Responses of pigment composition of the marine diatom *Thalassiosira weissflogii* to silicate availability during dark survival and recovery

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**Abstract:** Responses of light-harvesting and photoprotective pigments to the length of dark storage and silicate availability were determined for the marine diatom *Thalassiosira weissflogii* during dark survival and recovery. During 14-day’s darkness, no significant changes in the concentration of silicate were observed, whereas the cell density increased by 34% for 8 days and decreased afterward. All cellular pigment contents decreased sharply between days 3 and 5 and stayed at relatively constant levels on days 11 and 14. Once the cells were exposed to a light:dark cycle on days 3, 8, and 14 of the dark storage experiments, there was a significant loss of all cellular pigment contents during the first 24 h of the light:dark cycle, which corresponded to the log-phase of growth. Cellular photoprotective pigment levels then started to recover to levels similar to those observed on days 11 and 14 in the dark storage experiments. When the dark-acclimated cells were exposed to light:dark cycle conditions, cell growth was immediately restored. The growth rate decreased significantly with increasing length of dark storage but not with silicate availability. This observation may suggest that *T. weissflogii* trapped in ballast water are able to survive in total darkness and to restore cell division once they are released into ambient coastal waters within two weeks.

**Key words:** cell density, chlorophyll a, growth rate, light-harvesting pigments, photoprotective pigments

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**Introduction**

Since large merchant ships have been deployed in the last 50 years (Medcof 1975), ballast water has been a main concern with regards to the transport of non-indigenous species across the ocean (Mills et al. 1996, Ruiz et al. 2000, Bij et al. 2002, Gray et al. 2007). Two different situations have been recognized: the transfer of non-indigenous species in freshwater ports between two continents such as Europe and North America and the transfer in brackish or marine water between two coastal ports between continents. For freshwater ports, ballast water exchange has been proposed and exercised to prevent any transfer of non-indigenous species including phytoplankton (Wonham et al. 2001, Choi et al. 2005, Ruiz & Smith 2005). The effectiveness of ballast water exchange is expected to be greater in transit between freshwater ports than marine ports because any species remaining in tanks after ballast water exchange would experience a profound osmotic shock effect due to exposure to high-salinity oceanic water. For marine ports, ballast water exchange is less effective and no economically effective solution has yet been recognized. Therefore, problems for marine species between brackish or marine ports remain despite some attempt to prevent transfer of non-indigenous species. Marine phytoplankton are one of the most serious problems for spreading species on a global scale, as already recognized by harmful algal blooms (Baldwin 1992, Hallegraeff & Bosch 1992, Smayda 1997, Zhang & Dickman 1999, Hamer et al. 2000, Doblin et al. 2004, ). Moreover, marine temperate diatoms tend to survive for longer than two weeks in prolonged darkness (Peters 1996, Dickman & Zhang 1999). Little is known about how phytoplankton kept in total darkness and to restore cell division once they are released into ambient coastal waters within two weeks.

Dark survival of phytoplankton has been extensively studied (e.g. Smayda & Michell-Innes 1974, Antia 1976, Peters 1996, Murphy & Cowles 1997, Tang et al. 2009), and programmed cell death has been recognized in dinoflagellates (Franklin & Berger 2004). Several mechanisms for the survival of phytoplankton in dark conditions have been...
proposed. A reduction of respiratory activity has been proposed for Chlorophyceae (Dehning & Tilzer 1989); formation of resting cells is well established for coastal diatoms (e.g. Anderson 1976); heterotrophic nutrition is adopted by diatoms (e.g. Palmisano & Sullivan 1982); alteration of biochemical properties of energy acquisition modes is an active strategy of adaptation to darkness for cyanobacteria (White & Shilo 1975). Strategies for dark survival could be species specific, and one such strategy is likely related to cellular pigment contents and composition.

