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Combined effects of ocean acidification and warming on physiological response of the diatom *Thalassiosira pseudonana* to light challenges

Wubiao Yuan[a,1], Guang Gao[c,1], Qi Shi[a], Zhiguang Xu[b], Hongyan Wu[a,b,*]

[a] Hubei Provincial Cooperative Innovation Center of Industrial Fermentation, Hubei University of Technology, Wuhan 430068, China
[b] College of Life Science, Ludong University, Yantai 264025, China
[c] Marine Resources Development Institute of Jiangsu, Huaihai Institute of Technology, Lianyungang 222005, China

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ABSTRACT

Diatoms are one of the most important groups of phytoplankton in terms of abundance and ecological functionality in the ocean. They usually dominate the phytoplankton communities in coastal waters and experience frequent and large fluctuations in light. In order to evaluate the combined effects of ocean warming and acidification on the diatom's exploitation of variable light environments, we grew a globally abundant diatom *Thalassiosira pseudonana* under two levels of temperature (18, 24 °C) and pCO_2 (400, 1000 μatm) to examine its physiological performance after light challenge. It showed that the higher temperature increased the photoinactivation rate in *T. pseudonana* at 400 μatm pCO_2, while the higher pCO_2 alleviated the negative effect of the higher temperature on PSII photoinactivation. Higher pCO_2 stimulated much faster PsbA removal, but it still lagged behind the photoinactivation of PSII under high light. Although the sustained phase of nonphotochemical quenching (NPQs) and activity of superoxide dismutase (SOD) were provoked during the high light exposure in *T. pseudonana* under the combined pCO_2 and temperature conditions, it could not offset the damage caused by these multiple environmental changes, leading to decreased maximum photochemical yield.

1. Introduction

The ongoing anthropogenic emissions of carbon dioxide (CO_2) are expected to lead to the rise of CO_2 concentration in atmosphere to 800–1000 ppmv by the end of this century according to the "business as usual" CO_2 emission scenario (IPCC, 2001). During the past century, more than one third of the CO_2 released to the atmosphere has been absorbed by the ocean (Sabine et al., 2004), as a consequence, the carbonate buffer system in the surface ocean will be profoundly influenced (Wolf-Gladrow et al., 1999). Oceanic uptake of CO_2 will acidify the surface ocean by 0.3–0.4 pH units ($[H^+]$ increase by 100–150%) by the end of this century (Caldeira and Wickett, 2003). This decline in pH driven by increased CO_2 is termed ocean acidification (Doney et al., 2009). Ocean acidification is known to reduce calcification of coccolithophores (Beaufort et al., 2011; Riebesell and Tortell, 2011), downregulate the carbon concentrating mechanisms (CCMs) in diatoms (Li et al., 2014; Shi et al., 2017). A number of studies show stimulative effects on phytoplankton primary productivity (Hein and Sand-Jensen, 1997; Riebesell and Tortell, 2011), while unaffected (Boelen et al., 2011; Nielsen et al., 2012) and negative effects (Feng et al., 2009; Torstensson et al., 2012) of ocean acidification have also been observed.

The increasing levels of atmospheric CO_2 are concurrently driving ocean warming (Meehl et al., 2007). The global sea surface temperature increased at a rate of 0.121–0.124 °C per decade based on in situ data records from 1979 to 2012 (IPCC, 2013). The global ocean will continue to warm during the 21st century and the global mean sea surface temperature has been predicted to increase by 1.18–6.48 °C by the end of the 21st century (Meehl et al., 2007). The temperature rise will exert considerable direct and indirect influences on marine phytoplankton. Elevated temperature can affect the photosynthesis and growth of algae directly. For instance, a 6 °C increase of temperature increased the growth rate in *T. pseudonana* (Wu et al., 2012), and a 5 °C temperature rise increased photosynthesis and calcification of *Emiliania huxleyi* grown under Ca^{2+} sufficient conditions (Xu et al., 2011).

Diatoms are one of the most important groups of phytoplankton in terms of abundance and ecological functionality in the ocean. Marine diatoms carry out an estimated 40–45% of marine primary production, and thus play an integral role in the global carbon budget (Mann, 1999; Boyd and Hutchins, 2012). Marine diatoms usually dominate the phytoplankton assemblage particularly in turbulent coastal waters where they are exposed to frequent and large fluctuations in light due to fast

* Corresponding author. Hubei University of Technology, No.26 Nanli Road, Wuhan, 430068, China.
E-mail address: sdwuhongyan@126.com (H. Wu).
[1] Authors contributed equally to this work.

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vertical mixing through steep photic zone light gradients (Macintyre et al., 2000). Diatoms have developed interacting strategies to combat the potentially damaging effects of excess light. They can repair damaged PSII by replacing photo-damaged proteins in PSII with newly synthesized subunits (Wu et al., 2011; Komenda et al., 2012). Non-photochemical quenching (NPQ) is another important strategy of diatoms to cope with a sudden increase in irradiance (Lavaud et al., 2004; Lavaud and Lepetit, 2013). In addition, to scavenge reactive oxygen species (ROS) caused by excessive light diatoms have evolved an elaborate antioxidant network (Drábková et al., 2007; Li et al., 2015). For instance, the superoxide radical is converted enzymatically to H_2O_2 by superoxide dismutase (SOD).

Ocean warming and acidification are approaching simultaneously. However, little is known regarding the combined, interacting effects of these two global variables on photoinactivation, repair, and protection in diatoms. In this study we cultured the diatom *Thalassiosira pseudonana* under current and future temperature and pCO_2 conditions, and monitored its physiological performance after light challenge, to understand how ocean warming and acidification interact on the strong capacities of diatoms to exploit variable light.

