Photosynthetic activation of the dark-acclimated diatom *Thalassiosira weissflogii* upon light exposure

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Abstract: The photoresponse of chlorophyll *a* (Chl *a*) fluorescence and xanthophyll pigments to light was studied in the diatom *Thalassiosira weissflogii* with respect to various durations of dark storage. The light-limited slope (α) obtained from the electron transport rate (ETR) versus the irradiance curve remained steady, whereas the light-saturated rate (ETR_{max}) decreased gradually during dark storage. Consequently the light-saturation index ($E_k = ETR_{max}/\alpha$) decreased with time, suggesting that cells acclimate to dark conditions. Dark-acclimated cells were exposed to light-dark conditions with varied silicate availability. The maximum quantum yield of PSII (F_v/F_m), as well as the changes in the α and the ETR_{max} , increased immediately to the maximum on the first day and decreased for the second half of the light exposure period. The ratio of diatoxanthin (DT) to the xanthophyll pigment diadinoxanthin (DD) and DT ($DT/[DD+DT]$) represents thermal dissipation behavior through non-photochemical quenching (NPQ). This study indicates that F_v/F_m and E_k are a good index to follow photoacclimation during a transition from continuous dark conditions to the L-D cycle of saturated light. This study also indicates that dark-acclimated *T. weissflogii* can acclimate to the growth irradiance by the enhancement of photosynthetic activity with changes in xanthophyll pigments, in relation to the dark duration and silicate availability. Such a response to light suggests that the vegetative cells of *T. weissflogii* could possibly proliferate at ports in the coastal zone on a global scale after ejection into high light environments from the darkness of a ship's ballast water tank.

Key words: chlorophyll fluorescence, dark survival, diatoms, electron transport rates, photoacclimation, xanthophyll pigment

Introduction

Phytoplankton use light as an energy source via photosynthesis. Light availability is one of the major factors controlling phytoplankton growth. However, natural assemblages of phytoplankton may need to survive in unfavorable conditions. In some situations, phytoplankton may have to endure prolonged periods of near- or complete darkness. The vertical transport of surface water below the euphotic zone of oceans and lakes forces cells in the water to face light conditions below 1% of the levels present at the surface (Dehning & Tilzer 1989, Peters 1996, Murphy & Cowles 1997). In polar seas, phytoplankton are forced to acclimate to low light conditions by the thick surface ice and snow cover (e.g. Arrigo et al. 2012, Lund-Hansen et al. 2014).

Such situations of near-darkness or total darkness are

also induced by anthropogenic causes, such as transport in the ballast water of ships (Medcof 1975). Phytoplankton in ballast water are dispersed at ports in the coastal zone or lakes, promoting the invasion of non-indigenous species. Therefore, phytoplankton are among the most problematic spreading species on a global scale, as already recognized by harmful algal blooms (e.g. Doblin et al. 2004, Popels et al. 2007). Although it is important to consider multiple taxonomic groups when evaluating the efficacy of ballast water exchange, the response of representative species in typical taxonomic groups to ballast tank environments provides a better understanding. The present study focused on the response to light, which is one of the environmental variables in a ballast tank, in an effort to reveal the strategies of dark survival and recovery in phytoplankton.

Numerous species of phytoplankton are able to withstand the absence of light for weeks to months at a time (e.g. Antia 1976). Several mechanisms for the survival of phytoplankton under dark conditions have been proposed, including the formation of resting cells, heterotro-

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phic nutrition, and reduced respiratory activity (McMinn & Martin 2013). However, for some phytoplankton species, such as the ubiquitous diatoms *Asterionella japonica* and *Thalassiosira weissflogii*, the formation of resting cells and heterotrophy do not appear necessary for survival in prolonged darkness (Smayda 1958, McQuoid & Hobson 1996), although reduced respiratory activity may occur. After exposure to normal light conditions similar to the pre-incubation conditions before dark storage, growth recovery appears to occur within a few days in phytoplankton that survive prolonged darkness for several months (Bunt & Lee 1972, Palmisano et al. 1986, Peters 1996, Zhang et al. 1998, Lüder et al. 2002). Periods of darkness shorter than months occur in the ballast water of ocean liners travelling between continents, particularly between European countries and North America, by virtue of improved voyage speed. Even for such a short duration, the effects of a prolonged dark period on the physiology and photosynthesis of phytoplankton have been studied in only a limited number of taxa, in particular harmful algal bloom species (Popels et al. 2007).

Phytoplankton are noted for their ability to adjust their intracellular concentrations of light-harvesting and xanthophyll cycle pigments in response to ambient light conditions (Mouget et al. 1999). An important protection mechanism against increased light intensity is the thermal dissipation of excess energy by xanthophyll cycle pigments in their de-epoxidated state. In chromophyte algae, such as diatoms, dinoflagellates and prymnesiophytes, xanthophyll cycle pigments contain diadinoxanthin (DD) and diatoxanthin (DT) (Demers et al. 1991, Moisan et al. 1998). DT is involved in the process of excess energy dissipation, whereas DD is not. Thus, DD and DT interconvert via the addition/removal of an epoxide group, and the equilibrium between the two forms depends on the light conditions (Long & Humphries 1994).

Chlorophyll (Chl) fluorescence, which is largely derived from the Chl *a* associated with Photosystem II (PSII), can be used to estimate the response of photosynthetic activity in phytoplankton to light exposure. For example, the maximum quantum yield of PSII photochemistry (F_v/F_m) is commonly used to study the potential state of PSII in dark-acclimated cells. Individuals of the haptophyte *Phaeocystis antarctica* that are stored in the dark can recover their F_v/F_m upon being returned to light (Tang et al. 2009). This recovery of photosynthetic capacity is most likely the result of the recovery of chlorophyll synthesis. A similar recovery of photosynthetic capacity has been demonstrated in many other phytoplankton species, such as the diatoms *Phaeodactylum tricornutum*, *Anaulus australis* and *Aureococcus anophagefferens* (Pelagophyceae) (Griffiths 1973, Du Preez & Bate 1992, Popels et al. 2007, respectively).

Diatoms are a major component of the natural phytoplankton community, accounting for approximately 40% of marine primary production (Falkowski et al. 1998). Diatoms can utilize macronutrients, such as nitrogen, phos-

phorus, and silicate, for growth. Diatoms generally need to be able to synthesize the silicified cell wall in order to replicate (Graham & Wilcox 2000). Therefore, silicate availability is one of the key factors in the regulation of the growth of diatoms. Variations in the light-saturated photosynthetic rate (assessed by either ^{14}C or Chl fluorescence methods) have been correlated with silicate concentrations (Malone et al. 1980, Lippemeier et al. 1999) and have also been correlated with the cell cycle phase (Claquin & Martin-Jézéquel 2005). Particularly, the low concentration of silicate toward the end of batch incubation may influence the photosynthetic efficiency of the PSII reaction centers under light-limited conditions (Lippemeier et al. 2001).