Photoacclimation is typically manifested as a graded reduction of light-harvesting pigment content in response to increased irradiance (MacIntyre et al. 2002). Changes in the composition of light-harvesting pigments could be components of photoacclimation (Anderson et al. 1998). In response to environmental conditions, phytoplankton are notable for their ability to adjust their intracellular concentrations of light-harvesting and photoprotective pigments (Kana et al. 1997). Pigmentation is generally increased with decreasing light intensity, decreased under nutrient limitation and eventually lost in dark conditions. Dark acclimation is accomplished by changing the pigment composition. Change in pigment composition in both dark storage and light recovery is not related to nutrient availability. Within the light-harvesting pigments, the variability in the molar ratio of chlorophyll c (Chl c) or fucoxanthin (Fuco) to chlorophyll a (Chl a) is diminished under limited light conditions (MacIntyre et al. 2002), and it has also been shown to be limited regardless of the dark and/or light conditions (Dubinsky et al. 1986). Therefore, the enhancement of photoprotective pigment contents could occur in dark storage, whereas the enhancement of light-harvesting pigment contents could appear only in the early stage of light recovery conditions, when light intensity is not sufficiently low. A further increase of light intensity may enhance the relative photoprotective pigment contents.

Diatoms are one of the major groups reported in ballast water (Dickman & Zhang 1999). Diatoms contain a distinct pigment profile, including Chl a, Chl c, and Fuco as light-harvesting pigments (LHP) and didinoxanthin+dioxygen (DD+DT) and β·β carotene (Caro) as photoprotective pigments (PPP). In the present study Caro is defined as a photoprotective pigment because the central role of Caro is functionally protective (Schreiber 2003) as it may serve as energy quencher in the reaction center (MacIntyre et al. 2002) although it consists of photosynthetic accessory carotene and photoprotective carotene and both are contained in Photosystem I and II. Among diatoms, *Thalassiosira weissflogii* (Grunow) G. Fryxell and Hasle is one of the least vulnerable species to environmental changes, it is a relatively UV-tolerant species (Zudaire & Roy 2001), although this species does not seem to produce resting spores. Even though *T. weissflogii* loses cellular pigment contents during the dark storage period, the viable cells can resume growth once they are returned to light, in contrast to the diatom *Rhizosolenia setigera* Brightwell (Peters 1996). Therefore, it is an ideal species to determine the effect of dark-light transitions on pigment composition and contents of viable cells without the formation of resting spores.

The response of diatoms to nitrogen or phosphate deficiency is characteristically different from silicate limitation. For example, cells without an external nitrogen or phosphate supply continue to divide for more than three generations and then accumulate the photosynthetic products as long as silicate is supplied (Werner 1977). Although nitrogen and phosphate supply as well as salinity is highly variable among environments, this study is not intended to be comprehensive to cover all aspects but to manipulate silicate supply to study the response of diatoms under an ample supply of nitrogen and phosphate at a constant salinity. Therefore it is appropriate to determine the response of pigments to cell growth by using silicate to control the cell division. This study was designed to mimic two-week of dark conditions in the ballast water of merchant ships and was aimed to examine the responses of pigment composition and cellular contents of the marine diatom *T. weissflogii* to a total darkness, light-dark cycle condition, and silicate supply to study the strategies of their dark survival and recovery.

