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## **Photosystem II repair in marine diatoms with contrasting photophysiology**

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## Abstract

*S. costatum* and *P. tricornutum* are model marine diatoms with differing strategies for non-photochemical dissipation of excess excitation energy within photosystem II (PSII). We showed that *S. costatum*, with connectivity across the pigment bed serving PSII, and limited capacity for induction of sustained non-photochemical quenching (NPQ), maintained a large ratio of  $[PSII_{Total}] / [PSII_{Active}]$  to buffer against fluctuations in light intensity. In contrast, *P. tricornutum*, with a larger capacity to induce sustained NPQ could maintain a lower  $[PSII_{Total}] / [PSII_{Active}]$ . Induction of NPQ was correlated with an active PSII repair cycle in both species, and inhibition of chloroplastic protein synthesis with lincomycin lead to run away over-excitation of remaining  $PSII_{Active}$ , particularly in *S. costatum*. We discuss these distinctions in relation to the differing capacities, induction and relaxation rates for NPQ, and as strain adaptations to the differential light regimes of their originating habitats. The present work further confirms the important role for the light-dependent fast regulation of photochemistry by NPQ interacting with PSII repair cycle capacity in the ecophysiology of both pennate and centric diatoms.

*Key words:* diatoms; ecophysiology; photoprotection; non-photochemical fluorescence quenching; PsbA (D1) protein; Photosystem II repair.

*Abbreviations:* Chl *a*, Chlorophyll *a*; DD, diadinoxanthin; DT, diatoxanthin;  $\Delta pH$ , transthylakoidal proton gradient;  $F_V/F_M$ , maximum quantum yield of Photosystem II;  $k_{pi}$ , first order rate constant for photoinactivation of PSII;  $k_{PsbA}$ , first order rate constant for removal of PsbA protein of PSII; LHC, light-harvesting complex; NPQ, non-

photochemical Chl *a* fluorescence quenching; PSII, Photosystem II;  $\Phi_{\text{PSII}}$ , quantum yield of PSII electron transport;  $\sigma_{\text{PSII}}$ , effective absorbance cross section serving PSII photochemistry;  $\sigma_i$ , effective absorbance cross section driving PSII photoinactivation; XC, xanthophyll cycle.

## Introduction

Diatoms are a major group of eukaryotic microalgae ubiquitous in all marine and freshwater ecosystems. With  $> 10^5$  species (Kooistra et al. 2007; Mock and Medlin 2012), they are among the most significant photosynthetic organisms. They contribute to about 40% of the aquatic primary production making them essential for most marine food-webs (Armbrust 2009). Their contribution to several biochemical cycles (Tréguer and De La Rocha 2013; Sarthou et al. 2005) and unique biological, physiological and metabolic characteristics (Consalvey et al. 2004; Armbrust 2009; Mock and Medlin 2012; Obata et al. 2013; Lyon and Mock 2014; Wilhelm et al. 2014) contribute significantly to shaping the functions of contemporary oceans (Kooistra et al. 2007; Mock and Medlin 2012). As with most microalgae (MacIntyre et al. 2000), the photosynthetic productivity of diatoms strongly depends on the light climate (Depauw et al. 2012; Wilhelm et al. 2014). Planktonic as well as benthic diatoms tend to dominate ecosystems (i.e. coasts and estuaries) where the light climate is characterized by unpredictable high-frequency, large amplitude fluctuations in irradiance. Depending on light attenuation through water, ice or sediment, on the rate of water mixing, on the tidal cycle, and on the daily/seasonal changes of solar irradiation, diatoms can be exposed to episodic or chronic excess light, possibly generating stressful conditions that impair their photosynthetic efficiency and their productivity through photoinhibition (Blanchard et al. 2004; Lavaud et al. 2007; Wu et al. 2011; Wu et al. 2012).

In order to limit such situations, diatoms have evolved fast regulatory biological and physiological processes which comprise a 'photoprotective network' (Consalvey et al. 2004; Lavaud and Goss 2014; Wilhelm et al. 2014; Barnett et al. 2015; Laviale et al.

2015). They may 1) actively escape from excess light through motility, at least in benthic motile diatoms that inhabit the sediments of intertidal flats, 2) safely dissipate the excess of absorbed light energy, 2) balance the excess excitation energy within the photosynthetic apparatus and plastid, 3) scavenge reactive oxygen species, 4) repair damage to proteins, thus containing and countering net photodamage to the photosynthetic and plastid machinery. In diatoms, one of these regulatory processes, believed to be essential, is the fast (i.e. seconds-scale timing) development of a thermal dissipation of excess energy that can be detected thanks to a non-photochemical quenching of chlorophyll *a* (Chl *a*) fluorescence (NPQ) (Büchel 2014; Lavaud and Goss 2014). In diatoms, NPQ is controlled by i) the light-dependent build-up of the transthylakoidal proton gradient ( $\Delta\text{pH}$ ), ii) the  $\Delta\text{pH}$ -dependent conversion of the xanthophylls diadinoxanthin (DD) into diatoxanthin (DT) called ‘xanthophyll cycle’ (XC) (Brunet and Lavaud 2010; Lavaud and Goss 2014), and iii) the presence of specific polypeptides of the light-harvesting complex (LHC) antenna named Lhcx (Depauw et al. 2012; Büchel 2014). Recently, a working mechanistic model for diatom NPQ has been proposed (see Chukhutsina et al. 2014; Lavaud and Goss 2014; Derks et al. 2015; Goss and Lepetit 2015) with two mechanistic and kinetic quenching components (namely Q1 and Q2).