If diatoms can survive during dark storage periods in ballast tanks, how do diatoms respond physiologically and photosynthetically to the ambient light-dark cycle (L-D cycle) conditions in the surface water after the release of ballast water? The question is considered under the following light conditions in our study. The ambient light intensity is considered to be equal to the experimental light levels because the irradiance at the depth of the pump intake at the time of pumping into the ballast tank is assumed to be higher than the light-saturation index (E_k) of photosynthesis for cultures under nutrient-replete growth (MacIntyre et al. 2002). To answer the above questions, the following experiments were designed; a series of incubation experiments from the precondition to L-D cycle, the dark condition, the short exposure to the L-D cycle after the dark storage, and the subsequent exposure to the same L-D cycle were conducted (Katayama et al. 2011, this study). Cell division states in those four periods corresponded to dividing cells, no dividing cells, light-saturated dividing cells, and light-saturated non-dividing cells, respectively. Each sample was divided into two subsamples. In the first subsample, the response of pigment composition and growth rate to the continuous dark condition and then the subsequent L-D cycle condition was determined (Katayama et al. 2011). That study concluded that the vegetative cells of *Thalassiosira weissflogii* trapped in ballast water might be able to survive in total darkness for up to two weeks and still be able to undergo cell division once they were released into ambient coastal waters. In the second set of subsamples, photosynthetic activation of the dark-acclimated diatom *Thalassiosira weissflogii* upon light exposure was studied with respect to xanthophyll cycle pigments (this study). The dark-acclimated cells were returned to a L-D cycle with the addition of silicate (e.g. 10 μM or 130 μM), because the cells might exhaust the remaining silicate during dark storage before the end of the exposure to the L-D cycle experiment. The F_v/F_m , photosynthetic parameters derived from the electron transport rate (ETR) versus irradiance curve, and non-photochemical quenching (NPQ) were determined to gain further insight into their photosynthesis, and combined with data on the pigment composition and growth rate as determined in a previous study (Katayama et al. 2011). The four param-

ters enabled the detection and characterization of recovery from the dark condition during dark storage, and recovery from the combined stress of light and silicate availability during the L-D cycle.

Materials and Methods

Culture and experimental design

A culture of the marine diatom *Thalassiosira weissflogii* (CCMP1336) was obtained from the National Center for Marine Algae and Microbiota. Cells were maintained at 20°C at a salinity of 35 in filter-sterilized, aged seawater enriched with f/2 (Guillard & Ryther 1962) and illuminated with a 12-h light and 12-h dark cycle (L-D cycle) at a photosynthetic photon flux density of 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Irradiance was measured with a 4π sensor (Biospherical Instruments, QSL-100, USA). Our experimental design consisted of the following consecutive four incubations; the precondition to L-D cycle at 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, the dark condition, short exposure to the L-D cycle after dark storage, and subsequent exposure to the same L-D cycle. Cell division status in those periods corresponded to dividing cells, no-dividing cells, dividing cells, and light-saturated no-dividing cells. Cells were preconditioned in batch culture by transferring half of the initial culture during the exponential growth phase into new media every 2 days at least three times. Cells acclimatized in this way to a L-D cycle (i.e. 6 days) were inoculated into eighteen dark polycarbonate bottles in filter-sterilized, aged seawater enriched with new f/2 for the dark storage experiment (DS-Exp). The initial cell density was adjusted to $7\text{--}8 \times 10^3 \text{ cells ml}^{-1}$. Cells were incubated in the dark at 20°C for a maximum of 14 days in sterilized screw-top polycarbonate dark bottles; the cultures were slowly stirred, and the lids of the bottles were unscrewed to allow air in daily. Special attention was paid to maintaining complete darkness during the entire experimental period. Subsamples were taken for pigment and Chl *a* fluo-

rescence analyses on days 1, 3, 5, 8, 11, and 14 (Fig. 1). On each of days 1, 5, and 11, a set of three dark polycarbonate bottles was randomly selected from the DS-Exp. On each of days 3, 8, and 14, a set of three dark bottles was randomly selected and used in the recovery experiments (R-Exp) after subsamples were taken for measurements of xanthophyll cycle pigments and variable Chl *a* fluorescence during dark storage. The dark incubation period was to allow cells to recover from stress caused by the irradiance of 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ that they were exposed to during the pre-incubation. The R-Exp was initiated by transferring cells from triplicate dark bottles into triplicate transparent polycarbonate bottles and exposing these dark-acclimated cells to a L-D cycle of 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 20°C for three experiments, starting on days 3, 8, and 14, respectively. The three transparent polycarbonate bottles were inoculated with silicate in concentrations of 0, 10, or 130 μM , respectively. One set of three was incubated for 4 days, starting on days 3, 8, and 14, respectively, of the DS-Exp, giving a total of nine bottles used in the R-Exp. The stock solution of silicate was prepared by dissolving 0.284 g $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ into 100 ml distilled water. Subsequently, 0.74 ml and 9.6 ml, respectively, of the stock solution was added to bring the final concentrations to 10 and 130 μM of silicate, with both total volumes of medium being 740 ml. Changes in pH were minimal before and after the addition of the stock solution. These experiments were named the +10 μM -Exp and +130 μM -Exp, respectively. Media and polycarbonate bottles (18 dark, 9 transparent) were carefully autoclaved prior to the experiments. These experiments were designed based on the work of Katayama et al. (2011).

Sampling

On days 1, 3, 5, 8, 11, and 14 in the DS-Exp, triplicate subsamples were taken from three randomly-selected dark polycarbonate bottles. These subsamples were used for measurements of xanthophyll cycle pigments and variable Chl *a* fluorescence. On days 0, 1, 2, 3, and 4 in the R-Exp initiated on days 3, 8, and 14 of the DS-Exp, triplicate subsamples were taken from each set of three light polycarbonate bottles for the same measurements as in the DS-Exp at the middle of the light period. In addition, subsamples for the analysis of variable Chl *a* fluorescence were monitored over a short timescale at 1, 3, 6 hours.

Pigment analysis

Subsamples for the analysis of xanthophyll pigments were filtered through GF/F glass fiber filters (Whatman, England) within 5 min and stored at -80°C in a deep freezer until further analysis. A deep freezer appears equally as suitable as liquid nitrogen for flash-freezing samples immediately following filtration to prevent alteration of pigments due to rapid xanthophyll cycling (Southerton & Lewitus 2004). Cells collected on the filters were extracted with 2 ml *N,N*-dimethylformamide at -20°C for

Fig. 1. Experimental design of the present experiments. S indicates the sampling of measurements for pigments and Chl *a* fluorescence. Data from days 3, 8, and 14 under dark conditions were used as values for Day 0 in R-Exp.

