The xanthophyll-cycling pigment dynamics of *Isochrysis galbana* (Prymnesiophyceae) during light–dark transition

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Abstract: We investigated the photoresponses of diadinoxanthin (DD) and diatoxanthin (DT) using *Isochrysis galbana* cultures that had been acclimated to four different levels of photon flux density (PFD). DD can play a role as a photosynthetic light-harvesting pigment, whereas DT can play a role as a photoprotective pigment. The PFD ranged from limited light conditions (LL) to saturated light conditions (SL) for growth. The photoresponses of these pigments during light–dark transitions at each acclimation PFD were consistent. The photoresponses of these xanthophyll pigments, as normalized to either cell density or chlorophyll *a* (Chl *a*) concentration, to light–dark transitions varied with acclimation PFD. The stoichiometric variations between the DD or DT : Chl *a* ratio observed at the two higher acclimation PFDs suggested that these xanthophyll dynamics are controlled by xanthophyll cycling processes in addition to *de novo* processes and interconversion between DD and fucoxanthin under SL conditions. Significant linear relationships were observed between the levels of DD and/or DT normalized to either the cell density or Chl *a* concentration. Based on the cellular pigment content and the xanthophyll : Chl *a* ratio, the slopes of the linear relationships normalized to either the cell density or Chl *a* concentration were significantly related to the acclimation PFD linearly and sigmoidally, respectively. Similarly, the acclimation PFD at the intersection of the sigmoidal change and the transition point from the LL to SL condition suggests that these xanthophyll pigment dynamics can be characterized by acclimation to the light conditions. An examination of the slope may provide information leading to a better understanding of photoacclimation in the algal photosynthetic apparatus.

Key words: diadinoxanthin, diatoxanthin, photoacclimation, photon flux density, photoprotection

Introduction

The aquatic environment has a highly variable photon flux density (PFD), and algal cells are exposed to a range of PFD from 0 to 2000 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$ in natural conditions (Jerlov 1976, Cullen & Lewis 1988). The wide range in aquatic PFD is caused by day–night cycles and vertical mixing. The PFD is an important factor in determining the variability in the photosynthetic responses and growth rates of algae (Falkowski 1984, Falkowski & Dubinsky 1980, Palmisano et al. 1985, Harrison & Platt 1986, Cullen 1990). The acclimation of algae to the PFD, which is referred to as photoacclimation, follows rules that result in the optimization of photosynthesis within the constraints of the light conditions. This optimization involves a balance between maximizing the rate of photosynthesis

under a limiting PFD and minimizing the damage that can arise from saturating or excess PFD (Raven & Geider 2003). Photoacclimation operates on time scales of hours and days, due to changes in the macromolecular composition and function of the photosynthetic apparatus that occurs in response to the PFD variations. In the context of the light reactions, photoacclimation is accompanied by changes in the composition and cellular abundance of pigments and changes in the electron transfer chain components (for a review, see Falkowski & La Roche 1991).

Xanthophyll pigments are carotenoids that fulfill both photosynthetic light-harvesting and photoprotective functions in algae, and these pigments are essential for the survival (Polimene et al. 2012) and ecological success of algae in their natural environments (Eonson et al. 2003). In chlorophyll *c* (Chl *c*)-containing algae, such as diatoms, dinoflagellates and prymnesiophyceae, diadinoxanthin (DD) and fucoxanthin (Fuco) can transfer excitation en-