**Materials and Methods**

**Culture and experimental design**

*Thalassiosira weissflogii* was maintained at 20°C at a salinity of 35 in filter-sterilized, aged seawater enriched with f/2 medium (Guillard & Ryther 1962). A light intensity of 300 μmol photons m⁻² s⁻¹ was provided with cool white fluorescent tubes in a 12-h light and 12-h dark cycle (L:D cycle). This irradiance was adopted as a saturated but not inhibitory intensity for growth, as summarized in MacIntyre et al. (2002). Irradiance was measured with a 4r sensor (Biospherical Instruments, QSL-100, San Diego, USA). Cells were preconditioned in batch culture by transferring half of the initial culture during the exponential growth phase into new medium every three days at least three times. Cells acclimatized to a L:D cycle for six days were inoculated into nine 1-L and nine 2-L dark bottles in the filter-sterilized, aged seawater enriched with new f/2 medium for the dark storage experiment (DS-Exp). The initial cell density was adjusted to 7.8×10⁵ cells mL⁻¹. Dark incubation was performed at 20°C in a sterilized screw-top polycarbonate dark bottle and lasted for 14 days. Special attention was paid to maintaining complete darkness during the entire experimental period. On days 1, 5, and 11, a set of three 1-L dark bottles were taken randomly from the dark storage experiment only. On days 3, 8, and 14, a set of three 2-L dark bottles were picked randomly and employed for the recovery experiments after samples (0.84 L) were taken for dark storage measurements. Recovery experiments (R-Exp) were initiated by exposing the dark-acclimated cells in the three dark bottles to a L:D cycle of 300...
μmol photons m⁻² s⁻¹ at 20°C after the three bottles were inoculated with final concentrations of 0, 10, and 130 μM silicate on days 3, 8, and 14, respectively. The stock solution of silicate was prepared by dissolving 0.284 g Na₂SiO₃ · 9H₂O into 100 mL distilled water, and 0.74 mL of the solution was added to provide the addition of 10 and 130 μM of silicate with these experiments being named the +10 μM-Exp and +130 μM-Exp, respectively. All equipment and media were carefully autoclaved prior to the experiments.

**Sampling**

On days 1, 3, 5, 8, 11, and 14 in the DS-Exp, triplicate subsamples were taken from each set of three dark bottles for measurements of nutrient concentration, cell density, and pigment concentrations. On days 0, 1, 2, 3, 4, and 5 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 bottles for the same measurements as in the DS-Exp.

**Nutrient concentrations**

Subsamples for the analysis of silicate, nitrate, and phosphate were filtered through a glass fiber filter (Whatman, type GF/F, Maidstone, England) and the filtrates were stored in the dark at −20°C until further analysis. Concentrations of silicate, nitrate, and phosphate were determined using an auto-analyzer (Bran+Lubbe, AACS-II, Tokyo, Japan) following the methods of Parsons et al. (1984).

**Cell abundance**

Subsamples for cell counts were fixed with 5% formaldehyde solution and stored in the dark at 4°C until further analysis (Iwasawa et al. 2009). Cell density was determined on a haemocytometer (Erma, depth 0.1 mm, Tokyo, Japan) under a compound microscope (Olympus, IMT-2, Tokyo, Japan) at ×100 magnification by the method described by Guillard & Siercki (2005). Growth rate (μ, day⁻¹) was estimated using the following exponential growth equation (Guillard & Siercki 2005):

\[ μ = \frac{\ln(N_t/N_0)}{(t_1 - t_0)} \]  

where \( N_t \) and \( N_0 \) are the cell density in cells mL⁻¹ at the beginning of \( t_0 \) and 48 h after \( t_0 \) the exposure to a L:D cycle, respectively.

**Cellular pigments**

Subsamples for the analysis of pigments were filtered through a glass fiber filter (Whatman, GF/F, Maidstone, England) and stored at −60°C until further analysis. The cells collected on filters were extracted in 2 mL N,N-dimethylformamide (DMF) at −20°C for 24 h (Suzuki & Ishimaru 1990). The extracts were evaluated by high performance liquid chromatography (HPLC) (Beckman Coulter, 168 Diode Array Detector, C18 reversed-phase ultrasphere 3 μm column, Fullerton, USA) as described by Head & Horne (1993). The peaks were quantified using standards for Chl a, Chl c, Fuco, DD + DT, and Caro obtained from the International Agency for ¹⁴C determination. In the present study Caro was considered as a photoprotective pigment, as mentioned earlier, and the present analytical method did not distinguish between photosynthetic accessory carotenoids and photoprotective carotenoids.

**Statistical analysis**

The mean with one standard deviation was always calculated and is reported throughout the present study. The Student’s t-test and analysis of variance were conducted using the Sigma-Plot program (System Software, version 11.2, San Jose, USA).

