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A photosynthetic strategy for coping in a high-light, low-nutrient environment

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Abstract

Phytoplankton in high-light, low-nutrient ocean environments are challenged with maintaining high photosynthetic efficiency and simultaneously preventing photodamage that results from low levels of electron acceptors downstream of photosystem II (PSII). Here, we identify a process in open ocean picophytoplankton that preserves PSII activity by diverting electrons from the photosystem I (PSI) complex–mediated carbon assimilation to oxygen via a propyl gallate–sensitive oxidase associated with the photosynthetic electron transport chain. This process stabilizes diel photochemical efficiency of PSII, despite midday photoinhibition, by maintaining oxidized PSII reaction centers. Although measurements of the maximum photochemical efficiency of PSII, $F_v : F_m$ show midday photoinhibition, midday CO_2 fixation is not depressed. Moreover, CO_2 fixation saturates at low irradiances even though PSII electron flow is not saturated at irradiances of 1,985 μmol photons m^{-2} s^{-1}. This disparity between PSII fluorescence and CO_2 fixation is consistent with the activity of an oxidase that serves as a terminal electron acceptor, maintaining oxidized PSII reaction centers even when CO_2 fixation has saturated and the total number of functional reaction centers decreases because of photoinhibition (reflected in lower midday $F_v : F_m$ values). This phenomenon is less apparent in coastal phytoplankton populations, suggesting that it is a strategy particularly distinctive of phytoplankton in the oligotrophic ocean. Spatial variability in features of photosynthetic electron flow could explain biogeographical differences in productivity throughout the ocean and should be represented in models that use empirical photosynthesis and chlorophyll fluorescence measurements from a limited number of ocean sites to estimate the productivity of the entire ocean.

The open ocean presents numerous challenges to photosynthetic organisms. Physiological stresses imposed by a rapidly fluctuating light environment are exacerbated by oligotrophic nutrient conditions that limit the availability of iron, a nutrient required for maintenance and repair of the photosynthetic apparatus, and macronutrients, such as nitrogen and phosphorus, that are required for cell growth. Despite these challenges, picophytoplankton are remarkably well adapted to life in the open ocean. Recent estimates suggest that the dominant picocyanobacteria genera *Prochlorococcus* and *Synechococcus* are responsible for up to two thirds of primary production in the oceans or nearly one third of the total primary production on Earth (Field et al. 1998; Scanlan 2003). Picophytoplankton also comprise eukaryotes such as the Prasinophytes *Ostreococcus* and *Micromonas*. These organisms are located primarily in coastal areas where they can contribute up to 75% of total CO_2 fixation (Fouilland et al. 2004; Worden et al. 2004). However, they are also present in the oligotrophic oceans where they have successfully colonized the deep euphotic zone (Campbell and Vaulot 1993; Díez et al. 2001).

Iron is required for the synthesis of certain components of the photosynthetic apparatus. In eukaryotic phototrophs, the photosystem II (PSII) complex incorporates four iron atoms, whereas the comparatively iron-rich downstream electron acceptors cytochrome b_6f (cyt b_6f) and the photosystem I (PSI) complex require 6 and 12 iron atoms, respectively (Fig. 1A). The low amount of PSI relative to PSII in open ocean ecotypes of cyanobacteria (Bailey et al. 2008), green algae (P. Cardol, G. Finazzi, and F. A. Wolman pers. comm.), and diatoms (Strzepek and Harrison 2004) suggests that these organisms are evolutionarily adapted to coping with the limitations imposed by low iron availability. In the open ocean, photosynthetic CO_2 fixation saturates at relatively low irradiances (100–300 μmol quanta m^{-2} s^{-1}) compared with the maximum surface irradiance (~2,000 μmol quanta m^{-2} s^{-1}) (Partensky et al. 1993, 1999 and references therein; Li 1994), in

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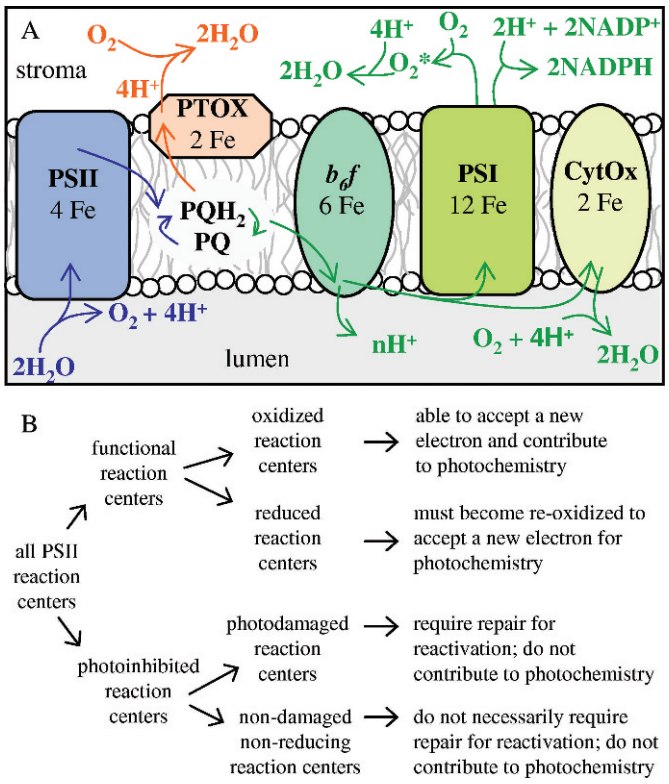

Fig. 1. (A) Schematic diagram of principle photosynthetic apparatus components, their electron flow paths, and iron requirements. Blue arrows denote shared pathways, green arrows denote pathways utilizing the iron-rich cyt b_6f (designated b_6f) or PSI complexes, and orange arrows denote the PTOX pathway, which bypasses cyt b_6f and PSI. The photosynthetic electron transport sequence is as follows: PSII to the plastoquinone pool (PQ, PQH_2, blue) to cyt b_6f to a mobile carrier (cytochrome of plastocyanin, not shown). (Protons are also transported to the lumen during the reduction of b_6f, helping to establish a ΔpH.) From b_6f, electrons passed to PSI can be used to reduce $NADP^+$ (nicotinamide adenine dinucleotide phosphate) for CO_2 fixation or to reduce oxygen via the Mehler reaction (green). The Mehler reaction comprises the following steps (not shown): (1) univalent reduction of O_2 to superoxide (designated O_2^*), (2) disproportionation of superoxide to H_2O_2, and (3) the reduction of H_2O_2 to H_2O (Asada et al. 1974; Asada 1999; Asada 2006). In prokaryotes, electron transport can also proceed from cyt b_6f to the respiratory cytochrome oxidase (green). Electrons can also be donated directly from the plastoquinone pool to a plastid terminal oxidase (PTOX, orange) upstream of cyt b_6f and PSI. In this pathway, protons are consumed from the stroma (via PQH_2) during the reduction of O_2 to H_2O, thereby helping to establish a ΔpH.) (B) Flow chart showing the terminology for PSII reaction center classification used in this study. A "functional" reaction center is one in which Q_A, the first stable PSII electron acceptor, is able to become reduced (on accepting an electron) and reoxidized (on donating the electron) while contributing to photochemistry. A "photoinhibited" reaction center does not refer to the oxidation state of the reaction center but denotes a center unable to perform photochemistry (e.g., after photodamage or down-regulation of PSII reaction centers.).

part because of a limitation in the maximum rate at which carbon can be incorporated into cellular biomass resulting from low nutrient availability and low rates of photosynthesis, possibly as a consequence of low levels of PSI (Strzepek and Harrison 2004; Bailey et al. 2008; P. Cardol, G. Finazzi, and F. A. Wolman pers. comm.). Low levels of electron acceptors downstream of PSII (e.g., scarcity of iron-rich PSI and cyt b_6f complexes) would ultimately restrict the flow of electrons away from PSII during light exposure (Fig. 1A). As a result of this impediment to efficient photochemical dissipation of PSII excitation energy, functional PSII reaction centers (i.e., those able to contribute to photochemistry; Fig. 1B) are more likely to remain reduced, which can lead to PSII photodamage (Adir et al. 2003). Phytoplankton residing in high-light, low-iron environments are therefore challenged with maintaining high photosynthetic efficiency while preventing photodamage stemming from low levels of electron acceptors downstream of PSII.

The development of mechanisms for maintaining oxidized PSII reaction centers and a high ΔpH across the thylakoid membranes would help prevent hyper-reduction of the electron carriers of the photosynthetic apparatus when PSI activity limits PSII electron flow, thereby decreasing the potential for photodamage and providing energy for cell maintenance and growth. A strategy that increases PSI light-harvesting efficiency through increased synthesis of the PSI antenna (IsiA) is used by some cyanobacteria (Boekema et al. 2001; Cadoret et al. 2004), although Ivanov et al. (2006, 2007) suggest that under Fe stress, IsiA does not increase the absorption cross section of PSI but rather acts as a quencher of excitation energy. Analysis of the genome sequence indicates that the marine *Synechococcus* WH8102 surface strain lacks the *isiA* gene (i.e., the PSI antenna polypeptide induced under iron starvation) (Palenik et al. 2003; http://bacteria.kazusa.or.jp/cyano/). Therefore, other strategies to cope with low levels of PSI under the high-light and low-nutrient conditions of the open ocean must exist.

In the classical *z*-scheme for photosynthetic electron flow, electrons passing through PSII are transported to PSI, where a second excitation results in the reduction of CO_2 (Fig. 1A). However, reduction of molecular oxygen can also occur at various points downstream of PSII. In the well-characterized Mehler reaction, oxygen is reduced at the acceptor side of PSI (Mehler 1951; Mehler and Brown 1952; Asada et al. 1974). Moreover, electrons from the photosynthetic electron transport chain can be diverted to the respiratory pathway, leading to cytochrome oxidase (Hart et al. 2005; Fig. 1A). A third pathway that, until recently, has not been explored as extensively involves the plastoquinol terminal oxidase (PTOX; Peltier and Cournac 2002; Josse et al. 2003; Hart et al. 2005), which uses electrons from the plastoquinone (PQ) pool to reduce oxygen and regenerate H_2O. The PTOX pseudo-cycle would alleviate PSII excitation pressure by passing electrons to oxygen, and at the same time bypassing the iron-rich cyt b_6f and PSI complexes of the photosynthetic apparatus (Fig. 1A). Thus, extracting electrons from the intersystem electron transport chain by specific oxidases could represent a clear advantage for open ocean organisms that contend with very low levels of iron and nutrients.

