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

TOMOYO KATAYAMA*, AI MURATA & SATORU TAGUCHI

Soka University, Faculty of Engineering, 1–236 Tanig-machi, Hachioji, Tokyo 192–8577, Japan

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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 ($\alpha$) 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 $\alpha$ 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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Diatoms can utilize macronutrients, such as nitrogen, phosphorus, 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 ($E_0$) 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 $\mu$M or 130 $\mu$M), because the cells might exhaust the remaining silicate during dark storage before the end of the exposure to the L-D cycle experiment. The $F_{m}/F_{m}^{\text{app}}$ photo-synthetic 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 parame-
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 μmol photons m⁻² s⁻¹. Irradiance was measured with a 4x sensor (Biospherical Instruments, QSL-100, USA). Our experimental design consisted of the following consecutive four incubations; the precondition to L-D cycle at 300 μmol photons m⁻² s⁻¹, the dark condition, short 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 7–8×10⁵ 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 in daily. Special attention was paid to maintaining complete darkness during the entire experimental period. Subsamples were taken for pigment and Chl a fluorescence 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 μmol photons m⁻² 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 μmol photons m⁻² 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 Na₂SiO₃·9H₂O 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 (Southerland & 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.](image-url)
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 \( (F_m) \) 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):

\[
\frac{F_v}{F_m} = \frac{(F_m - F_0)}{F_m}
\]

(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 µmol photons m\(^{-2}\) s\(^{-1}\). The PSII operating efficiency \( (\Delta F/F_m) \) was calculated by the following equation (Genty et al. 1989):

\[
\frac{\Delta F}{F_m} = \frac{(F_m - F)}{F_m}
\]

(2)

where \( \Delta F \) 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 µmol photons m\(^{-2}\) 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:

\[
ETR = \text{PPFD} \times a_{PSII} \times \Delta F/F_m'
\]

(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 \( \text{in vivo} \) 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 \( \alpha \) and the \( ETR_{\max} \) were calculated according to Webb et al. (1974):

\[
ETR = ETR_{\max} \left(1 - \exp[-\alpha \times \text{PPFD/ETR}_{\max}] \right)
\]

(4)

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

\[
E_v = \frac{ETR_{\max}}{a}
\]

(5)

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

\[
\text{NPQ} = \frac{(F_m - F_m')}{F_m'}
\]

(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 µmol photons m\(^{-2}\) 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 µmol photons m\(^{-2}\) 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 (Armbrecht et al. 1990). Therefore, the first 5-day dark storage period might 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