**Results**

**Nutrient concentrations**

Concentrations of silicate remained relatively constant in the DS-Exp (Fig. 1A). Temporal changes of nitrate and phosphate were similar to the silicate changes. The means with one standard deviation were 76±5.9 μM for silicate, 750±47 μM for nitrate, and 36±2.2 μM for phosphorus in the DS-Exp. When the cultures were returned to L:D cycle conditions in the R-Exp, silicate concentrations decreased with time (Fig. 2A–C). Concentrations of nitrate and phosphate decreased in a similar manner to the silicate. By the end of the R-Exp, the silicate concentrations decreased to about 0.6 μM in the +0 μM-Exp, whereas they also decreased and remained at 3.0 μM in the +10 μM-Exp and 96 μM in the +130 μM-Exp. Mean concentrations with one standard deviation of nitrate and phosphate at the end of the R-Exp were 580±109 and 22±1.3 μM, respectively. Neither nitrate nor phosphate was utilized at concentrations that limited cell growth.

**Cell abundance**

All cells were vegetative, and no empty frustules, damaged cells, or spore formation of *Thalassiosira weissflogii* were observed throughout the DS-Exp. Mean initial cell densities with one standard deviation on day 0 were 7.1±0.5×10⁵ cells mL⁻¹ in the DS-Exp (Fig 1B). Cell densities increased by 34% in 8 days, stayed at a fairly constant level until day 11, and then decreased by 5% on day 14 in the DS-Exp. When the dark-adapted cells were exposed to the L:D cycle in the R-Exp, the cells responded immediately and began to divide (Fig. 2D–F). The cell resumed active division, and a significant reduction in the growth rate was observed with increasing length of dark incubation from 3 to 14 days but not with increasing silicate availability (p<0.05, Table 1). The cell yields were the lowest at 68±2×10⁵ cells mL⁻¹ in the +0 μM-Exp, whereas they...
were $79\pm3\times10^3$ in the $+10\mu$M-Exp and $79\pm2\times10^3$ cells mL$^{-1}$ in the $+130\mu$M-Exp. The value in the $+0\mu$M-Exp was significantly lower than those in other experiments due to a lack of silicate supply ($p<0.01$).

Cellular light-harvesting pigments

This pigment group included Chl$\alpha$, Chl$c$, and Fuco. During the DS-Exp, cellular Chl$\alpha$ contents significantly decreased from 10.6 to 3.6 pg Chl$\alpha$ cell$^{-1}$ with increasing darkness exposure ($p<0.01$, Fig. 3A). Cellular Chl$c$ in the DS-Exp decreased similarly from 1.4 to 0.4 pg Chl$c$ cell$^{-1}$ (Fig. 3B). Cellular Fuco contents also showed a similar temporal decrease from 4.7 to 2.5 pg Fuco cell$^{-1}$, but the degree of decrease was less than for Chl$\alpha$ or Chl$c$ and only 50% of the initial value (Fig. 3C). Both the relative molar abundance of Chl$\alpha$ and Chl$c$ with respect to the total pigments decreased from 46 to 38% and from 8.4 to 4.8%, respectively, whereas that of Fuco increased from 28 to 37% (Table 2). Molar ratios of Chl$c$ to Chl$\alpha$ in the DS-Exp steadily decreased by 23% with increasing darkness exposure whereas the molar ratios of Fuco to Chl$\alpha$ showed a steady increase (Fig. 4A, B).

![Graph A](image1.png)
![Graph B](image2.png)

**Fig. 1.** Temporal change in the concentrations of silicate (A) and cell density (B) in the dark storage experiment (DS-Exp). Vertical bars indicate one standard deviation.

![Graph C](image3.png)
![Graph D](image4.png)

**Fig. 2.** Temporal change in the concentration of silicate in the $+0\mu$M-Exp (A), $+10\mu$M-Exp (B), and $+130\mu$M-Exp (C) and cell densities in the $+0\mu$M-Exp (D), $+10\mu$M-Exp (E), and $+130\mu$M-Exp (F) in the recovery experiments (R-Exp). Incubation was started on day 3 (solid circle), day 8 (open circle), and day 14 (closed versed triangle) of the dark storage experiment (DS-Exp).