2. Materials and methods

2.1. Culture conditions and seawater carbonate system

Thalassiosira pseudonana (CCMP1335) was obtained from the National Center for Marine Algae and Microbiota (NCMA). Cells were grown semi-continuously in F/2 medium (Guillard and Ryther, 1962), based on artificial seawater prepared according to Morel et al. (1979). The cultures were diluted by using pre-CO_2-equilibrated medium every 24 h, and the density was maintained at less than 10 μg chlorophyll *a* L^{-1}, so that the seawater carbonate chemistry parameters were stable (Table 1) with pH variations < 0.05 units. Two levels of pCO_2 (400, 1000 μatm) and temperature (18 °C, 24 °C) were imposed in the plant growth CO_2 chambers (HP 1000G-D, Ruihua, China). Cultures in the growth chamber were illuminated at 100 μmol photons m^{-2} s^{-1} provided by cool white fluorescent tubes with a 12h: 12h light: dark cycle. All cultures were grown through at least seven transfers of semi-continuous dilution to obtain steady growth rates under the given culture conditions to ensure full acclimation before use in subsequent experiments. There were four replicates under each treatment.

pH changes in the medium were determined with a pH meter (SevenMulti S40K, Mettler-Toledo) which was calibrated with National Bureau of Standard (NBS) buffers of pH 7.0 and 10.0 (Sigma-Aldrich). Dissolved inorganic carbon (DIC) was measured by using a Shimadzu Total Organic Carbon Analyzer (TOC-5000A, Japan) and total alkalinity was measured by potentiomentric titration. Subsequently, other parameters of the carbonate system were derived with CO_2SYS software (Pierrot et al., 2006) based on the known values of DIC, pH, salinity, alkalinity and temperature.

2.2. Upward light shift and recovery experiment

Culture replicates were split into two flasks and one was supplemented with a final concentration of 500 μg mL^{-1} lincomycin to block chloroplast protein synthesis (Bachmann et al., 2004), thereby inhibiting PSII repair (Tyystjärvi and Aro, 1996; Wu et al., 2012). Both flasks were placed in the dark for 10 min to allow the lincomycin to exert its effect and then exposed to fluorescent lamps (Philips, 21 W) at an intensity of 800 μmol photons m^{-2} s^{-1} for 120 min. Samples were collected prior to the onset of high light (plotted as time 0) and then after 15, 30, 60, 90, and 120 min exposure for chlorophyll fluorescence analyses and also for protein immunoblotting. After the high-light treatment, the remaining culture was returned to their growth light of 100 μmol photons m^{-2} s^{-1} for a 30-min recovery period followed by the final sampling and measurement.

2.3. Chlorophyll fluorescence measurement and parameterization

We used a portable pulse amplitude modulated fluorometer (WATER-PAM, Walz, Germany) to determine the chlorophyll fluorescence. At each sampling point, the samples were dark adapted for 5 min first to relax photosynthetic activity. The maximal efficiency of PSII photochemistry was determined as the ratio of variable to maximal chlorophyll fluorescence (F_V/F_M), where $F_V = (F_M - F_0)$, F_M and F_0 were the maximal and minimal fluorescence yield, respectively, of a dark-adapted suspension. F_0 was measured by using modulated measuring light (< 0.1 μmol m^{-2} s^{-1}) and F_M was determined at a 0.5 s saturating pulse of 4000 μmol m^{-2} s^{-1} in dark-adapted cells.

A sustained phase of NPQ, NPQs, which was induced over the course of the high-light treatment and persisted through the 5-min dark acclimation period just before measurement (Wu et al., 2012), was estimated as:

$$NPQs = (F_{Mt0} - F_M) / F_M.$$

F_{Mt0} is the measurement of F_M from dark-acclimated cells, taken at time T_0 just before the start of high-light treatment. F_M was taken at each measurement time point after 5 min dark relaxation. By definition, NPQs thus starts from 0 at T_0, and increases if the cells accumulate a sustained phase of NPQ (Lavaud et al., 2004). NPQs reflects an inducible 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 following the high light exposure.

A functional absorption cross-section driving the photoinactivation of PSII (σ_i, $Å^2$·quanta^{-1}) was estimated by plotting a single phase exponential decay through a plot of F_V/F_M (Campbell and Tyystjärvi, 2012), corrected for any influence of sustained non-photochemical quenching, versus the cumulative photons (quanta·$Å^{-2}$) applied during the 800 μmol photons m^{-2} s^{-1} light treatment. Multiplying σ_i by the applied photons $Å^{-2}$ s^{-1} generated a rate constant for photoinactivation (k_{pi}, s^{-1}) for the particular applied light level (Kok, 1956). The apparent rate constant for PSII repair (K_{rec}, s^{-1}) was estimated according to Campbell and Tyystjärvi (2012).

2.4. SDS-PAGE analysis and western blot

At each sampling point cells were harvested on glass fiber filters (25 mm diameter, binder-free glass fiber, Whatman), which were immediately flash frozen in liquid nitrogen and stored at -80 °C until later protein analyses. Total proteins were extracted by two thawing/

Table 1
Parameters of the seawater carbonate system in different cultures. DIC = dissolved inorganic carbon, TA = total alkalinity. Data are the means ± SE (n = 4). Different superscript letters represent significant differences ($P < 0.05$) among cultures.

Species	Temperature (°C)	pCO_2 (μatm)	pH_{NBS}	DIC (μmol kg^{-1})	HCO_3^- (μmol kg^{-1})	CO_3^{2-} (μmol kg^{-1})	CO_2 (μmol kg^{-1})	TA (μmol kg^{-1})
Thalassiosira pseudonana	18	386 ± 17 ^{a}	8.21 ± 0.01^b	2152 ± 66^a	1945 ± 60^a	194 ± 8^c	13.2 ± 0.6^b	2436 ± 72^a
	24	991 ± 23^b	7.85 ± 0.01^a	2338 ± 40^b	2216 ± 37^b	98 ± 3^a	33.8 ± 0.8^d	2469 ± 42^a
		376 ± 20 ^{a}	8.23 ± 0.02^b	2132 ± 74^a	1881 ± 65^a	240 ± 13^d	10.9 ± 0.6^a	2482 ± 86^a
		970 ± 48^b	7.88 ± 0.02^a	2319 ± 79^b	2168 ± 73^b	124 ± 7^b	28.2 ± 1.4^c	2481 ± 84^a

sonicating rounds in denaturing extraction buffer (Brown et al., 2008). The total protein concentration was determined using a Lowry protein assay kit (Bio-Rad DC Assay). Western blots were performed for PsbA, a core reaction center protein in Photosystem II. Two μg of total protein was loaded on 6%–12% polyacrylamide gel. Molar levels of PsbA (www.agrisera.se antibody AS05084; standard: AS01016S) were then determined with quantitative immunoblotting (Wu et al., 2011).