By significantly contributing to the cellular response under fluctuating light conditions (Wagner et al. 2006; Mills et al. 2010; Giovagnetti et al. 2014), NPQ is critical for maintaining the photosynthetic efficiency of both planktonic and benthic diatoms (Lavaud and Goss 2014; Barnett et al. 2015; Laviale et al. 2015). An increasing number of reports suggest that diatom/microalgal inter-species differences in NPQ are involved in

the differential colonization of marine habitats based on the light climate combined or not with other environmental factors including nutrients, temperature, and salinity (Strzepek and Harrison 2004; Lavaud et al. 2007; Dimier et al. 2007; Kropuenske et al. 2009; Bailleul et al. 2010; Mills et al. 2010; Petrou et al. 2011; van de poll et al. 2011; Wu et al. 2012; Lavaud and Lepetit 2013; Barnett et al. 2015; Juneau et al. 2015). Induction of NPQ nonetheless can impose opportunity costs upon microalgal productivity, since a sustained NPQ that persists after a downward shift in irradiance can lower achieved productivity at the new, lower light level (Raven 2011). In parallel to NPQ, capacities to repair the prime photosynthetic target of photodamage, the PsbA (D1) protein of photosystem II (PSII), was shown to differ among diatom species (Kropuenske et al. 2009; Key et al. 2010; Petrou et al. 2010; Wu et al. 2011; Wu et al. 2012; Campbell et al. 2013). In a series of previous studies (Lavaud et al. 2004, Lavaud et al. 2007; Lavaud and Lepetit 2013), we compared the photoprotective capacities and properties of two model marine diatom strains, *Phaeodactylum tricornutum* and *Skeletonema costatum*. We related NPQ and xanthophyll differences (i.e. *P. tricornutum* > *S. costatum*) to the differential light climates of the original habitats to which the strains were adapted (Lavaud and Lepetit 2013). We have not, however, to date, analyzed possible differential abilities to repair the PSII PsbA protein across these strains with differing photophysiology (Wu et al. 2011; Wu et al. 2012).

For that purpose, we grew both strains under low light (LL-50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) comparable to levels near the bottom of the photic zone, and then shifted both strains to moderate high light (HL-450  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) for 90 min, an intensity equivalent to the top 30% of the photic zone and sufficient to induce significant



photoinactivation/photoinhibition within a 90 min incubation (Lavaud et al. 2007). The HL incubations were in the presence or not of lincomycin, to inhibit the translation of chloroplast transcripts, notably *psbA*, the gene encoding the PsbA protein. Lincomycin thereby efficiently blocks the PSII repair cycle in diatoms (Wu et al. 2011; Wu et al. 2012; Campbell et al. 2013) allowing us to separate primary photoinactivation of PSII from the counteracting PSII repair cycle and to analyze the interaction of these processes with inductions of NPQ mechanisms. The results showed that both diatoms showed a comparable primary susceptibility to photoinactivation of PSII (Key et al. 2010; Wu et al. 2011; Wu et al. 2012) but that *S. costatum* showed a higher total content of PSII to maintain significantly higher capacity for PSII repair, in contrast to *P. tricornutum*, which relies more upon induction of NPQ to allow a smaller pool of PSII.

## **Material and Methods**

### *Culturing and light treatments*

Exponential cultures (500 mL) grown in glass Erlenmeyer flasks (*f*/2 medium, 50  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , 20°C, air bubbling) were split into two polystyrene flasks. One was supplemented with 500  $\mu\text{g mL}^{-1}$  lincomycin and both flasks were incubated in the dark for 10 min, to allow the antibiotic to penetrate the cells and inhibit ribosome function. The two flasks were then shifted for 90 min to 450  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (high light-HL) blue light (LEE Filter #183, Panavision; 455-479 nm peak transmission, 406-529 nm half-height width). Samples were collected at 15, 30, 60 and 90 min to measure biophysical properties and for later protein immunodetection. The sub-cultures were then

shifted back to their initial growth light (50  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , low light-LL) and sampled after 30, 60 and 180 min of recovery.

#### *Chlorophyll a (Chl a) fluorescence measurements*

Culture aliquots were dark-adapted for 10 min and a blue-green modulated measuring light (4 Hz; Xenon-PAM, Walz, Effetrich, Germany) was activated to measure the basal fluorescence  $F_0$ . A saturating white light pulse (4,000  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , 500 ms) was triggered in order to determine the maximal Chl *a* fluorescence level in the dark  $F_M$ . Actinic irradiance was then activated at HL level; after signal stabilisation ( $F_S$  level), a saturating light pulse was triggered to determine the light acclimated maximal fluorescence ( $F_M'$ ), and the actinic irradiance was briefly interrupted to measure  $F_0'$  with PSII centers open in the light acclimated state. The PSII inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was then added and after signal stabilisation, a light pulse was triggered again to determine the maximal fluorescence  $F_{Mm}$ . This measurement protocol was repeated for each time point along the time course experiments. The photochemical yield of PSII was calculated as:

$$F_V/F_M = (F_M - F_0) / F_M$$

For each time point we calculated the induction of non-photochemical quenching as:

$$\text{NPQ} = (F_M - F_M') / F_M'$$

To estimate the total cumulative non-photochemical down-regulation of PSII across the entire time course we then calculated  $\text{NPQ}_{\text{global}}$  as:

$$\text{NPQ}_{\text{global}} = (F_{Mm} - F_M') / F_M'$$

where  $F_M'$  was measured under the treatment HL level and  $F_{Mm}$  was taken as the highest measurement of  $F_{Mdark}$  at any point along the time course. This formulation assumes there was no significant culture growth over the course of the time course, which would increase  $F_{Mm}$  over time.

We determined the effective absorbance cross-section serving PSII photochemistry ( $\sigma_{PSII}$ ,  $A^2 \text{ quantum}^{-1}$ ) on culture aliquots illuminated by a saturating single turn-over flash of known irradiance (blue LED,  $455 \pm 20 \text{ nm}$ ; FIRE fluorimeter, Satlantic, Halifax, NS Canada) (Kolber et al. 1998; Barnett 2007). For comparison of the absorbance capacities driving PSII photochemistry and photoinactivation, we estimated an effective absorbance cross section for PSII photoinactivation ( $\sigma_i$ , ( $A^2 \text{ quantum}^{-1}$ )) (Campbell and Tyystjärvi 2012; Oliver et al. 2003) by plotting the exponential decay of the PSII quantum yield  $F_V/F_M$  in the absence of repair vs. the cumulative dose of photons  $A^{-2}$ . We estimated the electron transport rate through PSII following (Suggett et al. 2004; Suggett et al. 2009; Huot and Babin 2010) as:

$$ETR = E \times \sigma_{PSII} \times \Phi_{PSII} / (F_V/F_M)$$

where  $E$  is the instantaneous irradiance ( $\text{photons } A^{-2} \text{ s}^{-1}$ ),  $\Phi_{PSII} = (F_M' - F_S) / F_M'$  is the effective quantum yield for PSII photochemistry measured under irradiance (Genty et al. 1989), and  $F_V/F_M$  is the maximum quantum yield for PSII photochemistry after dark acclimation.

#### *PsbA immunodetection*

The procedure was previously described (Six et al. 2007; Brown et al. 2008). Briefly, cells were harvested on glass fibre filters (25mm, Whatman, UK) and the proteins were extracted by one thawing/sonicating round in LDS extraction buffer. The total protein concentration was determined (Lowry protein assay kit, Biorad, CA, USA, with BGG as a protein standard) and 2 µg of total protein were loaded on a 4-12% acrylamide precast NuPAGE gel (Invitrogen, CA, USA), along with PsbA (D1) protein standards (Agrisera, Sweden). Electrophoresis was run for 30 min at 200 V and the proteins were transferred to a PVDF membrane. The membranes were immersed in blocking solution (Amersham Biosciences, NJ, USA) for 1 h and successively incubated with primary antibodies directed against D1 (Agrisera, 1/50,000) in Tween-TBS in the presence of 2% blocking agent and anti-chicken secondary antibodies coupled with horseradish peroxidase (Biorad, 1/50,000). The membranes were developed by chemoluminescence using ECL Advance (Amersham biosciences) within a CCD imager (FluorSMax, Biorad). Target protein concentrations were determined by fitting the sample signal values on these curves to protein standard curves.

## Results and Discussion

### *Photosystem II (PSII) function, photoinactivation and repair*

In Figure 1 we present time courses for the maximum quantum yield for the PSII pool ( $F_V/F_M$ ) (A, C) and for the pool of the key PSII protein PsbA (B, D) for *S. costatum* (A, B) and *P. tricornutum* (C, D) shifted from growth under 50 µmol photons m<sup>-2</sup> s<sup>-1</sup> ( $T_0$ ) up to 450 µmol photons m<sup>-2</sup> s<sup>-1</sup> (0-90 min, high light-HL) and then back to growth light of 50 µmol photons m<sup>-2</sup> s<sup>-1</sup> for recovery (90-270 min, low light-LL). During the treatments cells

were incubated in the absence (open symbols) or presence (closed symbols) of lincomycin to inhibit translation of chloroplastic encoded proteins, including *psbA* gene, and thereby block the PSII repair cycle. To facilitate comparisons of changes in PSII function and protein content we plotted the data as %T<sub>0</sub>. Both *S. costatum* and *P. tricornutum* showed similar initial F<sub>V</sub>/F<sub>M</sub> values (Table 1), but under LL growth *S. costatum* showed a higher initial level of PsbA protein content, almost twice that of *P. tricornutum* (Table 1), as well as a slightly higher ETR<sub>PSII</sub> (25%, similar as reported before, Lavaud et al. 2007).