24 h (Suzuki & Ishimaru 1990). Cell extracts were evaluated by high-performance liquid chromatography (Beckman Coulter, 168 Diode Array Detector, C18 reversed-phase ultra-sphere 3 μm column, USA) using the method modified by Head & Horne (1993). The peaks were quantified using standards for Chl *a*, DD, and DT, obtained from the Danish Hydraulic Institute (Denmark).

Fluorescence analysis

Variations in chlorophyll fluorescence were measured with a pulse-amplitude modulated chlorophyll fluorometer (Water-PAM, Walz, Germany). Subsamples for fluorescence analysis were placed in a 15 mm diameter quartz cuvette, which was illuminated by a circular array of 14 red light-emitting diodes with a peak illumination at 655 nm.

The minimum (F_0) and maximum (F_m) fluorescence signals were determined on cells dark-adapted for 30 min. The maximum quantum efficiency of PSII (F_v/F_m) was calculated by the following equation (Schreiber et al. 1986):

$$F_v/F_m = (F_m - F_0)/F_m \quad (1)$$

where F_v is the difference between the F_0 and F_m .

The minimum and maximum fluorescence yields in the light-adapted state (F and F'_m) were measured with a 0.8 s saturation pulse at an intensity of 3,500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The PSII operating efficiency ($\Delta F/F'_m$) was calculated by the equation of Genty et al. (1989):

$$\Delta F/F'_m = (F'_m - F)/F'_m \quad (2)$$

where ΔF is the difference between the F and F'_m . The measurement of $\Delta F/F'_m$ was conducted by illuminating the subsamples in a stepwise manner, increasing actinic light by up to 419 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with 30 s illumination periods at each step (Parkins et al. 2006).

The electron transport rate (ETR) by PSII was obtained using the following equation:

$$\text{ETR} = \text{PPFD} \times 0.5 \times a_{\text{PSII}}^* \times \Delta F/F'_m \quad (3)$$

where PPFD is the photosynthetic photon flux density and 0.5 is a factor accounting for equal photons falling on PSI and PSII (Hartig et al. 1998, Gilbert et al. 2000). The a_{PSII}^* was calculated from the Chl *a* concentration and the *in vivo* absorption by PSII at 655 nm. Absorption was measured using a dual beam UV-visible spectrophotometer equipped with an integrating sphere (UV-2450, Shimadzu, Japan) following the quantitative filter technique method of Mitchell & Kiefer (1988).

The rapid light curves (RLCs) were measured by plotting the ETR against the actinic PPFD. The α and the ETR_{max} were calculated according to Webb et al. (1974):

$$\text{ETR} = \text{ETR}_{\text{max}} (1 - \exp[-\alpha \times \text{PPFD}/\text{ETR}_{\text{max}}]) \quad (4)$$

The light-saturation index (E_k), based on the observation of the ETR, was calculated using the following equation:

$$E_k = \text{ETR}_{\text{max}}/\alpha \quad (5)$$

Non-photochemical quenching (NPQ) was calculated by the following equation (Bilger & Björkman 1990):

$$\text{NPQ} = (F'_m - F'_m)/F'_m \quad (6)$$

where F'_m and F'_m were measured as described above. The F'_m for calculating NPQ was used as the value at an actinic light of 296 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, which was close to the irradiance in the R-Exp.

Statistical analysis

The mean and standard deviation of all parameters that were obtained in the present study were always calculated and are reported throughout this study. The two-way analysis of variance (ANOVA) was conducted to assess the effect of the three different dark incubation durations and the three silicate enrichments on the parameters. Student's *t*-test was performed using the Sigma-Plot software program (System Software, version 11.0, USA).

Results and Discussion

Dark-acclimation and recovery of photosynthesis

The F_v/F_m increased by 41% on Day 5, from 0.33 to 0.52, and then did not significantly change again until Day 14 during the DS-Exp (Fig. 2A). The steady increase in the F_v/F_m during dark storage is concordant with previous observations of *Thalassiosira weissflogii* (Van de Poll et al. 2006) and *Aureococcus anophagefferens* (Pelagophyte) (Popels et al. 2007). Low F_v/F_m values at the beginning of dark storage in the present study suggest that the cells had been stressed during the pre-incubation normal L-D cycle of 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (prior to the DS-Exp), as suggested by Popels et al. (2007). The stress could be caused by damage of PSII because the irradiance employed for the pre-incubation was higher than that of required for optimal growth reported for *T. weissflogii* (Armbrust et al. 1990). Therefore, the first 5-day dark storage period may have been a recovery period for the repair of the reaction centers, as suggested by Popels et al. (2007). Releasing extrinsic proteins (OECs) in PSII has been known to suppress the oxygen activity (Seidler 1996). The increase in F_v/F_m might be related to OECs. This should be confirmed in the future.

When the cells were transferred to the normal L-D cycle, the cell density immediately increased to the maximum and the cells stopped growing exponentially after 2 days (Fig. 2 in Katayama et al. 2011). A significant reduction in the growth rate was observed with an increasing length of dark incubation from 3 to 14 days (Table 1 in Katayama et al. 2011). During exposure to the normal L-D cycle in the R-Exp, F_v/F_m increased and reached its maximum value on either Day 1 or Day 2 (Fig. 3). Similar levels of F_v/F_m were observed on both days, with a mean and standard deviation of 0.62 ± 0.024 , regardless of the dark storage length but dependent on silicate availability (Table

Fig. 2. Temporal changes in the F_v/F_m (solid circles) and silicate concentration (μM , open circles) (A), cellular Chl *a* contents (pg cell^{-1} , solid circles) and the amounts of antenna pigments Fuco/Chl *c* ($\text{mol Fuco} [\text{mol Chl } c]^{-1}$, open circles) (B), the $DT/(DD+DT)$ (% , solid circles), $DD_{\text{Chl } a}$ ($\text{mol DD} [\text{mol Chl } a]^{-1}$, open circles) and $DT_{\text{Chl } a}$ ($\text{mol DT} [\text{mol Chl } a]^{-1}$, open triangles), and $(DD+DT)_{\text{Chl } a}$ ($\text{mol DD+DT} [\text{mol Chl } a]^{-1}$, open squares) (C), and the α ($\text{m}^2 [\text{mg Chl } a]^{-1} \mu\text{mol e}^- [\mu\text{mol photons}]^{-1}$, solid circles) and ETR_{max} ($\mu\text{mol e}^- [\text{mg Chl } a]^{-1} \text{s}^{-1}$, open circles), and E_k ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$, open triangles) (D) in the DS-Exp. Vertical bars indicate one standard deviation of the mean. The data of silicate concentration, cellular Chl *a* content, and $(DD+DT)_{\text{Chl } a}$ were derived from Katayama et al. (2011).

