/1.4	16.5±0.75	4.8	1.6	0.28
L1/3.9	12.3±1.11	6.1	1.2	0.31
L1/11.2	4.6±0.37	7.6	0.8	0.47
L1/43.2	3.4±0.33	7.2	0.5	0.68
L1/95	2.9±0.36	6.7	0.2	0.82
L3/13.4	8.7±2.55	7.5	1.2	0.32
L4/8.2	6.9±2.75	7.0	1.3	0.39

Accessory pigments: For the six constant I treatments, the ratio of photosynthetically active to photoprotective (PS : PP) pigments decreased from 4.3 to 1.1 with an in crease in TDLD from 0.7 to 95.0 mol(photon) m^{-2} (Fig. 2*B*) (the PS : PP ratio was not determined during sinusoidal *I* experiments). Similar to the results for Chl content (Fig. 2*A*), the fluctuating *I* regimes (L3, L4) yielded PS : PP ratios characteristic of growth at significantly lower than their average *I*, according to the baseline curve (Fig. 2*B*). Specifically, the PS : PP ratio of 3.3 for *I* treatment L3 corresponded to a baseline *I* of ~5 mol(photon) m^{-2}, while the PS : PP ratio for the treatment L4 of 3.7 corresponded to a baseline of ~2.8 mol(photon) m^{-2} (Fig. 2*B*). Similar to PS : PP, the ratio of Chl (*a*+*b*) to total carotenoids is a sensitive indicator of *I* adaptation of higher plants (Lichtenthaler and Buschmann 2001). In cultures grown under constant *I* this ratio varied between 0.2 [for TDLD of 95 mol(photon) m^{-2}] and 1.6 [for TDLD of 0.7 mol(photon) m^{-2}] (Table 1). Under fluctuating *I* this ratio was 1.2 and 1.3 for TDLD of 13.4 and 8.2, respectively. These results again indicate that growth of cells under fluctuating *I* induces adaptation to low *I*.

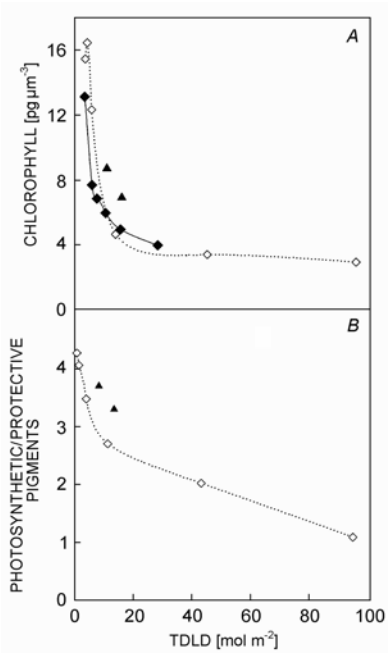

Fig. 2. **(***A***)** Chlorophyll per cell volume [pg μm^{-3} = mg m^{-3}] of *Dunaliella* cultures as a function of the growth irradiance (*I*) expressed as total daily *I* dose (TDLD) for *I* regimes L1 (◇) and L2 (–◆–), and for fluctuating *I* regimes L3 and L4 (▲). Data series for L1 and L2 present the average values of 3–5 measurements with standard deviations of 5–10 %, data for *I* regimes L3 and L4 were collected every 2 h during three days of the experiment and averaged value is plotted against the average *I* during the photoperiod. The standard deviation was ~5 %. (*B*) The *I* dependency of the ratio of photosynthetic (chlorophyll *a*, chlorophyll *b*, lutein, neoxanthin) to photoprotective (antheraxanthin, violaxanthin, zeaxanthin, β-carotene) pigments for *I* regime L1 (–◆–) and fluctuating *I* regimes L3 and L4 (▲).