Two recent laboratory-based studies provide evidence that a PTOX-like oxidase appears to prevent closure of

PSII reaction centers at high light intensities in photosynthetic marine prokaryotes (Bailey et al. 2008) and picoeukaryotes (P. Cardol, G. Finazzi, and F. A. Wolman pers. comm.). Bailey and coworkers found that *Synechoccocus* WH8102 (a photosynthetic picocyanobacterium from oligotrophic surface waters) appeared to have a low PSI to PSII ratio, indicative of constitutive low-iron adaptation. Furthermore, whereas CO_2 fixation saturated at low irradiance (~150 μmol photons m^{-2} s^{-1}) in this strain, PSII reaction centers remained open even at very high intensity illumination (~2,000 μmol photons m^{-2} s^{-1}), suggesting a flow of electrons to acceptors other than CO_2. This alternative electron transport out of PSII was abolished under anoxic conditions and in the presence of the oxidase inhibitor propyl gallate (pgal), suggesting that PSII excitation pressure is relieved via the reduction of oxygen by a pgal-sensitive oxidase, possibly PTOX (inhibitors of alternative quinol oxidases [Berry et al. 2002] had no effect). Similarly, the oligotrophic ocean picoeukaryote strain *Ostreococcus* RCC809 has low levels of PSI and cyt b_6f relative to PSII, and PSII photochemistry is pgal sensitive (P. Cardol, G. Finazzi, and F. A. Wolman pers. comm.). However, the coastal *Ostreococcus* OTH95 isolate did not exhibit pgal sensitivity and showed unremarkable PSI and cyt b_6f levels relative to PSII, suggesting that the photoprotective reduction of oxygen is a strategy distinctive of phytoplankton in the oligotrophic ocean where iron is scarce.

Gene sequences for PTOX are widespread among strains of cyanobacteria closely related to the high–light adapted *Prochlorococcus marinus* MED4, as well as *Synechococcus* in the oligotrophic Sargasso Sea (McDonald and Vanlerberghe 2004). To determine whether photoprotective strategies similar to those described above exist in natural assemblages of picophytoplankton in situ, we have explored the redox state of PSII over the diel cycle in environmental samples, its relationship to CO_2 fixation, and factors involved in PSII photochemistry in the oligotrophic waters of the open ocean. Chlorophyll fluorescence measurements were taken from surface waters in the Pacific and Atlantic oceans to assess diel variability in the maximum photochemical efficiency of PSII, variable to maximum fluorescence ($F_v:F_m$), and in the operating photochemical efficiencies of PSII under actinic irradiance (Φ_{PSII}) throughout the day. The photoprotective role of molecular oxygen reduction was investigated for open ocean phytoplankton from the surface and deep euphotic zone, as well as from coastal locations in the Atlantic and Pacific oceans.

Materials and methods

Site descriptions—Samples from the Pacific Ocean were collected onboard the research vessel R/V *Kilo Moana* from locations north of Hawaii within the North Pacific Subtropical Gyre (NPSG) at Station ALOHA (22°45′N, 158°W; Fig. 2A) from 07 to 11 November 2006 on Hawaiian Ocean Time-series (HOT) cruise No. 187 (http://hahana.soest.hawaii.edu/hot/hot_jgofs.html). Hawaiian coast samples were collected from the southeastern

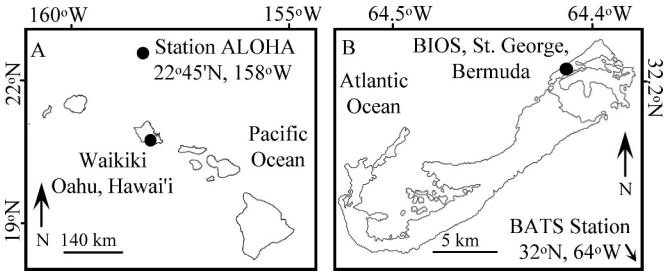

Fig. 2. Maps of sampling sites in the (A) Pacific and (B) Atlantic oceans.

coast of Oahu, south of Waikiki Beach, on 05 November 2006 (Fig. 2A). Samples from the Atlantic Ocean were collected onboard the research vessel R/V *Atlantic Explorer* south of Bermuda in the Sargasso Sea, in and around the Bermuda Atlantic Time-series Study (BATS; Fig. 2B) station (approximately 32°N, 64°W) from 21 to 25 November 2006 on cruise X0619 (http://www.bios.edu/research/bats.html). Bermuda coast samples were collected from Ferry Reach (Fig. 2B), the body of water separating St. David's Island and St. George's Island, at the dockside laboratory facility at the Bermuda Institute of Ocean Sciences (BIOS) on 26 and 27 November 2006.

Flow cytometry—Aliquots of seawater were removed throughout the sampling periods for flow cytometry and were fixed with gluteraldehyde (Sigma) at a final concentration of 0.1%. All flow cytometry samples were stored and shipped at −80°C, except the Pacific open ocean samples were stored briefly at −20°C during transport (roughly 6 h). Pacific coastal samples were not collected. Samples were analyzed on a FACSAria flow cytometer, and data analysis was performed with the use of FlowJo software (TreeStar) Absolute cell densities for picophytoplankton populations (cells < 2 μm in diameter, including *Prochlorococcus*, *Synechococcus*, and picoeukaryotes), were determined by spiking samples with a known volume and concentration of 1 μm of fluorescent yellow-green beads (Polysciences). *Prochlorococcus*, *Synechococcus*, and picoeukaryotes were identified on the basis of size (determined by right angle light scatter) and autofluorescence characteristics as described by Mackey et al. (2007). The coefficient of variation for phytoplankton cell densities determined from triplicate samples was <0.10 for all samples.

Chlorophyll fluorescence parameters and terminology—Energy absorbed but not used in photochemistry can either undergo nonphotochemical quenching (i.e., dissipation as heat, movement of the photosynthetic antennae from PSII to PSI in state transitions, or PSII photodamage) or fluorescence (re-emission of energy as light). Fluorescence analysis uses this energy balance to provide information about the efficiency of photochemistry on the basis of changes in PSII fluorescence under a range of different light treatments. In this study, maximum fluorescence in the dark-adapted state (F_m), minimum fluorescence in the dark-adapted state (F_o), maximum fluorescence in the

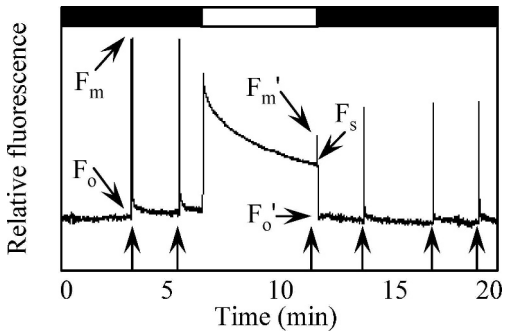

Fig. 3. Example chlorophyll fluorescence trace showing the fluorescence levels used for computing the fluorescence parameters in Table 1 from samples collected during diel monitoring. The thick line along the top indicates periods of no actinic light (black) and 1,985 μmol quanta m^{-2} s^{-1} actinic light (white). Arrows along the abscissa indicate the timing of the saturating pulses (saturating pulse intensity was 3,000 μmol quanta m^{-2} s^{-1} for a duration of 0.8 s). The saturating pulse during periods in which the cells were exposed to actinic light was delivered immediately before the actinic light was turned off.

light-adapted state (F'_m), minimum fluorescence in the light-adapted state (F'_o), and steady state fluorescence in the light-adapted state (F_s) values were extracted from fluorescence traces (Fig. 3), and all fluorescence parameters were calculated by standard equations (Campbell et al. 1998; Maxwell and Johnson 2000) (Table 1). $F_v : F_m$ reflects the maximum photochemical efficiency of PSII in the dark-adapted state, whereas Φ_{PSII} represents the actual photochemical efficiency of PSII after actinic irradiances of different light intensities. In contrast, the fraction of oxidized PSII reaction centers (qP) at specific light intensities (we used 1,985 μmol quanta m^{-2} s^{-1} to simulate natural midday irradiances) reflects the ability of the phytoplankton to cope with changing levels of light throughout the day.

Figure 1B gives the terminology used to classify PSII reaction centers in this study. In the initial steps of photosynthetic electron transport, energy is used to drive photochemical charge separation in which electrons are generated from splitting water at the level of PSII. These electrons are transferred from P680, the reaction center chlorophyll molecule of PSII, to the first stable PSII electron acceptor, Q_A. As long as Q_A is able to contribute to photochemistry, the PSII reaction center is considered functional. The fraction of the functional reaction centers that are in the reduced state at any given time will depend in part on the ambient light intensity (which determines the rate at which electrons are donated to Q_A) and the availability of competent electron acceptors downstream of PSII (which determines the rate at which electrons are accepted from Q_A) (Long et al. 1994). High light intensities, a limited availability of downstream electron acceptors, or both will limit how efficiently Q_A is able to become reoxidized, causing a higher fraction of Q_A to remain in the reduced state and therefore temporarily unable to accept electrons generated from the splitting of water. PSII reaction centers with reduced Q_A are considered to be "closed" to photochemistry; however, they are still considered functional because they can contribute to photochemistry immediately upon becoming reoxidized.

If energy from absorbed light is unable to be dissipated efficiently through photochemistry, as could be the case if a large fraction of PSII reaction centers were closed, the excess light energy can contribute to photoinhibition through damage to the D1 polypeptide that binds the P680, Q_A, and other cofactors involved in charge separation (Long et al. 1994). Photodamage renders PSII nonfunctional (i.e., unable to undergo photochemistry) until D1 repair is accomplished, typically on the order of hours. Photoacclimation via regulation of nondamaged, nonreducing centers also contributes to photoinhibition (Lavergne 1982; Guenther and Melis 1990; Oquist et al. 1992*b*), but these centers do not necessarily require repair to regain function. We note that a photoinhibited reaction center does not refer to the oxidation state of the reaction center but denotes a center unable to perform photochemistry. Photodamage and the presence of nondamaged, nonreducing centers causes a decrease in the overall number of functional reaction centers, thereby contributing to a decrease in the maximum photochemical efficiency of PSII ($F_v : F_m$), which is by definition photoinhibition (Maxwell and Johnson 2000). Thus, an increase in photoinhibition is evidenced by a decrease in the extent of the $F_v : F_m$ chlorophyll fluorescence parameter, and changes in the latter are commonly used to assess photoinhibition in vivo.