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ergy to chlorophyll *a* (Chl *a*) and play a role in the acquisition of light energy as a photosynthetic light-harvesting pigment, whereas diatoxanthin (DT) can absorb excitation energy from Chl *a* and play a role in thermal dissipation as a photoprotective pigment (Shreve et al. 1991, Young et al. 1997, Lohr & Wilhelm 1999, 2001, Harris et al. 2009, Polimene et al. 2012). The cellular content of Fuco exhibits the same variations as other chlorophyll pigments in response to the PFD intensity, however, the cellular content of DD and DT exhibits variation patterns that are different from other pigments (MacIntyre et al. 2002). The specific dynamics of DD and DT have been reported in previous studies (e.g., Ragni & D'alcalà 2007). The selective synthesis or degradation of DD and DT are known to be *de novo* synthesis or degradation processes (Olaizola et al. 1994). In addition to the *de novo* process, a reversible conversion between DD and DT is known in the xanthophyll cycling process (DD-cycle). The xanthophyll cycling process is one of the main processes regulating excessive light energy in the light-harvesting complexes of photosystems and represents an important photoprotection mechanisms in algae (for a review, see Goss & Jakob 2010). The conversion of DT to DD occurs under limited PFD and darkness, whereas a reverse conversion occurs under excess PFD. The DD-cycle is controlled by light–dark transition in addition to the PFD intensity (Hager 1980, Yamamoto 1985). Furthermore, an interconversion between Fuco and DD has been proposed in recent reports (e.g., Lohr & Wilhelm 1999, Harris et al. 2009, Polimene et al. 2012). The interconversion could be reversible depending on light conditions. Because the DD and DT dynamics can be affected by the light–dark transition and the intensity of the PFD, this could be of benefit in the highly variable underwater light climate, which requires high flexibility (Goss & Jakob 2010). The photoresponse of these xanthophyll pigments to light–dark transition and acclimation to PFD can provide useful information about the photoacclimation of the photosynthetic apparatus, in particular for phytoplankton.

The kinetics and capacity of the xanthophyll cycle can be used as a marker for the photoadaptive state of algal cells and their ecological niches (Dimier et al. 2009). Brunet & Lavaud (2010) pointed out that there is a need to increase the data set on xanthophyll cycle characteristics such as the light-dependent responses of xanthophyll-cycling pigment, in as many different microalgal species/groups as possible. There have been many studies on xanthophyll-cycling pigment dynamics in diatoms (e.g., Lavaud et al. 2004) but few studies in other algae. The aim of the present study was to gain additional insight into the relevance of the DD and DT dynamics and photoacclimation in the algal photosynthetic apparatus. The present study investigated the effect of the acclimation PFD on the photoresponse of DD and DT to repetitive light–dark transitions in cultures of *Isochrysis galbana* (Prymnesiophyceae). *Isochrysis galbana* has a simple shape (coccoid in fresh cells) and has division patterns typical of phytoplankton

(Chisholm 1981). This species has also been used for studies of photoacclimation (e.g., Falkowski et al. 1985, Fujiki & Taguchi 2002). *Isochrysis galbana* had previously been acclimated to four different PFD levels ranging from a limited light condition (LL) to a saturated light condition (SL) under cycles of light and dark, because photoacclimation responses depend on whether the PFD is light-limiting or light-saturating for growth (Raven & Geider 2003). As the interpretation of the photoacclimation response depends on the method by which phytoplankton mass is expressed (Leonardos 2008), the photoresponses of DD and DT were based on the xanthophyll : Chl *a* ratio and the cellular pigment content. Because Chl *a* is an essential pigment that transfers excitation energy to the reaction center of the photosystem (Scheer 2003), the photoresponses of the DD and/or DT : Chl *a* ratio can detect changes in the composition and function of the photosystem. The cellular DD and/or DT content also make intuitive sense for photoacclimation since a cell is the smallest population unit (Anning et al. 2000). The examination of xanthophyll-cycling pigment dynamics may provide a better understanding of photoacclimation in the algal photosynthetic apparatus.