We estimated a rate constant for the clearance of PsbA protein by plotting fmol PsbA μg total protein^{-1}, for cells incubated under the 800 μmol photons m^{-2} s^{-1} treatment in the presence of lincomycin to block the counteracting synthesis of PsbA through chloroplast translation. We fit this PsbA plot with a single-phase exponential decay over the period from 15 to 120 min of high-light incubation. This k_{PsbA} rate constant reflects the capacity for removal of PsbA protein from the PSII pool (Wu et al., 2012).

2.5. Assay of superoxide dismutase (SOD) activity

At each sampling point, cells were collected onto a polycarbonate membrane (0.22 μm, Whatman) and washed into a 1 mL centrifuge tube with phosphate buffer (pH 7.6). The enzyme extractions were carried out in 0.6 mL phosphate buffer (pH 7.6) that contained 50 mM KH_2PO_4, 1 mM ethylenediaminetetraaceticacid (EDTA), 0.1% Triton X-100 and 1% (w/v) polyvinylpolypyrrolidone. Afterwards the homogenized extract was centrifuged at 12000 *g* (4 °C) for 10 min, SOD activities were tested by using SOD Assay Kits (Nanjing Jiancheng Biological Engineering Company, China). One unit of SOD activity was defined as the amount of enzyme which resulted in a 50% inhibition of the rate of nitro-blue tetrazolium reduction at 560 nm.

2.6. Statistical analysis

Results are expressed as means of replicates ± standard error. Data were statistically analyzed using the software SPSS v.21. The data under every treatment conformed to a normal distribution (Shapiro-Wilk, $P > 0.05$) and the variances could be considered equal (Levene's test, $P > 0.05$). Two-way multivariate ANOVAs (MANOVAs) were conducted to assess the effects of temperature and pCO_2 on carbonate parameters in cultures. Tukey HSD was conducted for *post hoc* investigation. Repeated measures ANOVAs (RM-ANOVAs) were conducted to analyze the effect of exposure time on F_V/F_M, PsbA content, NPQs and SOD activity. Bonferroni post-tests were conducted for *post hoc* investigation. Two-way MANOVAs were used to analyze the effects of temperature, pCO_2 and species on F_V/F_M, PsbA content, NPQs and SOD activity in *T. pseudonana* at different exposure times. Two-way ANOVAs were used to analyze the effects of temperature, pCO_2 and species on photoninactivation rate constant and PsbA removal rate constant in *T. pseudonana*. Significance was determined at $P < 0.05$ for all tests.

3. Results

The carbonate system in the 1000 μatm pCO_2-grown cultures differed from that of 400 μatm pCO_2-grown cultures for both 18 °C and 24 °C (Table 1); DIC, HCO_3^- and CO_2 increased ($F_{(1,12)} > 85.214$, $P < 0.001$), CO_3^{2-} decreased ($F_{(1,12)} > 601.139$, $P < 0.001$) while total alkalinity was not significantly changed ($F_{(1,12)} < 0.193$, $P > 0.638$).

F_V/F_M in *T. pseudonana* grown under all conditions decreased with exposure time when the cells shifted from low light (100 μmol photons m^{-2} s^{-1}) to high light (800 μmol photons m^{-2} s^{-1}) ($F_{(5,60)} = 3736.875$, $P < 0.001$), even when PSII repair was active (white symbols, Fig. 1, $F_{(5,60)} = 443.873$, $P < 0.001$). The F_V/F_M at 24 °C and 1000 μatm for *T. pseudonana* was 48 ± 2% of the initial value. Temperature ($F_{(1,12)} < 1.198$, $P > 0.294$) or pCO_2 ($F_{(1,12)} < 2.182$, $P > 0.165$) did not affect the patterns of F_V/F_M in *T. pseudonana* during the high light shift. When lincomycin was added to block the PSII repair cycle (black symbols), a more severe decline was detected in F_V/F_M under each condition ($F_{(1,24)} = 6038.455$, $P < 0.001$). After the recovery at low light for 30 min, the F_V/F_M rose to 87.8–90.0% of the initial value in *T. pseudonana* ($F_{(1,12)} = 1474.278$, $P < 0.001$) in the absence of lincomycin.

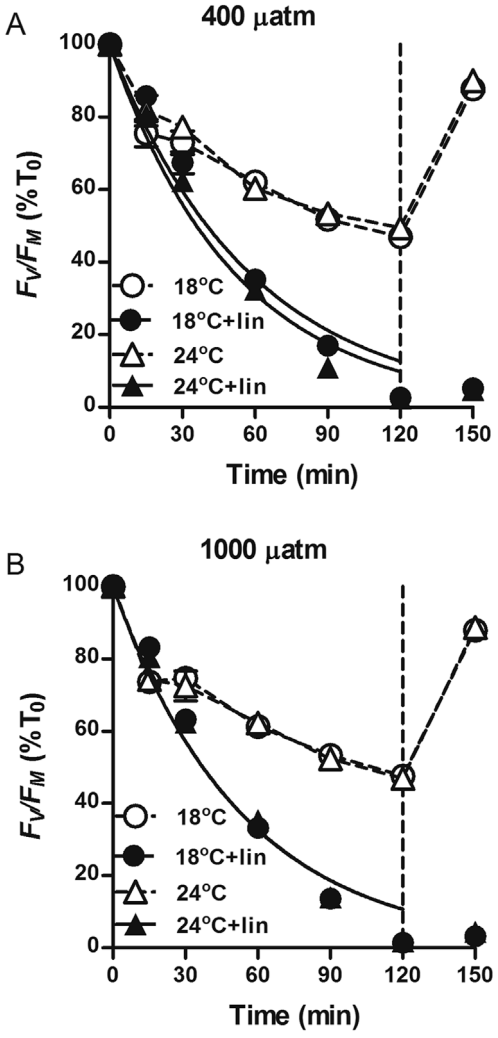

Fig. 1. Responses of PSII maximum photochemical yield (F_V/F_M) versus exposure time to high light in *Thalassiosira pseudonana* (A, B) cultures treated with (black symbols) or without (white symbols) the chloroplast protein synthesis inhibitor lincomycin. Cells were grown under two levels of temperature (18, 24 °C) and pCO_2 (400, 1000 μatm) at the light intensity of 100 μmol photons m^{-2} s^{-1}, then exposed to 800 μmol photons m^{-2} s^{-1} high light for 120 min, and then allowed to recover at 100 μmol photons m^{-2} s^{-1} for 30 min. The solid line shows the exponential decay fit of the F_V/F_M in the lincomycin-treated samples versus time. Data are the means ± SE (n = 4), most error bars within symbols.

