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Compensatory changes in Photosystem II electron turnover rates protect photosynthesis from photoinhibition

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Abstract

Exposure of algae or higher plants to bright light can result in a photoinhibitory reduction in the number of functional PS II reaction centers (*n*) and a consequential decrease in the maximum quantum yield of photosynthesis. However, we found that light-saturated photosynthetic rates (P_{max}) in natural phytoplankton assemblages sampled from the south Pacific ocean were not reduced despite photoinhibitory decreases in *n* of up to 52%. This striking insensitivity of P_{max} to photoinhibition resulted from reciprocal increases in electron turnover ($^1/\tau_{PSII}$) through the remaining functional PS II centers. Similar insensitivity of P_{max} was also observed in low light adapted cultures of *Thalassiosira weissflogii* (a marine diatom), but not in high light adapted cells where P_{max} decreased in proportion to *n*. This differential sensitivity to decreases in *n* occurred because $^1/\tau_{PSII}$ was close to the maximum achievable rate in the high light adapted cells, whereas $^1/\tau_{PSII}$ was initially low in the low light adapted cells and could thus increase in response to decreases in *n*. Our results indicate that decreases in plant productivity are not necessarily commensurate with photoinhibition, but rather will only occur if decreases in *n* are sufficient to maximize $^1/\tau_{PSII}$ or incident irradiance becomes subsaturating.

Abbreviations: PS II – Photosystem II; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea

Introduction

Although the detrimental effects of excessive light exposure on plant photosynthesis have been recognized for more than a century (Ewart 1895–1897), only during the past few decades has damage to Photosystem II (PS II) reaction centers been recognized as the fundamental mechanism responsible for photoinhibition (Kok 1956; Kyle 1987; Barber 1991, 1992a; Prasil et al. 1992). This light-dependent inactivation of PS II may either be rapidly reversible (Osmond 1994) or entail irreversible damage to core PS II reaction center proteins (D1), requiring *de novo* protein synthesis for repair (Prasil et al. 1992). For both phytoplankton and terrestrial plants, photodamage to PS II reaction centers can be detected with high sensitivity from changes in variable chlorophyll fluorescence (Björkman 1987a,b; Neale 1987; Baker et al. 1994; Long et al. 1994).[1] The ease with which variable fluorescence measurements can be made has led to their common usage as a diagnostic for photoinhibition, although the consequence of PS II inactivation on photosynthetic electron flow remains controversial.

In the field, diurnal patterns of variable fluorescence frequently exhibit midday depressions that are roughly symmetric relative to local noon. Full recovery from the midday minimum is often observed by late afternoon (Falkowski et al. 1994), but a mild

hysteresis may also be suggested by an incomplete recovery by nightfall (Baker et al. 1994). In either case, correspondence between decreases in variable fluorescence and photoinhibition is evidenced by similar diurnal patterns in the quantum yield of photosynthesis (Neale 1987; Long et al. 1994). However, in aquatic systems, short-term (0.5 to 2 h) ^{14}C-based photosynthesis-irradiance measurements often indicate maximum light-saturated photosynthetic rates (P_{max}) near noon (Doty and Oguri 1957; Yentsch and Ryther 1957; Sournia 1974; MacCaull and Platt 1977; Gargas et al. 1979; Malone et al. 1980; Cullen et al. 1992). This paradoxical co-occurrence of midday maxima in both photoinhibition and photosynthesis is inconsistent with a debilitating effect of PS II damage on carbon assimilation.

Theoretically, the effect of photoinhibition on carbon fixation should depend upon which step in the photosynthetic electron transport chain is rate limiting at a given incident irradiance. By definition, carbon fixation at subsaturating irradiance is rate limited by light absorption and excitation energy transfer to PS II reaction centers and thus is a near-linear function of irradiance, with an initial slope (α) defined by (Ley and Mauzerall 1982; Dubinsky et al. 1986; Falkowski and Raven 1997):

$$\alpha = \sigma_{\mathrm{PSII}} \times n, \tag{1}$$

where n is the number of functional PS II reaction centers and σ_{PSII} is their average effective absorption cross section. As suggested by Equation 1 and verified experimentally (Björkman 1987a; Baker et al. 1994), photoinhibition decreases α in proportion to changes in n (Figure 1). This correlation between n and α results because carbon fixation is limited by photochemistry at subsaturating irradiance and decreases in n do not induce reciprocal increases in σ_{PSII} [i.e. photon energy transfer from photodamaged to functional reaction centers is not significantly enhanced because the photodamaged centers can act as efficient nonphotochemical quenchers of excitation energy (Walters and Horton 1993)].

