Photosystem II Photoinactivation, Repair, and Protection in Marine Centric Diatoms1[OA]

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Diatoms are important contributors to aquatic primary production, and can dominate phytoplankton communities under variable light regimes. We grew two marine diatoms, the small Thalassiosira pseudonana and the large Coscinodiscus radiatus, across a range of temperatures and treated them with a light challenge to understand their exploitation of variable light environments. In the smaller T. pseudonana, photosystem II (PSII) photoinactivation outran the clearance of PSII protein subunits, particularly in cells grown at sub- or supraoptimal temperatures. In turn the absorption cross section serving PSII photochemistry was down-regulated in T. pseudonana through induction of a sustained phase of nonphotochemical quenching that relaxed only slowly over 30 min of subsequent low-light incubation. In contrast, in the larger diatom C. radiatus, PSII subunit turnover was sufficient to counteract a lower intrinsic susceptibility to photoinactivation, and C. radiatus thus did not need to induce sustained nonphotochemical quenching under the high-light treatment. T. pseudonana thus incurs an opportunity cost of sustained photosynthetic down-regulation after the end of an upward light shift, whereas the larger C. radiatus can maintain a balanced PSII repair cycle under comparable conditions.

To cope with the potentially damaging effects of light, and to maintain photosynthesis, diatoms, like all oxygenic photoautotrophs, must counter the photoinactivation of PSII with repair through proteolytic removal of photodamaged proteins (Silva et al., 2003; Nixon et al., 2010) and the coordinated insertion of newly synthesized subunits into the thylakoid membrane (Aro et al., 1993; Komenda et al., 2012). If photoinactivation outruns the rate of repair, the PSII pool suffers net photoinhibition (Aro et al., 1993; Nishiyama et al., 2005, 2006; Murata et al., 2007), leading ultimately to a decrease in photosynthetic quantum yield and/or capacity. In comparison with other marine phytoplankton groups, including cyanobacteria and prasinophyte green algae (Six et al., 2007, 2009), diatoms show a lower susceptibility to primary photoinactivation (Key et al., 2010), and also distinctive clearance patterns for the PsbA (D1), PsbD (D2), and PsbB (CP47) PSII subunits upon an increase in light, in comparison with most other taxa examined to date (Wu et al., 2011). Intriguingly, the diatom chloroplasts have thylakoids arranged in a triple layer without distinct grana stacked regions (Lepetit et al., 2012), indicating a lower capacity to counteract photoinactivation (Wu et al., 2011). To cope with the potentially damaging effects of light, and to maintain photosynthesis, diatoms, like all oxygenic photoautotrophs, must counter the photoinactivation of PSII with repair through proteolytic removal of photodamaged proteins (Silva et al., 2003; Nixon et al., 2010) and the coordinated insertion of newly synthesized subunits into the thylakoid membrane (Aro et al., 1993; Komenda et al., 2012). If photoinactivation outruns the rate of repair, the PSII pool suffers net photoinhibition (Aro et al., 1993; Nishiyama et al., 2005, 2006; Murata et al., 2007), leading ultimately to a decrease in photosynthetic quantum yield and/or capacity. In comparison with other marine phytoplankton groups, including cyanobacteria and prasinophyte green algae (Six et al., 2007, 2009), diatoms show a lower susceptibility to primary photoinactivation (Key et al., 2010), and also distinctive clearance patterns for the PsbA (D1), PsbD (D2), and PsbB (CP47) PSII subunits upon an increase in light, in comparison with most other taxa examined to date (Wu et al., 2011). Intriguingly, the diatom chloroplasts have thylakoids arranged in a triple layer without distinct grana stacked regions (Lepetit et al., 2012), indicating a lower capacity to counteract photoinactivation (Wu et al., 2011).
(Müller et al., 2001). Diatoms can dissipate excess light energy through distinct mechanisms of nonphotochemical quenching (NPQ) to limit overexcitation of their photosystems (Lavaud et al., 2002a, 2002b, 2004; Eisenstadt et al., 2008; Grouneva et al., 2008, 2009; Bailleul et al., 2010; Zhu and Green, 2010; Depauw et al., 2012; Lepetit et al., 2012).

Some NPQ mechanisms are associated with the operation of a xanthophyll cycle, which converts epoxidized to deepoxidized forms of certain xanthophylls. The carotenoid pigments in diatoms include fucoxanthin, ββ-carotene, and the xanthophyll-cycle pigment diadinoxanthin (Dd), which can be deepoxidized to form diatoxanthin (Dt). A pH gradient across the thylakoid membrane mediates NPQ formation (Lavaud and Kroth, 2006). Diatom NPQ can be linearly related to the Dt content (Lavaud et al., 2002a, 2002b, 2004, 2007), but species and ecotypes vary in their induction of NPQ (Lavaud et al., 2004; Schumann et al., 2007; Bailleul et al., 2010). Recently, Bailleul et al. (2010) and Zhu and Green (2010) showed evidence that the magnitude of NPQ induction is correlated with the expression level of Lhcx members of the chlorophyll a/c light-harvesting protein family.

The ability of diatoms to grow and dominate in light environments with large fluctuations in irradiance suggests an unusual photosynthetic flexibility, especially in harvesting, using or dissipating (Janknegt et al., 2009; Waring et al., 2010) variable levels of light energy over short time scales. Diatom growth and metabolism is also affected by temperature (Anderson, 2000), and the surface temperature of ocean waters is now expected to rise by 1°C to 7°C by 2100 (Houghton 2000), and the surface temperature of ocean waters is now expected to rise by 1°C to 7°C by 2100 (Houghton et al., 2001). Such an increase in temperature might influence diatom dominance of variable environments by modifying diatom responses to sudden increases in irradiance. We earlier showed that the primary susceptibility to the photoinactivation of PSII is inversely proportional to cell volume in diatoms (Key et al., 2010), so small and large diatoms have different balances between light-dependent photoinactivation and the counteracting temperature-dependent metabolic repair of PSII. We therefore quantitatively analyzed PSII photoinactivation, subunit turnover, pigment dynamics, and the kinetics of NPQ formation in two marine-centric diatoms, the small Thalassiosira pseudonana and the large Coscinodiscus radiatus, cultured under different temperatures and treated with a light challenge, to understand how PSII photoinactivation and repair, pigment dynamics, and Lhcx isoforms interact to generate the strong diatom capacity to exploit variable light.

RESULTS

Photoinhibition of the Photochemical Yield of PSII

T. pseudonana and C. radiatus cells were grown at different temperatures under 30 μmol photons m⁻² s⁻¹, approximating the bottom 10% of the photic zone. To assess their capacity to exploit variable light, we challenged them with a 90-min shift to 450 μmol blue photons m⁻² s⁻¹, approximating a rapid mixing up to the light field in the upper third of the photic zone. After the light challenge, cells were shifted back to their original low growth light to track recovery and relaxation processes.