After shifting to high light, in cells with ongoing PSII repair (white symbols) there was a slow decrease of PsbA content in *T. pseudonana* with exposure time ($F_{(5,60)} = 55.471$, $P < 0.001$, Fig. 2). The higher temperature alleviated the decline in PsbA ($F_{(1,12)} = 20.492$, $P = 0.001$) while higher pCO_2 led to a larger decline in comparison to 18 °C and 400 μatm ($F_{(1,12)} = 44.885$, $P < 0.001$). The addition of lincomycin resulted in a noticeable decrease of PsbA during the high light exposure ($F_{(1,24)} = 3398.056$, $P < 0.001$). PsbA content showed a small recovery for the samples grown at 24 °C and 1000 μatm pCO_2 after being shifted to low light for 30 min in the absence of lincomycin.

The photoinactivation rate constant (K_{pi}) in *T. pseudonana* (Fig. 3A) grown at different conditions was estimated by fitting a single-phase exponential decay to the F_V/F_M of the lincomycin-treated samples

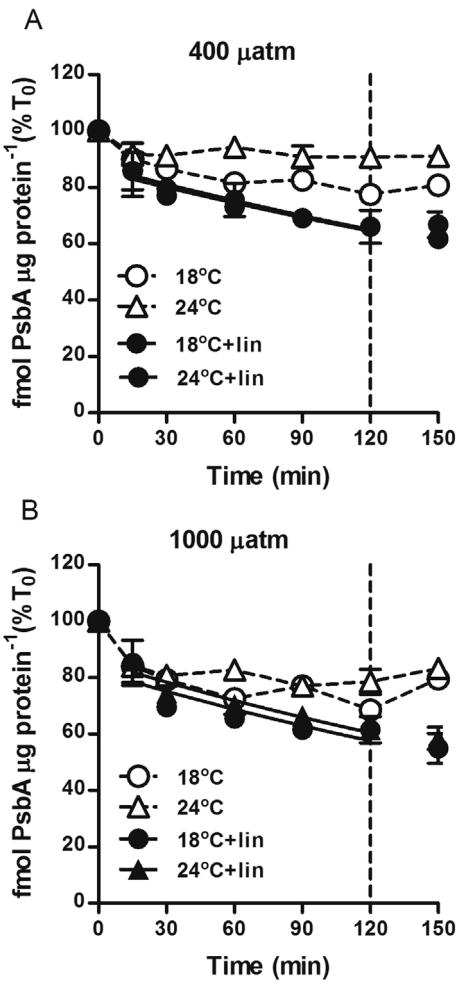

Fig. 2. Changes in PsbA protein content in *T. pseudonana* (A, B) cultures treated with (black symbols) or without (white symbols) the chloroplast protein synthesis inhibitor lincomycin. Cells were grown under two levels of temperature (18, 24 °C) and pCO_2 (400, 1000 µatm) at the light intensity of 100 µmol photons m^{-2} s^{-1}, then exposed to 800 µmol photons m^{-2} s^{-1} high light for 120 min, and then allowed to recover at 100 µmol photons m^{-2} s^{-1} for 30 min. The solid line shows the exponential decay fit of the PsbA content in the lincomycin-treated samples versus time. Data are the means ± SE (n = 4).

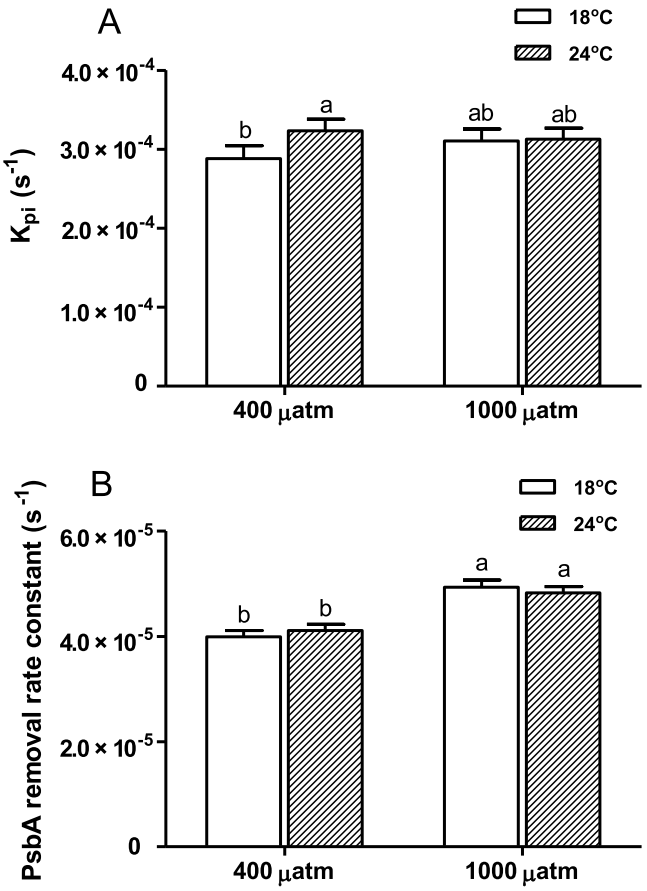

Fig. 3. Photoinactivation rate constant (K_{pi}, s^{-1}) (A) and PsbA removal rate constants (K_{PsbA}, s^{-1}) (B) in *T. pseudonana* grown under two levels of temperature (18, 24 °C) and pCO_2 (400, 1000 µatm) with the light intensity of 100 µmol photons m^{-2} s^{-1}. Rate constants were estimated from samples taken during 120 min of exposure to 800 µmol photons m^{-2} s^{-1} light intensity. Data are the means ± SE (n = 4). Different superscript letters indicate significance differences (Tukey HSD, $P < 0.05$) in K_{pi} or in K_{PsbA} among treatments.