At light saturation, photosynthesis is limited on the acceptor side of PS II, generally by the capacity of enzymatic processes in the Calvin-cycle (Stitt 1986; Sukenik et al. 1987). This 'down-stream' limitation ultimately restricts electron turnover through PS II. The light saturated rate of electron transport can be expressed as the product of n and $^1/\tau_{\mathrm{PSII}}$ (Herron and Mauzerall 1971; Myers and Graham 1971; Falkowski and Raven 1997):

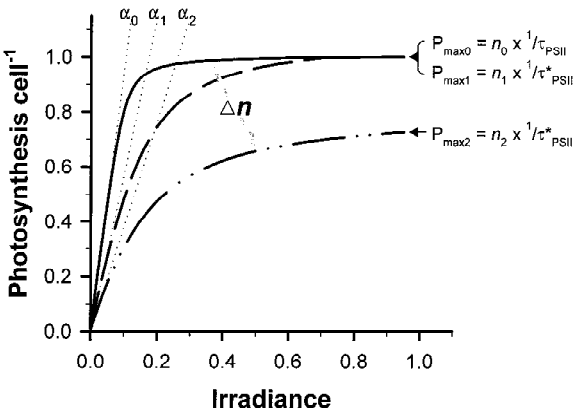

Figure 1. Theoretical effects of photoinhibition (Δn) on photosynthesis for a phytoplankter with an initial photosynthesis-irradiance relationship defined by the light-limited slope: $\alpha_0 = \sigma_{\mathrm{PSII}} \times n_0$, and light-saturated rate: $P_{max0} = n_0 \times {}^1/\tau_{\mathrm{PSII}}$. In this depiction, photoinhibition progressively decreases the number of functional PS II reaction centers from $n_0 \rightarrow n_1 \rightarrow n_2$. Consequently, α decreases proportionately from $\alpha_0 \rightarrow \alpha_1 \rightarrow \alpha_2$ and the photosynthesis-irradiance function changes from the solid line→dashed line→dash-dot line. Note that as $n_0 \rightarrow n_1$, P_{max} remains constant. This insensitivity of P_{max} to photoinhibition results from reciprocal increases in electron turnover through PS II from the initial rate ($^1/\tau_{\mathrm{PSII}}$) to a maximum achievable rate ($^1/\tau^*_{\mathrm{PSII}}$) (i.e. $P_{max} = n_0 \times {}^1/\tau_{\mathrm{PSII}} = n_1 \times {}^1/\tau^*_{\mathrm{PSII}}$). However, any additional photodamage (i.e. $n_1 \rightarrow n_2$) decreases P_{max} to P_{max2} because rate limitation of photosynthesis has shifted from the Calvin cycle reactions to electron transport through PS II. P_{max} thus remains uncoupled to photoinhibition until $^1/\tau^*_{\mathrm{PSII}}$ is achieved.

$$P_{max} = n \times {}^1/\tau_{\mathrm{PSII}}, \tag{2}$$

where $^1/\tau_{\mathrm{PSII}}$ is the steady-state, light-saturated rate of electron turnover through the functional PS II reaction centers. Initial rate limitation on the acceptor side of PS II implies that photoinhibition will not affect P_{max} until n is sufficiently decreased to cause limitation of carbon fixation by electron turnover through PS II (Figure 1). Thus, the critical difference between Equations 1 and 2 with respect to photoinhibition is that $^1/\tau_{\mathrm{PSII}}$ and n are independent variables that can vary inversely. The capacity for such reciprocal increases in $^1/\tau_{\mathrm{PSII}}$ to compensate for decreases in n will thus depend on the excess capacity of photosynthetic electron transport reactions relative to the Calvin cycle reactions. This capacity can be defined as the difference between the initial $^1/\tau_{\mathrm{PSII}}$ prior to photodamage and the maximum achievable electron turnover rate

($^1/\tau^*_{PSII}$) (i.e. that rate which would occur if limitation on the acceptor side of PS II was alleviated).

To investigate the influence of photoinhibitory decreases in *n* on photosynthesis, we followed diurnal changes in variable chlorophyll fluorescence, $^1/\tau_{PSII}$, σ_{PSII}, and photosynthesis-irradiance relationships in natural phytoplankton assemblages sampled in the South Pacific ocean. Results from this *in situ* study were further resolved by measuring changes in photosynthetic performance of *Thalassiosira weissflogii* (a marine diatom) as an increasing fraction of PS II reaction centers were functionally inhibited by the herbicide, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU).

Materials and methods

Field measurements

Diurnal cycles in F_v/F_o, σ_{PSII}, α, and P_{max} were measured in the tropical south Pacific at 5° S, 150° W and 16° S, 148° W between 18 and 28 November 1994 on the French research vessel, *L'Atalante*, during the OLIPAC research program. Samples were collected for fast-repetition-rate fluorometric determinations of F_v/F_o and σ_{PSII} (Kolber and Falkowski 1993; Falkowski and Kolber 1995; Kolber et al. 1998) at 2 to 4 h intervals from 5 to 55 m depth using trace-metal clean Niskin sampling bottles. For each sampling depth, 500 ml of sample were passed through a 1 ml Helma Cells quartz flow-through cuvette during the fluorometric measurements. A mean value of F_v/F_o and σ_{PSII} was calculated for each sample from 30 single turnover flash measurements.