Note that although Φ_{PSII} and qP both pertain to PSII photochemistry, qP incorporates information about steady state chlorophyll fluorescence levels during (F_s) and after (F'_o) exposure to actinic light. Throughout the day, the extent to which these steady state fluorescence levels (F_s and F'_o) change with respect to each other, and with respect

Table 1. Equations used in determining PSII fluorescence parameter values. Variables are defined in Fig. 4.

PSII fluorescence parameter	Calculation
Maximum (dark-adapted) efficiency of PSII photochemistry $\left(\frac{F_v}{F_m}\right)$	$\frac{F_m - F_o}{F_m}$
Actual (light-adapted) efficiency of PSII photochemistry (Φ_{PSII})	$\frac{F'_m - F_s}{F'_m}$
Photochemical quenching; fraction of oxidized PSII reaction centers (qP)	$\frac{F'_m - F_s}{F'_m - F'_o}$
Relative PSII electron transport rate (ETR)	$\Phi_{PSII} \times I_A$*

* I_A, actinic light intensity.

to maximum fluorescence (F'_m), reveals the degree of saturation of PSII photochemistry by light. It is this degree of saturation that reveals the fraction of oxidized PSII reaction centers that the photosynthetic apparatus maintains during exposure to a given actinic irradiance, which is by definition qP. By contrast, Φ_{PSII} does not incorporate steady state fluorescence after exposure to actinic light (F'_o), and as such it reflects the proportion of absorbed light energy that is used in PSII photochemistry. So, whereas Φ_{PSII} gives information about the efficiency with which PSII is able to use absorbed energy, qP gives information about how the degree of saturation (i.e., the fraction of centers that are oxidized) has altered this efficiency. However, the degree of saturation is only one factor influencing the photochemical efficiency of PSII, and as such, qP and Φ_{PSII} will not necessarily covary if the effects of other processes affecting PSII photochemistry, such as changes in the functionality of PSII reaction centers or the regulation of oxidase activity, are significant.

Chlorophyll fluorescence measurements—Chlorophyll fluorescence measurements were taken from natural phytoplankton assemblages in the Pacific and Atlantic oceans. Samples were preconcentrated in the dark at low pressure onto GF/F filters (Whatman) following in-line size fractionation with 20-μm mesh. Approximately 2–4 liters of seawater were collected onto each filter in the dark over a roughly 30-min interval. At sea, the in-line filtration assembly was connected to the ships' clean underway flow-through systems, which pumped surface water from 2 to 5 m depth. Samples from the deep chlorophyll maximum (DCM) were collected with a sampling CTD-Rosette (SeaBird) equipped with 12-liter Niskin bottles. The depth of the DCM was estimated from real-time fluorescence measurements taken during deployment of the rosette. Water was collected from 100 and 110 m in the Atlantic and Pacific open ocean sites, respectively, and was then transferred to large (20-liter), opaque polyethylene cubitainers that had been previously rinsed with sample water. These samples were collected at morning (~08:00 h), midday (~13:00 h), and midnight and were concentrated onto GF/F filters with the use of a low-pressure peristaltic pump. Samples from the Bermuda coast were collected by connecting the in-line filtration assembly to a clean flow-through system at the BIOS dockside laboratory facility that drew surface water from the adjacent inlet. Samples from the Hawaiian coast were collected in the surf zone from 0.5 m depth and were filtered under low pressure with a Mityvac II hand pump (Nalgene) after size fractionation as described above. In all locations, samples were kept in the dark during the entire filtration process, including when the filters were placed into the fluorometer chamber.

Chlorophyll fluorescence measurements were made with a WATER-PAM fluorometer and WinControl software (Heinz Walz GmbH). Sample filters were collected into glass cuvettes with approximately 3 mL of concurrently collected sample seawater and placed directly into the darkened sample chamber. For the diel measurements at the Atlantic open ocean and coastal sites and the Pacific open ocean site, an automated program was run to determine all chlorophyll fluorescence parameters. High-frequency diel measurements of chlorophyll fluorescence were not taken at the Pacific coastal site. Actinic irradiance of 1,985 μmol quanta m^{-2} s^{-1} was applied for 5 min for all diel samples. We note that the actinic light intensity used in the fluorescence traces was likely much higher than the light level to which cells from the DCM were acclimated. Oxygen deprivation experiments were performed as above with the following modifications. Each filter was placed in 3 mL of seawater within a foil-wrapped cuvette to eliminate exposure to light, and bubbling for 30 min with nitrogen gas was used to purge oxygen from the sample. (Note that because, in seawater, photoautotrophs obtain CO_2 in the form of aqueous bicarbonate, this process does not deprive cells of CO_2 for photosynthesis.) The cuvette was then placed within the darkened fluorometer chamber for 20 min to allow respiration to draw down any residual oxygen and regenerate some additional CO_2 in solution before initiating the automated program. For measurements of relative electron transport in the Pacific open ocean and coastal sites, an automated program was run in which the actinic irradiances were incrementally increased from 85 to 1,985 μmol quanta m^{-2} s^{-1} for 5 min at each irradiance, followed by a saturating pulse and 3 min in the dark. The relative electron transport rate was estimated as the product of light intensity and Φ_{PSII}. Note that electron transport rate is considered "relative" because no corrections have been made to account for the partitioning of absorbed light energy between the two photosystems in these calculations. When used, pgal was administered at a final concentration of 1 mmol L^{-1} followed by 5 min of dark incubation before collecting the fluorescence trace.

Photosynthesis–irradiance relationships—Photosynthesis–irradiance (PI) relationships were derived from the autotrophic incorporation of radiolabeled bicarbonate ($H^{14}CO_3^-$) into biomass at morning (~08:00 h) and midday (~13:00 h) for phytoplankton from surface and DCM waters at the Atlantic open ocean site. For each depth and time, twenty-four 20-mL aliquots of seawater were spiked with $H^{14}CO_3^-$ solution and incubated for 1 h within a temperature-controlled (24°C) photosynthetron (CHPT Manufacturing) that provided distinct irradiances of up to ~750 μmol quanta m^{-2} s^{-1} for each aliquot (Lewis and Smith 1983). Three randomly selected vials were subsampled immediately before incubation for total activity measurements. After incubation, the aliquots were filtered onto glass fiber filters, acidified with hydrochloric acid, and sealed within acid-washed vials. Within several days, filters were dried, resuspended in scintillation cocktail, and assessed for ^{14}C incorporation. Photosynthesis rates were normalized to corresponding chlorophyll *a* (Chl *a*) concentrations (data courtesy of M. Lomas), and the PI data were fitted to the following equation of Platt et al. (1980) with curve-fitting software in SigmaPlot (Version 10.0, Jandel Scientific).

$$P = P_s\left[1 - \exp\left(\frac{-\alpha}{P_s}E\right)\right]\exp\left(\frac{-\beta}{P_s}E\right) \quad (1)$$

Equation parameters determined by the program include: α, the initial slope of the best fit curve; β, the parameter used to express reduction in photosynthetic rates at high irradiances; and P_s, an estimate of what the sample's light-saturated photosynthetic rate would be if $\beta = 0$. The light-saturated rate of photosynthesis (P_m) and the saturation irradiance (E_k) were calculated from the following relationships in Eqs. 2 and 3 (Platt et al. 1980).

$$P_m = P_s\left(\frac{\alpha}{\alpha+\beta}\right)\left(\frac{\beta}{\alpha+\beta}\right)^{\beta/a} \quad (2)$$

$$E_k = \frac{P_m}{\alpha} \quad (3)$$

Results

Flow cytometry—Prochlorococcus was the dominant organism in Atlantic and Pacific open ocean surface waters at the time of our sampling (Fig. 4A,B). In the Atlantic open ocean, *Prochlorococcus* remained the dominant organism throughout the euphotic zone, whereas a substantial population of picoeukaryotes was encountered near the DCM in the Pacific open ocean. By contrast, *Synechococcus* was the dominant organism in the coastal Atlantic site, although measurable quantities of *Prochlorococcus* and picoeukaryotes were also present (Fig. 4C).

Diel chlorophyll fluorescence measurements—$F_v : F_m$, which is dependent on the total number and configuration of functional PSII reaction centers (Butler 1978), shows distinct diurnal variability with a midday minimum (indicative of the occurrence of photoinhibition) and rapid recovery throughout the afternoon and evening in open ocean samples (Fig. 5A,B). The midday $F_v : F_m$ minimum in the oceanic Atlantic and Pacific suggests a decrease in the total number of functional PSII reaction centers contributing to photochemistry. In contrast, the actual photochemical efficiency in the light (Φ_{PSII}), which indicates the portion of PSII excitation energy going to photochemistry (photosynthetic electrons used for either CO_2 fixation or the reduction of other electron acceptors), does not show a large midday depression. Hence, PSII photochemical efficiency remains relatively constant throughout the day despite photoinhibition. Analysis of open ocean photochemical quenching (qP) indicates that a large fraction of PSII reaction centers remain oxidized at midday when photoinhibition is greatest (Fig. 5D,E), showing that when the fewest functional reaction centers are present, the highest fraction of them remain oxidized.

By contrast, in a nearshore site along the Bermuda coast, photoinhibition is less pronounced at midday (Fig. 5C), but similar to the oligotrophic open ocean sites, the efficiency of PSII photochemistry in the light, Φ_{PSII}, remains relatively constant over a diel period, with a slight decrease occurring in the evening. The decreased level of photoinhibition in this nearshore site does not show a concurrent, pronounced midday increase in qP, the fraction of oxidized reaction centers (Fig. 5F).