(Fig. 1) after correction for the influence of sustained NPQ which relaxed during the final 30 min low light period, in the presence of lincomycin. Temperature and pCO_2 interacted ($F_{(1,12)} = 6.848$, $P = 0.023$) on K_{pi} and temperature had a main effect ($F_{(1,12)} = 5.040$, $P = 0.044$). The higher temperature increased K_{pi} at 400 µatm pCO_2 but did not affect it at 1000 µatm pCO_2, indicating that higher pCO_2 ameliorated the effect of the higher temperature on K_{pi}.

The PsbA removal rate constants (K_{PsbA}) in *T. pseudonana* cultured with varying temperature and pCO_2 treatments were also investigated (Fig. 3B). Temperature did not significantly affect the K_{PsbA} ($F_{(1,12)} < 0.001$, $P = 1.000$) but 1000 µatm pCO_2 significantly increased K_{PsbA} ($F_{(1,12)} = 44.741$, $P = 1.000$) compared to 400 µatm pCO_2.

The NPQs increased with high light exposure time ($F_{(5,60)} = 52.130$, $P < 0.001$, Fig. 4). After 120 min exposure, the high temperature decreased NPQs in *T. pseudonana* ($F_{(1,12)} = 6.208$, $P = 0.028$) compared to the low temperature. The addition of lincomycin led to larger increases in NPQs ($F_{(1,24)} = 36.342$, $P < 0.001$). During subsequent 30 min low light exposure, the NPQs in culture without lincomycin decreased to nearly zero but did not relax in the presence of lincomycin, suggesting different underlying mechanisms for the accumulation of NPQs in the presence of lincomycin compared to the absence of lincomycin.

In Fig. 5A we plotted the K_{PsbA} versus K_{pi}, for cells treated with lincomycin to block counteracting repair processes. Photoinactivation consistently outran the removal of PsbA. Although 1000 µatm CO_2 greatly improved K_{PsbA} ($t_{(30)} = -2.166$, $P < 0.001$), it still lagged behind K_{pi}. The plot of the apparent PSII repair rate constant (K_{rec}) versus K_{PsbA} (Fig. 5B) showed that functional recovery of PSII greatly exceeded K_{PsbA}. The stimulation of higher CO_2 on removal of PsbA was not enough to catch up with apparent K_{rec}.

After the shift to high light, the activity of SOD increased with exposure time for *T. pseudonana* grown under different conditions ($F_{(5,60)} = 8.173$, $P < 0.001$, Fig. 6). Temperature interacted with exposure time to affect SOD activity ($F_{(5,60)} = 2.492$, $P = 0.045$). Specifically, the stimulating effect of the higher temperature shrank with exposure time and it became insignificant at the end of 120 min of high light expsosure ($F_{(1,12)} = 2.955$, $P = 0.111$). The 30 min low light recovery period did not statistically decrease the activity of SOD ($F_{(1,12)} = 3.814$, $P = 0.075$).

4. Discussion

The PSII quantum yield (F_V/F_M) in *T. pseudonana* cells grown at all conditions decreased with exposure time when they were transferred from growth light to high light, indicating the inhibitive effect of high light on photosynthesis. Turnover of PsbA protein is required for PSII repair and restoration of PSII photochemical activity after photoinactivation (Aro et al., 1993; Edelman and Mattoo, 2008; Komenda

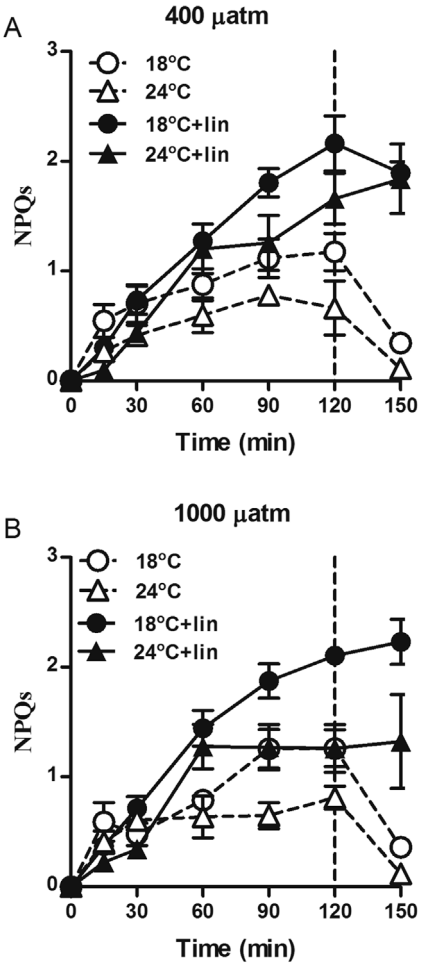

Fig. 4. Responses of sustained NPQ (NPQs) versus exposure time of high light in *T. pseudonana* (A, B) cultures treated with (black symbols) or without (white symbols) the chloroplast protein synthesis inhibitor lincomycin. Cells grown under two levels of temperature (18, 24 °C) and pCO_2 (400, 1000 µatm) with the light intensity of 100 µmol photons m^{-2} s^{-1} were exposed to 800 µmol photons m^{-2} s^{-1} high light for 120 min, and then allowed to recover at 100 µmol photons m^{-2} s^{-1} for 30 min. Data are the means ± SE (n = 4).