Table 1. Results of two-way analyses of variances in the Chl fluorescence parameters between the number of days under dark incubation conditions (DS-Exp) and silicate availability in the recovery experiment (R-Exp). DF=degrees of freedom, F=F value, p =probability. Significant values ($p<0.05$) are indicated in bold.

Source of Variation	Day 1			Day2			Day 3			Day 4		
	DF	F	p	DF	F	p	DF	F	p	DF	F	p
F_v/F_m												
Dark incubation (A)	2	1.86	0.19	2	1.91	0.18	2	9.20	<0.01	2	0.46	0.64
Silicate availability (B)	2	9.00	<0.01	2	9.97	<0.01	2	9.89	<0.01	2	2.39	0.12
A $\times$ B	4	3.60	<0.05	4	3.89	<0.05	4	3.08	<0.05	4	2.27	0.10
α												
Dark incubation (A)	2	8.40	<0.01	2	4.00	<0.05	2	14.81	<0.01	2	0.69	0.51
Silicate availability (B)	2	8.12	<0.01	2	39.95	<0.01	2	34.48	<0.01	2	3.17	0.066
A $\times$ B	4	2.71	0.063	4	6.33	<0.01	4	16.96	<0.01	4	1.85	0.16
ETR_{max}												
Dark incubation (A)	2	13.78	<0.01	2	33.60	<0.01	2	11.01	<0.01	2	1.59	0.23
Silicate availability (B)	2	1.38	0.28	2	8.59	<0.01	2	4.34	<0.05	2	2.46	0.11
A $\times$ B	4	1.93	0.15	4	5.85	<0.01	4	4.36	<0.05	4	2.03	0.13
E_k												
Dark incubation (A)	2	25.94	<0.01	2	19.96	<0.01	2	1.55	0.24	2	0.22	0.81
Silicate availability (B)	2	7.69	<0.01	2	3.35	0.058	2	1.96	0.17	2	2.29	0.13
A $\times$ B	4	3.17	<0.05	4	4.03	<0.05	4	2.82	0.056	4	1.14	0.37
NPQ												
Dark incubation (A)	2	3.77	<0.05	2	37.79	<0.01	2	0.62	0.55	2	0.78	0.48
Silicate availability (B)	2	24.47	<0.01	2	33.52	<0.01	2	2.78	0.089	2	0.03	0.97
A $\times$ B	4	0.26	0.90	4	0.17	0.95	4	2.39	0.089	4	4.12	<0.05

Fig. 3. Temporal changes in F_v/F_m in the +0 μM -Exp (white bar), +10 μM -Exp (hatched bar), and +130 μM -Exp (cross-hatched bar) incubations on Day 0 (A), Day 1 (B), Day 2 (C), Day 3 (D), and Day 4 (E) in the R-Exp. Incubation was started on days 3, 8, and 14 of DS-Exp. Error bars indicate one standard deviation of the mean.

1). After reaching these maximum levels, F_v/F_m began to decrease to similar levels to those on Day 0, concretely 0.47 ± 0.059 on Day 3 and 0.36 ± 0.044 on Day 4. On Day 3, F_v/F_m was influenced by both the length of dark storage and silicate availability (Table 1). On Day 4, both the effects of dark storage length and silicate availability on F_v/F_m were diminished, which might be associated with light-saturated growth under the L-D cycle of high irradiance at $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 2 in Katayama et al. 2011).

The higher values of F_v/F_m following the normal L-D cycle in the R-Exp than those for the DS-Exp may shed light on new aspects of photoacclimation by PSII. The observed maximum values of F_v/F_m fell within the range of

F_v/F_m of actively growing *T. weissflogii*, which have been reported to be approximately 0.6 to 0.7 (Lippemeier et al. 2001). Therefore, *T. weissflogii* was able to recover to a photosynthetically active state with maximum F_v/F_m even after up to 14 days of storage in dark conditions under an abundant supply of macronutrients, including silicate (Fig. 2A). The flexible response of F_v/F_m to either a continuous dark or normal L-D cycle indicates that F_v/F_m can be a useful indicator for studies of potential photosynthetic performance. Changes in F_v/F_m are believed to reflect the photosynthetic performance of cells and are most commonly interpreted in terms of photoacclimation to changing irradiance (e.g. Franklin et al. 2003).

Photoprotective xanthophyll pigments

Under dark conditions, the dominant form of xanthophyll pigment is the epoxidized DD form. During the DS-Exp, a significant decrease in cellular DD and DT contents was observed with a significant decrease in cellular Chl *a* contents, although the supply of macronutrients was in excess (Fig. 2A, B). Because Chl *a* is an essential pigment, transferring excitation energy to the reaction center of the photosystem (Scheer 2003), the interconversion of DD and DT during dark storage is most effectively illustrated using the ratios of DD to Chl *a* and/or DT to Chl *a*, although the pool size of xanthophyll cycle pigments ($\text{DD}_{\text{Chl } a} + \text{DT}_{\text{Chl } a}$) increased steadily by 1.34 during the entirety of the continuous dark incubation period (Fig. 2C). During the DS-Exp, the molar ratios of the xanthophyll pigments diadinoxanthin ($\text{DD}_{\text{Chl } a}$) and diatoxanthin ($\text{DT}_{\text{Chl } a}$) to Chl *a* changed significantly over time (Fig. 2C). The $\text{DD}_{\text{Chl } a}$ increased from 0.17 to 0.36 mol DD (mol Chl *a*)⁻¹ on Day 11 and then did not significantly change again until Day 14. The $\text{DT}_{\text{Chl } a}$ decreased by 61%, from 0.19 to 0.075 mol DT (mol Chl *a*)⁻¹ on Day 8, and then increased by 90% on Day 14 compared with Day 8. Therefore, the pool size of xanthophyll cycle pigments increased due to the increase of DD during darkness, which has also been observed by Kashino and Kudoh (2003). They suggested that the increase in the pool size in the dark might be in preparation to overcome the expected excess light conditions, such as those conditions experienced from the DS-Exp to the R-Exp in this study. The ratio of DT to DD+DT ($\text{DT}/[\text{DD}+\text{DT}]$), as well as the changes in the $\text{DT}_{\text{Chl } a}$, decreased by 62%, from 53 to 20% on Day 8 and then increased by 45% on Day 14 compared with Day 8 (Fig. 2C). The increase in $\text{DT}_{\text{Chl } a}$ as well as $\text{DT}/(\text{DD}+\text{DT})$ after 8 days of dark storage in this study may be due to chlororespiration (Bennoun 1982), where the cells use the thylakoid membrane for respiration to generate ATP. Chlororespiratory electron flow generates a trans-thylakoid proton gradient strong enough to activate the DD de-epoxidation of the xanthophyll cycle in the dark (Jakob et al. 1999). Thus, the proton gradient generated by chlororespiration in prolonged dark storage could induce the de-epoxidation of DD.