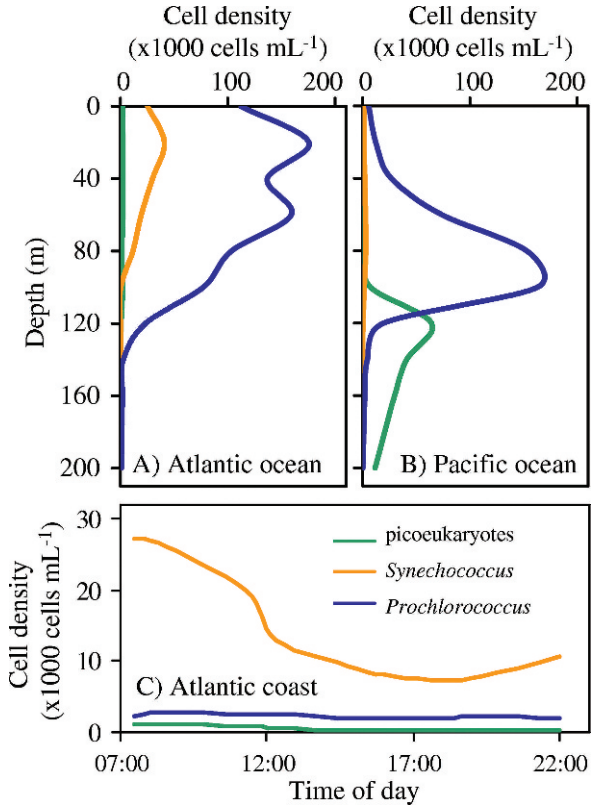

Fig. 4. Phytoplankton cell densities of *Prochlorococcus* (blue line), *Synechococcus* (orange line), and picoeukaryotes (green line). Depth profiles of the open ocean sites in the (A) Atlantic Ocean and (B) Pacific Ocean. (C) Cell abundances in surface populations from the Atlantic coastal site throughout the sampling period.

As observed for the diel patterns of $F_v : F_m$ and qP discussed above, fluorescence analysis of surface populations over a range of increasing actinic light intensities reveals that a higher proportion of PSII reaction centers remain open in the midday than in the midnight samples at any of the light intensities tested (Fig. 6). This midday rise in qP occurs in conjunction with a decrease in maximum photochemical efficiency of PSII ($F_v : F_m$ = 0.249 at midday, 0.523 at midnight). A similar midday decrease in $F_v : F_m$ relative to night ($F_v : F_m$ = 0.448 at midday, 0.700 at midnight) is also observed for the DCM samples. Thus, occurrence of some photoinhibition throughout the day, as reflected by the $F_v : F_m$ measurements, appears to be the inevitable consequence of light exposure in both populations, despite the extreme differences in ambient light intensity (Barber and Anderson 1992; Oquist et al. 1992*a*; Behrenfeld et al. 2006). The fraction of oxidized PSII centers in both surface and DCM samples, at any of the light intensities tested, is higher at midday (i.e., when the number of functional PSII centers is at its lowest). This suggests that natural samples from both the surface and the deep waters of oligotrophic oceans have conserved the capacity to maintain open PSII reaction centers at midday despite photoinhibition. However, the extent of this phenomenon is reduced in populations from the DCM,

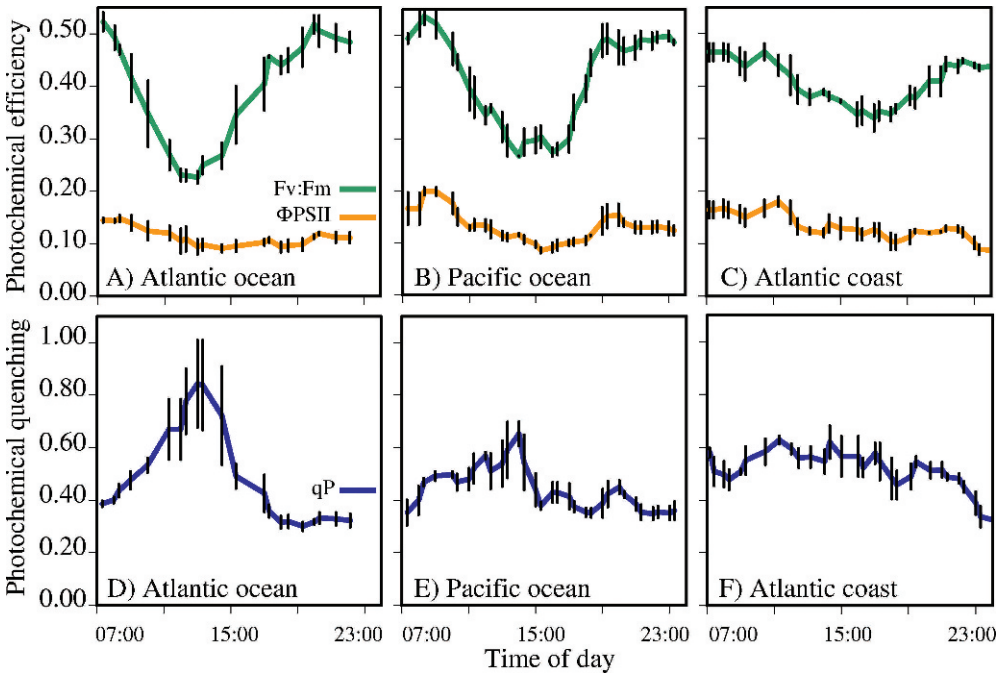

Fig. 5. Diel variation of the maximum ($F_v : F_m$, green line) and actual (Φ_{PSII}, orange line) PSII photochemical efficiencies in the (A) Atlantic open ocean site, (B) Pacific open ocean site, and (C) Atlantic coastal site. Diel variation of the proportion of oxidized PSII reaction centers (photochemical quenching, qP, blue line) of phytoplankton from the (D) Atlantic open ocean site, (E) Pacific open ocean site, and (F) Atlantic coastal site. Error bars show ±1 SEM.

where the absolute fraction of oxidized reaction centers remains low at midday compared with surface samples. Midday qP in DCM samples was largely insensitive to the inhibitory effect of oxygen removal (not shown), suggesting that other photoprotective mechanisms might also contribute to PSII reaction center oxidation in these cells.

In addition to the trends described above, diurnal F_o quenching was observed in samples at midday, and F'_m levels after termination of actinic light were generally lower than F_m levels, particularly at midday. Lack of total F'_m recovery could be due to (1) inactivation of PSII during actinic light treatment, (2) induction of a state transition that did not return to the initial dark-adapted state before illumination, or (3) inability of the pH gradient across the thylakoid membrane to relax completely during the dark relaxation period we measured (~8 min). Although identification of the mechanism for these observations is outside the scope of this text, it is possible that diurnal changes in the state 1 : state 2 ratio could occur (Schreiber et al. 1995) and could represent another adaptation to a low-Fe environment. Moreover, other processes affecting the redox state of the plastoquinone pool (Mi et al. 1994, 1995) might play an important role in determining the levels of F_o and F'_m throughout the day. More work is needed to clarify the primary source of this trend.

PI curves—PI parameters were determined for phytoplankton from the Atlantic surface ocean and the DCM at morning and midday (Fig. 7; Table 2). In surface samples, the light-saturated photosynthetic rate, P_m, was 8.4 g C (g Chl a)$^{-1}$ h^{-1} in the morning and 7.1 g C (g Chl a)$^{-1}$ h^{-1} at midday. Values of α were similar (0.06336 and 0.05416 g C [g Chl a]$^{-1}$ h^{-1} [μmol quanta m^{-2} s^{-1}]$^{-1}$); however, β values (which describe decreases in photosynthetic rates at high irradiances) differed considerably. In the morning, β was 0.000306 g C (g Chl a)$^{-1}$ h^{-1} (μmol quanta m^{-2} s^{-1})$^{-1}$, whereas cells that were exposed to approximately 5 h of natural, unattenuated sunlight before collection at midday when solar irradiance was greatest had a β equivalent to 0. Despite these differences, morning and midday surface samples had similar E_k values of 131

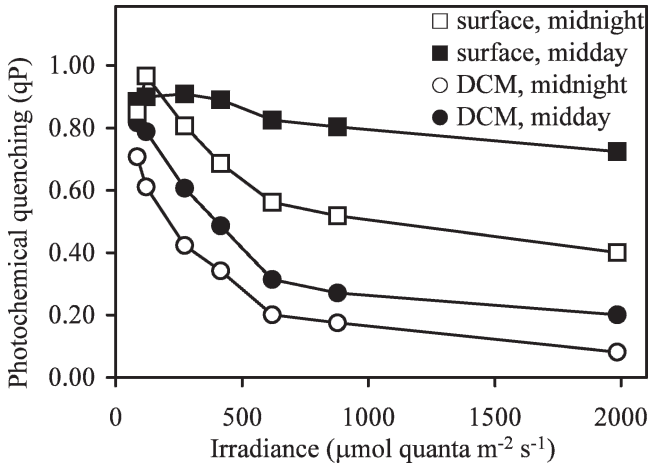

Fig. 6. Photochemical quenching at indicated actinic light levels in cells from the Atlantic open ocean surface waters (squares) and the deep chlorophyll maximum (circles) at midday (filled symbols) and midnight (open symbols).