Materials and Methods

Cultures of *Isochrysis galbana* (Prymnesiophyceae; NEPECC633) were obtained from the North East Pacific Culture Collection (NEPECC) at the University of British Columbia, Canada. The axenic stock cultures were routinely maintained at 25°C and at a PFD of 150 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$ under a 12 : 12 h light : dark cycle in filtered, aged seawater enriched with f/2 medium (Guillard & Ryther 1962) using the Eppley culture method (Eppley 1977). Cylindrical 3- or 6-L round-bottom borosilicate glass culture vessels were illuminated from the side. The illumination was provided by cool-white fluorescent tubes (FL30SEX-N, National, Tokyo, Japan) on a 12 : 12 h light–dark cycle at one of four PFDs: 45, 250, 425, and 1,370 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$. The spectral PFD of the fluorescence lamp showed six peaks (406, 436, 490, 545, 588 and 612 nm) from 400 to 700 nm. The maximum and minimum of these peaks were observed to be 545 and 406 nm, respectively. The PFD was determined from measurements taken in the center of a similar vessel containing 3 or 6 L of distilled water using a 4 π -scalar quantum sensor (QSL-100, Biospherical Instruments, San Diego, California, USA). The desired light intensities in the culture vessels were obtained by adjusting the number of fluorescent tubes. The cultures were mixed using air supplied through a 0.2- μm membrane filter and by a magnetic stirrer at the bottom of the culture vessel. The cultures were operated as continuous cultures for more than three weeks with the dilution rate dictated by the growth rate. The cell concentrations were monitored once per day at hour 6 of the light period. We attempted to maintain a relatively constant cell density at hour 6 of the light period by manually adjusting

the flow rate of the nutrient medium that was being added to the culture vessels with a peristaltic pump (MP-3A, Eyela, Tokyo, Japan). The steady state was operationally defined in our experiments to be when the cell density at hour 6 of the light period varied by less than 10% over at least three consecutive sampling times. The growth rate under the steady state was targeted to be 0.3 d^{-1} under all light conditions. The excess of nitrate was verified by measuring the nitrate concentrations of at least $50 \mu\text{M}$ of the out-flow from the culture vessels. The specific growth rate μ of the culture was calculated using the following equation:

$$\mu = (\ln N_1 - \ln N_0) \Delta t^{-1} + D \quad (1)$$

where N_0 and N_1 are the cell densities (cells mL^{-1}) at time t_0 and time t_1 , respectively; Δt is the length of the time (d^{-1}) interval ($t_1 - t_0$); and D is the dilution rate during Δt . The cultures were acclimated to each light condition for more than three weeks before the start of sampling. All of the incubation experiments were conducted for three light–dark transitions. The samples for the cell and pigment analysis were collected every 3 h during three light–dark transitions from continuous cultures at a steady state.

Cell density

The subsamples for measuring cell density were fixed using 5% formaldehyde and stored in the dark at 4°C (Iwasawa et al. 2009). The cell numbers were counted with a hemacytometer (model 35103: Erma, Tokyo, Japan) under a microscope (IMT-2, Olympus, Tokyo, Japan) at 400x magnification.

Pigment analysis

Triplicate subsamples for pigment analysis were filtered onto Whatman GF/A glass fiber filters (Whatman, Maidstone UK, USA) within 2 min of subsample collection under low vacuum pressure ($<100 \text{ mm Hg}$) and dark conditions. These filters were immediately frozen and kept in a deep-freezer at -60°C for further analysis. The filters containing cell materials were sonicated for 10 min in N,N -dimethylformamide and pigments were extracted at -20°C for 24 h in the dark (Suzuki & Ishimaru 1990). The extracts were analyzed on a Beckman HPLC (Model 168 Diode Array Detector, Fullerton, California, USA) with a C-18 reverse-phase column (Ultrasphere 3- μm ODS, Beckman, Fullerton, California, USA) using a solvent gradient system as described by Head & Horne (Head & Horne 1993). The integrated HPLC peak areas for Chl a , DD, and DT were quantified based on standards obtained from the Sigma Chemical Company (Saint Louis, Missouri, USA).

Statistical analysis

We used model II linear regression analysis. This regression analysis is the most accurate method for estimating the functional relationship between X and Y under conditions in which the X variable is uncontrolled and sub-

ject to error (Laws & Archie 1981). One-way ANOVA was employed to test for a difference in the cellular xanthophyll-cycling pigment content.