We used a multiple-turnover saturating flash and modulated fluorometer to estimate the maximum photochemical yield of PSII using the Fv/FM ratio. In the smaller species T. pseudonana (cell volume of 19 ± 0.6 μm³), Fv/FM was 0.66 ± 0.005 for cells growing at 12°C, and 0.67 ± 0.01 for cells growing at 18°C or 24°C. In T. pseudonana cells maintaining a PSII repair cycle, Fv/FM dropped significantly (P < 0.05) during the 90-min high-light challenge, but recovered (P > 0.05) during the subsequent growth light period regardless of the growth temperatures (Fig. 1, A–C). Compared with cells growing at 18°C, the sub- and supraoptimal growth temperatures of 12°C and 24°C led to significantly larger declines (P < 0.05) in Fv/FM over 30 to 60 min before stabilizing late in the high-light treatment. Blocking the PSII repair cycle by adding lincomycin showed a significant decline (P < 0.05) in Fv/FM at all growth temperatures, with the loss of PSII activity following a single-phase exponential decay. In contrast, in the larger species C. radiatus (cell volume of 138,000 ± 6,150 μm³) the maximum photochemical yield of PSII as measured by Fv/FM ratio was 0.81 ± 0.003 for cells growing at 18°C, and 0.79 ± 0.01 for cells growing at 24°C. C. radiatus also showed lower susceptibility to high-light treatments, with smaller, although still significant (P < 0.05; Fig. 3B), or remained (P > 0.05; Fig. 3B), or remained unchanged in PSII content in the control cells with active PSII repair cycles (Fig. 2, A–C), but lincomycin-treated cells suffered a progressive, significant drop in PsbA to about 75% of time 0 levels by the end of the 90-min high-light treatment (P < 0.05 at both temperatures). Similarly, high-light treatment did not cause any net loss of PsbA in the control cells of C. radiatus, but lincomycin-treated cells showed a significant net decline in PsbA content (Fig. 2, D and E; P < 0.05 at both temperatures).

In T. pseudonana cells with an active PSII repair cycle, PsbD varied but did not show significant change in cells grown at 12°C (Fig. 3A), declined somewhat in those grown at 18°C (P < 0.05; Fig. 3B), or remained nearly steady in those grown at 24°C (Fig. 3C). At all temperatures T. pseudonana cells treated with lincomycin showed a significant decline in PsbD (P < 0.05), which did not recover during the subsequent 30 min at the 90-min high-light exposure, but recovered (P > 0.05) during the subsequent growth light period regardless of the growth temperatures (Fig. 1, A–C). Compared with cells growing at 18°C, the sub- and supraoptimal growth temperatures of 12°C and 24°C led to significantly larger declines (P < 0.05) in Fv/FM over 30 to 60 min before stabilizing late in the high-light treatment. Blocking the PSII repair cycle by adding lincomycin showed a significant decline (P < 0.05) in Fv/FM at all growth temperatures, with the loss of PSII activity following a single-phase exponential decay. In contrast, in the larger species C. radiatus (cell volume of 138,000 ± 6,150 μm³) the maximum photochemical yield of PSII as measured by Fv/FM ratio was 0.81 ± 0.003 for cells growing at 18°C, and 0.79 ± 0.01 for cells growing at 24°C. C. radiatus also showed lower susceptibility to high-light treatments, with smaller, although still significant (P < 0.05), decreases in Fv/FM in the cells treated without or with lincomycin at both 18°C and 24°C (Fig. 1, D and E).

Turnover of PSII Subunits

We quantified the levels and variation in the content of key PSII proteins PsbA and PsbD during the 90-min high-light exposure and the subsequent 30-min recovery, for cells grown across a range of temperatures. T. pseudonana showed no significant change in total PsbA content in the control cells with active PSII repair cycles (Fig. 2, A–C), but lincomycin-treated cells suffered a progressive, significant drop in PsbA to about 75% of time 0 levels by the end of the 90-min high-light treatment (P < 0.05 at all temperatures). Similarly, high-light treatment did not cause any net loss of PsbA in the control cells of C. radiatus, but lincomycin-treated cells showed a significant net decline in PsbA content (Fig. 2, D and E; P < 0.05 at both temperatures).
lower light (Fig. 3, A–C). *C. radiatus* showed a significant accumulation of PsbD content upon a shift to high light (*P* < 0.05), which was blocked by the addition of lincomycin both at 18°C and 24°C (Fig. 3, D and E).

Overall, the changes in the pools of PsbA and PsbD show significant departures in magnitude and even in direction from the patterns of PSII activity shown in Figure 1.

Changes in Pigmentation

The major pigments in *T. pseudonana* and *C. radiatus* are chlorophyll *a*, chlorophyll *c*₂, fucoxanthin, diadinoxanthin (Dd), diatoxanthin (Dt), and β,β-carotene. Growth temperature did not cause large changes in the pigment content in either species under the low growth light level of 30 µmol m⁻² s⁻¹ (Table I). The levels of chlorophyll *a*, chlorophyll *c*₂, fucoxanthin, and β,β-carotene were steady throughout the high-light shift and the subsequent recovery periods in both species, both in the absence and the presence of lincomycin (data not shown), so our cultures neither lost

![Figure 1. Responses of PSII maximum photochemical yield (*F*₀/ *F*ₐ) versus time in *T. pseudonana* (A–C) and *C. radiatus* (D and E) cultures treated with (closed symbols) or without (open symbols) the chloroplast protein synthesis inhibitor lincomycin. Both species were grown at 30 µmol photons m⁻² s⁻¹ at 12°C (A), 18°C (B and D), or 24°C (C and E), then exposed to 450 µmol photons m⁻² s⁻¹ blue light for 90 min, and then allowed to recover at 30 µmol photons m⁻² s⁻¹ for 30 min. *n* = 4 to 5 independent culture experiments, ±SE; most error bars within symbols.](image1)

![Figure 2. Changes in PsbA content in *T. pseudonana* (A–C) and *C. radiatus* (D and E) cultures treated with (closed symbols) or without (open symbols) the chloroplast protein synthesis inhibitor lincomycin. Both species were grown at 30 µmol photons m⁻² s⁻¹ at 12°C (A), 18°C (B and D), or 24°C (C and E), then exposed to 450 µmol photons m⁻² s⁻¹ blue light for 90 min, and then allowed to recover at 30 µmol photons m⁻² s⁻¹ for 30 min. *n* = 3, ±SE.](image2)
nor accumulated net light-harvesting pigments during our short high-light treatments.