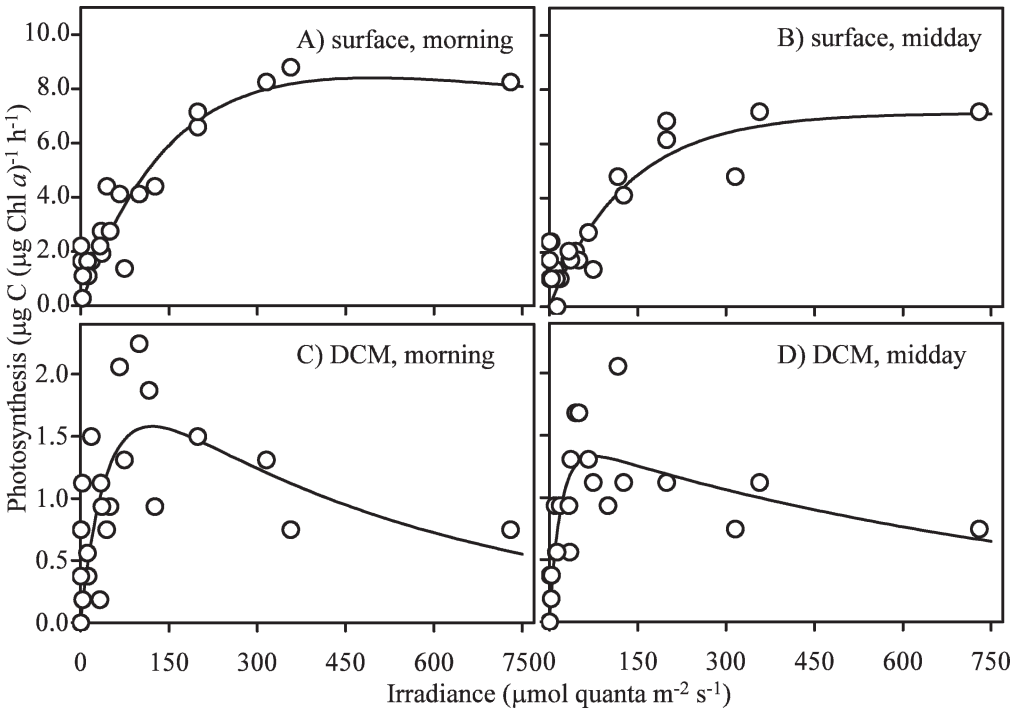

Fig. 7. Photosynthesis–irradiance curves from the Atlantic open ocean site for (A) surface samples in the morning, (B) surface samples in midday, (C) DCM samples in the morning, and (D) DCM samples in midday.

and 132 μmol quanta m^{-2} s^{-1}, respectively, showing that the irradiance at which the rate of photosynthesis saturated remained similar between morning and midday.

Photosynthetic parameters derived from PI curve data from the DCM differed from surface samples, with lower light-saturated photosynthetic rates (P_m) of 1.3 g C (g Chl a)$^{-1}$ h^{-1} and 1.6 g C (g Chl a)$^{-1}$ h^{-1} for morning and midday samples, respectively. Values of α from the DCM at morning (0.07496 g C [g Chl a]$^{-1}$ h^{-1} [μmol quanta m^{-2} s^{-1}]$^{-1}$) were nearly twice as high as at midday (0.04459 g C [g Chl a]$^{-1}$ h^{-1} [μmol quanta m^{-2} s^{-1}]$^{-1}$). In contrast, values of β from the DCM at morning (0.00163 g C [g Chl a]$^{-1}$ h^{-1} [μmol quanta m^{-2} s^{-1}]$^{-1}$) were roughly half as great as at midday (0.00384 g C [g Chl a]$^{-1}$ h^{-1} [μmol quanta m^{-2} s^{-1}]$^{-1}$). Values of E_k for DCM samples were an order of magnitude lower than in surface samples and were half as high at morning (18 μmol quanta m^{-2} s^{-1}) than at midday (35 μmol quanta m^{-2} s^{-1}).

Comparison of the relative PSII electron transport rates and PI curves in surface cells at midday indicate that electron flow through PSII remains high even at irradiances for which CO_2 fixation is saturated (Fig. 8). Electron flow to carbon saturates near 131 μmol quanta m^{-2} s^{-1}, whereas the relative electron transport rate through PSII remains unsaturated up to 1,985 μmol quanta m^{-2} s^{-1} (not shown).

Oxygen deprivation experiments—As shown in Fig. 9, oxygen is necessary for open ocean picophytoplankton to maintain PSII reaction centers in an oxidized state. Even under actinic irradiances of 1,985 μmol quanta m^{-2} s^{-1}, a large portion of PSII reaction centers remained oxidized in aerobic samples, as indicated by the high value of F'_m relative to F_s (Fig. 9A). By contrast, if oxygen is eliminated from the samples, the PSII reaction centers are completely closed (reduced) at 1,985 μmol quanta m^{-2} s^{-1}, and F'_m

Table 2. Photosynthesis–irradiance parameters determined for Atlantic open ocean samples from the surface of the euphotic zone and the deep chlorophyll maximum (DCM).

	P_s (g C [g Chl a]$^{-1}$ h^{-1})	α ([g C {g Chl a}$^{-1}$ h^{-1}]/[μmol quanta m^{-2} s^{-1}])	β ([g C {g Chl a}$^{-1}$ h^{-1}]/[μmol quanta m^{-2} s^{-1}])	P_m (g C [g Chl a]$^{-1}$ h^{-1})	E_k (μmol quanta m^{-2} s^{-1})	R^2
Surface morning	10.219	0.06336	0.00306	8.4	132	0.85
Surface midday	7.126	0.05416	0.00000	7.1	131	0.76
DCM morning	1.478	0.07496	0.00163	1.3	18	0.69
DCM midday	2.134	0.04459	0.00384	1.6	35	0.38

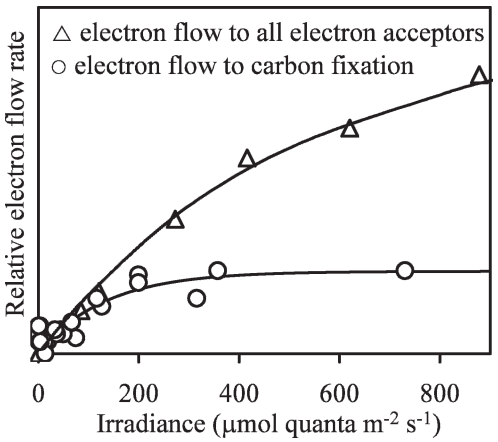

Fig. 8. In phytoplankton from the Atlantic open ocean surface waters, the electron flow to carbon (open circles) saturates near 131 μmol quanta m^{-2} s^{-1}, whereas the relative electron transport rate (ETR) through PSII (open triangles) remains unsaturated. Relative ETR and CO_2 fixation (photosynthesis) are scaled to facilitate comparison; *y*-axes use arbitrary units.

does not exceed F_s (Fig. 9B). This decrease in the proportion of oxidized PSII reaction centers when the samples are made anoxic is reflected in a significantly decreased photochemical efficiency of PSII, Φ_{PSII} ($p < 0.0001$, $n = 3$). We note that observation of variable fluorescence under oxic conditions during exposure to 1,985 μmol quanta m^{-2} s^{-1} actinic light, as shown in Fig. 9A, does not indicate a high capacity for carbon incorporation but rather indicates a high capacity for maintaining PSII reaction centers in an oxidized state. Accordingly, measurements of qP from the anoxic trace (Fig. 9B) do not suggest that oxygen is required to initiate CO_2 fixation because CO_2 fixation becomes saturated at 131 μmol quanta m^{-2} s^{-1} and the actinic light intensity we used in these experiments (1,985 μmol quanta m^{-2} s^{-1}) exceeded this level. Moreover, phytoplankton have a range of carbon concentrating mechanisms that compensate for the relatively low affinity of ribulose bisphosphate carboxylase/oxygenase (Rubisco) for CO_2, thereby allowing acclimation to a wide range of CO_2 concentrations (Kaplan and Reinhold 1999). Therefore, the absence of variable fluorescence from the anoxic trace in Fig. 9B does not indicate limitation for CO_2, but rather limitation for oxygen.

Inhibitor experiments—Using pgal, we tested whether electrons were being used to reduce oxygen through the activity of PTOX or a PTOX-like oxidase in natural populations of open ocean phytoplankton. The proportion of photochemical electron flow inhibited by pgal was estimated by the difference in the relative PSII electron transport rate (ETR) between control and pgal-treated samples. As shown in Fig. 9C, in the Pacific coastal site, inhibition of electron transport by pgal remains below 15% across all actinic irradiances. By contrast, in the oceanic Pacific site, a large portion of the photochemical ETR is abolished in the presence of pgal. This effect is particularly

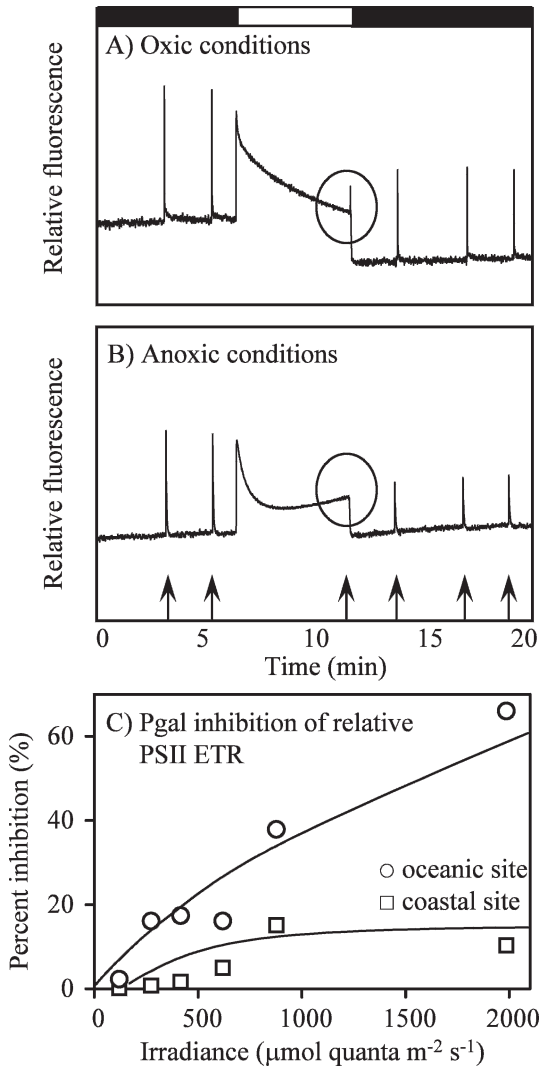

Fig. 9. Chlorophyll fluorescence traces of cells from the surface Atlantic open ocean under (A) oxic and (B) anoxic conditions show that the portion of oxidized reaction centers (indicated by circled areas on the traces) are substantially lower in anoxic conditions than in oxic conditions during exposure to high light. The thick line along the top indicates periods of no actinic light (black line segments) and 1,985 μmol quanta m^{-2} s^{-1} actinic light (white line segment) for (A) and (B). Arrows along the abscissa indicate timing of the saturating pulses (saturating pulse intensity was 3,000 μmol quanta m^{-2} s^{-1} for a duration of 0.8 s). The saturating pulse during actinic light was delivered immediately before the actinic light was turned off. (C) Percent decrease in the relative PSII electron transport rate in the presence of 1 mmol L^{-1} pgal for natural populations in the surface Pacific open ocean site (open circles) and the Pacific coastal site (open squares) after 30 min of dark adaptation.

remarkable during exposure to high light, where relative ETR is more than twice as high in control samples than in cells treated with pgal.