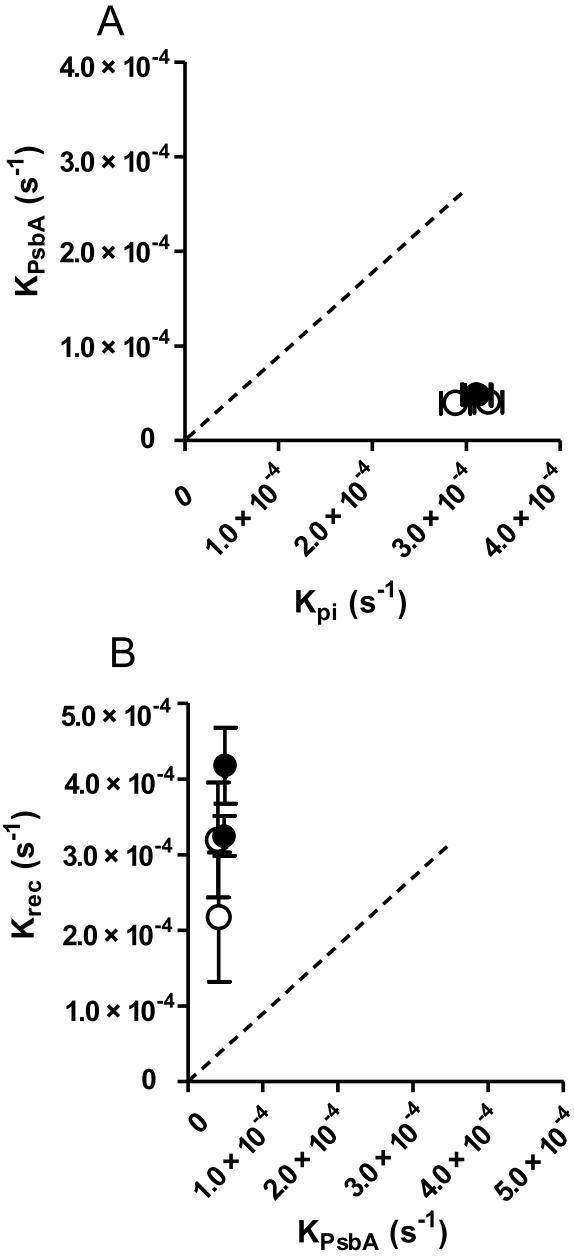

Fig. 5. Correlation of K_{PsbA} (s^{-1}) and K_{pi} (s^{-1}) (A), and correlation of apparent PSII repair rate constant (K_{rec} s^{-1}) and K_{pi} (s^{-1}) (B) for *T. pseudonana* cells grown under two levels of temperature (18, 24 °C) and pCO_2 (400 µatm, white symbols; 1000 µatm, black symbols). Rate constants were estimated from samples taken during 120 min exposure to 800 µmol photons m^{-2} s^{-1} light. Dotted line indicates 1:1 ratio. Data are the means ± SE (n = 4).

et al., 2012). We found that the pool of PsbA in *T. pseudonana* decreased during the high-light exposure but the decline in PsbA was much smaller than the decline of F_V/F_M when the repair cycle was active. These results indicate that maintenance of the PsbA protein pool did not alone suffice for these cells to maintain their pool of active PSII (Wu et al., 2011). When PSII repair is blocked by lincomycin in *T. pseudonana* the upward light shift provoke photoinacitivation of PSII to a larger extent as compared with that in cells without lincomycin. A larger decline was found in both F_V/F_M and in pools of PsbA, showing the critical role of PsbA synthesis in repair and maintenance of active PSII.

Cells grown at the higher temperature suffered a higher photoinactivation rate constant when pCO_2 is 400 µatm. However, the high temperature did not significantly increase photoinactivation rate when cells were grown at 1000 µatm pCO_2, indicating that the negative effect of the higher temperature upon PSII activity was somewhat alleviated by higher pCO_2 in the present study. This could be attributed to the accelerated net clearance of PsbA from a pool of photoinactivated PSII centers at the higher pCO_2. Our previous study with *T. pseudonana* showed that PSI activity in cells grown under high pCO_2 could be enhanced to support an increase in ATP synthesis by cyclic electron transfer (Shi et al., 2017). The proteolytic removal of PsbA subunits by the membrane-bound FtsH protease requires ATP (Nagao et al., 2012; Campbell et al., 2013), thus the extra ATP generation might support faster removal of PsbA under high pCO_2 conditions. Although growth under high pCO_2 stimulated the removal of PsbA, it still lagged behind the photoinactivation of PSII under high light, indicating that cells are building up a growing pool of inactivated PsbA during the high light treatment.

The exposure of phytoplankton cells to high irradiances requires the dissipation of excess energy to prevent damage of PSII (Gao et al., 2018). After the upward light shift, NPQs in the cells increased with exposure time, suggesting the activation of sustained photoprotection mechanisms to function against excess light energy. The diatoms induce NPQs when photoinactivation outruns removal of PsbA protein (Wu et al., 2012a), *T. pseudonana* showed a two-phase response to light shifts in this study, with initial acceleration of PSII repair followed by

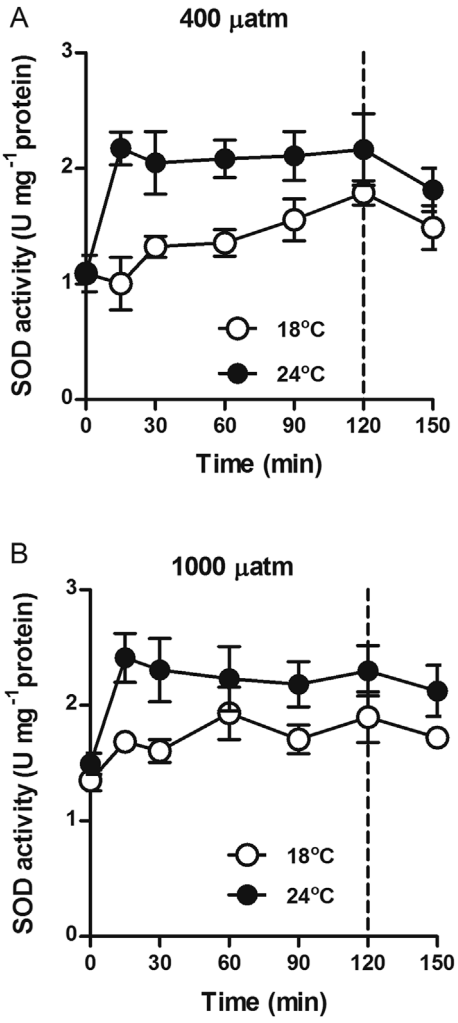

Fig. 6. Changes in superoxide dismutase (SOD) activity in *T. pseudonana* (A, B). Cells were grown under two levels of temperature (18, 24 °C) and pCO_2 (400, 1000 μatm) under a light intensity of 100 μmol photons m^{-2} s^{-1}, then exposed to 800 μmol photons m^{-2} s^{-1} high light for 120 min, and then allowed to recover at 100 μmol photons m^{-2} s^{-1} for 30 min. Data are the means ± SE (n = 4).