Samples for α and P_{max} determinations were collected from 5 and 55 m at the 5° S station and from 5 m at the 16° S station using trace-metal clean Niskin sampling bottles. For each depth, 50 ml subsamples were dispensed into 12 polystyrene tissue culture flasks, inoculated with 0.5 μCi ml^{-1} $H^{14}CO_3^-$, and incubated for 120 to 180 min in a radial photosynthetron (described in Babin et al. 1994). An incubation irradiance gradient ranging from 5 to 400 μmol quanta m^{-2} s^{-1} (integrated from 400 to 700 nm) was created by stacking the 12 culture flasks in front of a 250 W arc lamp (Osram, HQI-T250WD). Following incubation, samples were passed through a 25 mm glass fiber filter (Whatman GF/F), acidified with concentrated HCl to remove inorganic carbon, and total ^{14}C activity (counts min^{-1}) determined by liquid scintillation counting. Carbon fixation was calculated from the measured total activity, after correcting for scintillation counter background and quenching, following methods described by Parsons et al. (1984). PAR (400 to 700 nm) was measured at all flask positions with a calibrated Biospherical Instruments Model QSL100 4π quantum sensor. For each depth and time increment, $^1/\tau_{PSII}$ was calculated from the ^{14}C and fluorometric measurements as: $\sigma_{PSII} \times (P_{max}/\alpha)$ (Falkowski 1992).

Laboratory measurements

Stock cultures of *Thalassiosira weissflogii* (Grunow) Hasle (Brookhaven clone T. vic.) were grown in batch mode at 18 °C in artificial seawater enriched with f/2 nutrients and trace metals (Guillard and Ryther 1962). Cultures were kept optically thin by periodic dilutions with fresh medium and were continuously bubbled with filtered air. *T. weissflogii* was acclimated for > 4 days to a growth irradiance of either 150 or 1000 μmol quanta m^{-2} s^{-1}. Growth irradiance was measured as scalar PAR with a calibrated Biospherical Instruments Model QSL100 4π quantum sensor.

For each growth irradiance, the photosynthetic response to decreases in *n* was measured by progressively blocking electron transport through an increasing fraction of PS II reaction centers using DCMU (Heber et al. 1988) and following changes in α and P_{max}. Replicate DCMU experiments were conducted for both growth irradiances. For each experiment, between 6 and 7 subsamples of 20 ml were collected from the stock culture and inoculated with DCMU (stock concentration = 10^{-3} M) and/or ethanol. Total inoculum volume was kept at 200 μl (1:1000 dilution). Final DCMU concentrations ranged from 0 to 0.3 μM. Each 20 ml sample was gently bubbled with N_2 to remove dissolved O_2. Aliquots were then drawn for fluorescence, chlorophyll, cell concentration, and photosynthesis-irradiance measurements.

For each DCMU concentration, P_{max} and α were calculated from measurements of O_2 evolution in 7 ml samples exposed to irradiances ranging from 30 to 1050 μmol quanta m^{-2} s^{-1}. Sample temperature was controlled ($\pm$ 0.01 °C) with a thermostated water bath (Dubinsky et al. 1986). Irradiance was supplied as a collimated beam from a tungsten source passed through a heat filter and attenuation filters. Irradiance was measured using a Licor Model LI-189 light meter equipped with a calibrated cosine corrected quantum sensor attached to the rear wall of the sample chamber. The sample was stirred constantly