In *T. pseudonana*, Dd was rapidly deepoxidized to Dt when cells were shifted to higher light (Fig. 5, A–C; *P* < 0.05), particularly in cells at 12°C and 24°C. Our earliest pigment time point was at 15 min, but in similar treatments Zhu and Green (2010) found that this conversion of Dd to Dt was largely complete within 2 min of high-light treatment. When cultures were shifted to low growth light for recovery, Dt was epoxidized back to Dd within 30 min (*P* < 0.05), and perhaps sooner. The pool size of Dd + Dt increased (*P* < 0.05) in all *T. pseudonana* cultures in the presence and absence of lincomycin, but deepoxidation of Dt was partially inhibited in cultures treated with lincomycin (data not shown; Bachmann et al., 2004). For *C. radiatus* the pool size of Dd + Dt increased (*P* < 0.05), indicating de novo synthesis, but the cells showed only slight, although statistically significant (*P* < 0.05) accumulations of Dt, much smaller than for *T. pseudonana* (Fig. 5, D and E).

**NPQ Induction and PSII Functional Absorption Cross Section**

Diatoms have significant capacities to induce different phases of NPQ, which lower the achieved photochemical yield of PSII. Figure 6 shows the dynamic NPQd phase, which relaxes within 5 min of dark incubation and is reinduced during a brief exposure to the treatment light; the sustained NPQs phase that persists beyond 5 min of dark incubation, and the total NPQt = NPQd + NPQs. In *T. pseudonana* cells with an active PSII repair cycle, those grown and treated at 12°C and 24°C showed a decrease in NPQd across the period of high-light exposure, but with a mirror increase in NPQs (Fig. 6, A–C). This shift from NPQd to NPQs reversed during the low-light recovery period. In *T. pseudonana* cells growing at their optimal temperature of 18°C NPQd was steady and there was only a limited, though significant (*P* < 0.05) accumulation of NPQs, over the 90-min high-light treatment. For both *T. pseudonana* and *C. radiatus*, NPQd accumulated and subsequently relaxed even when chloroplastic protein synthesis was blocked by lincomycin (data not shown). In *T. pseudonana* the total NPQ was highest in cells grown at 12°C, and increased significantly (*P* < 0.05) during the high-light treatment. In *C. radiatus* (Fig. 6, D and E) the level of NPQ was much lower and there was only a slight accumulation of NPQs that did not

![Figure 3](image-url)  
**Figure 3.** Changes in PsbD content in *T. pseudonana* (A–C) and *C. radiatus* (D and E) cultures treated with (closed symbols) or without (open symbols) the chloroplast protein synthesis inhibitor lincomycin. Both species were grown at 30 µmol photons m⁻² s⁻¹ at 12°C (A), 18°C (B and D), or 24°C (C and E), then exposed to 450 µmol photons m⁻² s⁻¹ blue light for 90 min, and then allowed to recover at 30 µmol photons m⁻² s⁻¹ for 30 min. *n* = 3, ±SE.

![Figure 4](image-url)  
**Figure 4.** Correlation of PsbA removal rate constant (s⁻¹) and photo-inactivation rate constant of PSII (kpi,s⁻¹) in *T. pseudonana* cells grown at 30 µmol photons m⁻² s⁻¹, 12°C (open circle), 18°C (open upward triangle, diamond, downward triangle), or 24°C (open square) and *C. radiatus* cells grown at 18°C (closed upward triangle, diamond, downward triangle) or 24°C (closed square). Rate constants were estimated from samples taken during 90 min of exposure to 450 µmol photons m⁻² s⁻¹ blue light (circles, upward triangles, squares) or 450 µmol photons m⁻² s⁻¹ red light (diamonds), or 1,400 µmol photons m⁻² s⁻¹ white fluorescent light (downward triangles). *n* = 3 to 5, ±SE for both x and y axes. Dotted line indicates 1:1 ratio; solid line shows a hyperbolic tangent Michaelis-Menten curve fit to the data, showing saturation of the PsbA removal rate constant.
reach the threshold of statistical significance during the high-light treatment. Because our experiments involved short-term light-shift experiments to track PSII photoinactivation, we did not apply longer-term relaxation periods to estimate $q_E$ and $q_I$ according to Farber et al. (1997), as done by Zhu and Green (2010), although we have the 30-min low-light incubation at the end of the treatments, to track final relaxation of $q_E$ and $q_I$ phases.

Dark-adapted cells of the smaller species *T. pseudonana* had a larger functional absorption cross section serving PSII photochemistry ($\sigma_{\text{PSII}}$) than did the larger cells of *C. radiatus* (compare Fig. 7, A–C to D and E; species difference significant at $P < 0.05$ in a Bonferroni posttest after one-way ANOVA), consistent with pigment packaging or self-screening effects in the larger cells (Morel and Bricaud, 1981; Finkel, 2001; Key et al., 2010). In *T. pseudonana* there was a weak influence of growth temperature on $\sigma_{\text{PSII}}$ with cells growing at 12°C showing a $\sigma_{\text{PSII}}$ of 250 A$^2$ quanta$^{-1}$, whereas cells growing at 18°C or 24°C showed a slightly larger $\sigma_{\text{PSII}}$ of 260 A$^2$ quanta$^{-1}$ (temperature difference significant at $P = 0.05$ in a Bonferroni posttest after one-way ANOVA). *C. radiatus* did not show a significant effect of growth temperature on $\sigma_{\text{PSII}}$. These $\sigma_{\text{PSII}}$ estimates for dark-adapted cells are very close to the $\sigma_{\text{PSII}}$ estimated for cells measured under their low growth light (data not presented). For *T. pseudonana*, with an active PSII repair cycle, $\sigma_{\text{PSII}}$ decreased slightly at all temperatures during the 90-min high-light treatment, but recovered to close to initial values after 30 min of recovery (Fig. 7, A–C). In *T. pseudonana* cells incubated with lincomycin to block the PsbA repair cycle, $\sigma_{\text{PSII}}$ increased 20% as the cells suffered approximately 50% to 60% photoinhibition of PSII (compare Fig. 7, A–C with Fig. 1, A–C). When the functional absorption cross section of PSII was measured under the treatment light level of 450 µmol blue photons m$^{-2}$ s$^{-1}$, $\sigma_{\text{PSII}}$ decreased to half the value of $\sigma_{\text{PSII}}$ from dark-acclimated cells, consistent with the induction of NPQ by the treatment light (Fig. 6; significant at $P < 0.05$ using a Bonferroni posttest after one-way ANOVA). In contrast, $\sigma_{\text{PSII}}$ was stable across the light-shift treatments in *C. radiatus* cells with or without lincomycin at both temperatures, and there was no significant down-regulation of $\sigma_{\text{PSII}}$ measured under the treatment light level of 450 µmol blue photons m$^{-2}$ s$^{-1}$, in comparison to the $\sigma_{\text{PSII}}$ measured from dark-acclimated cells (Fig. 7, D and E).