During the HL treatment period, we fitted the decline in F<sub>V</sub>/F<sub>M</sub> in the presence of lincomycin (Figure 1A, C, closed symbols) with a single phase exponential decay to extract a first order rate constant for photoinactivation of PSII in the absence of counteracting repair ( $k_{pi}$ , s<sup>-1</sup>) (Table 1). These curve fits were not significantly different between *S. costatum* and *P. tricornutum* which therefore showed a common rate constant for photoinactivation under 450  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>. To generalize this irradiance-dependent rate constant to a generalized susceptibility to photoinactivation, we converted  $k_{pi}$  to a target size formulation (Campbell and Tyystjärvi 2012) of the effective absorbance cross section for photoinactivation,  $\sigma_i$  (A<sup>2</sup> quantum<sup>-1</sup>) (Table 1) (Oliver et al. 2003; Key et al. 2010; Campbell and Tyystjärvi 2012), giving a value typical for small diatoms (Key et al. 2010; Wu et al. 2011; Wu et al. 2012; Li and Campbell 2013). In parallel, we fitted the decline in PsbA protein in the presence of lincomycin (Figure 1B, D, closed symbols) with single phase exponentials to extract apparent first order rate constant for clearance of PsbA,  $k_{PsbA}$  (s<sup>-1</sup>) (Table 1) (Wu et al. 2011; Wu et al. 2012).

Unexpectedly,  $k_{\text{PsbA}}$  was three times lower for *S. costatum* than for *P. tricornutum* (Table 1).

Over the same initial HL treatment we fitted  $F_V/F_M$ , our measure of the pool of  $[\text{PSII}_{\text{Active}}]$  from the cultures treated in the absence of lincomycin (Figures 1A, C, open symbols) following (Kok 1956; Oliver et al. 2003):

$$F_V/F_{Mt} = F_V/F_M \times ((k_{\text{rec}} + (k_{\text{pi}} \times ((\exp(-(k_{\text{pi}} + k_{\text{rec}}) \times t)))))) / (k_{\text{pi}} + k_{\text{rec}}))$$

where  $F_V/F_{Mt}$  was measured at treatment time  $t$ ,  $F_V/F_M$  was the initial pre-treatment, maximal level of  $F_V/F_M$  measured after 10 min of dark adaptation from the growth LL,  $k_{\text{pi}}$  was imported from the single phase exponential fits in the presence of lincomycin, and  $k_{\text{rec}}$  was fit as the apparent first order rate constant for repair of photoinactivated PSII  $[\text{PSII}_{\text{Inactive}}]$  to  $[\text{PSII}_{\text{Active}}]$ .

During the succeeding LL recovery period, we assumed the PSII photoinactivation rate constant fell to near 0 ( $\sigma_i \times I \sim 0$ ) and followed (Oliver et al. 2003) to fit  $F_V/F_M$  as:

$$F_V/F_{Mr} = F_V/F_{Mi} + \{(F_V/F_{Mm} - F_V/F_{Mi}) \times (1 - e^{-k_{\text{rec}} \times t})\}$$

where  $F_V/F_{Mr}$  was measured at recovery time  $r$ ,  $F_V/F_{Mi}$  was the maximally inhibited  $F_V/F_M$  measured at the start of the recovery period, coincident with the end of the HL treatment period,  $F_V/F_{Mm}$  was the maximal  $F_V/F_M$  measured during the time course. These formulations follow the assumptions of (Kok 1956) that the rate of PSII recovery depends upon accumulated  $[\text{PSII}_{\text{Inactive}}]$ , and that drops in  $F_V/F_M$  reflect drops in  $[\text{PSII}_{\text{Active}}]$  (Wu et al. 2012), mirrored by increases in  $[\text{PSII}_{\text{Inactive}}]$ . Although these fits generated good approximations of the experimental data, they contained surprises; the

apparent  $k_{\text{rec}}$  estimates were far higher than the measured  $k_{\text{PsbA}}$  (Table 1) for clearance of photoinactivated PsbA protein, particularly for *S. costatum*. Furthermore, during the LL recovery period, *S. costatum* showed an increase in its pool of PsbA protein (Fig. 1B, open squares), while *P. tricornutum* showed no increase in PsbA pool (Fig. 1D, open circles) even though  $F_V/F_M$  recovered over the same period (Fig. 1C, open circles). Since clearance of photoinactivated PsbA is a key step in the PSII repair cycle, we sought to reconcile these discrepancies.

The models stemming from (Kok 1956), including (Oliver et al. 2003; Lee et al. 2001) assume that at the start of the HL treatment the content of  $[\text{PSII}_{\text{Inactive}}]$  is negligible, so that the  $[\text{PSII}_{\text{Inactive}}]$  substrate for repair accumulates only during the HL treatment period, enabling the repair process parameterized by  $k_{\text{rec}}$  to start action upon the accumulating pool of  $[\text{PSII}_{\text{Inactive}}]$ . If the light level is not excessive, at some point, loss of  $[\text{PSII}_{\text{Active}}]$  and accumulation of  $[\text{PSII}_{\text{Inactive}}]$  will result in a steady state where:

$$[\text{PSII}_{\text{Active}}] \times k_{\text{pi}} = [\text{PSII}_{\text{Inactive}}] \times k_{\text{rec}}$$