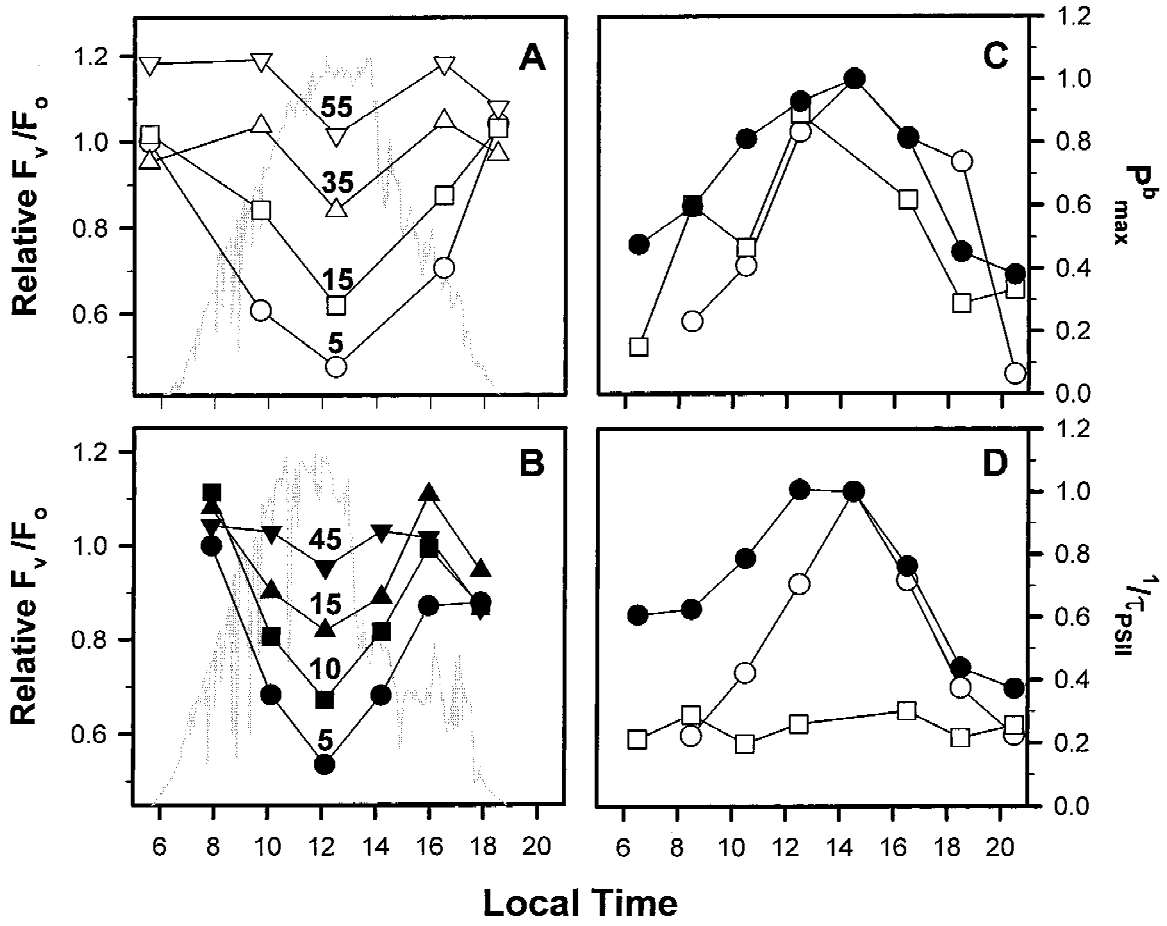

Figure 2. Diurnal changes in F_v/F_o, biomass-normalized light-saturated carbon fixation rates (P^b_{max}), and $^1/\tau_{PSII}$ measured in natural phytoplankton assemblages collected at (A) 5° S, 150° W and (B) 16° S, 148° W in the tropical south Pacific ocean. (A, B) Midday decreases in F_v/F_o are indicative of photoinhibition. Numbers adjacent to each curve indicate sample depths (m), with progressively increasing depths symbolized by ●, B , ▲, and ▼. F_v/F_o for each depth has been normalized to the morning value at 5 m. Light solid line = surface solar PAR (max. 2700 μmol quanta m^{-2} s^{-1} on both days). (C) Diurnal changes in P^b_{max}. Circles = 5 m samples. Squares = 55 m samples. P^b_{max} ranged from 0.05–0.85 μgC μgChl^{-1} h^{-1} at the 5° S station (open symbols) and from 0.08–0.21 μgC μgChl^{-1} h^{-1} at the 16° S station (solid symbols) and thus have been normalized to the maximum value at 5 m for each station. (D) Diurnal changes in $^1/\tau_{PSII}$ calculated as: $\sigma_{PSII} \times (P_{max}/\alpha)$ and normalized to the maximum value at 5 m for each station. Symbols as in (C).

with a magnetic spinbar. Dissolved oxygen concentrations were measured with a Clark type polarographic electrode. Gross photosynthesis was calculated as O_2 evolution at a given irradiance plus respiratory O_2 utilization measured in the dark prior to light exposure. Photosynthesis-irradiance curves were fit to the gross photosynthesis data using methods described by Platt et al. (1980) and Leverenz(1994).

A fast-repetition-rate fluorometer was used to measure σ_{PSII} for each DCMU concentration (Kolber et al. 1998). The fractional reduction in *n* from DCMU addition was evaluated from fluorescence decay rates following single turnover flashes. For each DCMU concentration, $^1/\tau_{PSII}$ was calculated as: $\sigma_{PSII} \times (P_{max}/\alpha)$ (Falkowski 1992). Chlorophyll concentrations were determined spectrophotometrically from 15–25 ml samples filtered onto 25 mm glass fiber filters (Whatman GF/F), homogenized in 90% acetone with a Teflon-glass tissue grinder, and refiltered and reextracted with fresh acetone. The resultant clarified extract was then scanned from 375–750 nm with a Hewlett Packard HP8451A diode array spectrophotometer. Cell concentrations were measured microscopically and with a Coulter Counter (Coulter Elec-

tronics Inc., Model TAII). Mean cell volumes were determined with a Coulter Counter.