| Growth rate (µd$^{-1}$) | Day 3 | Day 8 | Day 14 |
|-------------------------|-------|-------|--------|
| Experiment              | Mean±S.D. | Mean±S.D. | Mean±S.D. |
| $+0\mu$M-Exp            | 1.03±0.030 | 0.952±0.023 | 0.937±0.031 |
| $+10\mu$M-Exp           | 1.10±0.055 | 0.977±0.041 | 0.870±0.042 |
| $+130\mu$M-Exp          | 0.979±0.064 | 0.932±0.025 | 0.914±0.022 |

**Table 1.** Growth rates of *Thalassiosira weissflogii* in the recovery experiments (R-Exp). Mean±one standard deviation of growth rates was observed on the cells exposed to the L:D cycles in the $+0\mu$M-Exp, $+10\mu$M-Exp, and $+130\mu$M-Exp on day 3, day 8, and day 14 of the dark storage experiment (DS-Exp).
Once cells were transferred to the L:D cycle on day 3 of the DS-Exp, a significant decrease in the cellular Chl \(\alpha\) contents from 10 to 0.38 to 0.04 pg Chl \(\alpha\) cell\(^{-1}\) was observed within 24 h \((p < 0.01)\), without any noticeable difference among the three silicate availability conditions (Fig. 5A–C). The lowest cellular Chl \(\alpha\) contents were usually observed on day 1 within the narrow range of 3 and 4 pg Chl \(\alpha\) cell\(^{-1}\), regardless of the length of dark storage and/or silicate availability. After the minimum, Chl \(\alpha\) contents increased by 67\% by the end of the R-Exp. The degree of recovery was highest in the +0 \(\mu\)M-Exp.

A significant decrease in the cellular Chl \(c\) content was also observed for the R-Exp started on day 3 (Fig. 5D–F). The decrease in Chl \(c\) for the R-Exp started on other dates was less than the decrease observed on day 3 because of the already low cellular Chl \(c\) contents on the other starting dates. The lowest cellular Chl \(c\) contents were also observed on day 1 within the narrow range of 0.3 and 0.5 pg Chl \(c\) cell\(^{-1}\), regardless of the length of dark storage and/or silicate availability. Following the minimum, Chl \(c\) contents increased by 82\% by the end of the R-Exp. The recovery of cellular Chl \(c\) contents was also highest in the +0 \(\mu\)M-Exp and was usually close to or higher than those obtained on day 0 in the experiments started on days 8 and 14.

Cellular Fuco contents showed different patterns of de-
crease between the +0 μM-Exp and the other two experiments (Fig. 5G–I). Cellular Fuco contents in the +0 μM-Exp decreased gradually until day 4. Cellular Fuco contents in the +10 μM-Exp and +130 μM-Exp decreased sharply to around 1 pg cell$^{-1}$ on day 1 and then increased gradually. Cellular Fuco contents in the other two experiments also significantly decreased within the first day ($p<0.01$) because of the relatively gradual decrease under dark storage in comparison with the cellular Chl $a$ and Chl $c$ contents (Fig. 3A, B). The lowest cellular Fuco contents were observed on either day 1 or 2, with a narrow range of 0.98 and 1.6 pg Fuco cell$^{-1}$. Following the minimum, Fuco contents increased by 55±20% by the end of the R-Exp. The degree of recovery was highest in the +0 μM-Exp and usually close to those observed on day 0 in the experiments started on day 8 and 14, except for the +130 μM-Exp.

The relative molar abundance of each pigment indicated a different response among the Chl $a$, Chl $c$, and Fuco pigments depending on days 3, 8, or 14 when the exposure experiment was conducted (Table 2). The most significant change from the beginning of each R-Exp (day 0) was observed in the experiment started on day 14. Both Chl $a$ and Chl $c$ increased whereas Fuco decreased at the end of the R-Exp. The molar ratio of Chl $c$ to Chl $a$ indicated a general pattern of increase within the first day of the L:D cycle, and it stayed at a relatively constant level (Fig. 6A–C). The molar ratios of Fuco to Chl $a$ on day 14 in the R-Exp decreased within the first day and the ratios on day 3 remained relatively unchanged and the ratio on day 14 decreased sharply compared to day 1 (Fig. 6D–F).