Results

Cell density and pigment response to the light–dark transition

The cell density and cellular Chl a content exhibited a repetitive photoresponse during the light–dark transitions at each acclimation PFD (Fig. 1). During the light-to-dark transition, the cell density decreased at the end of the first 3 or 6 h of the dark period due to cell dilution, and subsequently increased in the remaining dark period until the end of the first 3 or 6 h of the light period, due to cell division prevailing against cell dilution, for all PFD conditions except for the lowest acclimation PFD. Under the lowest acclimation PFD, the cell density was relatively constant throughout the light–dark transition. When the cell densities were low, the cellular Chl a levels were high and *vice versa* (Fig. 1). The cellular Chl a level began to increase during the first 9 h or by the end of the light period, due to pigment synthesis. The Chl a level began to decrease toward the end of the dark period, due to cell division, and later increased again at the dark-to-light transition for all

Fig. 1. Cell density (upper) and cellular Chl a content (lower) under light–dark transition. Acclimation PFD levels: 45 (circles), 250 (squares), 425 (triangles), and 1370 (diamonds) $\mu\text{mol photons m}^{-2} \text{ sec}^{-1}$. Grey boxes indicate dark phase.

Fig. 2. Photoresponses of the cellular content of xanthophyll pigments to light–dark transition at 45, 250, 425 and 1,370 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$. Open symbols of circles, squares and triangles indicate DD_{cell} , DT_{cell} and $(\text{DD}+\text{DT})_{\text{cell}}$, respectively. Grey boxes indicate dark phase.

PFD conditions except for in the highest acclimation PFD. Under the highest acclimation PFD almost no response was observed with regards to the cellular Chl *a* content.

The photoresponses of xanthophyll pigments to light–dark transitions differed between the acclimation PFDs. Under the two lower acclimation PFDs, the pool of cellular xanthophyll pigments $[(\text{DD}+\text{DT})_{\text{cell}}]$ and the cellular DD content (DD_{cell}) continued to decrease during the first half of the light period and subsequently began to increase from the middle of the light period to the end of the light period or through the first 3 h of the dark period (Fig. 2). In contrast to the $(\text{DD}+\text{DT})_{\text{cell}}$ and DD_{cell} levels, the cellular DT levels (DT_{cell}) under the two lower acclimation PFDs were relatively constant under the light–dark transition. Under 425 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$, the $(\text{DD}+\text{DT})_{\text{cell}}$ and the DD_{cell} continued to increase during the light period and through the first 3 h of the dark period and later decreased at the end of the dark period. The DT_{cell} increased during the light period and decreased during the dark period. Under the highest acclimation PFD, the $(\text{DD}+\text{DT})_{\text{cell}}$ continued to increase during the light period and through the middle of the dark period and subsequently decreased at the end of the dark period. In contrast to the $(\text{DD}+\text{DT})_{\text{cell}}$, the DD_{cell} was relatively constant during the light period and increased during the first 3 h of the dark period; it began to decrease thereafter from the middle of the dark period to the end of the dark period. The DT_{cell} increased

during the light period and decreased during the dark period.

The photoresponses of the DD and/or DT : Chl *a* ratios ($[(\text{DD}+\text{DT})_{\text{Chl } a}]$, $\text{DD}_{\text{Chl } a}$, $\text{DT}_{\text{Chl } a}$) were similar to the cellular xanthophyll pigment responses at each acclimation PFD (Fig. 3). Stoichiometric variations were observed between the $\text{DD}_{\text{Chl } a}$ and $\text{DT}_{\text{Chl } a}$ during the first 3 h of the light to dark or dark to light transitions at the two higher acclimation PFDs. The magnitude of the stoichiometric variations under 1,370 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$ was 10 times as large as that under 425 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$.