Quantitative information on content and composition of extracted pigments from cells exposed to fluctuating *I* regimes was confirmed by absorption and fluorescence emission spectroscopy. Since the room temperature Chl fluorescence excitation spectra were recorded at an emission wavelength of 683 nm, the spectra provided

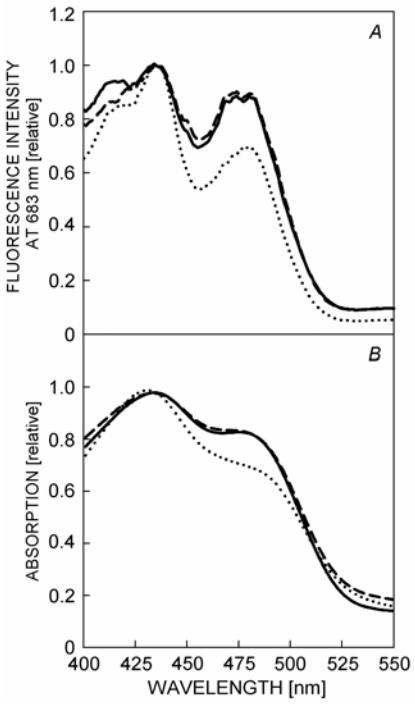

Fig. 3. Chlorophyll (Chl) fluorescence excitation (*A*) and absorption (*B*) spectra in the 400–550 nm region of whole cells grown either under fluctuating L4 regime (*solid curve*) or L1 regime with average *I* of 1 100 µmol(photon) m^{-2} s^{-1} (*dotted line*) or 45 µmol(photon) m^{-2} s^{-1} (*dashed line*). For the fluorescence excitation spectra, the emission was set to 683 nm. Both the Chl fluorescence excitation and the absorption spectra were normalized to maxima at 435 nm.

information about photosynthetic pigments that transfer energy to reaction centre of PS2. On the other hand, absorption spectra of whole cells are formed by all pigments, PS and PP. Both the fluorescence excitation (Fig. 3*A*) and absorption (Fig. 3*B*) spectra of cells exposed to fluctuating *I* were almost identical to corresponding spectra of cells acclimated to low *I*. Both spectra were clearly distinct from similar spectra of cells acclimated to the highest (Fig. 3) or to the average *I* (data not shown). For clarity, only data from the highest and lowest constant *I* are present in Fig. 3. From these results we conclude that under fluctuating *I*, the increased cellular content of both PP and PP pigments is incorporated into pigment-protein complexes associated with PS2 in a similar manner as in cells acclimated to low *I*. During fluctuating *I* regimes, some changes in the size of the peak at 480 nm of fluorescence excitation spectra were observed, corresponding to changes in the effective size of PS2 antenna. In addition to the near 30 % decrease of the peak during the photoperiod, smaller (~10 %) changes in the antenna emitting at 480 nm were observed in response to *I* fluctuations during the photoperiod (data not shown).

The diurnal time-course of the xanthophyll cycle pigments (*i.e*. zeaxanthin, antheraxanthin, and violaxanthin) exhibited fluctuations paralleling changes in *I* (Fig. 4*A*). Interestingly, the role of zeaxanthin in photoprotection appeared to differ between the constant and fluctuating *I* treatments. In the constant *I* experiment, the de-epoxidation state of cells (DPS) varied strongly with growth *I*, while under fluctuating *I* (L3, L4), DPS varied over a much more restricted range (Fig. 4*B*).

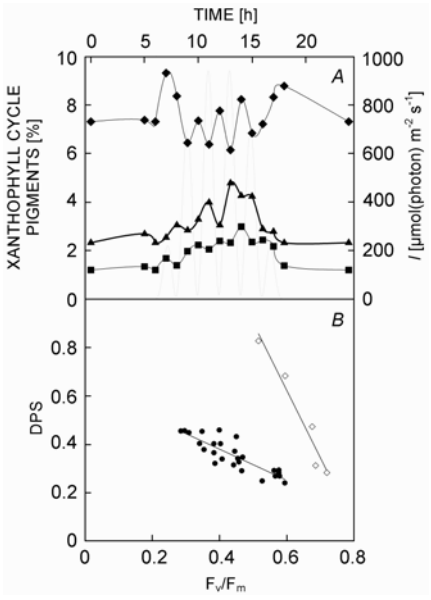

Fig. 4. (*A*) Diurnal course of xanthophyll cycle pigments [%] during the irradiance (*I*) regime L3: violaxanthin (◆), zeaxanthin (▲), and antheraxanthin (■), and time course of *I* during the photoperiod (*gray line, right y-axis*). (*B*) Correlation between the de-epoxidation state of cells (DPS) and quantum yield of photosystem 2 measured by multiple turnover excitation (F_v/F_m) for constant *I* in L1 (◇) and fluctuating *I* regimes L3, L4 (◆).

Since the pigment content of cells growing under fluctuating *I* indicated acclimation of cells to *I* lower than average, we also followed other physiological parameters that are *I*-dependent.