**Cellular photoprotective pigments**

This pigment group included DD+DT and Caro. Both cellular DD+DT and Caro contents showed a similar pattern of 50% decrease with the duration of dark conditions from 2.5 to 1.2 pg DD+DT cell$^{-1}$ and 0.26 to 0.13 pg Caro cell$^{-1}$ (Fig. 3D, E). Molar ratios of DD+DT to
Chl $a$ and Caro to Chl $a$ in the DS-Exp steadily increased by >40% with the dark duration (Fig. 4C, D).

When the cells were exposed to the L:D cycle, a significant decrease in cellular DD+DT content was observed within one day, particularly for the sample exposed on day 3 ($p<0.01$, Fig. 7A–C). The cellular DD+DT contents reached the lowest amounts on either day 1 or 2 and then increased with time. The delayed recovery of cellular DD+DT contents after day 1 was observed in the exposure experiment started on day 14 regardless of silicate availability. Some delay pattern was also observed for Caro (Fig. 7D–F). The relative molar abundance of DD+DT and Caro with respect to the total pigments fell into a narrower range at the end of incubation (day 4) than at the beginning of incubation (day 0) regardless of the starting dates of the DS-Exp (Table 2). The molar ratio of DD+DT to Chl $a$ significantly decreased within the first day of the experiments started on days 3 and 8, whereas the decrease continued until the second day of the experiment started on day 14 (Fig. 8A–C). After the occurrence of the minimum, cellular DD+DT contents increased gradually. The molar ratio of Caro to Chl $a$ in the experiments started on days 3 showed a different pattern of temporal change from the molar ratios of DD+DT to Chl $a$, whereas that in the experiment started on day 14 showed a general decay curve (Fig. 8D–F).

**Relative abundance of light harvesting pigments (LHP)**

The percent molar LHP in the DS-Exp decreased significantly from 87% on day 3 to 47% on day 5 and increased to 54% on day 14 ($p<0.05$, Fig. 9A). When the cells were exposed to the L:D cycle in the R-Exp, the percent molar LHP with respect to the total pigments had a very similar pattern of temporal change between the three silicate availability conditions (Fig. 9B–D). They reached a maximum of about 90% on day 1 or 2 and then decreased to 85%. The significant increase on day 1 corresponded to the first half of the log-phase in the growth curve (Fig. 2D–F). A gradual decrease after the occurrence of the maximum ranged from 10% to 6% without any significant trends correlated to silicate availability in the R-Exp.

**Discussion**

**Dark survival and recovery**

The occurrence of cell division during the first week of darkness is common among diatoms (e.g. Handa 1969, Peters 1996, Peters & Thomas 1996, Berges & Falkowski...
1998, Parker et al. 2004) as well as other taxa. Other patterns of cell division during the first week of darkness have also been recognized (see, Furusato & Asaeda 2009). Our results, however, are dissimilar to those obtained for a similar strain of *Thalassiosira weissflogii* from the North Pacific Culture Collection, University of British Columbia (Murphy & Cowles 1997), although the incubation conditions were somewhat different. The different conditions of maintaining the culture in an individual laboratory for years could be a possible source of the difference. The immediate cell division without any lag phase upon exposure to the L:D cycle confirms the previous finding that growth is possible as long as the length of dark storage is less than 2 weeks (Peters 1996). The growth rate obtained within 5 days of commencement of the L:D cycle in the present study may confirm that *T. weissflogii* can restore growth when they are returned to a L:D cycle within 14 days of darkness, because this species has been shown to be able to restore growth even after 2 months (Murphy & Cowles 1997). Our estimate of the growth rate is more than double that of 0.43 d$^{-1}$ estimated after 2 months of darkness. The significant reduction of the growth rate during 2 weeks of darkness ($p < 0.01$, Table 1) and also between 2 weeks and 2 months may suggest that the photochemical apparatus and biochemical carbon fixation functions remain intact in cells but are affected by two possible phenomena, depending on the length of darkness. The first effect could be the initial immediate loss of cellular pigments within the first day with highest cell growth on day 3 (Table 1), as observed in the present study, and the second effect could be a gradual decrease of cellular pigments after the initial loss as observed by others (e.g., Murphy & Cowles 1997). Because the reduction in growth rate is significantly related to the length of dark storage regardless of silicate availability (Table 3), the different volumes of silicate solution added to the +10 µM and +130 µM-Exp seemed to be insignificant.