In marine diatoms, this assumption of a negligible initial content of  $[\text{PSII}_{\text{Inactive}}]$  may not be generally true (Wu et al. 2011; Wu et al. 2012), since even under low light growth, the cells may retain a significant pool of PSII repair cycle intermediates awaiting recycling into  $[\text{PSII}_{\text{Active}}]$  (Campbell et al. 2013). In this case, the model fit generates an exaggerated estimate for  $k_{\text{rec}}$  because the initial  $[\text{PSII}_{\text{Inactive}}]$  is larger than assumed by the model. To test this possibility, we had a direct measure of  $k_{\text{pi}}$  ( $\text{s}^{-1}$ ) (Table 1) from our lincomycin treated cultures, and we had  $k_{\text{PsbA}}$  ( $\text{s}^{-1}$ ) (Table 1) for clearance of PsbA

protein, as a proxy for the rate-limiting step in the overall PSII repair cycle in diatoms (Campbell et al. 2013). Therefore, we rearranged the steady state equation to:

$$k_{\text{PsbA}} / k_{\text{pi}} = [\text{PSII}_{\text{Active}}] / [\text{PSII}_{\text{Inactive}}]$$

This allowed us to estimate  $[\text{PSII}_{\text{Active}}] / [\text{PSII}_{\text{Inactive}}]$  for the HL steady state condition (Table 1), which we reached within our 90 min incubation under  $450 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (Figure 1). We then used the measured drop in  $F_V/F_M$  as a proxy for the decline in  $[\text{PSII}_{\text{Active}}]$  (Vassiliev et al. 1994; Wu et al. 2012) (Fig. 1A, C, open symbols) from  $T_0$  to the steady state level, to backcast to the estimated  $[\text{PSII}_{\text{Active}}] / [\text{PSII}_{\text{Inactive}}]$  under growth LL conditions (Table 1), at 0.29 ( $[\text{PSII}_{\text{Active}}] \sim 22\%$  of  $\text{PSII}_{\text{Total}}$ ) for *S. costatum*, and 0.94 ( $[\text{PSII}_{\text{Active}}] \sim 48\%$  of  $\text{PSII}_{\text{Total}}$ ) for *P. tricornutum*. These estimates reconcile the apparent discrepancy that upon an upward shift in light *S. costatum* suffered a smaller drop in PSII function ( $F_V/F_M$ ), even though its capacity to clear PsbA protein was lower than in *P. tricornutum*. *S. costatum* started HL treatment with a larger initial reservoir of  $\text{PSII}_{\text{Inactive}}$ , awaiting reassembly into  $\text{PSII}_{\text{Active}}$ . This difference between the strains was consistent with the higher initial content of PsbA protein in *S. costatum* (Table 1), since our detection of PsbA protein includes both PsbA from  $\text{PSII}_{\text{Active}}$  and PsbA from PSII complexes that have been photoinactivated but which are awaiting disassembly (Campbell et al. 2013). In combination with similar findings from the diatoms *Thalassiosira pseudonana* and *Coscinodiscus radiatus* (Wu et al. 2011; Wu et al. 2012), we suggest this pattern of a reservoir of  $\text{PSII}_{\text{Inactive}}$  is a common feature buffering PSII dynamics in marine diatoms (Behrenfeld et al. 1998), particularly in strains such as *S. costatum* with, compared to *P. tricornutum*, a relatively limited capacity for non-photochemical dissipation of excess excitation energy within PSII, including NPQ and



PSII cyclic electron transfer (PSII CET) (Lavaud et al. 2004; Lavaud et al. 2007; Lavaud and Lepetit 2013).

In Figure 2, we present a conceptual summary of these arguments. The dotted lines define thresholds for the PSII repair capacity, measured as the  $k_{\text{PsbA}}$  ( $\text{s}^{-1}$ , Y axis) required to maintain a given steady state  $[\text{PSII}_{\text{Total}}] / [\text{PSII}_{\text{Active}}]$  cellular content (X axis), at a given rate constant  $k_{\text{pi}}$  ( $\text{s}^{-1}$ ) for photoinactivation provoked by light level, E:

$$k_{\text{PsbA}} / k_{\text{pi}} = [\text{PSII}_{\text{Active}}] / [\text{PSII}_{\text{Inactive}}]$$

$$k_{\text{PsbA}} = \{[\text{PSII}_{\text{Active}}] / [\text{PSII}_{\text{Inactive}}]\} \times k_{\text{pi}}$$

$$k_{\text{PsbA}} = \{[\text{PSII}_{\text{Active}}] / [\text{PSII}_{\text{Inactive}}]\} \times (\sigma_i \times E)$$

$$k_{\text{PsbA}} = \{([\text{PSII}_{\text{Total}}] / [\text{PSII}_{\text{Active}}]) - 1\}^{-1} \times (\sigma_i \times E)$$