When the dark-acclimated cells were exposed to the

normal L-D cycle in the R-Exp, the $DD_{Chl\ a}$ decreased by almost half on Day 1 in the $+0\ \mu\text{M}$ - and $+10\ \mu\text{M}$ -Exp, whereas it stayed at similar levels to those on Day 0 in the $130\ \mu\text{M}$ -Exp (Fig. 4). The initial decreases in the $DD_{Chl\ a}$ could be caused by rapid cell division at the beginning of the normal L-D cycle (R-Exp), which was accompanied by a decrease in cellular Chl *a* contents (Fig. 5 in Katayama et al. 2011). The further decreases in $DD_{Chl\ a}$ in the second half of the L-D cycle at $300\ \mu\text{mol photons m}^{-2}\text{ s}^{-1}$ could be influenced by both the length of dark storage and silicate availability (Table 2). All $DD_{Chl\ a}$ values converged to approximately $0.2\ \text{mol DD (mol Chl } a)^{-1}$ by Day 4. The $DT_{Chl\ a}$, as well as the changes in the $DD_{Chl\ a}$, decreased to less than $0.05\ \text{mol DT (mol Chl } a)^{-1}$ on Day 1 (Fig. 5) due to the rapid cell division at the beginning of the normal L-D cycle. Afterwards the $DT_{Chl\ a}$ increased to $0.20 \pm 0.022\ \text{mol DT (mol Chl } a)^{-1}$ by the end of the R-

Exp, as well as the cellular DD and DT contents increasing (Fig. 7 in Katayama et al. 2011). The observed values of $DT_{Chl\ a}$ ($0.20\ \text{mol DT [mol Chl } a]^{-1}$) at the end of the R-Exp were within the ranges of previously reported studies on diatoms ($0.033\text{--}0.28\ \text{mol DT [mol Chl } a]^{-1}$) after their exposure to high-light from low-light conditions (Olaizola et al. 1994, Lavaud et al. 2004). The $DT_{Chl\ a}$ was significantly correlated to the length of dark storage and the silicate availability until Day 2, whereas $DD_{Chl\ a}$ was correlated on Days 3 and 4 (two-way ANOVA, see Table 2).

The $DT/(DD+DT)$ reached the lowest level within a range of 8.0 to 14% on either Day 1 or Day 2 (Fig. 6). Thereafter, the $DT/(DD+DT)$ increased to $49 \pm 3.0\%$ by the end of the R-Exp. The changes in the $DT/(DD+DT)$ during the whole period of R-Exp were controlled by both the dark storage period duration and silicate availability (Table 2). An increase in xanthophyll cycle activity induces

Fig. 4. Temporal changes in $DD_{Chl\ a}$ ($\text{mol [mol Chl } a]^{-1}$) in the R-Exp. See Fig. 3 for the panel numbers, bars, and the legends for the x-axis.

Fig. 5. Temporal changes in $DT_{Chl\ a}$ ($\text{mol [mol Chl } a]^{-1}$) in the R-Exp. See Fig. 3 for the panel numbers, bars, and the legends for the x-axis.

Table 2. Results of two-way analysis of variance in the $DD_{Chl\ a}$, $DT_{Chl\ a}$, and $DT/(DD+DT)$ between the number of days under dark incubation conditions (DS-Exp) and silicate availability in the recovery experiment (R-Exp). DF=degrees of freedom, F=F value, p =probability. Significant values ($p<0.05$) are indicated in bold.

Source of Variation	Day 1			Day 2			Day 3			Day 4		
	DF	F	p	DF	F	p	DF	F	p	DF	F	p
$DD_{Chl\ a}$												
Dark incubation (A)	2	33.36	<0.01	2	28.63	<0.01	2	36.23	<0.01	2	42.26	<0.01
Silicate availability (B)	2	0.70	0.51	2	2.43	0.12	2	4.70	<0.05	2	8.94	<0.01
A $\times$ B	4	7.92	<0.01	4	0.76	0.56	4	8.60	<0.01	4	13.24	<0.01
$DT_{Chl\ a}$												
Dark incubation (A)	2	13.61	<0.01	2	29.97	<0.01	2	9.19	<0.01	2	0.39	0.68
Silicate availability (B)	2	11.99	<0.01	2	9.11	<0.01	2	0.071	0.93	2	5.91	<0.05
A $\times$ B	4	0.94	0.46	4	3.29	<0.05	4	13.02	<0.01	4	3.10	<0.05
$DT/(DD+DT)$												
Dark incubation (A)	2	6.519	<0.01	2	7.749	<0.01	2	41.248	<0.01	2	12.457	<0.01
Silicate availability (B)	2	16.336	<0.01	2	8.443	<0.01	2	4.179	<0.05	2	10.157	<0.01
A $\times$ B	4	0.358	0.84	4	2.994	<0.05	4	48.528	<0.01	4	1.719	0.19

Fig. 6. Temporal changes in $DT/(DD+DT)$ (%) in the R-Exp. See Fig. 3 for the panel numbers, bars, and the legends for the x-axis.

the thermal dissipation of excess energy and photoprotection through non-photochemical quenching (Lavaud et al. 2004). The energy dissipation capacity of the xanthophyll cycle in diatoms is greater than in green algae or more complex plants (Ruban et al. 2004, Goss et al. 2006). Thus, the marine diatom *Thalassiosira weissflogii* can increase its photoprotective repertoire against sudden light fluctuations after a few days, such as in the case of the sudden exposure to light of cells that were dark-acclimated for 3, 8 or 14 days in this study.

Electron transport rate of photosynthesis

During the DS-Exp, the α increased by 56% on Day 5 and then did not significantly change again until Day 14 (Fig. 2D). The increase in the α corresponded to the increase in the respective amounts of the antenna pigments fucoxanthin/Chl *c* (Fig. 1B), though cellular Chl *a* contents, representing the antenna size, decreased during dark storage (Fig. 2B). The unchanged α during the second half of dark storage in the present study was similar to that found in previous reports in shade-acclimated plants (Ihnken et al. 2010). The ETR_{max} increased by 37% on Day 5 and then decreased by 26% on Day 14 compared to Day 5 (Fig. 2D). The decrease in the ETR_{max} observed during the second half of dark storage was concomitant with a decrease in the cellular content of light-harvesting pigments, including Chl *a*, Chl *c*, and fucoxanthin (Fig. 3 in Katayama et al. 2011). Previous studies on micro- and macroalgae have reported a decrease in the light-saturated photosynthetic rate during near-darkness or complete darkness using the ^{14}C and Chl fluorescence methods (Palmisano & Sullivan 1982, White & Critchley 1999, Lüder et al. 2002, Lazzara et al. 2007, Wulff et al. 2008) as well as a positive linear relationship between the Chl *a* content and the ETR_{max} of macroalgae in the darkness (Lüder et al. 2002). The cells may decrease energy transfer from the light-harvesting antennae to the reaction centers of PSII by decreasing light-harvesting pigment content.