Growth rates: Specific daily growth rates (μ) for the six sinusoidal *I* treatments (L2) increased from 0.29 to 0.67 d^{-1} (Fig. 5). μ of cells grown under fluctuating *I* was 0.66±0.1 d^{-1}, and corresponded to maximum growth rates observed at relatively high *I* during the L2 experiments. Since cells grown under fluctuating *I* regimes were well synchronized in growth and cell divisions, as observed from diurnal changes of the cell size and numbers (data

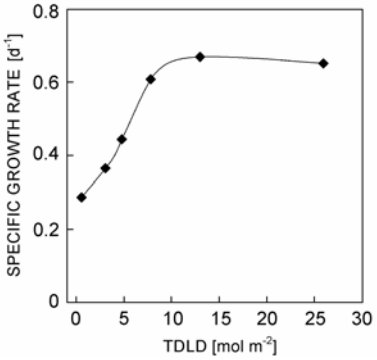

Fig. 5. Specific growth rate per day (μ) as a function of total daily irradiance (*I*) dose (TDLD) for *I* regime L2.

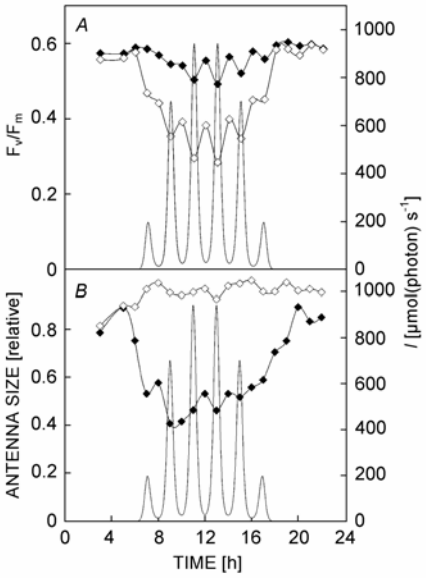

Fig. 6. (*A*) Diurnal course of photosystem 2 quantum yield (F_v/F_m) during the irradiance (*I*) regime L4 measured by single (◆) or multiple (◇) turnover method. (*B*) Relative changes in the functional antenna size (◆) and chlorophyll content (◇) during the *I* regime L4. Averages of 3 consecutive days, standard deviations were generally less than 10 %. Time course of *I* during the photoperiod (*gray line, right y-axis*).

not shown), μ values were compared only with those of sinusoidal *I* treatments.

Photochemical activity: Reproducible *I*-dependent changes in photochemical activities were observed during the photoperiod of the fluctuating *I* regimes (Fig. 6*A*). The diurnal pattern of maximal photochemical yield of PS2, measured as F_v/F_m – ST (single turnover) or F_v/F_m – MT (multiple turnover) as derived from respective F_0 and F_m showed that at any time during the photoperiod F_v/F_m was inversely correlated with *I* (Fig. 6*A*). The dynamics of F_v/F_m were symmetrical within each oscillation and over the entire photoperiod. F_v/F_m – ST varied only slightly during the photoperiod between 0.5 and 0.6, while considerably larger decreases (~50 %) were observed in F_v/F_m – MT at high *I*.

A significant decrease (~45 %) in the effective size of the light-harvesting antenna (σ_{PS2}) was observed during the first 4 h of the light period in the fluctuating regimes (L3, L4), followed by an increase in σ_{PS2} during the remaining *I* period (Fig. 6*B*; data shown for the regime L4). These changes were not due to changes in Chl content, as it remained constant during the photoperiod.

Discussion

Despite the many studies previously conducted on photoacclimation in variable *I* regimes, a consensus has yet to be achieved regarding the acclimation 'status' or 'condition' of cells exposed to variable *I*. In this study, our approach has been to construct 'baseline' acclimation curves for relatively stable *I* regimes and then compare acclimation in fluctuating *I* to these reference baselines. With respect to cellular pigmentation (Fig. 2, Table 1) and pigment incorporation into pigment-protein complexes (Fig. 3), this approach consistently indicated that photoacclimation states in fluctuating *I* corresponded to baseline growth *I* substantially (approx. 3 times) lower than the average *I* of the fluctuating regime. This result points to the predominant influence of low *I* stress over the high *I* stress in physiological adjustments to variable *I*.