Discussion

Cells that are adapted to oligotrophic, low-iron conditions by maintaining low levels of PSI and cyt b_6f relative

to PSII are particularly vulnerable to the effects of high light because inefficient dissipation of PSII excitation energy can lead to PSII photodamage (Long et al. 1994; Adir et al. 2003). The development of mechanisms for maintaining oxidized PSII reaction centers would relieve PSII excitation pressure when CO_2 fixation is limited by low nutrient availability and low levels of PSI and cyt b_6f, thereby decreasing the potential for PSII photodamage. In surface populations from the open ocean, $F_v:F_m$ and qP showed strong diel periodicity, whereas Φ_{PSII} remained relatively constant (Fig. 5A,B,D,E). This suggests that at midday, when the fewest functional reaction centers are present (low $F_v:F_m$), a larger fraction of them remain oxidized (high qP), which allows for the maintenance of high PSII photochemical efficiency (reflected in a stable Φ_{PSII}) and likely prevents cells from incurring lethal levels of photodamage from high midday irradiances. By contrast, in the morning when irradiances are low and the maximum number of functional PSII reaction centers are present (high $F_v:F_m$), a smaller fraction of the reaction centers remain oxidized during exposure to high light (low qP). This configuration still allows for the maintenance of high PSII photochemical efficiency, resulting in a stable Φ_{PSII}. The maintenance of a higher fraction of oxidized PSII reaction centers at midday is therefore likely to help open ocean picophytoplankton survive despite incurring photoinhibition, a potential consequence of performing photosynthesis in a high-light, low-nutrient environment.

In contrast to the open ocean, coastal populations showed less variability in $F_v:F_m$ and qP, while still maintaining a relatively stable Φ_{PSII} (Fig. 5C,F). This shows that a greater proportion of the PSII reaction centers remain functional at midday in the coastal relative to the open ocean samples and that a smaller fraction of the centers need to remain oxidized to achieve constant photochemical efficiency throughout the day. In other words, different strategies are used in the open ocean and coastal sites to keep a nearly constant diel photochemical efficiency. In the open ocean, picophytoplankton maintain photochemical efficiency at midday by enhancing oxidation of those reaction centers that are still functional and simultaneously contending with a decrease in the total number of functional PSII reaction centers from photoinhibition. By contrast, in coastal waters in which photosynthesis is less limited by iron and macronutrient availability, more reaction centers remain functional throughout the day, with a smaller fraction of them remaining in the oxidized state. This might reflect a more favorable PSII to PSI ratio in the coastal organisms, allowing balanced electron flow through the two photosystems (Strzepek and Harrison 2004).

Understanding the mechanism by which open ocean picophytoplankton maintain a large fraction of oxidized PSII reaction centers during the period when there appears to be maximal photoinhibition is critical for understanding how these organisms cope in this high-light, oligotrophic environment. In open ocean surface waters, photosynthetic CO_2 fixation saturates at relatively low irradiances (100–300 μmol quanta m^{-2} s^{-1}) compared with the maximum surface irradiances (2,000 μmol quanta m^{-2} s^{-1}) (Partensky et al. 1993, 1999 and references therein; Li 1994). Comparison of PI relationships and the relative PSII ETRs of natural populations of phytoplankton (Fig. 8) indicate that, although CO_2 fixation rates saturate at irradiances of 131 μmol quanta m^{-2} s^{-1} (Fig. 7C,D), PSII electron flow saturates at much higher light intensities (Fig. 8). Interestingly, the relative PSII ETR does not saturate in the phytoplankton populations from surface waters of the open ocean even at intensities of 1,985 μmol quanta m^{-2} s^{-1} (data not shown). Similar results were observed in laboratory cultures of both prokaryotic (Bailey et al. 2008) and eukaryotic (P. Cardol, G. Finazzi, and F. A. Wolman pers. comm.) picophytoplankton isolated from the oligotrophic open ocean. These data show that electron flow through PSII at irradiances greater than 131 μmol quanta m^{-2} s^{-1} must be used to reduce an electron acceptor other than CO_2.

Because oxygen has been shown to be a major electron acceptor in laboratory strains of open ocean phytoplankton (Bailey et al. 2008; P. Cardol, G. Finazzi, and F. A. Wolman pers. comm.), we tested whether electrons were being used to reduce oxygen in natural populations of open ocean phytoplankton. Under oxic conditions, the photochemical efficiency of PSII in cells exposed to high light remains high, and a substantial fraction of PSII reaction centers are oxidized (Fig. 9A). These results show that at irradiances above those that saturate photosynthetic CO_2 fixation, electrons can still exit PSII. However, when oxygen is removed, the photochemical efficiency of PSII drops significantly and none of the PSII reaction centers remain oxidized during exposure to high light (Fig. 9B). This oxygen-dependent transition suggests that excess PSII excitation energy is rerouted to the reduction of molecular oxygen.

The decrease in PSII electron flow during treatment with pgal (Fig. 9C) shows that the activity of a pgal-sensitive oxidase is necessary to keep PSII reaction centers oxidized, and that the activity of such an oxidase appears to be particularly important in high-light, low-nutrient waters of the open ocean. Our in situ data are further supported by studies performed with laboratory-grown cells using a range of inhibitors specific to various oxidases (Bailey et al. 2008; P. Cardol, G. Finazzi, and F. A. Wolman pers. comm.). A pgal-sensitive oxidase is critical for maintaining open PSII reaction centers under high-light conditions for both *Synechococcus* WH8102 (Bailey et al. 2008) and the open ocean ecotype of *Ostreococcus*, RCC809 (P. Cardol, G. Finazzi, and F. A. Wolman pers. comm.), both of which have low PSI : PSII and cyt b_6f : PSII ratios (Bailey et al. 2008; P. Cardol, G. Finazzi, and F. A. Wolman pers. comm.). This oxidase activity is negligible in a coastal ecotype of *Ostreococcus* (OTH95), which does not show features of low iron adaptation (P. Cardol, G. Finazzi, and F. A. Wolman pers. comm.).

Pgal might have some nonspecific physiological effects; in addition to inhibiting PTOX, it can also inhibit the alternative oxidase of the mitochondrial electron transport chain in eukaryotes. However, it has negligible effects on other components of the photosynthetic electron transport chain in *Synechococcus* (Bailey et al. 2008). Although the

photoprotective process identified in this study cannot be definitively attributed to PTOX per se, the sensitivity of the process to pgal together with the high abundance of cyanobacterial PTOX sequences within the environmental metagenomic data set for the oligotrophic Sargasso Sea (McDonald and Vanlerberghe 2004) lend credence to the notion of a photoprotective role for PTOX in open ocean plankton.

Diverting PSII photogenerated electrons from PSI to molecular oxygen might represent an essential and widespread mechanism to alleviate PSII excitation and prevent PSII photodamage in the open ocean. Moreover, the pathway would generate a large ΔpH across the thylakoid membrane via the release of H^+ in the lumen during the splitting of H_2O by PSII, as well as by the consumption of H^+ in the cytoplasm (or stroma in eukaryotes) during the oxidase-mediated reduction of oxygen to H_2O (Fig. 1A). Maintenance of this pH gradient under conditions of limited CO_2 fixation (i.e., low nutrient and PSI levels) would provide the adenosine triphosphate (ATP) needed for cell maintenance and nutrient acquisition. Furthermore, it would allow the cell to maintain favorable ATP : nicotinamide adenosine dinucleotide phosphate ratios, a photoprotective role filled by PSI in plants (Munekage et al. 2002, 2004). Therefore, by implementing oxygen reduction in response to high light, photosynthetic efficiency is optimized, photo-oxidative stress is minimized, and the energetic demands of the cell are satisfied (Kuntz 2004).

In cyanobacteria, both the PTOX and the cyt *c* oxidases are present in the same membrane. Therefore electron diversion to either PTOX or cyt *c* oxidase can bypass the iron-rich PSI complex. This is not true for the Mehler reaction, in which electrons used to reduce molecular oxygen are generated at the reducing side (i.e. downstream) of PSI (Mehler 1951; Mehler and Brown 1952; Asada et al. 1974). On the other hand, the photosynthetic electron transport chain is physically separated from respiratory electron transport in eukaryotes. Rerouting electrons towards the cyt *c* oxidase complex has to be mediated by metabolite exchanges through the cytoplasm and mitochondrial membrane and requires both the cyt b_6f complex and PSI activities (Hart et al. 2005), as does the Mehler reaction. Therefore, under conditions of iron limitation, wherein both cyt b_6f and PSI levels can be significantly lower, both of these processes are less advantageous than an oxidase pathway located upstream of PSI and cyt b_6f, such as the proposed PTOX pathway (Peltier and Cournac 2002; Kuntz 2004).

The IMMUTANS (IM) protein is a thylakoid membrane–associated oxidase in vascular plants that accepts electrons from the plastoquinol pool, potentially protecting PSI from photodamage. However, Rosso et al. (2006) showed that IM does not act as an electron valve to regulate the redox state of the PQ pool via the reduction of O_2 during stress and acclimation in *Arabidopsis*. By measuring the PSI reaction center redox state, they noted that expression of *IM* did not alter the flux of PSII-generated electrons to the PSI reaction center. In contrast to the results for *Arabidopsis*, Bailey et al. (2008) used a similar method to show that a pgal-sensitive oxidase, possibly PTOX, appears to compete with PSI reaction centers for electrons generated from the oxidation of water by PSII in *Synechococcus*, supporting the concept that in cyanobacteria the oxidase might have a photoprotective role. While the cause of this difference is not certain, we speculate that the PSI electron flow in *Synechococcus* is sufficiently low that an efficient electron valve upstream of PSI represents a critical photoprotective mechanism in the high-light environment of the surface ocean where Fe and nutrient levels are extremely low and can limit CO_2 fixation rates. However, the question of how different photosynthetic organisms have come to regulate photosynthetic excitation energy while minimizing photodamage is clearly complex (Bibby et al. 2003; Asada 2006; Ivanov et al. 2006), and more work is needed to clarify these evolutionary distinctions.