### Table I. Growth rate, PSII reaction center protein, and pigment content of *T. pseudonana* and *C. radiatus* cells cultured at different temperatures and 30 µmol photons m$^{-2}$ s$^{-1}$

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>T. pseudonana</em></th>
<th><em>C. radiatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12°C</td>
<td>18°C</td>
</tr>
<tr>
<td></td>
<td>(µmol Chl a$^{-1}$ mg protein$^{-1}$)</td>
<td>(µmol Chl a$^{-1}$ mg protein$^{-1}$)</td>
</tr>
<tr>
<td>Proteins (µmol µg protein$^{-1}$)</td>
<td>0.8 (±0.01)</td>
<td>1.1 (±0.01)</td>
</tr>
<tr>
<td>PsbA</td>
<td>51 (±7)</td>
<td>71 (±14.5)</td>
</tr>
<tr>
<td>PsbD</td>
<td>137 (±10)</td>
<td>139 (±5)</td>
</tr>
<tr>
<td>Chlorophyll a (mol cell$^{-1}$)</td>
<td>0.3 (0.01)</td>
<td>0.3 (0.01)</td>
</tr>
<tr>
<td>Pigments (mol 100 mol Chl a$^{-1}$)</td>
<td>48 (±1)</td>
<td>53 (±0.5)</td>
</tr>
<tr>
<td>Fucoxanthin: Chl a</td>
<td>5 (±1)</td>
<td>5 (±1)</td>
</tr>
<tr>
<td>β,β-Carotene: Chl a</td>
<td>12 (±0.4)</td>
<td>10 (±0.2)</td>
</tr>
</tbody>
</table>

$n = 3$ to 5, ± se.
In *T. pseudonana*, NPQ was significantly correlated with the deepoxidation state (DES) of the xanthophyll cycle pigments, calculated as DES = Dt/(Dd + Dt) (Fig. 8B; *F* test for slopes nonzero at *P* < 0.015 for cells growing at 12°C, 18°C, and 24°C; *R*^2^ for regression lines 0.98 at 12°C, 0.89 at 18°C, and 0.99 at 24°C). *T. pseudonana* cells grown at 12°C and 24°C, however, induced much more NPQ at a given DES level than did cells at the optimal growth temperature of 18°C (slopes at 12°C and 24°C significantly higher than at 18°C, *P* < 0.0001). In *C. radiatus* DES remained low (Fig. 5, D and E) across the light treatment, as did NPQ (Fig. 6, D and E) so we did not plot regressions of these data.

*T. pseudonana* showed significant temperature and light induction patterns for the Lhcx1 (Fig. 9A) and Lhcx6 (Fig. 9B) chlorophyll proteins. Lhcx1 levels were markedly higher (*P* < 0.05) in cells growing at 12°C than at 18°C or 24°C (Bonferroni posttests *P* < 0.05 after two-way ANOVA), but then increased further in cells shifted to higher light for 90 min under all three growth temperatures (Fig. 9A; Bonferroni posttests *P* < 0.05 after two-way ANOVA). In agreement with our previous work (Zhu and Green, 2010), Lhcx6 could not be detected under low light at 18°C, but was detected in cells grown at 12°C, suggesting a cold stress or excitation pressure (Huner et al., 1998) response (Fig. 9B). The high-light-induced increase was also stronger at lower temperature (Bonferroni posttests *P* < 0.05 after two-way ANOVA), again consistent with an excitation-pressure response. Since both the anti-Lhcx1 and anti-Lhcx6 antibodies were raised to gene-specific C-terminal peptides (Zhu and Green, 2010), we did not detect bands in total protein extracts from *C. radiatus* (data not presented). Under the same treatments, DES induction in *T. pseudonana* increased

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**Figure 6.** Responses of rapidly reversible, dynamic NPQ (open circles; NPQd = \(\frac{F_M - F_{M9}}{F_M}\)); sustained NPQ (closed squares; NPQs = \(\frac{F_{M0} - F_{M}}{F_{M}}\)), and total NPQ (closed circles; NPQt = NPQs + NPQd) versus time in *T. pseudonana* (A–C) and *C. radiatus* (D and E) cultures with active PSII repair. Both species were grown at 30 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) at 12°C (A), 18°C (B and D), and 24°C (C and E), then exposed to 450 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) blue light for 90 min, and then allowed to recover at 30 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) for 30 min. *n* = 4 to 5 separate culture experiments, ±SE.

**Figure 7.** Changes in PSII effective absorption cross section (\(\sigma_{\text{PSII}}\)), estimated from flash fluorescence rise kinetics, in *T. pseudonana* (A–C) and *C. radiatus* (D and E) cultures treated with (closed symbols) or without (open symbols) the chloroplast protein synthesis inhibitor lincomycin. Both species were grown at 30 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) at 12°C (A), 18°C (B and D), or 24°C (C and E), then exposed to 450 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) blue light for 90 min, and then allowed to recover at 30 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) for 30 min. Circles represent \(\sigma_{\text{PSII}}\) measured in the dark, squares represent \(\sigma_{\text{PSII}}\) measured under the treatment light of 450 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) blue light. *n* = 4 to 5 independent culture experiments, ±SE; most error bars within symbols. Downward arrows indicate light-driven down-regulation of \(\sigma_{\text{PSII}}\) to \(\sigma_{\text{PSII}}^t\) in *T. pseudonana*. 

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with increasing temperature (Fig. 9C; two-way ANOVA with Bonferroni posttests $P < 0.05$). NPQ$_t$ was lowest in cells growing at the optimal 18°C (Fig. 9D), although the upward light shift still provoked a significant increase in NPQ$_t$ (two-way ANOVA with Bonferroni posttests $P < 0.05$).

DISCUSSION

PSII Photoinactivation Can Outrun PSII Protein Turnover

Turnover of the PsbA protein is required for PSII repair and restoration of PSII photochemical activity after photoinactivation (Aro et al., 1993; Murata et al., 2007; Edelman and Mattoo, 2008; Nixon et al., 2010; Komenda et al., 2012), but PSII repair is a separate, multistep process that shows kinetic departures from PSII photoinactivation (Edelman and Mattoo, 2008). Both $T$. pseudonana and $C$. radiatus were able to maintain or increase their total pools of PsbA and PsbD proteins when the repair cycle was active (Figs. 2, A–C and 3, A–C), demonstrating active synthesis of the PsbA and PsbD proteins. In $T$. pseudonana at 18°C this maintenance of PsbA protein levels was consistent with nearly stable PSII quantum yields during the shift to higher light (Fig. 1B), similar to Zhu and Green (2010). $T$. pseudonana grown at 12°C and 24°C also maintained PsbA protein levels (Fig. 2, A and C) but the PSII quantum yield declined (Fig. 1, A and C) during the high-light exposure period, showing that maintenance of PsbA protein pools (Fig. 2, A and C)
Diatom PSII Function and Repair

Figure 10. Sustained NPQ accumulates when PSII photoinactivation outruns repair. Accumulated NPQ, plotted versus the ratio of the photoinactivation rate constant for PSII \(k_p\) and the PsbA removal rate constant, for \(T.\) pseudonana cells grown at 30 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\), 12°C (open circle), 18°C (open upward triangle, diamond, downward triangle), or 24°C (open square) and \(C.\) radiatus cells grown at 18°C (closed upward triangle, diamond, downward triangle) or 24°C (closed square). NPQs and rate constants were estimated from samples taken during 90 min of exposure to 450 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) blue light (circles, upward triangles, squares) or 450 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) red light (diamonds), or 1,400 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) white fluorescent light (downward triangles). \(n = 3\) to 5, ±SE for both x and y axes. Dotted line shows linear regression, \(R^2 = 0.62.\)

did not alone suffice for these cells to maintain their pool of active PSII, and that cells can accumulate subpools of PsbA and PsbD beyond their pools of active PSII.