Comparison with other laboratory studies: Previous studies, where the influence of fluctuating *I* on phytoplankton photoacclimation has been directly measured, have generally compared responses to variable *I* with a single 'control' *I* of the same average daily *I* (*e.g.* Kromkamp and Limbeek 1993, Flameling *et al.* 1997, Fietz and Nicklisch 2002) or the same daily maximum (Ibelings *et al.* 1994). A direct comparison of results from these various studies is complicated by differences in the organisms tested, frequencies of *I* oscillations, and *I* maxima. Two pronounced acclimation characteristics are changes in Chl amount with irradiance and changes in the complement of photoprotective pigments. Consistent with our results, *Phaeocystis globulosa, Thalassiosira weissflogii, Microcystis aeruginosa,* and *Planktothrix agardhii* also exhibited higher Chl content in fluctuating *I* regimes than in the more stable *I* treatments with the same average *I* (Kromkamp and Limbeek 1993, Ibelings *et al.* 1994, Flameling and Kromkamp 1997, Fietz and Nicklisch 2002). In contrast to these results, Chl contents in *Stephanodiscus neoastraea* grown under variable *I* matched that of cells grown under the same average constant *I*, while the Chl to carotenoids ratio was higher for the variable *I* regime (Fietz and Nicklisch 2002). *Scenedesmus protruberans*, on the other hand, had lower Chl content in fluctuating *I* than in the more stable *I* regimes and exhibited no difference in its Chl/carotenoids ratio (Ibelings *et al.* 1994, Flameling and Kromkamp 1997).

Our results are thus consistent with the bulk of studies described above, but notable exceptions exist. The basis of these discrepancies remains unresolved and appears to be species dependent, but differences in experimental design still cannot be ruled out. In our experiments we have used the 'baseline' acclimation curves developed from relatively stable *I* regimes to assess acclimation states of cells grown under fluctuating *I* regimes. This approach proved instructive for choosing an appropriate variable *I* regime and for interpreting our results. For example, if the average *I* of the fluctuating *I* regime falls into the range of the baseline curve where the acclimation characteristic changes slightly with *I*, then acclimation to the highest or average *I* may by difficult to distinguish. We submit that our 'baseline' comparative approach can enhance the rigor of photoacclimation state evaluations relative to studies involving only a single constant or sinusoidal 'control' treatment.

Time scales of acclimation: One of the fascinating aspects of photoacclimation is the diversity of time scales at which different mechanisms operate. Relative to changes in Chl content, *I*-dependent changes in the epoxidation-de-epoxidation of xanthophylls (violaxanthin–antheraxanthin–zeaxanthin) and changes in functional states of PS2 reaction centres (F_v/F_m) respond to more rapid time scale changes in *I* (Kroon 1994). Accordingly, these later two parameters paralleled the short time scale changes of our fluctuating *I* regimes (Figs. 4 and 6*A*). Unlike some studies on photoinhibition (Neale 1987, Long *et al.* 1994), the changes in photochemical quantum yield of PS2 (F_v/F_m) observed during this study indicated a lack of any hysteresis effect following exposures to high *I* (Fig. 4*A*). The short time-scale and symmetry of this response indicates that the associated rate constants for PS2 repair were equivalent to or exceeded the extant rates of photodamage. *I* fluctuations modify the function of the acceptor side of PS2 (electron transfer from Q_A to Q_B and then to the plastoquinone pool) as can be seen from the

higher decrease of F_v/F_m measured by the multiple turnover (MT) protocol than by the ST protocol (Fig. 4*A*). The exact nature of the modification is not known, but conformational changes in the Q_B pocket are the probable target (Ohad *et al*. 1988).

Under fluctuating *I*, *D. tertiolecta* synthesized relatively less photoprotective pigments of the xanthophyll cycle compared to the constant *I* treatment. Consequently, cells in the fluctuating regime were more sensitive to high *I*, as evidenced by changes in photochemical activity of PS2 (Fig. 4*B*). As a testable hypothesis, we propose that this shift between the highly fluctuating and more stable *I* reflects a fundamental difference in acclimation strategies, where the former represents an active employment of the excess capacity of PS2 at saturating *I* as a secondary photoprotective mechanism (Behrenfeld *et al.* 1998, 2002).