In surface populations from the open ocean, $F_v : F_m$ was not strongly correlated with Φ_{PSII} (Fig. 5A,B), a measure of the efficiency with which energy absorbed by PSII is used to drive photochemistry (i.e., to reduce electron acceptors downstream of PSII). Similarly, our data show that changes in $F_v : F_m$ do not necessarily correlate with the maximum rate of CO_2 fixation (Fig. 7A,B; Table 2). At first glance, these metrics seem to offer conflicting perspectives about photoinhibition, in large part, however, because the term is used to describe many distinct yet closely related processes (Long et al. 1994; Adir et al. 2003). In linear photosynthetic electron flow, photoinhibition would manifest as a concurrent decrease in $F_v : F_m$, oxygen evolution and CO_2 fixation (Long et al. 1994); however, these parameters would not necessarily covary if alternative PSII photoacclimation strategies were employed (Oquist et al. 1992*b*) or if alternative pathways were used for a substantial portion of the electron flow. Indeed, our findings in the surface of the Atlantic open ocean show midday $F_v : F_m$ depressions consistent with photoinhibition (Fig. 5A), whereas CO_2 fixation in the same samples is maintained during exposure of the cells to high light (Fig. 7B; Table 2). This indicates that the physiological status of the cells, including the status of all PSII reaction centers and the regulation of oxidase activity, result in a greater fraction of the functional reaction centers remaining oxidized (thereby maintaining nonphotoinhibited CO_2 fixation rates at high light intensities), even while the total number of functional reaction centers decreases, suggesting photoinhibition (which is reflected in lower values of $F_v : F_m$).

In contrast to the high-light, low-nutrient surface environment, life at greater depths in the open ocean presents a different set of challenges to photosynthetic organisms. Specifically, scattering and absorption attenuate the light intensity with depth and decrease the range of photosynthetically active wavelengths compared with those of surface waters. On the other hand, macronutrient levels tend to increase with depth because of the activity of the heterotrophic bacterial community, which recycles nutrients in sinking organic material (Laws et al. 1984; Azam and Cho 1987). These inverse gradients of light and nutrients generate a different niche at depth compared with surface waters, and different ecotypes of picoeukar-

yotes and picocyanobacteria with larger photosynthetic antennae and a higher ratio of Chl *b* to Chl *a* are more abundant (Partensky et al. 1997; Urbach et al. 1998; West and Scanlan 1999).

Because many physiological differences have been shown to exist between high- and low-light picocyanobacterial ecotypes (Moore et al. 1995, 1998; Bibby et al. 2003), we tested whether natural populations of picocyanobacteria from the DCM are also able to reduce oxygen during exposure to high light as a means of avoiding photodamage. In surface and DCM samples, the fraction of oxidized PSII centers increases at midday (i.e., when the number of functional PSII centers is lowest) (Fig. 6). However, unlike the surface samples, the light-saturated photosynthetic rate, P_m, is markedly lower in DCM samples, and photoinhibition during exposure to high light is observed in CO_2 fixation experiments (Fig. 7B,D; Table 2). This observation is consistent with other studies involving low-light ecotypes of *Prochlorococcus* that measured lower CO_2 fixation rates at the base of the euphotic zone compared with surface waters (Li 1994) and shows that photoinhibition occurs in low-light ecotypes at irradiances that are optimal for the growth of high-light ecotypes isolated from the surface ocean (Moore et al. 1995, 1998; Urbach et al. 1998). Although we cannot rule out that natural samples from the deep waters of oligotrophic oceans could also have an alternative oxidase pathway, the extent of this phenomenon is reduced in the populations we sampled from the DCM (primarily *Prochlorococcus*; Fig. 4A), in which photoinhibition is observed at the level of CO_2 fixation (Fig. 7C,D; Table 2) and the fraction of oxidized reaction centers does not increase at midday when compared with surface samples (Fig. 5D). More work is needed to identify photoprotective mechanisms in low–light adapted cells from the DCM.

Of the different marine locations (i.e., Pacific, Atlantic, coastal, oceanic, surface, deep) examined in this study, all appear to undergo some degree of photoinhibition at midday. Photodamage, which contributes to photoinhibition (Long et al. 1994; Fig. 1B), can occur at all levels of illumination that generate a PSII charge separation; even low irradiances can lead to photodamage (Barber and Anderson 1992; Adir et al. 2003). Note that the number of photoinhibited reaction centers is influenced by both photodamage and other PSII photoacclimation processes (Oquist et al. 1992*b*; *see* Fig. 1B.) Therefore, changes in photoinhibition throughout the day do not necessarily exclusively reflect photodamage of PSII. The extent of photoinhibition is greatest in surface populations from the high-light, low-nutrient open ocean where oxygen appears to be a major electron acceptor. However, photoinhibition in these populations would likely be even greater if the process of oxygen reduction were absent. Despite the substantial degree of photoinhibition observed from chlorophyll fluorescence measurements at midday in the open ocean, this study indicates that, through oxygen reduction, the cells are still able to maintain stable photochemical efficiencies and CO_2 fixation rates, as well as to recover from photoinhibition rapidly (with complete recovery occurring by evening, as also reported by Behrenfeld et al. [2006]). Oxygen reduction is therefore likely to play a photoprotective role that gives certain phytoplankton a competitive edge in the challenging environment of the surface open ocean.

Besides its physiological relevance for photosynthetic life in the open ocean, these observations have other important global oceanographic implications. Marine primary productivity models use direct measurements of primary productivity on the basis of ^{14}C assimilation experiments from various locations throughout the world's oceans, many of which are coastal (Longhurst et al. 1995; Antoine et al. 1996; Behrenfeld and Falkowski 1997), to establish algorithms that convert remotely derived chlorophyll (ocean color) data to productivity estimates (Behrenfeld and Falkowski 1997). Discrepancies in the actual and the modeled CO_2 fixation rates in the open ocean have been observed (Behrenfeld et al. 2006), prompting the suggestion of incorporating a scaling factor that is based on $F_v : F_m$ to account for photosynthetic variability in the open ocean (Behrenfeld et al. 2006). However, our data suggest that $F_v : F_m$ does not necessarily reflect the actual photochemical efficiency of natural phytoplankton populations. In particular, the processes driving variability in $F_v : F_m$ are not necessarily the same processes as those responsible for the observed discrepancy in actual and modeled open ocean CO_2 fixation rates. Comparison of the light-saturated photosynthetic CO_2 fixation rates (P_m) during morning and midday indicates a P_m decrease of approximately 15% (Fig. 7A,B; Table 2), which is much smaller than the 57% decrease in the concurrently measured $F_v : F_m$ values (Fig. 5A, green line). Together, the greater variability in $F_v : F_m$ relative to P_m and the observation that photochemical efficiency (Φ_{PSII}) is stable throughout the day (Fig. 5A, orange line) suggest that productivity is not necessarily directly or universally correlated with $F_v : F_m$.

The reduction of oxygen by phytoplankton in the open ocean could be another source of the discrepancy between actual and predicted productivity rates. Applications of empirical photosynthesis and chlorophyll fluorescence data from locations where oxygen reduction represents a minor fraction of electron flow to those areas in which it is a major process are likely to overestimate oceanic primary productivity. Specifically, empirical data that are weighted toward nutrient-rich coastal waters in which electron flow to oxygen does not appear to be a common adaptation might not accurately represent the open ocean. More work is needed to fully understand processes underlying carbon sequestration in oligotrophic waters and to represent them mechanistically in models of marine primary productivity by taking into account the significantly different features of photosynthetic electron flow and CO_2 fixation associated with the different oceanic habitats. A mechanistic understanding of how picophytoplankton in oligotrophic oceans manage the absorption of excess light energy will help clarify their contribution to marine primary productivity, provide a greater understanding of the features of the marine environment that limit growth and CO_2 fixation in situ, and enable us to more accurately predict how they might affect and be affected by future environmental and climatic changes.

References

Adir, N., H. Zer, S. Shochat, and I. Ohad. 2003. Photoinhibition—a historical perspective. Photosynth. Res. **76:** 343–370.

Antoine, D., J. Andre, and A. Morel. 1996. Oceanic primary production. 2. Estimation at global scale from satellite (coastal zone color scanner) chlorophyll. Glob. Biogeochem. Cycles **10:** 57–69.

Asada, K. 1999. The water–water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. Annu. Rev. Plant. Physiol. Plant. Mol. Biol. **50:** 601–639.

———. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol. **141:** 391–396.

———, K. Kiso, and K. Yoshikawa. 1974. Univalent reduction of molecular oxygen by spinach chloroplasts on illumination. J. Biol. Chem. **249:** 2175–2181.

Azam, E., and B. C. Cho. 1987. Bacterial utilization of organic matter in the sea, p. 261–281. *In* M. Fletcher, T. R. G. Gray and J. G. Jones [eds.], Ecology of microbial communities. Cambridge University Press.

Bailey, S., A. Melis, K. R. M. Mackey, P. Cardol, G. Finazzi, G. van Dijken, G. M. Berg, K. Arrigo, J. Shrager, and A. Grossman. 2008. Alternative photosynthetic electron flow to oxygen. Biochim. Biophys. Acta. **1777:** 269–276. doi: 10.1016/j.bbabio.2008.01.002.

Barber, J., and B. Andersson. 1992. Too much of a good thing: Light can be bad for photosynthesis. Trends Biochem. Sci. **17:** 61–66.

Behrenfeld, M. J., and P. G. Falkowski. 1997. Photosynthetic rates derived from satellite-based chlorophyll concentration. Limnol.Oceanogr. **42:** 1–20.

———, K. Worthington, R. M. Sherrell, F. P. Chavez, P. Strutton, M. McPhaden, and D. M. Shea. 2006. Controls on tropical Pacific Ocean productivity revealed through nutrient stress diagnostics. Nature **442:** 1025–1028.

Berry, S., D. Schneider, W. F. Vermaas, and M. Rogner. 2002. Electron transport routes in whole cells of *Synechocystis* sp. Strain PCC 6803: The role of the cytochrome bd-type oxidase. Biochem. **41:** 3422–3429.