Relationship between DD or DT and DD+DT

Linear relationships between DD or DT and DD+DT were obtained at all four experimental PFDs, whether the data were normalized to the cell density (Table 1) or to Chl *a* concentration (Table 2) during both light and dark periods (Fig. 4). A highly significant relationship ($p < 0.001$) was observed between DD_{cell} and $(\text{DD}+\text{DT})_{\text{cell}}$ for all PFD conditions, except for in the light phase at the highest acclimation PFD (Table 1). At the two lower acclimation PFDs, the slopes for these relationships were approximately >0.9 during both the light and dark phases. During the light phase, the slopes ranged from approximate unity at the lowest acclimation PFD to 0.3 at the highest acclimation PFD. The slopes obtained during the dark phase were less variable than the slopes obtained during the light

Fig. 3. Photoresponses of the xanthophyll : Chl *a* ratio to light–dark transition at 45, 250, 425 and 1370 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$. Open symbols of circles, squares and triangles indicate $\text{DD}_{\text{Chl } a}$, $\text{DT}_{\text{Chl } a}$ and $(\text{DD}+\text{DT})_{\text{Chl } a}$, respectively. Grey boxes indicate dark phase.

Table 1. Linear regression analyses of the relationships between DD or DT and (DD+DT) on a cell basis.

PFD	Light				Dark			
	Slope	Intercept	n	r^2	Slope	Intercept	n	r^2
DD								
45	0.978	−0.0003	12	0.99	0.969	0.0015	13	0.99
250	0.892	0.0048	12	0.99	0.932	0.0026	13	0.99
425	0.608	0.0226	12	0.94	0.843	0.0068	13	0.99
1370	0.298	0.0303	12	0.28 ^{ns}	0.705	0.0140	12	0.92
DT								
45	0.022	0.0003	12	0.52	0.031	−0.0015	13	0.33
250	0.113	−0.0051	12	0.90	0.065	−0.0024	13	0.88
425	0.393	−0.0227	12	0.88	0.158	−0.0069	13	0.83
1370	0.710	−0.0309	12	0.70	0.301	−0.0146	12	0.68

ns indicates no significance ($p > 0.05$).

phase. Significant relationships were also observed between the DT_{cell} and $(\text{DD}+\text{DT})_{\text{cell}}$ during both the light and dark periods at all PFD levels ($p < 0.05$) (Table 1). These slopes increased with increasing acclimation PFD from 0.02–0.03 at the lowest acclimation PFD to 0.7 during the light phase and 0.3 during the dark phase at the highest acclimation PFD. Except for the light period at the lowest acclimation PFD, the *X*-axis intercept ($\text{DT}=0$) of all of the lines converged around 0.049 ± 0.008 (mean $\pm$ one standard

deviation) $\text{fmol (DD+DT) cell}^{-1}$ during the light period and 0.044 ± 0.005 $\text{fmol (DD+DT) cell}^{-1}$ during the dark period (Fig. 4).

Relationships similar to those observed for the cell density-specific values were also obtained for the xanthophyll : Chl *a* ratio during both the light and dark periods (Table 2). The slopes for the relationships between $\text{DD}_{\text{Chl } a}$ and $(\text{DD}+\text{DT})_{\text{Chl } a}$ were 1.0 or close to 1.0 during both the light and dark periods at the lowest acclimation PFD and

Table 2. Linear regression analyses of the relationships between DD or DT and (DD+DT) on a Chl *a* basis.

PFD	Light				Dark			
	Slope	Intercept	n	r ²	Slope	Intercept	n	r ²
DD								
45	1.00	-0.0019	12	0.99	0.989	-0.0008	13	0.99
250	0.931	0.0041	12	0.97	0.821	0.0269	13	0.93
425	0.189	0.152	12	0.27 ^{ns}	0.784	0.0299	13	0.98
1370	0.201	0.407	12	0.28 ^{ns}	0.758	0.0917	12	0.93
DT								
45	0.004	0.0019	12	0.02 ^{ns}	0.002	0.0019	13	0.00 ^{ns}
250	0.068	-0.0040	12	0.14 ^{ns}	0.179	-0.0268	13	0.46
425	0.811	-0.152	12	0.87	0.216	-0.0298	13	0.80
1370	0.799	-0.407	12	0.86	0.242	-0.0917	12	0.56

ns indicates no significance ($p > 0.05$).