Given the low activity of the xanthophyll cycle under the fluctuating *I*, other mechanisms were apparently important in adjusting the effective size of the light-harvesting antenna complexes (LHC) during the high *I* exposure (Fig. 6*B*). These mechanisms apparently function by dissipating excess energy within the LHC, rather than in reaction centres themselves. This conclusion is evidenced by the modest decreases in photochemical activity of PS2 (~20 %) and significant parallel decreases (45 %) in the effective antenna sizes. State transitions may have contributed to the daytime, *I*-dependent changes in energy quenching and have been shown to function under fluctuating *I* (Kroon 1994). However, according to Long *et al*. (1994) state transitions are responsible for a maximum decrease in σ_{PS2} of about 25 %. In *D. tertiolecta*, a combination of mechanisms probably operates at short time scales to maintain a large capacity for non-photochemical dissipation that does not interfere with other photoacclimation attributes (*e.g*. pigment synthesis) exhibiting low-*I* acclimation characteristics (Fig. 4*B*). The combination of increased light-harvesting capacity with highly effective photoprotection and rapid repair of photodamage is suggestive of an acclimation strategy geared toward optimizing fitness under a fluctuating *I* environment and maximizing growth rates (Fig. 5).

Kinetic models of photoacclimation: A classic approach to modelling photoacclimation in the surface mixed layer is to use kinetic rates of photoacclimation measured in laboratory *I*-shift experiments and applying these rates to modelled underwater *I*. Falkowski and Wirick (1981) combined *I*-shift kinetics and a Monte Carlo random-walk description of cell movement within the surface mixed layer to predict that vertically integrated parameters (Chl/carbon) would not differ for stratified and mixing conditions. In other words, their conclusions suggested that phytoplankton in a mixed layer acclimates to the average *I* of that layer. An important assumption of their model was that the rate constants for photoacclimation were similar for a shift-up and a shift-down in *I*. Cullen and Lewis (1988) suggested an alternative description of photoacclimation under variable *I*, where the time scale of change in acclimation parameters was dependent on (*1*) the relative magnitude of the change in *I*, (*2*) the starting conditions, and (*3*) the direction of the *I* shift. In their logistic model of unbalanced synthesis and accumulation of cellular components, photoacclimation to an increase in *I* was predicted to require a longer time period than a reciprocal decrease of *I*. Consequently, this model indicated that cells within the mixed layer would acclimate to *I* somewhat lower than the average. Although their conclusion is in agreement with our results, the mechanistic basis is not necessarily correct. The 'dynamic' photoacclimation model of Geider *et al.* (1996) is based on the description of energy and mass fluxes in cell and the regulated partitioning of photosynthate during *I* acclimation. According to this model, the adaptation to high *I* will be much more rapid than adaptation to low *I*. In addition, brief exposures to high *I* will outweigh much longer exposures to low *I*, resulting in photoacclimation state characteristics of cells grown in *I* higher than the average *I* of the mixed layer (Geider *et al.* 1996).

One of the complications in these kinetic based photoacclimation models is that they require the extrapolation of rate constants derived from shifts between different steady state conditions to predict algal responses to a fluctuating *I* regime. More specifically, cells are energetically acclimated to a given constant *I* and then shifted to a very different but also constant *I*. In contrast, cells growing for multiple generations under highly variable *I* must constantly cope with prolonged periods of low *I* exposure interspersed by short periods of high *I*. Thus, unlike the laboratory conditions, the mixed-layer condition requires simultaneous acclimation to both high and low *I*.

We propose that the key to this acclimation lies in the varied rate constants for different classes of photoacclimation such that brief high *I* exposures are accommodated through rapid changes in non-photochemical quenching mechanisms, while exposure to low *I* drives increases in light-harvesting capacity. This interpretation seems consistent with our observed changes in xanthophyll intermediates and photochemical quantum yields of PS2 (F_v/F_m) and the higher Chl content under fluctuating *I* is then anticipated from our baseline stable *I* regime relationships.

Conclusions: We applied a novel approach to characterizing photoacclimation in *D. tertiolecta* exposed to variable *I* regimes. Our results suggest that, for the test species studied, photoacclimation under fluctuating *I* entails an interesting simultaneous physiological preparation for both high and low *I* exposures, not simply an acclimation to the average *I*. A consequence of this physiological tuning is that it would enable *D. tertiolecta* to maintain an enhanced fitness state and maximal growth rate in the relatively unpredictable *I* environment of turbid waters.

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