When PSII repair is blocked in \(T.\) pseudonana the upward light shifts provoke photoinactivation of PSII (Fig. 1, A–C) that can outrun the clearance of PsbA protein (Figs. 2, A–C and 4). Edelman and Mattoo (2008) have reviewed similar data from other taxa showing that turnover of PsbA protein shows light saturation at moderate light, much different from photoinactivation rates that increase with increasing light. Conversely, in \(T.\) pseudonana elevated red light activates removal of PsbA that can outrun photoinactivation, at least transiently (Wu et al., 2011), showing that even under low light \(T.\) pseudonana can maintain pools of PSII repair cycle intermediates aside from the pool of active PSII. In \(T.\) pseudonana grown under low light the clearance of PsbA protein is light saturated at \(<450\ \mu\)mol photons m\(^{-2}\) s\(^{-1}\) (Wu et al., 2011; H. Wu and D. Campbell, unpublished data), with a maximum rate constant of about \(8 \times 10^{-3}\) s\(^{-1}\) (Fig. 4). We initially anticipated that clearance of PsbA, as a process of protein metabolism, would show a positive correlation with growth temperature. In fact, the rate constant for removal of PsbA (Fig. 4) and the growth rate of \(T.\) pseudonana (Table I) both peaked at 18°C. Intriguingly, the growth rate of \(T.\) pseudonana is supersaturated by light at \(<450\ \mu\)mol photons m\(^{-2}\) s\(^{-1}\) (G. Li and D. Campbell, unpublished data), suggesting that cells reach a mechanistic upper limit on their capacity to clear PsbA from photoinactivated PSII.

The larger, slower-growing cells of \(C.\) radiatus present a contrast since under the given light treatment \(C.\) radiatus suffers less photoinactivation, a manifestation of the negative size scaling of diatom susceptibility to photoinactivation (Key et al., 2010), which probably reflects light screening in larger cells (Finkel, 2001). Even though \(C.\) radiatus grows more slowly (Table I) it achieves rate constants for removal of PsbA similar to \(T.\) pseudonana (Figs. 2, D and E and 4), and thus PsbA removal can keep pace with the slower photoinactivation in \(C.\) radiatus (Fig. 4). We suspect that the saturation of the rate constant for removal of PsbA at around \(8 \times 10^{-3}\) s\(^{-1}\) (Fig. 4) reflects a fundamental property of the PSII repair cycle in these diatoms, with their triply stacked thylakoids and lack of apparent grana/stroma regions (Lepetit et al., 2010), rather than a size-dependent variable related to size scaling of growth rate or metabolic rate (Finkel, 2001). These differences in the balance between photoinactivation and PsbA and PsbD turnover in \(T.\) pseudonana and \(C.\) radiatus prove to have important consequences for the different induction patterns of NPQ and antenna function in the two species under comparable light treatments.

Antenna Function and Induction of NPQ

Algae can respond to sustained high light by changing the size of the PSII antenna by modifying the composition and the arrangement of pigments in relation to the PSII reaction center content, a strategy termed \(\sigma\)-type acclimation (Falkowski and Owens, 1980; Dubinsky and Stambler, 2009). In this study, except for the xanthophyll cycle pigments, neither species showed short-term changes in their contents of accessory pigments or chlorophyll \(a\) (data not shown), and the functional absorption cross sections \((\sigma_{\text{PSII}}\) and \(\sigma_{\text{PSII}}')\) of the two species remained nearly stable during the 90-min high-light exposure (Fig. 7). For \(T.\) pseudonana cultures that suffered progressive loss of PSII function in the presence of lincomycin (Fig. 1, A–C, closed symbols), the \(\sigma_{\text{PSII}}\) of the remaining PSII centers showed a modest increase after the 90-min high-light exposure (Fig. 7, Table II. Definitions of chlorophyll fluorescence parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Equation</th>
<th>Similar Parameter</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(F_v/F_M) (= (F_{Mv} - F_0)/F_M)</td>
<td></td>
<td>NPQ</td>
<td>van Kooten and Snel (1990)</td>
</tr>
<tr>
<td>NPQd</td>
<td>(F_M - F_{Mv})/(F_M)</td>
<td>NPQ</td>
<td>Bilger and Björkman (1990)</td>
</tr>
<tr>
<td>NPQc</td>
<td>(F_{S0} - F_0)/(F_M)</td>
<td>(q_2)</td>
<td>Nilkens et al. (2010)</td>
</tr>
</tbody>
</table>
A–C), consistent with limited connectivity across the antennae serving the active PSII centers (Mauzerall, 1982). In T. pseudonana \( \sigma_{\text{PSII}} \) measured under the growth light of 30 \( \mu \text{mol} \text{photons m}^{-2} \text{s}^{-1} \) was very close to the \( \sigma_{\text{PSII}} \) measured in the dark (data not presented) while \( \sigma_{\text{PSII}} \) measured under the treatment light level of 450 \( \mu \text{mol} \text{photons m}^{-2} \text{s}^{-1} \) dropped to half the size of \( \sigma_{\text{PSII}} \) measured in dark-acclimated cells (Fig. 7, A–C), showing strong short-term down-regulation of antenna function in the face of increasing light. The magnitude of this down-regulation is mirrored by light induction of NPQ (Fig. 6), which is mediated at least in part by the diatom xanthophyll cycle (Lavaud et al., 2002b; Goss and Jakob, 2010). In C. radiatus \( \sigma_{\text{PSII}} \) remained much closer to \( \sigma_{\text{PSII}} \) (Fig. 7, D and E), consistent with very limited induction of NPQ, and lower overall NPQ, under our treatment conditions.