Fig. 4. Relationship between cellular DT content and cellular (DD+DT) content in light and dark phases. Acclimation PFD levels: 45 (circles), 250 (squares), 425 (triangles), and 1370 (diamonds) $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$.

decreased to 0.20 during the light period and 0.76 during the dark period. The slopes for the relationships between $\text{DT}_{\text{Chl } a}$ and $(\text{DD}+\text{DT})_{\text{Chl } a}$ were the lowest (<0.004) at low PFDs and generally increased to 0.80 with increasing acclimation PFD during the light period and to 0.24 during the dark period.

Fig. 5. Changes in the slopes of the relationships between DD and (DD+DT) (open symbols) and the relationships between DT and (DD+DT) (closed symbols) to PFD during the light phase on the basis of the cellular pigment content (upper) and the xanthophyll : Chl *a* ratio (lower). Acclimation PFD levels: 45 (circles), 250 (squares), 425 (triangles), and 1370 (diamonds) $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$.

Discussion

The patterns of the photoresponse according to cell density and cellular Chl *a* content under light–dark cycles obtained in this study are similar to those found in previous

studies (e.g., Post et al. 1984, DuRand et al. 2002, Harris et al. 2009). The photoresponses, measured by cell density and cellular Chl *a* content in *Isochrysis galbana* during the light–dark transition, were consistent with the concept that cell division during the dark phase is common among phytoplankton (Chisholm 1981, Jacquet et al. 2001) and that cellular Chl *a* content increases with pigment synthesis during the light phase and decreases during the dark phase upon cell division (Kohata & Watanabe 1988, Ragni & D'Alcala 2007). Photoacclimation is commonly demonstrated as a reduction in the cellular Chl *a* content in response to increased PFD (e.g., MacIntyre et al. 2002). The decline in the level of cellular Chl *a* content with increasing acclimation PFD that was observed in the present study may represent evidence of success in acclimation to each growth PFD.

Repetitive photoresponses of cellular xanthophyll pigment content and xanthophyll pigment : Chl *a* ratio under light–dark cycles have been reported in the diatom *Pheodactylum tricornutum* (Ragni & D'Alcala 2007). For *I. galbana*, the cellular xanthophyll pigment content and the xanthophyll pigment : Chl *a* ratio exhibited a repetitive response to the light–dark transition. It was clear that acclimation PFD affected the xanthophyll pigment : Chl *a* ratio of *I. galbana*. In general, xanthophyll pigment : Chl *a* ratio increases with the growth PFD due to both an increase in cellular xanthophyll pigment content and a decrease in the cellular Chl *a* content with an increased growth PFD (MacIntyre et al. 2002). However, the level of the cellular xanthophyll pigment content of *I. galbana* changed slightly compared with the decrease of cellular Chl *a* content over the range of acclimation PFDs. This finding suggests that the increase in the level of xanthophyll pigment : Chl *a* ratio of *I. galbana* with acclimation PFD results from the simultaneous decline in the level of cellular Chl *a* content in response to increasing acclimation PFD. Because the decline in the level of cellular Chl *a* content lowers the excess of energy reaching the photosystems, these mechanisms can be photoprotective and/or photoacclimatory (Harris et al. 2009). In *I. galbana*, the xanthophyll pigment : Chl *a* ratio depends on cellular Chl *a* content.

The patterns of the photoresponses of the cellular xanthophyll pigment content and the xanthophyll pigment : Chl *a* ratio in *I. galbana* under light–dark transition depended on the acclimation PFD. The dependence in the patterns of these photoresponses to the acclimation PFD indicates an effect of the acclimation PFD on these pigment dynamics during light–dark transitions. Dynamics of DD and DT during light–dark transition are controlled by three processes (Olaizola et al. 1994, Lavaud et al. 2004, Harris et al. 2009, Polimene et al. 2012). The first process is *de novo* synthesis or the degradation of DD and DT (Olaizola et al. 1994, Förster et al. 2009, Harris et al. 2009, Polimene et al. 2012). The *de novo* process represents a fundamental process controlling the xanthophyll pigment