induction of NPQs. The increase was larger with the addition of lincomycin, indicating that NPQs plays a more important role in maintaining PSII activity or limiting photoinactivation when PSII repair is blocked. It is worth noting that NPQ is mostly related to xanthophyll cycle pigments, which is unlikely to be affected by lincomycin theoretically. The apparently large NPQs values in the presence of lincomycin may be due to the mixup of sustained NPQ with photoinhibitory damage that was enhanced by lincomycin. *T. pseudonana* grown at 18 °C showed more reliance on induction of NPQs at both pCO_2 conditions to cope with a less-effective maintenance of PsbA pools as compared with that at 24 °C. It was reported that the induction of NPQs is consistent with the kinetics of induction of expression of the Lhcx6, Lhcx4 and Lhcx1 transcripts in *T. pseudonana* (Zhu and Green, 2010), diatoms might possess overlapping means to dissipate excessive light energy.

Apart from NPQ, in the present study, high light exposure stimulated the activity of SOD in *T. pseudonana*. Activation of SOD, which converts the superoxide radical to H_2O_2, is commonly used to assess the antioxidative defence (Butow et al., 1997; Rijstenbil, 2001). Cells grown at the higher temperature produced higher induction of SOD activity upon the upward light. This finding would imply that high temperature enhanced the cell's scavenging capacities to excess light treatment and thus help protect PSII repair against ROS. Therefore, the increased activity of SOD induced by the higher light and temperature might partially explain the higher K_{rec} compared to K_{psbA}. In contrast to light and temperature, pCO_2 did not affect the activity of SOD, suggesting that the capacity to detoxify superoxide was not affected by the ocean acidification.

Planktonic diatoms, such as *T. pseudonana*, are likely to be subject to rapid changes in irradiance when present in a mixed water column (Ross et al., 2008; Van de Poll and Buma, 2009). Our findings indicate that ocean warming will increase the photoinactivation in *T. pseudonana* when it suffers a high light challenge. Ocean acidification could help alleviate the negative effect of ocean warming through increasing the PsbA removal rate. Although NPQ and SOD activity were stimulated upon upward light, it was not enough to fully offset the damage caused by warming and high light exposure.

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References

Aro, E.-M., McCaffery, S., Anderson, J.M., 1993. Photoinhibition and D1 protein degradation in peas acclimated to different growth irradiances. Plant Physiol 103, 835–843.
Bachmann, K.M., Ebbert, V., Adams Iii, W.W., Verhoeven, A.S., Logan, B.A., Demmig-Adams, B., 2004. Effects of lincomycin on PSII efficiency, non-photochemical quenching, D1 protein and xanthophyll cycle during photoinhibition and recovery. Funct Plant Biol 31, 803–813.
Beaufort, L., Probert, I., De Garidel, T., Bendif, E.M., Ruiz-Pino, D., Metzl, N., Goyet, C., Buchet, N., Coupel, P., Grelaud, M., Rost, B., Rickaby, R.E.M., De Vargas, C., 2011. Sensitivity of coccolithophores to carbonate chemistry and ocean acidification. Nature 476, 80–83.
Boelen, P., van de Poll, W.H., van der Strate, H.J., Neven, I.A., Beardall, J., Buma, A.G., 2011. Neither elevated nor reduced CO_2 affects the photophysiological performance of the marine Antarctic diatom *Chaetoceros brevis*. J Exp Mar Biol Ecol 406, 38–45.
Boyd, P.W., Hutchins, D.A., 2012. Understanding the responses of ocean biota to a complex matrix of cumulative anthropogenic change. Mar Ecol Prog Ser 470, 125–135.
Brown, C.M., MacKinnon, J.D., Cockshutt, A.M., Villareal, T.A., Campbell, D.A., 2008. Flux capacities and acclimation costs in *Trichodesmium* from the Gulf of Mexico. Mar Biol 154, 413–422.
Butow, B.J., Wynne, D., Tel-Or, E., 1997. Superoxide dismutase activity in *Peridinium gatunense* in Lake Kinneret: effect of light regime and carbon dioxide concentration. J. Phycol. 33, 787–793.
Caldeira, K., Wickett, M.E., 2003. Oceanography: anthropogenic carbon and ocean pH. Nature 425 365–365.
Campbell, D.A., Hossain, Z., Cockshutt, A.M., Zhaxybayeva, O., Wu, H., Li, G., 2013. Photosystem II protein clearance and FtsH function in the diatom *Thalassiosira pseudonana*. Photosynth. Res. 115, 43–54.
Campbell, D.A., Tyystjärvi, E., 2012. Parameterization of photosystem II photoinactivation and repair. Biochim. Biophys. Acta 1817, 258–265.
Doney, S.C., Fabry, V.J., Feely, R.A., Kleypas, J.A., 2009. Ocean acidification: the other CO_2 problem. Annu. Rev. Mater. Sci. 1, 169–192.
Drábková, M., Admiraal, W., Marsálek, B., 2007. Combined exposure to hydrogen peroxide and light-selective effects on cyanobacteria, green algae, and diatoms. Environ. Sci. Technol. 41, 309–314.
Edelman, M., Mattoo, A.K., 2008. D1-protein dynamics in photosystem II: the lingering enigma. Photosynth. Res. 98, 609–620.
Feng, Y., Hare, C.E., Leblanc, K., Rose, J.M., Zhang, Y., Ditullio, G.R., Lee, P.A., Wilhelm, S.W., Rowe, J.M., Sun, J., Nemcek, N., Gueguen, C., Passow, U., Benner, I., Brown, C., Hutchins, D.A., 2009. Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. I. The phytoplankton community and biogeochemical response. Mar Ecol Prog Ser 388, 13–25.
Gao, G., Shi, Q., Xu, Z., Xu, J., Campbell, D.A., Wu, H., 2018. Global warming interacts with ocean acidification to alter PSII function and protection in the diatom *Thalassiosira weissflogii*. Environ. Exp. Bot. 147, 95–103.
Guillard, R.R., Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (cleve) Gran. Can. J. Microbiol. 8, 229–239.
Hein, M., Sand-Jensen, K., 1997. CO_2 increases oceanic primary production. Nature 388, 526–527.
IPCC, 2001. Climate Change 2001: the Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 881.
IPCC, 2013. Climate change 2013: the physical science basis. In: Stocker, T.F., Qin, D.,

Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge Univ Press, New York, pp. 6–10.
Kok, B., 1956. On the inhibition of photosynthesis by intense light. Biochim. Biophys. Acta 21, 234–244.
Komenda, J., Sobotka, R., Nixon, P.J., 2012. Assembling and maintaining the Photosystem II complex in chloroplasts and cyanobacteria. Curr. Opin. Plant Biol. 15, 245–251.
Lavaud, J., Lepetit, B., 2013. An explanation for the inter-species variability of the photoprotective non-photochemical chlorophyll fluorescence quenching in diatoms. Biochim. Biophys. Acta 1827, 294–302.
Lavaud, J., Rousseau, B., Etienne, A.L., 2004. General features of photoprotection by energy dissipation in planktonic diatoms (Bacillariophyceae). J. Phycol. 40, 130–137.
Li, W., Gao, K., Beardall, J., 2015. Nitrate limitation and ocean acidification interact with UV-B to reduce photosynthetic performance in the diatom *Phaeodactylum tricornutum*. Biogeosciences 12, 2383–2393.
Li, Y., Xu, J., Gao, K., 2014. Light-modulated response of growth and photosynthetic performance to ocean acidification in the model iatom Phaeodactylum tricornutum. PLoS One 9, de96173.
Macintyre, H.L., Kana, T.M., Geider, R.J., 2000. The effect of water motion on short-term rates of photosynthesis by marine phytoplankton. Trends Plant Sci. 5, 12–17.
Mann, D.G., 1999. The species concept in diatoms. Phycologia 38, 437–495.
Meehl, G., Stocker, T., Collins, W., 2007. Global climate projections. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K., Tignor, M., Miller, H. (Eds.), Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth 16 Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, pp. 747–845.
Morel, F.M.M., Rueter, J.G., Anderson, D.M., Rrl, G., 1979. Aquil: a chemically defined phytoplankton culture medium for trace metal studies. J. Phycol. 15, 135–141.
Nagao, R., Tomo, T., Noguchi, E., Suzuki, T., Okumura, A., Narikawa, R., Enami, I., Ikeuchi, M., 2012. Proteases are associated with a minor fucoxanthin chlorophyll a/c-binding protein from the diatom, *Chaetoceros gracilis*. Biochim. Biophys. Acta 1817, 2110–2117.
Nielsen, L.T., Hallegraeff, G.M., Wright, S.W., Hansen, P.J., 2012. Effects of experimental seawater acidification on an estuarine plankton community. Aquat. Microb. Ecol. 65, 271–285.
Pierrot, D., Lewis, E., Wallace, D.W.R., 2006. MS Excel Program Developed for CO2 System Calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee.
Riebesell, U., Tortell, P.D., 2011. Effects of ocean acidification on pelagic organisms and ecosystems. In: Gattuso, J.P., Hansson, L. (Eds.), Ocean Acidification. Oxford University Press, UK, pp. 99–116.
Rijstenbil, J.W., 2001. Effects of periodic, low UVA radiation on cell characteristics and oxidative stress in the marine planktonic diatom *Ditylum brightwellii*. Eur. J. Phycol. 36, 1–8.
Ross, O.N., Moore, C.M., Suggett, D.J., Macintyre, H.L., Geider, R.J., 2008. A model of photosynthesis and photo-protection based on reaction center damage and repair. Limnol. Oceanogr. 53, 1835–1852.
Sabine, C.L., Feely, R.A., Gruber, N., Key, R.M., Lee, K., Bullister, J.L., Wanninkhof, R., Wong, C.S., Wallace, D.W.R., Tilbrook, B., Millero, F.J., Peng, T.H., Kozyr, A., Ono, T., Rios, A.F., 2004. The oceanic sink for anthropogenic CO_2. Science 305, 367–371.
Shi, Q., Xiahou, W., Wu, H., 2017. Photosynthetic responses of the marine diatom *Thalassiosira pseudonana* to CO_2-induced seawater acidification. Hydrobiologia 788, 361–369.
Torstensson, A., Chierici, M., Wulff, A., 2012. The influence of increased temperature and carbon dioxide levels on the benthic/sea ice diatom *Naviculadirecta*. Polar Biol. 35, 205–214.
Tyystjärvi, E., Aro, E.-M., 1996. The rate constant of photoinhibition, measured in lincomycin-treated leaves, is directly proportional to light intensity. Proc. Natl. Acad. Sci. U.S.A. 93, 2213–2218.
Van de Poll, W.H., Buma, A.G., 2009. Does ultraviolet radiation affect the xanthophyll cycle in marine phytoplankton. Photochem. Photobiol. Sci. 8, 1295–1301.
Wolf-Gladrow, D., Bijma, J., Zeebe, R.E., 1999. Model simulation of the carbonate chemistry in the microenvironment of symbiont bearing foraminifera. Mar. Chem. 64, 181–198.
Wu, H., Cockshutt, A.M., Mccarthy, A., Campbell, D.A., 2011. Distinctive photosystem II photoinactivation and protein dynamics in marine diatoms. Plant Physiol 156, 2184–2195.
Wu, H., Roy, S., Alami, M., Green, B.R., Campbell, D.A., 2012a. Photosystem II photoinactivation, repair, and protection in marine centric diatoms. Plant Physiol 160, 464–476.
Wu, X., Gao, G., Giordano, M., Gao, K., 2012. Growth and photosynthesis of a diatom grown under elevated CO_2 in the presence of solar UV radiation. Fund Appl Limnol 180, 279–290.
Xu, K., Gao, K., Villafañe, V.E., Helbling, E.W., 2011. Photosynthetic responses of *Emiliania huxleyi* to UV radiation and elevated temperature: roles of calcified coccoliths. Biogeosciences 8, 1441–1452.
Zhu, S.H., Green, B.R., 2010. Photoprotection in the diatom *Thalassiosira pseudonana*: role of LI818-like proteins in response to high light stress. Biochim. Biophys. Acta 1797, 1449–1457.