The fine dotted line defines the threshold for cells when E is a low light of 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , near the bottom of the photic zone, or at dawn or dusk. Under low light, photoinactivation is slow, and the cells require only a low capacity to remove photoinactivated PsbA proteins ( $k_{\text{PsbA}}$ ) in the PSII repair cycle, in order to maintain a given  $[\text{PSII}_{\text{Total}}] / [\text{PSII}_{\text{Active}}]$ . The dashed line defines the threshold for cells under a moderately high light of 450  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , equivalent to the upper 30% of the photic zone. Under this high light the rate of photoinactivation is faster, and to maintain a steady  $[\text{PSII}_{\text{Active}}]$ , the cells need a higher capacity for PSII repair (increase in  $k_{\text{PsbA}}$  on the Y axis) or must accumulate a larger  $[\text{PSII}_{\text{Total}}]$  (shift to the right on the X axis). If the cells fall below the threshold, they suffer net photoinactivation of the PSII pool, driving  $[\text{PSII}_{\text{Total}}] / [\text{PSII}_{\text{Active}}]$  to the right until they reach or surpass the threshold. Alternately, cells below the threshold can induce more rapid PSII repair with a higher  $k_{\text{PsbA}}$ , or

possibly induce protective screening or dissipatory mechanisms (NPQ and/or PSII CET for instance) to increase their tolerance threshold by slowing the rate of PSII photoinactivation under a given light level. These threshold lines are related to  $E_{MAX}$  estimates (Neale et al. 2014) of the maximum tolerable irradiance before the onset of net photoinhibition.

Our measurement of  $k_{PsbA}$  was made upon cells treated under  $450 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , but grown under  $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . *S. costatum* (squares) achieved a  $k_{PsbA}$  of  $3.8 \times 10^{-5} \text{ s}^{-1}$  within 30-90 min of the shift to HL. For growth under LL, this  $k_{PsbA}$  combined with a  $[\text{PSII}_{Total}] / [\text{PSII}_{Active}]$  of  $\sim 4.5$  to place the cells well above their tolerance threshold (Fig. 2, fine dotted line), with ample reserve capacity to tolerate an upward fluctuation in light. Upon the shift to HL, *S. costatum* shifted towards a yet higher  $[\text{PSII}_{Total}] / [\text{PSII}_{Active}]$ , to move to the new threshold (Fig. 2, dashed line). *P. tricornutum* showed a much higher  $k_{PsbA}$  and a corresponding lower initial  $[\text{PSII}_{Total}] / [\text{PSII}_{Active}]$ , which shifted only slightly upwards upon the upward shift in light.

#### *Non-photochemical Chl fluorescence quenching (NPQ)*

In Figure 3 we present time courses for induction of NPQ. Figures 3A and 3C compare the development of NPQ, tracking down regulation of PSII relative to  $F_M$  measured for each time point after 5 min in the dark, prior to measurement of  $F_M'$  during re-exposure to the treatment light. *S. costatum* (Fig. 3A, open squares) showed a progressive induction of NPQ, with subsequent partial relaxation during LL recovery, consistent with a readily reversible NPQ mechanism. This induction was inhibited when chloroplastic protein

synthesis was inhibited by lincomycin (Fig. 3A, closed squares). *P. tricornutum* showed a higher initial NPQ as reported previously (Lavaud et al. 2004; Lavaud et al. 2007; Lavaud and Lepetit 2013), but no further increase over the course of the HL treatment. Instead, during subsequent LL recovery, *P. tricornutum* showed a large accumulation of NPQ (Fig. 3C, open circles). Again, inhibition of chloroplastic protein synthesis by lincomycin blocked NPQ induction (Fig. 3C, closed circles).

We also estimated  $\text{NPQ}_{\text{global}}$ , the cumulative global non-photochemical down regulation of PSII across the experiment time course by comparing  $F_M'$  measured under illumination at each time point, to the highest level of  $F_M$  achieved by the culture across the entire experiment time course. In the absence of lincomycin  $\text{NPQ}_{\text{global}}$  increased significantly during the HL incubation (Fig. 3B, D, open symbols) and relaxed during subsequent LL recovery, particularly in *S. costatum*. Inhibition of chloroplast protein synthesis by lincomycin blocked most (*S. costatum*; Fig. 3B, closed squares) or all (*P. tricornutum*, Fig. 3D, closed circles) of this light and time dependent increase. We are as yet unsure whether NPQ and  $\text{NPQ}_{\text{global}}$  have direct and specific dependencies upon active chloroplastic protein synthesis, or whether accumulation of  $\text{NPQ}_{\text{global}}$  is blocked as a secondary effect of the blockage of the PSII repair cycle by lincomycin. The two species both achieved comparable peak levels of  $\text{NPQ}_{\text{global}}$  after 60-90 min of higher light incubation, i.e. NPQ ~1.4, a value similar to previous reports for *P. tricornutum* but higher for *S. costatum* (Lavaud et al. 2007), likely due to a higher DT content (see Lavaud and Lepetit 2013). The NPQ induction was nevertheless significantly more sustained in *P. tricornutum* (Fig. 3C), both during and subsequent to HL incubation.

These patterns are consistent with the studies showing induction of sustained quenching centres under prolonged HL exposure in *P. tricornutum* (i.e. persistence of a higher number of Q1 NPQ sites due to the persistence of DT molecules in the PSII LHC antenna) (see Lavaud and Goss 2014).