Fig. 7. Temporal changes in α ($\text{m}^2 [\text{mg Chl } a]^{-1} \mu\text{mol e}^- [\mu\text{mol photons}]^{-1}$) in the R-Exp. See Fig. 3 for the panel numbers, bars, and the legends for the x-axis.

When the dark-acclimated cells were exposed to the normal L-D cycle in the R-Exp, the increase in the α on Day 1 was influenced by the duration of the dark storage period and silicate availability (Fig. 7, Table 1), whereas the ETR_{max} increased by approximately 2.0 times on Day 1, regardless of silicate availability (Fig. 8, Table 1). An increase in ETR_{max} to maximum levels, caused by exposing cells to light immediately after dark storage, has also been confirmed in terrestrial plants, based on the Chl fluorescence method (White & Critchley 1999). Previous studies have reported an increase in both the light-saturated photosynthetic rate (P_m^B) and the light-limited slope (α^B), based on the ^{14}C method, in marine phytoplankton during light exposure following dark conditions (Harding et al. 1981, Erga & Skjoldal 1990). The increases in the α in the first day of the normal L-D cycle were more influenced by both the length of darkness and silicate availability than

Fig. 8. Temporal changes in ETR_{max} ($\mu\text{mol e}^- [\text{mg Chl } a]^{-1} \text{s}^{-1}$) in the R-Exp. See Fig. 3 for the panel numbers, bars, and the legends for the x-axis.

the ETR_{max} (Table 1). The electron transport rates of photosynthesis were able to immediately reach their maximum following the availability of light energy after returning to normal L-D cycle conditions.

The maximum α on Day 1 fell within the range of 0.0011 to 0.0016 $\text{m}^2 (\text{mg Chl } a)^{-1} \mu\text{mol e}^- (\mu\text{mol photons})^{-1}$ (Fig. 7). The maximum ETR_{max} on Day 1 fell within the range of 0.17 to 0.24 $\mu\text{mol e}^- (\text{mg Chl } a)^{-1} \text{s}^{-1}$ (Fig. 8). The decreases in the α and the ETR_{max} on Days 2 and 3 were controlled by both the duration of the dark storage period and silicate availability, although the effect of both factors disappeared on Day 4 (Table 1) when the cells reached saturated levels (Fig. 2 in Katayama et al. 2011).

During the DS-Exp, the E_k decreased from 169 ± 14 on Day 1 to $111 \pm 6.7 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ on Day 14 (Fig. 2D). The initial increase in the α and the ETR_{max} re-

sulted in a decrease in the E_k during the first half of the DS-Exp, suggesting that cells were acclimating to dark conditions. During the R-Exp, the E_k decreased from 138 ± 25 to $92 \pm 16 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ with time (Fig. 9). The decreases in the E_k on Days 1 and 2 were due to the combined effects of dark storage duration and silicate availability, although the effects of both factors were undetectable on Days 3 and 4 (Table 1) when the cell growth reached saturated levels (Fig. 2 in Katayama et al. 2011). The decrease in both α and ETR_{max} after Day 2, following exposure to the normal light-dark cycle, may be indicative of a photoprotective mechanism to regulate the energy flow required for photosynthesis. These decreases are accompanied by a dissipation of excess energy in the cellular photophysiological state known as the non-photochemical quenching (NPQ) state (Genty et al. 1990).

After exposure to the normal L-D cycle in the R-Exp,

the NPQ, as well as the changes in the cellular DD+DT content (Fig. 7 in Katayama et al. 2011) and DT/(DD+DT), decreased on Day 1, and then increased (Fig. 10). Previous studies have reported a positive linear relationship between the DT/(DD+DT) and NPQ of diatoms under high irradiance (e.g. Jesus et al. 2008). The NPQ reached the lowest level on Day 1 following exposure to the normal L-D cycle. These levels fell within the range of 0.22 to 0.47 on Day 1, although the NPQ on Days 1 and 2 was influenced by the effects of dark storage duration and silicate availability (Table 1). Thereafter, the NPQ increased and stayed within a relatively small range between Days 3 and 4 at 0.77 ± 0.071 . On both days the NPQ was not influenced by the dark storage duration or silicate availability (Table 1). In this study, there was a discrepancy between changes in NPQ and changes in DT/(DD+DT) and $\text{DT}_{\text{Chl } a}$ on Day 0 in the R-Exp. The values of DT/(DD+DT) (Fig. 6A) and

Fig. 9. Temporal changes in E_k ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) in the R-Exp. See Fig. 3 for the panel numbers, bars, and the legends for the x-axis.

Fig. 10. Temporal changes in NPQ in the R-Exp. See Fig. 3 for the panel numbers, bars, and the legends for the x-axis.

DT_{Chl a} (Fig. 5A) on Day 0 in the R-Exp were lower on Day 8 of the DS-Exp than on Days 3 and 14 of the DS-Exp, whereas the NPQ on Day 0 was higher on Day 8 of the DS-Exp (Fig. 6A). The changes in DT during dark storage might be influenced by chlororespiration. Although chlororespiratory electron flow could activate the xanthophyll cycle by generating a trans-thylakoid proton gradient (Jakob et al. 1999), it might not induce the enhancement of the NPQ state.

The E_k estimated in this study, which is analogous to a similar index employed for the traditional P versus I curve based on the end product of photosynthesis, indicates the domination of photochemical or non-photochemical quenching under light conditions as follows: photochemical quenching dominates below the irradiance at E_k , whereas non-photochemical quenching dominates the fluorescence quenching above the irradiance at E_k (Henley 1993). Therefore, after exposure to the second half of incubation under the normal L-D cycle, *Thalassiosira weissflogii* may engage in more non-photochemical quenching by photoprotection through xanthophyll cycle activity, such as with DT/(DD+DT), than photochemical quenching, regardless of the duration of dark storage and/or silicate availability (Table 1). The response of E_k to both the duration of the dark incubation period and silicate availability disappeared in the second half of the L-D cycle after the cells reached saturated levels. Therefore, the E_k as obtained in the present study may be a good index to follow photoacclimation during the transition from dark to light regimes.