Bibby, T. S., I. Mary, J. Nield, F. Partensky, and J. Barber. 2003. Low-light-adapted *Prochlorococcus* species possess specific antennae for each photosystem. Nature **424:** 1051–1054.

Boekema, E., and others. 2001. A giant chlorophyll–protein complex induced by iron deficiency in cyanobacteria. Nature **412:** 745–748.

Butler, W. L. 1978. Energy distribution in the photochemical apparatus of photosynthesis. Annu. Rev. Plant Physiol. **29:** 345–378.

Cadoret, J. C., R. Demouliere, J. Lavaud, H. J. van Gorkom, J. Houmard, and A. L. Etienne. 2004. Dissipation of excess energy triggered by blue light in cyanobacteria with CP43' (isiA). Biochim. Biophys. Acta **1659:** 100–104.

Campbell, D., V. Hurry, A. K. Clarke, P. Gustafsson, and G. Oquist. 1998. Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. Microbiol. Mol. Biol. Rev. **62:** 667–683.

Campbell, L., and D. Vaulot. 1993. Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). Deep-Sea Res. Part I Oceanogr. Res. Pap.) **40:** 2043–2060.

Díez, B., C. Pedrós-Alió, T. L. Marsh, and R. Massana. 2001. Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques. Appl. Environ. Microbiol. **67:** 2942–2951.

Field, C. B., M. J. Behrenfeld, J. Y. Randerson, and P. Falkowski. 1998. Primary production of the biosphere: Integrating terrestrial and oceanic components. Science **281:** 237–240.

Fouilland, E., C. Descolas-Gros, C. Courties, Y. Collos, A. Vaquer, and A. Gasc. 2004. Productivity and growth of a natural population of the smallest free-living eukaryote under nitrogen deficiency and sufficiency. Microb. Ecol. **48:** 103–110.

Guenther, J. E., and A. Melis. 1990. The physiological significance of photosystem II heterogeneity in chloroplasts. Photosynth. Res. **23:** 105–109.

Hart, S. E., B. G. Schlarb-Ridley, D. S. Bendall, and C. J. Howe. 2005. Terminal oxidases of cyanobacteria. Biochem. Soc. Trans. **33:** 832–835.

Ivanov, A. G., and others. 2007. The induction of CP43' by iron-stress in *Synechococcus* sp. PCC 7942 is associated with carotenoid accumulation and enhanced fatty acid unsaturation. Biochim. Biophys. Acta **1767:** 807–813.

———, and others. 2006. Iron deficiency in cyanobacteria causes monomerization of photosystem I trimers and reduces the capacity for state transitions and the effective absorption cross section of photosystem I in vivo. Plant Physiol. **141:** 1436–1445.

Josse, E. M., J. P. Alcaraz, A. M. Laboure, and M. Kuntz. 2003. In vitro characterization of a plastid terminal oxidase (PTOX). Eur. J. Biochem. **270:** 3787–3794.

Kaplan, A., and L. Reinhold. 1999. CO_2 concentrating mechanisms in photosynthetic microorganisms. Annu. Rev. Plant Physiol. Plant Biol. **50:** 539–570.

Kuntz, M. 2004. Plastid terminal oxidase and its biological significance. Planta **218:** 896–899.

Lavergne, J. 1982. Two types of primary acceptors in chloroplasts photosystem II. Photobiochem. Photobiophys. **3:** 257–285.

Laws, E. A., and others. 1984. High phytoplankton growth rates in oligotrophic Hawaiian coastal waters. Limnol. Oceanogr. **29:** 1161–1169.

Lewis, M. R., and J. C. Smith. 1983. A small-volume, short-incubation-time method for measurement of photosynthesis as a function of incident irradiance. Mar. Ecol. Prog. Ser. **13:** 99–102.

Li, W. K. W. 1994. Primary productivity of prochlorophytes, cyanobacteria, and eucaryotic ultraphytoplankton: Measurements from flow cytometric sorting. Limnol. Oceanogr. **39:** 169–175.

Long, S. P., S. Humphries, and P. G. Falkowski. 1994. Photoinhibition of photosynthesis in nature. Annu. Rev. Plant Physiol. Plant Mol. Biol. **45:** 633–662.

Longhurst, A., S. Sathyendranath, T. Platt, and C. Caverhill. 1995. An estimate of global primary production in the ocean from satellite radiometer data. J. Plankton Res. **17:** 1245–1271.

Mackey, K. R. M., R. G. Labiosa, M. Calhoun, J. H. Street, A. F. Post, and A. Paytan. 2007. Phosphorus availability, phytoplankton community dynamics, and taxon-specific phosphorus status in the Gulf of Aqaba, Red Sea. Limnol. Oceanogr. **52:** 875–885.

Maxwell, K., and G. N. Johnson. 2000. Chlorophyll fluorescence—a practical guide. J. Exp. Bot. **51:** 659–668.

McDonald, A. E., and G. C. Vanlerberghe. 2004. Alternative oxidase and plastoquinol terminal oxidase in marine prokaryotes of the Sargasso Sea. Gene **349:** 15–24.

Mehler, A. H. 1951. Studies on reactions of illuminated chloroplasts. I. Mechanism of the reduction of oxygen and other Hill reagents. Arch. Biochem. Biophys. **33:** 65–77.

———, AND A. H. BROWN. 1952. Studies on reactions of illuminated chloroplasts. III. Simultaneous photoproduction and consumption of oxygen studied with oxygen isotopes. Arch. Biochem. Biophys. **38:** 365–70.

MI, H., T. ENDO, T. OGAWA, AND K. ASADA. 1995. Thylakoid membrane-bound, NADPH-specific pyridine nucleotide dehydrogenase complex mediates cyclic electron transport in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Cell Physiol. **36:** 661–668.

———, T. ENDO, U. SCHREIBER, T. OGAWA, AND K. ASADA. 1994. NAD(P)H dehydrogenase-dependent cyclic electron flow around photosystem I in the cyanobacterium *Synechocystis* PCC 6803: A study of dark-starved cells and spheroplasts. Plant Cell Physiol. **35:** 163–173.

MOORE, L. R., R. GOERICKE, AND S. W. CHISHOLM. 1995. Comparative physiology of *Synechococcus* and *Prochlorococcus*: Influence of light and temperature on growth, pigments, fluorescence and absorptive properties. Mar. Ecol. Prog. Ser. **116:** 259–275.

———, G. ROCAP, AND S. W. CHISHOLM. 1998. Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. Nature **393:** 464–468.

MUNEKAGE, Y., M. HASHIMOTO, C. MIYAKE, K. TOMIZAWA, T. ENDO, M. TASAKA, AND T. SHIKANAI. 2004. Cyclic electron flow around photosystem I is essential for photosynthesis. Nature **429:** 579–582.

———, M. HOJO, J. MEURER, T. ENDO, M. TASAKA, AND T. SHIKANAI. 2002. PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in Arabidopsis. Cell **110:** 361–371.

OQUIST, G., J. M. ANDERSON, S. MCCAFFERY, AND W. S. CHOW. 1992*a*. Mechanistic differences in photoinhibition of sun and shade plants. Planta **188:** 422–431.

———, W. S. CHOW, AND J. M. ANDERSON. 1992*b*. Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. Planta **186:** 450–460.

PALENIK, B., AND OTHERS. 2003. The genome of a motile marine *Synechococcus*. Nature **424:** 1037–1042.

PARTENSKY, F., W. R. HESS, AND D. VAULOT. 1999. *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. Microbiol. Mol. Biol. Rev. **63:** 106–127.

———, N. HOEPFFNER, W. K. W. LI, O. ULLOA, AND D. VAULOT. 1993. Photoacclimation of *Prochlorococcus* sp. (Prochlorophyta) strains isolated from the North Atlantic and the Mediterranean Sea. Plant Physiol. **101:** 295–296.

———, J. LAROCHE, K. WYMAN, AND P. G. FALKOWSKI. 1997. The divinyl-chlorophyll *a*/*b*-protein complexes of two strains of the oxyphototrophic marine prokaryote *Prochlorococcus*—characterization and response to changes in growth irradiance. Photosynth. Res. **51:** 209–222.

PELTIER, G., AND L. COURNAC. 2002. Chlororespiration. Ann. Rev. Plant. Biol. **53:** 523–550.

PLATT, T., C. L. GALLEGOS, AND W. G. HARRISON. 1980. Photoinhibition of photosynthesis in natural assemblages of marine-phytoplankton. J. Mar. Res. **38:** 687–701.

ROSSO, D., AND OTHERS. 2006. IMMUTANS does not act as a stress-induced safety valve in the protection of the photosynthetic apparatus of Arabidopsis during steady-state photosynthesis. Plant Physiol. **142:** 574–585.

SCANLAN, D. J. 2003. Physiological diversity and niche adaptation in marine *Synechococcus*. Adv. Microb. Physiol. **47:** 1–64.

SCHREIBER, U., T. ENDO, H. MI, AND K. ASADA. 1995. Quenching analysis of chlorophyll fluorescence by the saturation pulse method: Particular aspects relating to the study of eukaryotic algae and cyanobacteria. Plant Cell Physiol. **36:** 873–882.

STRZEPEK, R. F., AND P. J. HARRISON. 2004. Photosynthetic architecture differs in coastal and oceanic diatoms. Nature **431:** 689–692.

URBACH, E., D. J. SCANLAN, D. L. DISTEL, J. B. WATERBURY, AND S. W. CHISHOLM. 1998. Rapid diversifcation of marine picophytoplankton with dissimilar light-harvesting structures inferred from sequences of *Prochlorococcus* and *Synechococcus* (cyanobacteria). J. Mol. Evol. **46:** 188–201.

WEST, N. J., AND D. J. SCANLAN. 1999. Niche-partitioning of *Prochlorococcus* populations in a stratified water column in the eastern North Atlantic ocean. Appl. Environ. Microbiol. **65:** 2585–2591.

WORDEN, A. Z., J. K. NOLAN, AND B. PALENIK. 2004. Assessing the dynamics and ecology of marine picophytoplankton: The importance of the eukaryotic component. Limnol. Oceanogr. **49:** 168–174.

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