Results

Field measurements

Physical, chemical, and biological characteristics differed between the two oceanographic sampling stations. Equatorial upwelling enhanced nutrient concentrations (inorganic nitrogen > 2 μM) at the 5° S station and consequently supported phytoplankton concentrations ranging from 0.09 to 0.13 μg l^{-1} at 5 m and 0.12 to 0.28 μg l^{-1} at 55 m. In contrast, thermal stratification within the upper water column at the 16° S station resulted in both diminished nutrient (inorganic nitrogen < 0.01 μM) and phytoplankton (< 0.04 μg l^{-1}) concentrations.

Diurnal patterns in F_v/F_o were similar at both stations and indicated maximum decreases in *n* of up to 52% in samples collected from 5 m depth (Figures 2A, B). At greater depths, midday decreases in *n* were diminished in direct proportion to the attenuation of light through the water column (Figures 2A, B). At all depths, diurnal patterns in F_v/F_o were symmetric relative to local noon. Despite significant photoinhibition at 5 m (Figures 2A, B), corresponding biomass-normalized, light-saturated carbon fixation rates (P^b_{max}) exhibited midday maxima at both stations (Figure 2C). This insensitivity of P^b_{max} to photoinhibition was accomplished through reciprocal increases in $^1/\tau_{PSII}$ (Figure 2D). In contrast, samples collected at 55 m did not exhibit significant changes in F_v/F_o or $^1/\tau_{PSII}$ (Figure 2D), despite similar midday increases in P^b_{max} (Figure 2C). These results clearly illustrate the interdependence between increases in $^1/\tau_{PSII}$ and decreases in *n* observed in the 5 m samples.

Laboratory measurements

T. weissflogii was chosen as the test species because its photoadaptive response to changes in growth irradiance has been well described (Falkowski et al. 1985; Dubinsky et al. 1986; Kolber et al. 1988). Combining our data with those of Dubinsky et al. (1986), increases in growth irradiance from 30 to 1000 μmol quanta m^{-2} s^{-1} result in an exponential decrease in cellular concentrations of PS II reaction centers (r^2 = 0.99) and a factor of 3.6 increase in $^1/\tau_{PSII}$ (r^2 = 0.95) (Figure 3). Lower initial values of $^1/\tau_{PSII}$ at diminishing growth irradiances were thus anticipated to

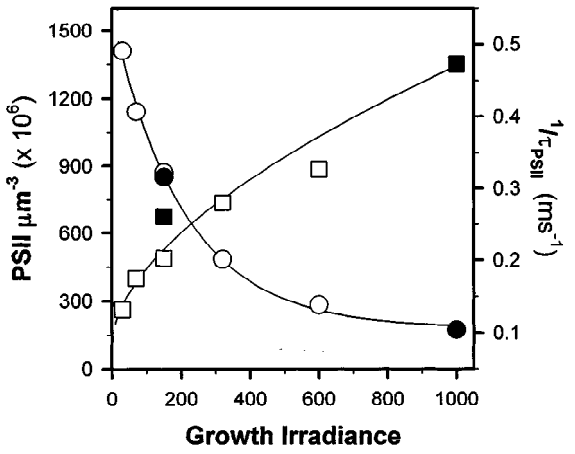

Figure 3. Changes in $^1/\tau_{PSII}$ (ms^{-1}) (squares) and cellular PS II concentrations ($\mu m^{-3} \times 10^6$) (circles) in *Thalassiosira weissflogii* for growth irradiances ranging from 30 to 1000 μmol quanta m^{-2} s^{-1}. Solid symbols = data from the current study. Open symbols = data from Dubinsky et al. (1986). For the current study, $^1/\tau_{PSII}$ was calculated as: $\sigma_{PSII} \times (P_{max}/\alpha)$, where σ_{PSII} was determined from fluorescence measurements (Kolber and Falkowski 1993; Falkowski and Kolber 1995; Kolber et al. 1998) and P_{max} and α were determined from O_2 evolution measurements. PS II concentrations (●) were calculated from the relationship (r^2 = 0.99) between Chl:PS II and growth irradiance reported by Dubinsky et al. (1986).

confer a reduced sensitivity of P_{max} to decreases in *n*, due to the increased capacity for reciprocal changes in $^1/\tau_{PSII}$ (Equation (2); Figure 1). These expectations were verified by the DCMU experiments.

Titration with DCMU enables a variable fraction of PS II reaction centers to be functionally inhibited, thereby permitting a controlled simulation of photoinhibition. For *T. weissflogii* grown at 1000 μmol quanta m^{-2} s^{-1}, addition of DCMU decreased both α and P_{max} (Figures 4A, B). Decreases in α were linearly related to the percentage decrease in *n* (Figure 4B), whereas P_{max} exhibited a slight shouldering at the lowest DCMU concentrations (Figure 4B) reflecting a 35% increase in $^1/\tau_{PSII}$ (Figure 4C). This modest increase in $^1/\tau_{PSII}$ was not sufficient, however, to prevent decreases in P_{max} even at the lowest DCMU concentrations (Figure 4B).