Cell yields are controlled by either silicate concentration or light intensity in the present study as observed by Taguchi et al. (1987). Low cell yields without a further supply of silicate in the +0 µM-Exp were interpreted to be due to limitation by the total amount of silicate, which was

### Table 3. Results of two-way analysis of variance in growth rate among the length of day in the dark incubation (DS-Exp) and silicate availability in the recovery experiment (R-Exp). DF=degree of freedom, SS=sum of square, MS=mean of square, F=F value, $p=$ probability.

| Source of variation | DF | SS   | MS   | F    | p    |
|---------------------|----|------|------|------|------|
| Silicate availability (A) | 2  | 0.00786 | 0.00393 | 2.615 | 0.101 |
| Dark incubation (B) | 2  | 0.0763 | 0.0382 | 25.393 | <0.001 |
| A×B                 | 4  | 0.0254 | 0.00635 | 4.222 | 0.014 |
| Residual            | 18 | 0.0271 | 0.00150 |       |      |
| Total               | 26 | 0.137  | 0.00525 |       |      |

Fig. 8. Temporal changes in the molar ratios of diadinoxanthin+ diatoxanthin to chlorophyll a in the +0 µM-Exp (A), +10 µM-Exp (B), and +130 µM-Exp (C) and β·β carotene to chlorophyll a in the +0 µM-Exp (D), +10 µM-Exp (E), and +130 µM-Exp (F) in the recovery experiment (R-Exp). See Fig 2 for symbols.

Fig. 9. Temporal changes in the relative abundance of molar light-harvesting pigments (LHP) in the dark storage experiment (A) and in the +0 µM-Exp (B), +10 µM-Exp (C), and +130 µM-Exp (D) of the recovery experiment (R-Exp). See Fig 2 for symbols.
available for cell growth saturation without light limitation. High cell yields with an ample supply of silicate in the 
+10 μM-Exp and +130 μM-Exp suggested that the saturation of cell growth occurred due to light limitation.

Photosynthetic carbon metabolism leads to an accumulation of carbohydrates or lipids during the photo-phase. At night the reserves are invariably used in maintenance respiration, and some reserves are consumed to provide the carbon skeletons and energy for net synthesis of protein during balance growth (Post et al. 1985). Prolonged exposure to darkness prevents photosynthetic carbon metabolism. A significant decrease in the cellular pigment contents during dark storage may suggest that the cells could utilize pigments after metabolizing carbohydrates or lipids to survive in darkness, because no energy is provided through photosynthesis. Diatoms have been known to survive for extended periods in darkness, slowly consuming their reserves (Falkowski & Raven 2007). Such a strategy as adopted by diatoms facilitates outbursts of rapid growth when the environmental conditions become favorable, such as after release from ballast water, and this could be a similar situation to that of the spring bloom.

**Pigment acclimation**

Induction of a loss in cellular pigment contents during the two-week dark storage period as observed in the present study differs from observations obtained for a similar period using a similar strain of *Thalassiosira weissflogii* (Murphy & Cowles 1997). The pigment composition in their experiment remained relatively unchanged during dark storage, whereas dark storage induced a considerable variation in the cellular pigment composition in the present study. The decrease in the relative abundance of cellular Chlα, Chl c, and Caro contents and the increase in cellular DD+DT and Fuco contents during dark storage may suggest that dark acclimation in the pigment composition is accompanied with a reduction in cellular pigment content. This combination of acclimation could result in a decrease in the molar ratio of Chl c to Chl a and an increase in the molar ratio of DD+DT or Caro to Chl a. Dark induction of an enhancement in the molar ratios of Fuco to Chl a within two weeks was not observed and was relatively constant (0.56±0.03 as a mean±one standard deviation, Murphy & Cowles 1997). This value was similar to the initial value found in the present study although their cells were grown at an irradiance of 100 μmol photons m⁻² s⁻¹. Photo-acclimation of Fuco is not considered to be similar to photo-acclimation of LHP, although Fuco is considered to be a principal accessory pigment in diatoms (Jorgensen 1977). Dark induction of an enhancement in the molar ratios of Fuco to Chl a (63%) might be due to differences in the loss rate during dark incubation between Fuco and Chl a. This could be speculated to be because a higher energy cost is required for Fuco metabolism than for Chl a. The enhanced molar ratio of Fuco to Chl a at the end of dark incubation in the present study was similar to the unity obtained for the diatom *Cyclotella maneghiniana* Kützing, which indicated a high efficiency in absorbing and/or transferring excitation energy to Chl a (Gildenhoff et al. 2010).