In T. pseudonana Dd deepoxidized rapidly to Dt when cells were shifted to high light, and the pool size of Dd + Dt increased significantly during the high-light exposure in both diatom species (Fig. 5). In diatoms one Dd pool representing 40% to 60% of the total has a very low turnover (Goericke and Welschmeyer, 1992), is not convertible into Dt (Lohr and Wilhelm, 2001; Lavaud et al., 2004; Fig. 5), and may contribute to structural stabilization of pigment-protein complexes (Pascal et al., 1998). The other diatom Dd pool, representing 50% to 60% of the total, has a much higher turnover (Goericke and Welschmeyer, 1992), is converted into Dt, and may also serve as the precursor for fucoxanthin synthesis (Goericke and Welschmeyer, 1992; Lohr and Wilhelm, 1999, 2001). In our study the net fucoxanthin content was stable across the higher-light treatment and the subsequent low-light recovery period. However, fast accumulation of Dt from the deepoxidation of Dd was clear in T. pseudonana, especially over the first 2 to 30 min of high-light exposure (Fig. 5; Zhu and Green, 2010). In T. pseudonana the deepoxidation of Dd to Dt (DES) was not correlated with the rapidly reversible NPQd (Fig. 8; Zhu and Green, 2010), but DES was linearly correlated with the extent of sustained NPQs (Fig. 8B) and with the total NPQ (Fig. 8C). Our NPQ phase is consistent with the \( q_s \) estimates of Zhu and Green (2010) and appears similar to the \( q_s \) quenching recently defined by Nilsken et al. (2010), which correlated with deepoxidation of xanthophyll pigments in Arabidopsis (Arabidopsis thaliana).

Diatoms show complex regulation of NPQ (Lohr and Wilhelm, 1999; Lavaud et al., 2002a, 2002b, 2004; Goss et al., 2006; Dimier et al., 2007; Lavaud, 2007; Eisenstadt et al., 2008; Grouneva et al., 2008, 2009; Goss and Jakob, 2010; Lepetit et al., 2010, 2012). The differences among studies reflect physiological distinctions in the rates and magnitudes of induction and relaxation of NPQ phases across taxa (e.g. Fig. 6) or growth conditions (e.g. Fig. 8), but also differences in the experimental sequences and durations of light, dark, and low-light incubations (Goss et al., 2006; Zhu and Green, 2010).

The slope of the correlation between NPQ induction and DES varied with the growth temperature in T. pseudonana (Fig. 8B). For the same amount of DES, more NPQs was induced in cells at the suboptimal growth temperature of 12°C (Fig. 8C), with less induction of NPQs at the optimal growth temperature of 18°C. Cells at 24°C achieved higher DES (Fig. 8, B and C). Variations in the efficacy of quenching induction in response to Dt accumulation occur among species (Lavaud et al., 2004) and across different ecotypes of Phaeodactylum tricornutum (Bailleul et al., 2010). A lower quenching efficiency of Dt could result if the newly synthesized Dt pool is located in a lipid shield surrounding the fucoxanthin chlorophyll proteins and is not necessarily protein bound (Lepetit et al., 2010, 2012). In addition, studies by Eisenstadt et al. (2008, 2010) showed that changes in the functional organization of the diatom PSII reaction center under higher excess light could lead to the formation of NPQ independently of Dt.

Levels of members of the Lhcx pigment protein family are implicated in modulating the magnitude of induction of NPQ in diatoms. Zhu and Green (2010) showed that the expression of the Lhcx6 protein is associated with modulation of NPQ induction in T. pseudonana, and Bailleul et al. (2010) showed a correlation between NPQs and one of the Lhcx1 homologs in P. tricornutum. Therefore, diatoms possess...
overlapping means to dissipate excessive light energy. In Figure 9 we show that *T. pseudonana* has significant levels of Lhcx1 and Lhcx6 proteins during growth at 12°C. These 12°C cultures also show the highest baseline levels of total NPQ, (Fig. 8C). Within 90 min of an upward shift to 450 μmol photons m⁻² s⁻¹ *T. pseudonana* induced further expression of Lhcx1 and Lhcx6, with the magnitude of induction negatively correlated with growth temperature (Fig. 9, A and B), a pattern consistent with regulation of Lhcx1 and Lhcx6 by excitation pressure (Huner et al., 1998). The light induction of Lhcx1 and Lhcx6 coincides with the conversion of rapidly reversible NPQd to sustained NPQ. In contrast to the negative temperature correlation of Lhcx induction, induction of DES is highest at the highest growth temperature of 24°C. Overall, the differential inductions of Lhcx isoforms and xanthophyll deepoxidation mediate the highest accumulation of NPQ, at 12°C, where *T. pseudonana* suffered the biggest gap between photoinactivation and clearance of PsbA protein (Figs. 4 and 10).

CONCLUSION

Small and large centric diatom species react differentially to a comparable increase in light intensity (Key et al., 2010; Wu et al., 2011; Figs. 1–3 and 5–7). Figure 10 summarizes our findings by plotting the accumulation of sustained NPQ, versus the ratios of the rate constants for photoinactivation (kₜ) and for removal of PsbA protein. In the smaller *T. pseudonana* PSII photoactivation outran removal of PsbA protein (Figs. 1, 3, 4, and 10), the cells induced strong expression of Lhcx1 and Lhcx6 proteins (Fig. 9), and deepoxidized their xanthophyll cycle pigments (Fig. 5) to induce a sustained NPQ, phase of NPQ, showing the highest accumulation of NPQ, at 12°C, where *T. pseudonana* grown at sub- or supraoptimal temperatures exhibited the highest accumulation of NPQ, at 12°C, where *T. pseudonana* induced strong expression of Lhcx1 and Lhcx6 proteins (Fig. 9), and deepoxidized their xanthophyll cycle pigments (Fig. 5) to induce a sustained NPQ, phase of NPQ, showing the highest accumulation of NPQ, at 12°C, where *T. pseudonana* grown at sub- or supraoptimal temperatures showed that this broad-band blue-light treatment affects the cells similarly to a high-light treatment with unfiltered fluorescent light (Wu et al., 2011). Samples were collected prior to the onset of high light (plotted as time 0) and after 15, 30, 60, and 90 min for chlorophyll fluorescence analyses and for filtration onto glass fiber filters, which were flash frozen for later protein immunoblotting and pigment analyses. Following the high-light treatment, the remaining culture volumes were returned to their initial growth light of 30 μmol photons m⁻² s⁻¹ for a 30-min recovery period followed by the final sampling.