This study indicates the feasibility of using F_v/F_m and E_k to follow photoacclimation in the marine diatom *Thalassiosira weissflogii* during transitions from and between the L-D cycle, continuous dark conditions, and an L-D cycle of high irradiance. The variable Chl fluorescence in this study was observed over short timescales of 1 hour to 6 hours after the transition to the L-D cycle (data not shown). After exposure to the second half of the normal L-D cycle, *T. weissflogii* may engage in more non-photochemical quenching by photoprotection through xanthophyll cycle activity than photochemical quenching, regardless of the duration of the dark storage period and/or the silicate availability (Table 1). The ability to resume photosynthesis upon exposure to high light conditions is maintained regardless of the duration of continuous darkness. Longer exposure to high light conditions, which is more likely expected for cells released from ballast water, may cause cells to go through a photoprotection process, such as activation of the xanthophyll cycle and non-photochemical quenching.

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References

- Antia NJ (1976) Effects of temperature on the darkness survival of marine microplanktonic algae. *Microb Ecol* 3: 41–54.
- Armbrust EV, Chisholm SW, Olson RJ (1990) Role of light and the cell cycle on the induction of spermatogenesis in a centric diatom. *J Phycol* 26: 470–478.
- Arrigo KR, Perovich DK, Pickart R S, Zachary W, Brown ZW, van Dijken GL, Lowry KE, Mills MM, Palmer MA, Balch WM, Bahr F, Bates NR, Benitez-Nelson C, Bowler B, Brownlee E, Ehn JK, Frey KE, Garley R, Laney SR, Lubelczyk L, Mathis J, Matsuoka A, Mitchell BG, Moore GWK, Ortega-Retuerta E, Pal SM, Polashenski CM, Reynolds RA, Schieber B, Sosik HM, Stephens M, Swift JH (2012) Massive phytoplankton blooms under Arctic sea ice. *Science* 336: 1408.
- Bennoun P (1982) Evidence for a respiratory chain in the chloroplast. *Proc Acad Sci USA* 79: 4352–4356.
- Bilger W, Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynth Res* 25: 173–185.
- Bunt JS, Lee CC (1972) Data on the composition and dark survival of four sea ice microalgae. *Limnol Oceanogr* 17: 458–461.
- Claquin P, Martin-Jézéquel V (2005) Regulation of the Si and C uptake and of the soluble free-silicon pool in a synchronized culture of *Cylindrotheca fusiformis* (Bacillariophyceae): effects on the Si/C ratio. *Mar Biol* 146: 877–886.
- Dehning I, Tilzer MM (1989) Survival of *Scenedesmus acuminatus* (Chlorophyceae) in darkness. *J Phycol* 25: 509–515.
- Demers S, Roy S, Gagnon R, Vignault C (1991) Rapid light-induced changes in cell fluorescence and in xanthophyll-cycle pigments of *Alexandrium excavatum* (Dinophyceae) and *Thalassiosira pseudonana* (Bacillariophyceae): a photoprotection mechanism. *Mar Ecol Prog Ser* 76: 185–193.
- Doblin MA, Popels LC, Coyne KJ, Hutchins DA, Cary SC, Dobbs FC (2004) Transport of the harmful bloom alga *Aureococcus anophagefferens* by oceangoing ships and coastal boats. *Appl Environ Microbiol* 70: 6495–6500.
- Du Preez DR, Bate GC (1992) Dark survival of the surf diatom *Anaulus australis* Drebes et Schulz. *Bot Mar* 35: 315–319.
- Erga SR, Skjoldal HR (1990) Diel variations in photosynthetic activity of summer phytoplankton in Lindåspollene, western Norway. *Mar Ecol Prog Ser* 65: 73–85.
- Falkowski PG, Barber RT, Smetacek V (1998) Biogeochemical controls and feedbacks on ocean primary production. *Science* 281: 200–206.
- Franklin LA, Osmond CB, Larkum AWD (2003) Photoinhibition, UV-B and algal photosynthesis. In: *Photosynthesis in algae* (eds Larkum AWD, Douglas SE, Raven JA). Kluwer Academic Publishers, Dordrecht, pp. 351–384.
- Genty B, Briantais J, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta* 990: 87–92.