The cellular pigment contents decreased due to cell division when they were exposed to a light:dark cycle. This was most pronounced on day 3 in the R-Exp due to the shorter length of dark storage (Popels & Huchins 2002). Although the cells resumed growth, the growth rates were slower than the optimal growth for *T. weissflogii* determined in batch mode under light:dark conditions (e.g., Costello & Chisholm 1981). The slower growth rates observed in the present study, particularly in the experiment started on day 14, might be possibly due to a low photosynthetic rate per cell although the photosynthetic apparatus remained intact in the cells. Our observation suggests that recovery in the growth rate could be expected because the recovery is not related to pigment composition but rather to cellular pigment content. The cellular pigment contents are related to the availability of resources such as light and nutrients (MacIntyre et al. 2002).

The cellular pigment composition in the light:dark cycle is related to photoacclimation, as reported by Rodriguez et al. (2006). Reductions in the cellular contents of accessory LHP are commonly observed in response to increasing irradiance (MacIntyre et al. 2002). Immediate recovery in percent LHP to 90% within one day may indicate that photoacclimation is not related to the immediate availability of silicate but rather to light. The dark-acclimated cells may maintain the photochemical apparatus and biochemical carbon fixation remain functional even after 14 days of darkness. After the initial recovery, a reduction of percent LHP occurred in a similar manner, regardless of the kind of limitation caused either by silicate in the +0 μM-Exp or light in the +10 and +130 μM-Exp. The further reduction in percent LHP with respect to the total pigment content after the cells were exposed for five days might suggest that the irradiance of 300 μmol photons m⁻² s⁻¹ was saturating for growth, and that cells were beginning to protect themselves from excess light. The light saturation index for the growth of *T. weissflogii* has been reported to be approximately 100 μmol photons m⁻² s⁻¹ (e.g. Falkowski & Raven 2007) and 133 μmol photons m⁻² s⁻¹ is the optimal growth irradiance (Costello & Chisholm 1981). The irradiance level of our study should be considered to be a saturated but not inhibitory level for growth. Acclimation to increased irradiance is typically accompanied by a decrease in cellular LHP (MacIntyre et al. 2002) and an increase in cellular PPP to protect the cells from excess light (Siefermann-Harms 1985). Alteration of cellular LHP is a mechanism enabling the balancing of energy captured from light absorption with the energy demand for growth (Geider et al. 1998).
Implications

Cellular pigment contents and composition are simple, basic characteristics in algal physiology. The responses of cellular pigment contents and composition, derived from analysis of prolonged dark exposure for 14 days, provide significant insight into the regulation of the material balance of LHP and PPP in the cells. Even in total darkness, cellular pigment composition may change; a significant decrease in LHP occurred in the early period, followed by a gradual increase as cellular pigment contents substantially decreased. After the cells were transferred to a light:dark cycle, a balance in the partition between LHP and PPP seemed to be affected when the cell growth was saturated, regardless of the presence of light or silicate. The present study suggests that *Thalassiosira weissflogii* trapped in ballast waters are well adapted to survive extended periods of total darkness for at least two weeks and to restore cell division without any lag phase by maintaining the ability of the photosynthetic apparatus to take advantage of any light that becomes available once they are released into ambient coastal waters, as long as other factors are favorable. It remains to be determined whether this feature of *T. weissflogii* is related to the ecological characteristic of it not producing a resting spore.

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