Fluorescence Measurement and Photooxidation Parameterization

Chlorophyll fluorescence data were collected using a Xe-PAM fluorometer (Walz) connected to a temperature-controlled cuvette holder (Walz). At each sampling point, a sample of culture was dark adapted for 5 min to relax photosynthetic activity. A modulated (4 Hz) blue-light measuring beam was used to measure F₀, followed by a 500-ms saturating white-light pulse of 4000 μmol photons m⁻² s⁻¹ to measure Fₚ, and F₀, and Fₚ, were measured in a light-acclimated sample, was measured in a light-acclimated sample, was measured. Another saturating pulse was then applied to measure the maximum fluorescence in the light (Fₚ). The maximum quantum yield of PSII photochemistry (van Kooten and Snel, 1990) was then estimated as:

\[\varphi_{PSII} = \frac{(Fₚ - F₀) / Fₚ}{(Fₚ - Fₚ_{dark}) / Fₚ_{dark}}\]

Two kinetic phases of NPQ were estimated (Table II). Dynamic NPQ, NPQ, that relaxed within the 5-min dark period before measurement, and was then reinduced within the short measuring period was estimated as:

\[NPQ_δ = \frac{(Fₚ - Fₚ_{dark}) / Fₚ_{dark}}{(Fₚ - F₀) / Fₚ)}\]

This NPQ is equivalent to the NPQ estimated by Zhu and Green (2010) following Bilger and Bjorkman (1990).

MATERIALS AND METHODS

Culture Conditions and Growth Rates Calculation

The diatoms *Thalassiosira pseudonana* National Center for Marine Algae and Microbiota (NCMA, formerly CCMIP) 1014 and *Coscinodiscus radiatus* NCMA 312 (both obtained from Provasoli-Guillard National Center for Marine Algae and Microbiota) were grown in semicontinuous batch cultures using K medium (Keller et al., 1987) in polystyrene flasks (Corning) at 12°C (for *T. pseudonana* only), 18°C, or 24°C. Cultures were grown under continuous light of 30 μmol photons m⁻² s⁻¹ provided by fluorescent tubes (Sylvania) and measured in the culture flasks using a microspherical quantum sensor (US-SQ6; Walz) connected to a LiCor light meter (Li250; Li-Cor). The cultures were agitated manually twice daily. Cell densities were monitored by cell counts using a Beckman counter (Multiscorer 3) for *T. pseudonana* NCMA 1014. The cell counter also provides an equivalent spherical volume estimate for the counted cells. We used a Sedgewick-Rafter counting chamber under a light microscope to count *C. radiatus* NCMA 312, and estimated the cell volume by approximating the cells as cylinders, and measuring radius and cylinder height. Growth rate (μ) was estimated as:

\[μ = ln(N₂)/t₂ - ln(N₁)/t₁\]

where N is the number of cells at time t and N is the number of cells at time 0. All cultures were grown through at least four transfers of semicontinuous dilution with fresh media under the given light level, and went through more than 24 generations under the given light level to ensure full acclimation before use in subsequent experiments.

Upward Light Shift and Recovery Experiment

Culture replicates from exponential growth phase were split into two flasks, with 500 μg ml⁻¹ lincomycin added to one flask to block chloroplast protein synthesis (Bachmann et al., 2004), thereby inhibiting PSII repair (Baroli and Melis, 1996; Tysjvärvi and Arro, 1996; Key et al., 2010). Both flasks were incubated in the dark for 10 min to allow the lincomycin to exert its effect and then exposed to broad-band blue light (LEE filter no. 183, 455- to 479-nm peak transmission, 406- to 529-nm half-height width) of 450 μmol photons m⁻² s⁻¹ for 90 min. This blue-light treatment was chosen to approximate a marine light-field quality, and to match the spectral band for our determinations of the functional absorption cross section serving PSII photochemistry (\(φ_{PSII,Å²} \) quanta). We previously showed that this broad-band blue-light treatment affects the cells similarly to a high-light treatment with unfiltered fluorescent light (Wu et al., 2011). Samples were collected prior to the onset of high light (plotted as time 0) and after 15, 30, 60, and 90 min for chlorophyll fluorescence analyses. Following the high-light treatment, the remaining culture volumes were returned to their initial growth light of 30 μmol photons m⁻² s⁻¹ for a 30-min recovery period followed by the final sampling.
A more sustained phase of NPQ, NPQs, which was induced over the course of the high-light treatment, and which persisted through the 5-min dark acclimation period just before measurement, was estimated as:

\[ \text{NPQ} = \frac{F_{\text{m}'}}{F_{\text{m}}} \]

where \(F_{\text{m}'}\) is the measurement of \(F_{\text{m}}\) from dark-acclimated cells, taken at time 0 just before the start of high-light treatment. \(F_{\text{m}}\) is taken at each measurement time point. By definition, NPQ thus starts at 0 at \(F_{\text{m}}\) and increases as the cells accumulate a sustained phase of NPQ. Because our repeated time course measurements were conducted rapidly during short-term light-shift experiments to track PSII photo-inactivation, we did not apply longer-term relaxation periods (Goss et al., 2006), as used by Zhu and Green (2010) to estimate \(q_{\infty}\) and \(q_{\infty}\) according to Farber et al. (1997). NPQ reflects an indeterminate increase in the relaxation time for a fraction of NPQ, persisting beyond a 5-min dark period, but largely relaxing over a 30-min period of low light, even when chloroplastic protein synthesis is blocked. NPQ is thus similar to the \(q_{\infty}\) defined by Nilkens et al. (2010). Finally, we calculated the total accumulated NPQ as the sum of NPQs.

We estimated a functional absorption cross section driving the photo-inactivation of PSII (\(\sigma_{\text{fl}}\) \(\AA^2\) quanta \(^{-1}\)) by gently centrifuging and resuspension in 5 mL of the residual growth media for the particular applied light level and resuspended in 5 mL of the residual growth media for the particular applied light level and resuspended in 5 mL of the residual growth media for the particular applied light level and resuspended in 5 mL of the residual growth media for the particular applied light level.

**Validation of \(F_{\text{m}}/F_{\text{m}'}\) by Oxygen Flash Yield Quantification of the Content of Active PSII**

As a validation and cross check of the rapid, low-volume sample \(F_{\text{m}}/F_{\text{m}'}\) measures we used to track changes in PSII function during the light-shift experiments presented in Figures 1, 4, and 10, we subsequently conducted a series of measurements on nine different \(T. pseudonana\) cultures grown at 18°C under 30 (n = 6), 90 (n = 1), 180 (n = 1), and 270 (n = 1) \(\mu\text{mol photons m}^{-2}\text{s}^{-1}\) for multiple rounds of media transfer and many generations of growth. We then shifted the cultures to combinations of 90 (n = 1), 180 (n = 1), 270 (n = 1), 450 (n = 6), 1,000 (n = 2), 1,400 (n = 2), 1,900 (n = 1), or 2,200 (n = 1) \(\mu\text{mol photons m}^{-2}\text{s}^{-1}\) for 30 to 90 min, with or without lincomycin.