For *T. weissflogii* grown at 150 μmol quanta m^{-2} s^{-1}, addition of progressively higher concentrations of DCMU caused α to decrease in proportion to the percentage decrease in *n* (Figures 5A, B). However, no change in P_{max} was observed until $> 50\%$ of the PS II reaction centers were functionally inhibited (Figure 5B). Thus, DCMU additions sufficient to reduce α up to 50% had no discernible effect on P_{max}. As observed in the natural phytoplankton assemblages

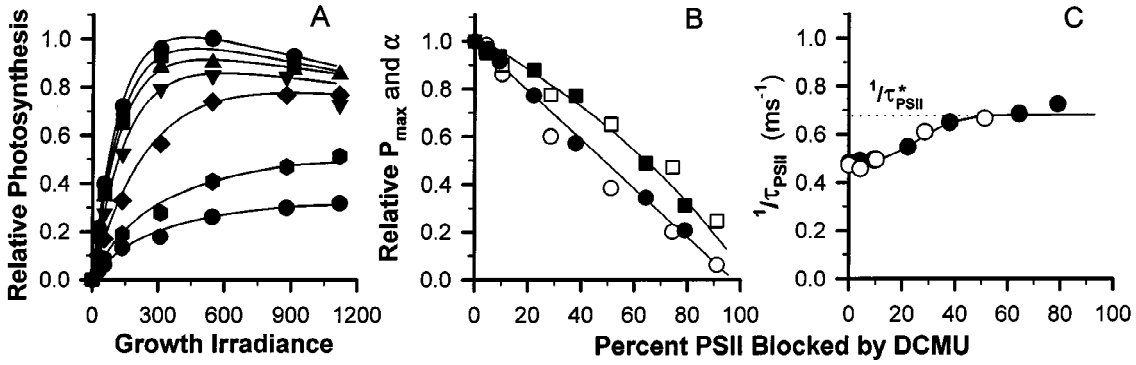

Figure 4. Changes in *Thalassiosira weissflogii* photosynthesis following DCMU addition for cells acclimated to a growth irradiance of 1000 μmol quanta m^{-2} s^{-1}. (A) Photosynthesis-irradiance relationships measured for one of the replicate DCMU-titration experiments. DCMU additions of 0, 10^{-9}, 3×10^{-9}, 10^{-8}, 3×10^{-8}, 10^{-7}, and 3×10^{-7} M resulted in a ● = 0%, B = 4%, ▲ = 9%, ▼ = 22%, ♦ = 38%, ⬢ = 64%, and lower ● = 79% decrease in the number of functional PS II reaction centers, respectively. Photosynthetic rates are normalized to P_{max} in the control treatment (DCMU = 0). (B) Relationship between the percentage of PS II reaction centers functionally blocked by DCMU and the light-limited slope (α) and light-saturated rate (P_{max}) of photosynthesis. Circles = α. Squares = P_{max}. Solid symbols = data in (A). Open symbols = results for the second replicate experiment. (C) Relationship between $^1/\tau_{PSII}$ and the percentage of PS II functionally blocked by DCMU. $^1/\tau_{PSII}$ was calculated as: $\sigma_{PSII} \times (P_{max}/\alpha)$, where σ_{PSII} = 690 Å^2 photon^{-1}. Dotted line = estimated value for $^1/\tau^*_{PSII}$. Symbols as in (B).

(Figure 2), this marked insensitivity of P_{max} to decreases in *n* was accomplished through a factor of 2 increase in $^1/\tau_{PSII}$ (Figure 5C). Decreases in α without subsequent changes in P_{max} imply an increase in the irradiance necessary for saturation, E_k (Talling 1957). Thus, in low light adapted *T. weissflogii*, E_k increased in proportion to changes in *n* until $^1/\tau_{PSII} = {}^1/\tau^*_{PSII}$ [as anticipated from the relationship: $E_k \times \sigma_{PSII} = {}^1/\tau_{PSII}$ (Falkowski and Raven 1997)].

Discussion

Insensitivity of light-saturated photosynthesis (P_{max}) to significant decreases in the number of functional PS II reaction centers in natural phytoplankton populations (Figure 2), laboratory algal cultures (Figure 5B) (Kok 1956; Leverenz et al. 1990), and terrestrial plants (Weinbaum et al. 1979; Heber et al. 1988) clearly illustrates that overall electron transport can remain virtually unaltered despite substantial PS II photodamage. The basis for the uncoupling between the number of PS II reaction centers and rates of electron flow is that photoinhibition does not directly impact the rate limiting step for photosynthesis at light saturation. Thus, changes in carbon fixation will not be observed until rate limitation is shifted from the Calvin cycle reactions to electron transport through PS II.