dynamics during light–dark transitions, regardless of the acclimation PFD. The occurrence of the *de novo* process as related to xanthophyll pigments is demonstrated by the changes in the pool size of the xanthophyll pigments ($[(DD+DT)_{Chl\ a}]$) (Olaizola & Yamamoto 1994, Mewes & Richter 2002, Lavaud et al. 2004). The second process is the light-dependent interconversion between DD and Fuco (Harris et al. 2009, Polimene et al. 2012). The interconversion also leads to changes in the $DD_{Chl\ a}$ and $(DD+DT)_{Chl\ a}$. The third process is the xanthophyll cycling process, which is the reversible conversion between DD and DT (Hager & Stransky 1970). The xanthophyll cycling process results in a stoichiometric increase in $DT_{Chl\ a}$ and decrease in $DD_{Chl\ a}$ during high PFD exposure and *vice versa* during low PFD exposure or during the dark phase. Therefore, $(DD+DT)_{Chl\ a}$ remains constant in the xanthophyll cycling process when the *de novo* process and interconversion between DD and Fuco are excluded. The dependence of the xanthophyll cycling process on the PFD acclimation (Hager 1980, Yamamoto 1985) suggests that the effect of the xanthophyll cycling process on the DD and DT dynamics during light–dark transitions varies with the PFD. Because the rate and degree of de-epoxidation are greater at higher PFDs (Lavaud et al. 2004), the effect of the xanthophyll cycling process on xanthophyll pigment dynamics during light–dark transitions may become larger at higher PFDs. No stoichiometric changes were observed between $DD_{Chl\ a}$ and $DT_{Chl\ a}$ at 45 or 250 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$, suggesting that these pigment dynamics might be controlled by the *de novo* process and interconversion between DD and Fuco, rather than the xanthophyll cycling process. In contrast, the xanthophyll pigment dynamics at the two higher acclimation PFDs might be controlled by all three processes. The occurrence of the *de novo* process and interconversion between DD and Fuco for these pigments was demonstrated by the change in $(DD+DT)_{Chl\ a}$ in the light and dark phases and the decrease in $DD_{Chl\ a}$ during the last 6 h of the dark phase under 425 and 1,370 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$. Additionally, the occurrence of the xanthophyll cycling process was shown by stoichiometric variations between $DD_{Chl\ a}$ and $DT_{Chl\ a}$ during the first 3 h of the light to dark or dark to light transitions at 425 and 1,370 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$. Furthermore, the large stoichiometric variations between $DD_{Chl\ a}$ and $DT_{Chl\ a}$ at the higher acclimation PFDs suggest that the role of the xanthophyll cycling process in xanthophyll pigment dynamics is significant at high acclimation PFDs. This idea is consistent with the concept that phytoplankton activate the xanthophyll cycle reaction at high PFDs as an important process related to the photoprotection of the PSII (Müller et al. 2001). The dependence of xanthophyll pigment dynamics on the acclimation PFD suggests that it is associated with both *de novo* and xanthophyll cycling processes.

The existence of the *X*-axis intercept in the relationship between the DT_{cell} and $(DD+DT)_{cell}$ under all acclimation PFDs (Fig. 4) suggests the persistent existence of a fraction

of the DD that may not be directly associated with DT synthesis. Because DT is converted from DD through de-epoxidation, this fraction may be non-convertible, regardless of the acclimation PFD. The non-convertible fraction of DD was estimated to be approximately $0.046 \text{ fmol DD cell}^{-1}$ for *I. galbana* in the present study. This non-convertible fraction could be a constitutive portion of the light-harvesting complex (LHC) as suggested by Lohr & Wilhelm (2001). According to Lohr & Wilhelm (2001), the percentage of the constitutive DD within the LHC (DD_{LHC}) was estimated to be 6% of the total carotenoids, corresponding to 37% of DD+DT for the diatom *P. tricornutum* at steady-state growth in low light conditions ($40 \mu\text{mol photons m}^{-2} \text{ sec}^{-1}$). If the non-convertible fraction of the DD estimated in the present study is assumed to be the DD_{LHC} , then a similar percentage (39%) of DD+DT is obtained for *I. galbana* grown at the lowest acclimation PFD ($45 \mu\text{mol photons m}^{-2} \text{ sec}^{-1}$). A percentage similar to that found in diatoms may be explained by the coincident fact that prymnesiophytes and diatoms have a similar LHC that contains fucoxanthin-chlorophyll *a/c*-protein (Fawley et al. 1987, Macpherson & Hiller 2003). The de-epoxidation state (DES), defined conventionally as the ratio of the DT to DD+DT concentrations (e.g., Janknegt et al. 2008), could potentially be underestimated, due to the presence of the non-convertible fraction of the DD (Lavaud et al. 2004). Estimating the DES considering only the convertible fraction of the DD enabled us to assess the functional de-epoxidation state accurately.