Before, and after, light-shift treatments we used a modulated fluorometer to measure \(F_{\text{m}'}/F_{\text{m}}\) from culture samples, as described above. At the same times we took 100-mL culture samples and concentrated them 30X by gentle centrifugation at 4,000g and resuspension in 5 mL of the residual growth media supernatant (not fresh media). This concentration step was needed to give sufficient culture density to provoke detectable, short-term, changes in oxygen concentration in the media. We loaded 2.5 mL of the resulting concentrated culture into a plastic 1-cm path length spectrophotometer cuvette and measured the oxygen content of the culture samples using a Fosy R oxygen sensor (Ocean Optics; Bacon and Demas, 1987), mounted in a lab-built epoxy gas-tight cuvette plug that incorporates the oxygen sensor, a temperature probe, and a thermostatted temperature control loop, which we set to 18°C. The entire assembly was then mounted into the SuperHead unit of a PSI FL3500 fluorometer (PSI). This fluorometer unit contains LED light sources that can provide repetitive trains of blue- or red-light flashes of up to 90,000 \(\mu\text{mol}\) photons \(\text{m}^{-2}\text{s}^{-1}\) with durations as short as 2 \(\mu\text{s}\). Before any oxygen yield experiments were carried out on a given day, the oxygen sensor was calibrated. Distilled water was shaken for 2 min to give air-saturated seawater, at an oxygen content of 238 \(\mu\text{mol O}_2\) L \(^{-1}\) (YSI model 51B dissolved oxygen meter instructions, YSI). The oxygen-saturated seawater medium was measured with the oxygen sensor, the reading was allowed to stabilize, and the reading of the oxygen sensor was recorded. The oxygen sensor was then immersed in 2 mol L \(^{-1}\) sodium dithionite (Flinn Scientific Inc.), which consumes oxygen, rendering the oxygen content of the solution effectively zero (Jhaeveri and Sharma, 1968). The reading was allowed to stabilize and the reading of the oxygen sensor was recorded, to establish the response range of the instrument. After initial stabilization the temperature in the cuvette assembly was maintained within 0.2°C throughout subsequent readings.

Concentrated culture samples were loaded into the cuvette assembly and were then kept in the dark for 5 min while the temperature stabilized. Note that during prior centrifugation and resuspension the cells were in darkness or low light for 5 min prior to oxygen measurements. Once the oxygen content and expected steady downward slope in the levels of oxygen in the culture suspensions we used the PSI Superhead system to apply 1 min of low-level (50 \(\mu\text{mol}\) photons \(\text{m}^{-2}\text{s}^{-1}\) of combined red and blue light) continuous pre-illumination, to ensure induction of electron transport through both PSI and PSII during the subsequent flash train (Kuvykin et al., 2008). Following the 1-min preillumination, the oxygen content of the samples was tracked while we used the Superhead to apply a flash train of 3,000 red-light flashes of 20 \(\mu\text{s}\) duration, approximately 8,000 \(\mu\text{mol}\) photons \(\text{m}^{-2}\text{s}^{-1}\), spaced by 50-ms dark intervals, giving a flash train lasting 150 s in total. We used multiple test samples to verify that this flash train was indeed sufficient to provide saturating, single-turnover flashes by varying the level, duration, and dark spacing of the flashes. Following the flash train, the sample was kept in the dark once more while oxygen consumption was measured to account for the base rate of cellular respiration that took place during the flash train. We then extracted total chlorophyll from the concentrated sample culture under 90% acetone, measured absorbance, and used the equations of Jeffrey and Humphrey (1975) to estimate the chlorophyll content of the concentrated culture suspensions. We followed the method of Chow et al. (1989) to use the change in oxygen concentration provoked by the series of single-turnover saturating flashes to estimate the content of active PSII per chlorophyll in the suspension as:

\[
(\text{mol O}_2 \text{L}^{-1} \text{s}^{-1}) \times (5 \times 10^{-5} \text{ s flash cycle}^{-1}) \times (1 \text{ flash cycle mol PSI} \text{ mol e}^{-1})^{-1} \times (1 \text{ L mol Chl}^{-1})^{-1}
\]

In Figure 11 we present the results of this cross validation by plotting \(F_{\text{m}'}/F_{\text{m}}\) from the cultures treated under high light, with or without lincomycin, as percent of growth level \(F_{\text{m}'}/F_{\text{m}}\) versus the content of active PSII in the same cultures, as percent of active PSII in the same culture under growth conditions. The slopes of the linear regressions for measurements with (closed circles) or without (open circles; some data points obscured) lincomycin were not statistically significantly different (\(F\) tests) so we present a pooled linear regression for the combined data from culture samples treated with or without lincomycin. Note the strong correlation between \(F_{\text{m}'}/F_{\text{m}}\) and the content of active PSII in the cultures. A particular concern of reviewers was that sustained phase(s) of NPQ could suppress \(F_{\text{m}'}/F_{\text{m}}\) measures, leading to overestimations of the extent of photo-inactivation of PSII in our treatments. In fact, Figure 11 shows that as the content of active PSII in the culture declines toward zero under strongly photo-inhibitory conditions, there is a y intercept of \(F_{\text{m}'}/F_{\text{m}}\) of 33% of control levels of \(F_{\text{m}'}/F_{\text{m}}\). Therefore, in strongly photo-inhibited cultures the \(F_{\text{m}'}/F_{\text{m}}\) measure we used somewhat underestimates the loss of PSII activity, rather than overestimating the loss of activity. We are thus confident that our estimates of rate constants for photoinactivation (Fig. 4) are conservative, rather than exaggerated, and that photo-inactivation rate constants can indeed exceed rate constants for clearance of PsbA (Figs. 2 and 4), as reviewed by Edelman and Matteo (2008).
Quantitation of Proteins by Immunoblotting

Cells were harvested on glass fiber filters (0.7-μm effective pore size, 25-mm diameter, binder-free glass fiber, Whatman), which were immediately flash frozen in liquid nitrogen and stored at −80 °C for later protein analyses. We quantified molar levels of PsbA and PsbD from samples taken during the high-light treatment time courses. Total proteins were extracted by two thawing/sonicating rounds in denaturing extraction buffer (Brown et al., 2008). The total protein concentration was determined (Lowry protein assay kit, BioRad-DC Assay). One microgram of total protein was loaded on 4% to 12% acrylamide gel (AgriSera, 1:50,000) were applied, followed by an anti-rabbit secondary antibody coupled with horseradish peroxidase. The membranes were developed by chemiluminescence using ECL Advance (Amerham biosciences) and a CCD imager (BioRad VersaDoc 4000MP). PsbA and PsbD protein contents were determined by fitting the sample signal values to the protein standard curves, taking care that all sample signals fell within the range of the protein standard curve, and that no band signals were saturated. We estimated a rate constant for taking care that the band densities for quantitation of proteins by immunoblotting

LITERATURE CITED


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An error in composition resulted in numerous errors in the presentation of the figures in this article. Figures 1 to 3 and 5 to 11 contain incorrect panels. In addition, the letters “pi” were incorrectly changed to the Greek symbol “π” in the legend for Figure 10 and the “Conclusion” and “Materials and Methods” sections.

The online version of the article has been corrected.