$\sigma_{PSII}$ , the effective absorbance cross section serving PSII photochemistry

$\sigma_{PSII}$  measured in dark-adapted samples was initially similar in the two species as reported before under similar growth conditions, (Lavaud et al. 2007), at  $253 \text{ A}^2 \text{ quantum}^{-1}$  (95% C.I.  $\pm 5$ ) for *S. costatum* and  $267 \text{ A}^2 \text{ quantum}^{-1}$  (95% C.I.  $\pm 10$ ) for *P. tricornutum* (Table 1, Figure 4A, B, open symbols) when measured using the blue excitation LED of the FIRE fluorometer. When chloroplastic protein synthesis was running, the PSII repair cycle was operational, and both species were able to induce NPQ (Fig. 3), they held  $\sigma_{PSII}$  steady over the course of HL treatment and LL recovery, (Figure 4A, B, open symbols) even though *P. tricornutum* suffered a 25% drop in  $F_V/F_M$  and subsequent recovery over this time period.

In contrast, when lincomycin was added,  $\sigma_{PSII}$  increased significantly over the course of the HL treatment, with only variable decline during the subsequent LL recovery period. This increase was consistent with some antenna connectivity, so that as PSII centres were inactivated, their antenna capacity was left to increase the  $\sigma_{PSII}$  of the remaining PSII active centres. Interestingly, the connectivity parameter  $\rho$  extracted from the fluorescence induction curves used to estimate  $\sigma_{PSII}$  was near zero in *P. tricornutum* ( $0.02 \pm 0.03$ ) but significant in *S. costatum* ( $0.16 \pm 0.08$ ), consistent with the more effective control of excitation energy by NPQ in *P. tricornutum*, as long as PSII repair was active. The

increase in dark  $\sigma_{\text{PSII}}$  was also consistent with the inhibition of NPQ induction in the presence of lincomycin, so that in the absence of lincomycin, an increase in NPQ (Figure 3, open symbols) countered the tendency towards an increase in  $\sigma_{\text{PSII}}$  whereas in the presence of lincomycin, loss of NPQ capacity allows  $\sigma_{\text{PSII}}$  to increase excessively as PSII centres became photoinactivated.

After growth and treatment of *P. tricornutum* at higher lights Koblizek et al. (2001) found a strong correlation between induction of NPQ and a decrease in  $\sigma_{\text{PSII}}$  for red light. Their conditions of higher growth and treatment lights were sufficient to drive NPQ to  $\sim 1.7$  within 100 s, whereas our lower light cultures and treatments did not reach that level of NPQ within 3600 s of treatment. Koblizek et al. (2001) finding's are thus consistent with our works (Lavaud et al. 2002; Ruban et al. 2004) on the fast NPQ induction in *P. tricornutum* under higher light, where recent NPQ model (Chukhutsina et al. 2014; Lavaud and Goss 2014; Goss and Lepetit 2015) predict that detachment of FCP units from PSII (Q1 quenching site) should decrease  $\sigma_{\text{PSII}}$ .

Figure 5 summarizes these critical interactions among PSII repair, NPQ and control of excitation delivery to PSII under moderately high light. In *S. costatum* cells with some connectivity across the PSII antenna bed, inhibition of the PSII repair cycle and parallel limitation on the induction of NPQ (Fig. 5A, closed squares) suffered large increases in the  $\sigma_{\text{PSII}}$ ' measured under treatment irradiance. In *P. tricornutum*, with little connectivity across the PSII antenna bed, and higher basal NPQ, cells were able to maintain better control of  $\sigma_{\text{PSII}}$ ', even in the face of inhibition of the PSII repair cycle. We connect these patterns back to our estimates of  $[\text{PSII}_{\text{Total}}] / [\text{PSII}_{\text{Active}}]$ , showing that the general strong

NPQ capacities of *P. tricornutum* confer a lower-cost PSII repair cycle upon the cells (Fig. 2), since they can maintain their [PSII<sub>Active</sub>] with a smaller pool of [PSII<sub>Total</sub>], even in the face of upward fluctuations in light. Such feature fits very well with the adaptation of *P. tricornutum* to a highly fluctuating light climate regularly punctuated with excess light exposures as encountered in its original habitat (Lavaud et al. 2007; Lavaud and Lepetit 2013). This is in contrast to *S. costatum* which originates from a habitat where light changes are slower and of lower amplitude (Lavaud et al. 2007; Lavaud and Lepetit 2013). The present work further confirms the important role for the light-dependent fast regulation of photochemistry by NPQ in coordination with PsbA synthesis and PSII repair cycle capacity in the ecophysiology of both pennate and centric diatoms (Key et al. 2010; Wu et al. 2011; Wu et al. 2012). It supports the ecological diversity of diatoms as well as their ubiquity and ecological success in modern oceans (Armbrust 2009; Kooistra et al. 2007; Mock and Medlin 2012).