The slopes of the relationship between the DD or DT and DD+DT on either a cell or chlorophyll basis indicate a quantitative index of the extent of DD and DT dynamics. The change in the slopes of the relationship between the DD or DT and DD+DT in response to the variation of acclimation PFD suggests that DD and DT dynamics vary with the acclimation PFD. The change in the slope of the relationship between the DD or DT and DD+DT of *I. galbana* during the light phases in response to the variation of acclimation PFD was characterized by either a linear or sigmoidal function, respectively (Fig. 5, Obata 2010). The different functions arise from the different physiological bases of the estimation, i.e., measurements coming from either a whole cell or the Chl *a* mass. Examining the photoacclimation parameters at a cellular level makes intuitive sense (Anning et al. 2000). The linear change in the cell-specific slopes in response to the variation of the acclimation PFD (Fig. 5) suggests that the DD and DT dynamics during the light-dark transition can be directly determined by the acclimation PFD. Alternatively, the sigmoid change in the Chl *a*-specific slopes in response to the variation of the acclimation PFD may be determined by the light conditions. Based on the relationship between the algal growth rate and the acclimated PFD (MacIntyre et al. 2002), light conditions for algal growth can be categorized into two types: limited light conditions (LL), where the growth rate depends on the PFD, or saturated light conditions (SL),

where the growth rate is at its maximum. For *I. galbana*, there may be a range of LL conditions corresponding to PFDs below approximately $200\text{--}300 \mu\text{mol photons m}^{-2} \text{ sec}^{-1}$ and a range of SL conditions at higher PFDs, because their growth rate becomes saturated at around approximately $200\text{--}300 \mu\text{mol photons m}^{-2} \text{ sec}^{-1}$ (Jokiel & York 1984, Falkowski et al. 1985). Examining the photoacclimation parameters on the basis of Chl *a* provides insight into the antenna of the photosystem because Chl *a* transfers excitation energy to the reaction center of the photosystem (Scheer 2003). The high slopes based on the $\text{DD}_{\text{Chl } a}$ and low slopes based on the $\text{DT}_{\text{Chl } a}$ under the LL conditions suggests that algae may have enhanced light harvesting under LL conditions. The opposite situation, i.e., low slopes based on $\text{DD}_{\text{Chl } a}$ and high slopes based on $\text{DT}_{\text{Chl } a}$, under the SL conditions suggests that algae may have enhanced photoprotective energy dissipation. The suggestions obtained from examining the two slopes based on $\text{DD}_{\text{Chl } a}$ and $\text{DT}_{\text{Chl } a}$ to the light conditions of LL vs. SL in the present study agree with the findings of previous studies (e.g., Stolte et al. 2000) that demonstrated the consequences of photoacclimation through the relative abundances of photosynthetic and photoprotective pigments.

In addition, the position of the intersection between the sigmoid changes in Chl *a*-specific slopes in response to the variation of the acclimation PFD in the present study is close to the "hinge point" that was observed for the photoacclimation of microalgae with respect to the relative abundance of photosynthetic and photoprotective pigments (MacIntyre et al. 2002). The slopes of the relationship between DD or DT and DD+DT on a Chl *a* basis during the light-dark transition may be useful as an index of the relevant roles of these xanthophyll-cycling pigments in light harvesting or photoprotection and as a marker of the physiological status of phytoplankton with respect to PFD. An examination of the slope may provide information for a better understanding of photoacclimation in the photosynthetic apparatus of algae.

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