Comparison of results for *T. weissflogii* grown at constant irradiances of 150 (Figure 5) and 1000 μmol quanta m^{-2} s^{-1} (Figure 4) indicates that the capacity to withstand decreases in functional PS II reaction centers without subsequent changes in P_{max} depends upon the initial value of $^1/\tau_{PSII}$ relative to $^1/\tau^*_{PSII}$ (Figure 3). When acclimated to high light, electron turnover through PS II is high in *T. weissflogii*, while light harvesting capacity is relatively low (Figure 3). This initially high electron turnover rate causes limitation of photosynthesis to be rapidly shifted to the light reactions upon only minor decreases in *n* (Figure 4B). In contrast, acclimation to low light in *T. weissflogii* leads to a greater excess capacity of the photosynthetic light reactions relative to the Calvin cycle capacity (Figure 5) and lower initial rates of light saturated electron turnover (Figure 3). Consequently, decreases in the population of functional PS II of up to 50% can be compensated by increases in $^1/\tau_{PSII}$. Similar insensitivity of P_{max} to decreases in *n* ranging from 40 to 65% has been reported previously (Kok 1956; Weinbaum et al. 1979; Heber et al. 1988; Leverenz et al. 1990). It therefore appears that the capacity for reciprocal changes in *n* and $^1/\tau_{PSII}$ is common, if not ubiquitous, among oxygenic photoautotrophs.

The large excess capacity of phytoplankton sampled near the ocean surface [range: 46–52% (Figure 2)] suggests a photoacclimated state characteristic of relatively low light exposure. If responses of *T.*

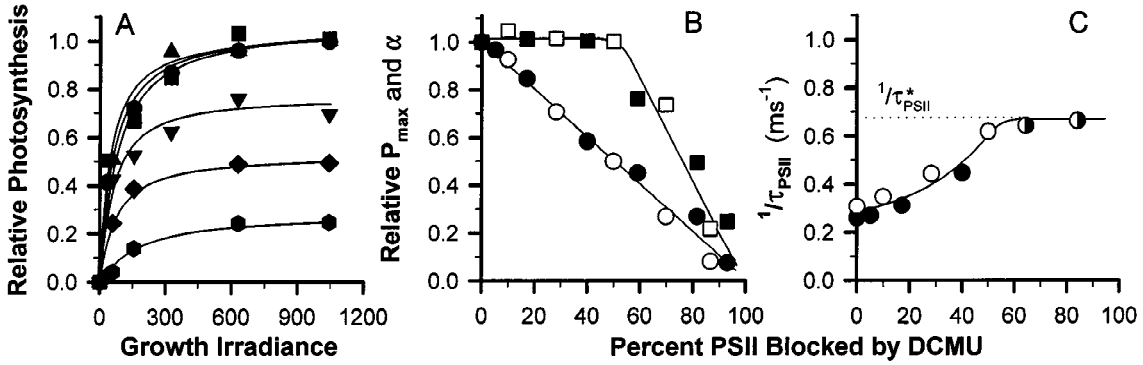

Figure 5. Changes in *Thalassiosira weissflogii* photosynthesis following DCMU addition for cells acclimated to a growth irradiance of 150 μmol quanta m^{-2} s^{-1}. (A) Photosynthesis-irradiance relationships measured for one of the replicate DCMU-titration experiments. DCMU additions of 0, 3×10^{-9}, 10^{-8}, 3×10^{-8}, 10^{-7}, and 3×10^{-7} M resulted in a ● = 0, B = 17%, ▲ = 40%, ▼ = 59%, ♦ = 81%, and ⬢ = 93% decrease in the number of functional PS II reaction centers, respectively. Photosynthetic rates are normalized to P_{max} in the control treatment (DCMU = 0). (B) Relationship between the percentage of PS II reaction centers functionally blocked by DCMU and the light-limited slope (α) and light-saturated rate (P_{max}) of photosynthesis. Circles = α. Squares = P_{max}. Solid symbols = data in (A). Open symbols = results for the second replicate experiment. (C) Relationship between $^1/\tau_{PSII}$ and the percentage of PS II functionally blocked by DCMU. $^1/\tau_{PSII}$ was calculated as: $\sigma_{PSII} \times (P_{max}/\alpha)$, where σ_{PSII} = 808 $Å^2$ $photon^{-1}$. Dotted line = estimated value for $^1/\tau^*_{PSII}$. Symbols as in (B). Half-shaded symbols represent average values for the two replicate experiments. Average values are shown for these treatments with large decreases in functional PS II because small errors in α and P_{max} estimates can cause large variability in calculated $^1/\tau_{PSII}$ values.

weissflogii are used as a model (Figure 3), then the excess capacity of PS II for the natural phytoplankton assemblages would correspond to an acclimated state characteristic of cells grown at a constant irradiance of $<$ 270 μmol quanta m^{-2} s^{-1}, which is far lower than even the average surface light intensity ($>$ 1,300 μmol quanta m^{-2} s^{-1}) during the study period. Why then do phytoplankton populations that periodically experience irradiance exceeding 2000 μmol quanta m^{-2} s^{-1} exhibit such photoacclimation characteristics?