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1 **Table 1 Photosystem II (PSII) photoinactivation and repair parameters**

Species	$F_V/F_M$	$\sigma_{PSII}$ ( $A^2$ quantum <sup>1</sup> )	$ETR_{PSII}$ ( $e^- PSII^{-1} s^{-1}$ )	$k_{pi}$ ( $s^{-1}$ )	$\sigma_i$ ( $A^2$ quantum <sup>-1</sup> )	$K_{PsbA} (s^{-1})$	$k_{rec} (s^{-1})$	initial fmol PsbA ( $\mu g \text{ protein}^{-1}$ )	HL steady state [PSII <sub>Active</sub> ] / [PSII <sub>Inactive</sub> ] = $K_{PsbA} / k_{pi}$	Growth LL [PSII <sub>Active</sub> ] / [PSII <sub>Inactive</sub> ]
<i>Sc</i>	0.67 $\pm 0.03$	253 $\pm 5$	252 $\pm 25$	1.6 x 10 <sup>-4</sup> $\pm 3 \times 10^{-5}$	6.1 x 10 <sup>-5</sup> $\pm 1.1 \times 10^{-25}$	3.8 x 10 <sup>-5</sup> $\pm 2.7 \times 10^{-5}$	$\geq 2.1 \times 10^{-3}$	65 $\pm 19$	0.24	~0.29
<i>Pt</i>	0.67 $\pm 0.003$	267 $\pm 10$	201 $\pm 7$			1.1 x 10 <sup>-4</sup> $\pm 6.6 \times 10^{-5}$	5.4 x 10 <sup>-4</sup> $\pm 1.9 \times 10^{-4}$	38 $\pm 6.8$	0.69	~0.94

2

3 *Sc*, *Skeletonema costatum*; *Pt*, *Phaeodactylum tricornutum*;  $F_V/F_M$ , maximum quantum yield of PSII;  $\sigma_{PSII}$ , effective absorbance cross  
4 section serving PSII photochemistry;  $ETR_{PSII}$  PSII electron transport rate at 450  $\mu mol \text{ photons m}^{-2} s^{-1}$ ;  $k_{pi}$ , rate constant for  
5 photoinactivation of PSII at 450  $\mu mol \text{ photons m}^{-2} s^{-1}$ ;  $\sigma_i$ , effective absorbance cross section for photoinactivation of PSII;  $k_{psba}$ , rate  
6 constant for clearance of PsbA protein;  $k_{rec}$ , apparent Kok rate constant for recovery of PSII; HL, high light; LL, low light. Parameters  
7 are means of measures from three independent cultures,  $\pm 95\%$  C.I.

## 8 **Figure legends**

9 **Figure 1.** Photosystem II (PSII) function and protein dynamics. Maximum quantum yield  
10 of PSII photochemistry  $F_V/F_M$  (A, C) or PsbA protein content (B, D) *vs.* time under 450  
11 (0-90 min) or 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (90-270 min) in *Skeletonema costatum* (squares; A,  
12 B) or *Phaeodactylum tricornutum* (circles; C, D) cultures in the absence (open symbols,  
13 no lincomycin) or presence (closed symbols, with lincomycin) of PSII repair.  $n = 3$   
14 independent cultures  $\pm$  S.D. 95% confidence intervals on non-linear regression fits are  
15 shown with fine dotted lines.  $T_0$  values for  $F_V/F_M$  and fmol PsbA  $\mu\text{g protein}^{-1}$  are shown  
16 in Table 1.

17  
18 **Figure 2.** Rate constant,  $k_{\text{PsbA}}$  ( $\text{s}^{-1}$ ) for clearance of PsbA protein plotted *vs.* estimated  
19 ratio of  $[\text{PSII}_{\text{Total}}] / [\text{PSII}_{\text{Active}}]$ .  $k_{\text{PsbA}}$  was estimated from the single phase exponential  
20 curve fits of the decline in PsbA protein in the presence of lincomycin (Figs. 1B, D).  
21  $[\text{PSII}_{\text{Total}}] / [\text{PSII}_{\text{Active}}]$  was estimated from a simple kinetic model of the PSII repair  
22 cycle. *S. costatum* (squares, rightward arrow indicates shift from 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$   
23 growth LL up to HL-450  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) or *P. tricornutum* (circles). Dotted lines  
24 show the threshold between steady state (below line) maintenance of  $[\text{PSII}_{\text{Active}}]$  and net  
25 photoinhibition of PSII (above the line) for 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  growth LL while the  
26 dashed line shows the threshold to maintain a steady  $[\text{PSII}_{\text{Active}}]$  in the face of accelerated  
27 photoinactivation under 450  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  growth HL.

28  
29 **Figure 3.** Non-photochemical quenching-NPQ (A, C) and cumulative global NPQ-  
30  $\text{NPQ}_{\text{global}}$  (B, D) *vs.* time under 450 (0-90 min) or 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (90-270 min)



in *S. costatum* (squares, A, B) or *P. tricornutum* (circles, C, D) cultures in the absence (open symbols, no lincomycin) or presence (closed symbols, with lincomycin) of PSII repair. n = 3 independent cultures  $\pm$  S.D.

**Figure 4.** Effective absorbance cross section serving PSII photochemistry ( $\sigma_{\text{PSII}}$ ) vs. time under 450 (0-90 min) or 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (90-270 min) in *S. costatum* (squares, A) or *P. tricornutum* (circles, B) cultures in the absence (open symbols, no lincomycin) or presence (closed symbols, with lincomycin) of PSII repair. n = 3 independent cultures  $\pm$  S.D.

**Figure 5.** Effective absorbance cross section serving PSII photochemistry ( $\sigma_{\text{PSII}}$ ) measured under HL (450  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) vs. NPQ in *S. costatum* (squares, A) or *P. tricornutum* (circles, B) cultures in the absence (open symbols, no lincomycin) or presence (closed symbols, with lincomycin) of PSII repair. Individual paired data points plotted from n = 3 independent cultures.