- Genty B, Harbinson J, Briantais JM, Baker NR (1990) The relationship between non-photochemical quenching of chlorophyll fluorescence and the rate of photosystem 2 in leaves. *Photosynth Res* 25: 249–257.
- Gilbert M, Domin A, Becker A, Wilhelm C (2000) Estimation of primary productivity by chlorophyll *a* *in vivo* fluorescence in freshwater phytoplankton. *Photosynthetica* 38: 111–126.
- Goss R, Ann Pinto E, Wilhelm C, Richter M (2006) The importance of a highly active and Δ pH-regulated diatoxanthin epoxidase for the regulation of the PS II antenna function in diadinoxanthin cycle containing algae. *J Plant Physiol* 163: 1008–1021.
- Graham LE, Wilcox LW (2000) Ochrophytes I: Introduction to the Ochrophytes and focus on diatoms. In: *Algae* (eds Graham LE, Wilcox LW). Prentice-Hall, Inc., U.S.A., pp. 232–268.
- Griffiths DJ (1973) Factors affecting the photosynthetic capacity of laboratory cultures of the diatom *Phaeodactylum tricornutum*. *Mar Biol* 21: 91–97.
- Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleave) Gran. *Can J Microbiol* 8: 229–239.
- Harding LW Jr, Meeson BW, Prézélin BB, Sweeney BM (1981) Diel periodicity of photosynthesis in marine phytoplankton. *Mar Biol* 61: 95–105.
- Hartig P, Wolfstein K, Lippemeier S, Colijn F (1998) Photosynthetic activity of natural microphytobenthos populations measured by fluorescence (PAM) and 14 C-tracer methods: a comparison. *Mar Ecol Prog Ser* 166: 53–62.
- Head EJ, Horne EPW (1993) Pigment transformation and vertical flux in an area of convergence in the North Atlantic. *Deep-Sea Res II* 40: 329–346.
- Henley WJ (1993) Measurement and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. *J Phycol* 29: 729–739.
- Ihnken S, Eggert A, Beardall J (2010) Exposure times in rapid light curves affect photosynthetic parameters in algae. *Aquat Bot* 93: 185–194.
- Jakob T, Goss R, Wilhelm C (1999) Activation of diadinoxanthin de-epoxidase due to a chlororespiratory proton gradient in the dark in the diatom *Phaeodactylum tricornutum*. *Plant Biol* 1: 76–82.
- Jesus B, Mouget JL, Perkins RG (2008) Detection of diatom xanthophyll cycle using spectral reflectance. *J Phycol* 44: 1349–1359.
- Kashino Y, Kudoh S (2003) Concerted response of xanthophyll-cycle pigments in a marine diatom, *Chaetoceros gracilis*, to shifts in light condition. *Phycological Res* 51: 168–172.
- Katayama T, Murata A, Taguchi S (2011) Responses of pigment composition of the marine diatom *Thalassiosira weissflogii* to silicate availability during dark survival and recovery. *Plankton Benth Res* 6: 1–11.
- Lavaud J, Rousseau B, Etienne A-L (2004) General features of photoprotection by energy dissipation in planktonic diatoms (Bacillariophyceae). *J Phycol* 40: 130–137.
- Lazzara L, Nardello I, Ermanni C, Mangoni O, Saggiomo V (2007) Light environment and seasonal dynamics of microalgae in the annual sea ice at Terra Nova Bay, Ross Sea, Antarctica. *Antarct Sci* 19: 83–92.
- Lippemeier S, Hartig P, Colijn F (1999) Direct impact of silicate on the photosynthetic performance of the diatom *Thalassiosira weissflogii* assessed by on- and off-line PAM fluorescence measurements. *J Plankton Res* 21: 269–283.
- Lippemeier S, Hintze R, Vanselow K, Hartig P, Colijn F (2001) In-line recording of PAM fluorescence of phytoplankton cultures as a new tool for studying effects of fluctuating nutrient supply on photosynthesis. *Eur J Phycol* 36: 89–100.
- Long SP, Humphries S (1994) Photoinhibition of photosynthesis in nature. *Annu Rev Plant Physiol Plant Mol Biol* 45: 633–662.
- Lüder UH, Wiencke C, Knoetzel J (2002) Acclimation of photosynthesis and pigments during and after six months of darkness in *Palmaria decipiens* (Rhodophyta): A study to simulate Antarctic winter sea ice cover. *J Phycol* 38: 904–913.
- Lund-Hansen LC, Hawes I, Sorrell BK, Nielsen MH (2014) Removal of snow cover inhibits spring growth of Arctic ice algae through physiological and behavioral effects. *Polar Biol* 37: 471–481.
- MacIntyre HL, Kana TM, Anning T, Geider RJ (2002) Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. *J Phycol* 38: 17–38.
- Malone TC, Garside C, Neale PJ (1980) Effects of silicate depletion on photosynthesis by diatoms in the plume of the Hudson River. *Mar Biol* 58: 197–204.
- McMinn A, Martin A (2013) Dark survival in a warming world. *Proc R Soc B* 280: 2012–2909.
- McQuoid MR, Hobson LA (1996) Diatom resting stages. *J Phycol* 32: 889–902.
- Medcof JC (1975) Living marine animals in a ship's ballast water. *Proc Nat Shellfish Ass* 65: 11–12.
- Mitchell BG, Kiefer DA (1988) Chlorophyll *a* specific absorption and fluorescence excitation spectra for light-limited phytoplankton. *Deep-Sea Res* 35: 639–663.
- Moisan TA, Olaizola M, Mitchell BG (1998) Xanthophyll cycling in *Phaeocystis antarctica*: changes in cellular fluorescence. *Mar Ecol Prog Ser* 169: 113–121.
- Mouget J-L, Tremblin G, Morant-Manceau A, Morancals M, Robert J-M (1999) Long-term photoacclimation of *Haslea ostrearia* (Bacillariophyta): effect of irradiance on growth rates, pigment content and photosynthesis. *Eur J Phycol* 125: 1558–1566.
- Murphy AM, Cowles TJ (1997) Effects of darkness on multi-excitation *in vivo* fluorescence and survival in a marine diatom. *Limnol Oceanogr* 42: 1444–1453.
- Olaizola M, La Roche J, Kolber Z, Falkowski PG (1994) Non-photochemical fluorescence quenching and the diadinoxanthin cycle in a marine diatom. *Photosynth Res* 41: 357–370.
- Palmisano AC, Soohoo JB, Soohoo SL, Kottmeier ST, Craft LL, Sullivan CW (1986) Photoadaptation in *Phaeocystis pouchetii* advected beneath annual sea ice in McMurdo Sound, Antarctica. *J Plankton Res* 8: 891–906.
- Palmisano AC, Sullivan CW (1982) Physiology of sea ice diatoms. I. Response of three polar diatoms to a simulated summer-winter transition. *J Phycol* 18: 489–498.
- Parkins RG, Mouget J-L, Lefebvre S, Lavaud J (2006) Light response curve methodology and possible implications in the application of chlorophyll fluorescence to benthic diatoms. *Mar*

- Biol 149: 703–712.
- Peters E (1996) Prolonged darkness and diatom mortality: II. Marine temperate species. *J Exp Mar Biol Ecol* 207: 43–58.
- Popels LC, MacIntyre HL, Warner ME, Zhang Y, Hutchins DA (2007) Physiological responses during dark survival and recovery in *Aureococcus anophagefferens* (Pelagophyceae). *J Phycol* 43: 32–42.
- Ruban VA, Lavaud J, Rousseau B, Guglielmi G, Horton P, Etienne A-L (2004) The super-excess energy dissipation in diatom algae: comparative analysis with higher plants. *Photosynth Res* 82: 165–175.
- Scheer H (2003) The pigments, In: *Light-Harvesting Antennae in Photosynthesis* (eds Green BR, William WP). Kluwer Academic Publisher, Netherlands, pp. 29–81.
- Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth Res* 10: 51–62.
- Seidler A (1996) The extrinsic polypeptides of Photosystem II. *Biochim Biophys Acta* 1277: 35–60.
- Smayda TJ (1958) Biogeographical studies of marine phytoplankton. *Oikos* 9: 29–32.
- Southerland HA, Lewitus AJ (2004) Physiological responses of estuarine phytoplankton to ultraviolet light-induced fluoranthene toxicity. *J Exp Mar Biol Ecol* 298: 303–322.
- Suzuki R, Ishimaru T (1990) An improved method for the determination of phytoplankton chlorophyll using N,N-dimethylformamide. *J Oceanogr* 46: 190–194.
- Tang KW, Smith WO, Shields AR, Elliot DT (2009) Survival and recovery of *Phaeocystis antarctica* (Prymnesiophyceae) from prolonged darkness and freezing. *Proc R Soc B* 276: 81–90.
- Van de Poll WH, Alderkamp A-C, Janknegt PJ, Roggeveld J, Buma AGJ (2006) Photoacclimation modulates excessive photosynthetically active and ultraviolet radiation effects in a temperate and an Antarctic marine diatom. *Limnol Oceanogr* 51: 1239–1248.
- Webb WL, Newton M, Starr D (1974) Carbon dioxide exchange of *Alnus rubra*: a mathematical model. *Oecologia* 17: 281–291.
- White AJ, Critchley C (1999) Rapid light curves: A new fluorescence method to assess the state of the photosynthetic apparatus. *Photosynth Res* 59: 63–72.
- Wulff A, Roleda M, Zacher K, Wiencke C (2008) Exposure to sudden light burst after prolonged darkness—a case study on benthic diatoms in Antarctica. *Diatom Res* 23: 519–532.
- Zhang Q, Gradinger R, Spindler M (1998) Dark survival of marine microalgae in the High Arctic (Greenland Sea). *Polarforsch* 65: 111–116.