Phytoplankton in the surface mixed layer undergo tremendous variability in daily irradiance, due not only to changes in incident solar radiation but also turbulence-driven changes in their vertical position within the water column. To optimize carbon fixation under such conditions, cells must balance the use of scarce resources, such as nitrogen or trace metals, between the Calvin cycle capacity (which risks insufficient carbon fixation under prolonged subsaturating light) and light harvesting capacity (which increases susceptibility to photodamage). Investing resources in light harvesting components is often advantageous in aquatic systems because irradiance is generally subsaturating (Falkowski and Raven 1997).

Light harvesting capacity is enhanced by increasing either the number of PS II reaction centers or their effective absorption cross sections (Falkowski et al. 1981), the latter requiring fewer resources than the former. This difference in resource requirements may partially explain the characteristically higher absorption cross-sections observed in phytoplankton deep within the water column, as these populations run little risk of exposure to photodamaging light.

In the upper mixed layer, however, increases in absorption cross-sections will lead to overexcitation of PS II upon exposure to high light, thereby exacerbating susceptibility to photodamage (Barber and Andersson 1992a). In contrast, an increase in the number of PS II reaction centers with relatively low absorption cross-sections will increase light harvesting at low light, while enhancing the capacity for compensatory changes in electron turnover upon PS II photodamage. Stated otherwise, the large excess capacity observed in near-surface phytoplankton populations (Figure 2) can be regarded as a resource-optimization strategy that permits a constant rate of photosynthesis over a maximum duration of the photoperiod by saturating carbon fixation at relatively low light intensities, while simultaneously preventing losses in photosynthesis under photoinhibiting light intensities.

In accordance with this proposed acclimation strategy, the symmetric diurnal pattern in variable fluorescence (Figure 2) may be interpreted as a feedback interaction between changes in light intensity and PS II repair. Specifically, repair of photodamaged PS II

reaction centers through turnover of D1 is inhibited when photosynthetic electron transport is saturated and stimulated when subsaturated (Gong and Ohad 1991; Prasil et al. 1992). Thus, decreases in *n* prior to noon would not stimulate D1 turnover because simultaneous increases in light intensity maintain overall light-saturated electron transport. After local noon (or upon physical transport to greater depth), transient subsaturation of electron flow stimulates PS II repair and subsequently leads to the resaturation of electron transport until irradiance decreases further. This postulated feedback system based on electron turnover through the light reactions [possibly detected by changes in the redox state of the plastoquinone pool (Gong and Ohad 1991; Escoubas et al. 1995)] would thus help maintain virtually constant carbon assimilation throughout the photoperiod.

The evolutionary precursors of the PS II reaction center are the purple bacteria (Michel and Deisenhofer 1988), which date back at least 3.6 billion years to the Archean ocean. The structure of the core D1 reaction center protein is highly conserved in all oxygenic photoautotrophs (Barber 1992b), including higher plants and over 18 phylogenetic divisions of extant algae, yet D1 has remained highly susceptible to photodamage in all oxygenic photoautotrophs. Clearly, evolutionary selection has not minimized susceptibility to photodamage of PS II reaction center proteins. By definition, such selection could only exist if photodamage decreased fitness. Our analysis suggests that losses in PS II capacity are not necessarily coupled with decreases in carbon acquisition and hence may have a negligible influence on overall growth in comparison with other factors that influence the tempo of evolution. In contrast, selection pressures have clearly been pronounced for providing strategies which enhance the acquisition of solar radiation. The energetic cost of replacing photodamaged D1 proteins appears to be less than the cost of not acclimating to low light (Raven 1994), assuming availability of the required substrates. However, energetic costs of photoinhibition may be substantially increased if additional stresses are imposed or a rapid decrease in irradiance causes photodamaged cells to become light-limited, as either of these conditions can result in significant decreases in carbon fixation (Krause 1994; Baker et al. 1994; Ort et al. 1994).

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Note

[1]Variable fluorescence is typically expressed as the difference (F_v) between initial (F_o) and maximal (F_m) fluorescence normalized to either F_o or F_m (Kyle et al. 1984; Krause and Weis 1991; Crofts et al. 1993). When nonphotochemical quenching (NPQ) in PS II antennae is constant, the fraction of functional PS II reaction centers (*n*) is linearly related to F_v/F_o (Crofts et al. 1993) and hyperbolically related to F_v/F_m [note: $F_v/F_m = (F_v/F_o) / (1 - F_v/F_o)$]. However, this relationship between F_v/F_o and *n* can deviate from linearity when NPQ is highly variable.

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