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Fig. 2. Temporal changes in the $F_v/F_m$ (solid circles) and silicate concentration ($\mu$M, open circles) (A), cellular Chl $a$ contents (pg cell$^{-1}$, solid circles) and the amounts of antenna pigments Fuco/Chl $c$ (mol Fuco [mol Chl $c$]$^{-1}$, open circles) (B), the DT/(DD+DT) (%) (C), and $\alpha$ (mol e$^{-}$ [mol Chl $a$]$^{-1}$ s$^{-1}$, open circles) and ETR$_{\text{max}}$ (mol e$^{-}$ [mg Chl $a$]$^{-1}$ s$^{-1}$, open squares) (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) 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 | Day 2 | Day 3 | Day 4 |
|---------------------|-------|-------|-------|-------|
|                     | DF    | $F$   | $p$   | DF    | $F$   | $p$   | DF    | $F$   | $p$   | DF    | $F$   | $p$   |
| $F_v/F_m$ (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  | $<0.01$ | 4     | 3.89  | $<0.01$ | 4     | 3.08  | $<0.01$ | 4     | 2.27  | 0.10  |
| $\alpha$ (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$_{\text{max}}$ (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  |
| $E_k$ (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  | $<0.01$ | 4     | 4.03  | $<0.05$ | 4     | 2.82  | 0.056  | 4     | 1.14  | 0.37  |
| NPQ (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  | $<0.05$ |
Effects of dark storage length and silicate availability on photosynthetic performance 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 (DP$_{Chl\,a}$ + DT$_{Chl\,a}$) 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 (DD$_{Chl\,a}$) and diatoxanthin (DT$_{Chl\,a}$) to Chl $a$ changed significantly over time (Fig. 2C). The DD$_{Chl\,a}$ 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 DT$_{Chl\,a}$ 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 (DT/(DD+DT)), as well as the changes in the DT$_{Chl\,a}$, 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 DT$_{Chl\,a}$ as well as DT/(DD+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 $DD_{\text{Chl} a}$ decreased by almost half on Day 1 in the $+0 \mu M$- and $+10 \mu M$-Exp, whereas it stayed at similar levels to those on Day 0 in the $130 \mu M$-Exp (Fig. 4). The initial decreases in the $DD_{\text{Chl} a}$ 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 $DD_{\text{Chl} a}$ in the second half of the L-D cycle at $300 \mu$mol photons m$^{-2}$ s$^{-1}$ could be influenced by both the length of dark storage and silicate availability (Table 2). All $DD_{\text{Chl} a}$ values converged to approximately 0.2 mol DD (mol Chl $a$)$^{-1}$ by Day 4. The DT$_{\text{Chl} a}$, as well as the changes in the $DD_{\text{Chl} a}$, decreased to less than 0.05 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$_{\text{Chl} a}$ increased to $0.20 \pm 0.022$ 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$_{\text{Chl} a}$ (0.20 mol DT [mol Chl $a$]$^{-1}$) at the end of the R-Exp were within the ranges of previously reported studies on diatoms (0.033–0.28 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$_{\text{Chl} a}$ was significantly correlated to the length of dark storage and the silicate availability until Day 2, whereas $DD_{\text{Chl} a}$ 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...
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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 14C 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.

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 × 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 × 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     | 6.19  | <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 × 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.
When the dark-acclimated cells were exposed to the normal L-D cycle in the R-Exp, the increase in the $\alpha$ on Day 1 was influenced by the duration of the dark storage period and silicate availability (Fig. 7, Table 1), whereas the ETR$_{\text{max}}$ increased by approximately 2.0 times on Day 1, regardless of silicate availability (Fig. 8, Table 1). An increase in ETR$_{\text{max}}$ 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$_{m}$) and the light-limited slope ($\alpha$), 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 $\alpha$ in the first day of the normal L-D cycle were more influenced by both the length of darkness and silicate availability than the ETR$_{\text{max}}$ (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 $\alpha$ on Day 1 fell within the range of 0.0011 to 0.0016 m$^2$ (mg Chl a$^{-1}$ $\mu$mol e$^{-}$ ($\mu$mol photons)$^{-1}$ (Fig. 7). The maximum ETR$_{\text{max}}$ on Day 1 fell within the range of 0.17 to 0.24 $\mu$mol e$^{-}$ (mg Chl a$^{-1}$ s$^{-1}$) (Fig. 8). The decreases in the $\alpha$ and the ETR$_{\text{max}}$ 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 $E_0$ decreased from 169±14 on Day 1 to 111±6.7 $\mu$mol photons m$^{-2}$ s$^{-1}$ on Day 14 (Fig. 2D). The initial increase in the $\alpha$ and the ETR$_{\text{max}}$ 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 $138 \pm 25$ to $92 \pm 16 \mu$mol photons m$^{-2}$ 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 $\alpha$ and $ETR_{\text{max}}$ 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 DT$_{Chl\ a}$ on Day 0 in the R-Exp. The values of $DT/(DD+DT)$ (Fig. 6A) and

![Fig. 9. Temporal changes in $E_k$ (μmol photons m$^{-2}$ s$^{-1}$) in the R-Exp. See Fig. 3 for the panel numbers, bars, and the legends for the x-axis.](image)

![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.](image)
DT$_{\text{Chl}}$ (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 (Ja-kob 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 $E_k$ (Heny-ley 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 $F_r/F_m$ 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 wa-ter, may cause cells to go through a photoprotection process, such as activation of the xanthophyll cycle and non-photochemical quenching.

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