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Relationship between light absorption and the xanthophyll-cycle pigments in marine diatoms

TETSUICHI FUJIKI & SATORU TAGUCHI

Laboratory of Biological Oceanography, Department of Bioengineering, Faculty of Engineering, Soka University, 1–236 Tangi-cho, Hachioji 192–8577, Japan

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Abstract: The relative contribution of the xanthophyll-cycle pigments, diadinoxanthin plus diatoxanthin, to total in vivo pigment absorption was examined in 3 species of marine diatoms grown at 6 irradiances between 90 and 750 μmol $m^{-2}s^{-1}$. These diatoms included *Phaeodactylum tricornutum*, *Chaetoceros gracilis* and *Thalassiosira weissflogii*. The pigment ratios of diadinoxanthin plus diatoxanthin to chlorophyll *a* (Chl *a*) for all 3 species increased with increasing growth irradiance. For *P. tricornutum*, the ratio was 5.5 times higher in cells grown under 750 μmol $m^{-2}s^{-1}$ compared to those grown at 90 μmol $m^{-2}s^{-1}$. As the growth irradiance varied from 90 to 750 μmol $m^{-2}s^{-1}$, the relative contribution of absorption by diadinoxanthin plus diatoxanthin to total in vivo pigment absorption (for wavelengths from 400 to 700 nm [PAR]) increased from 4.5 to 17% for *P. tricornutum*, 5.8 to 19% for *C. gracilis* and 13 to 30% for *T. weissflogii*. The high contribution of absorption by diadinoxanthin plus diatoxanthin to total in vivo pigment absorption was observed between 450 and 500 nm, in which the contribution of Chl-*a* absorption was minimal. The maximum contribution of absorption by diadinoxanthin plus diatoxanthin to total in vivo pigment absorption was 57% at 462 nm in *T. weissflogii* grown under 750 μmol $m^{-2}s^{-1}$. For all species, a significant negative relationship was obtained between absorption of diadinoxanthin plus diatoxanthin and quantum yield for growth. This relationship may suggest that the xanthophyll cycle dissipates the excess light energy and helps to maintain growth rate under high light conditions.

Key words: absorption spectra, quantum yield, spectral reconstruction technique, xanthophyll cycle

Introduction

Phytoplankton can adapt to changes in light quality (intensity and/or spectrum) by varying the total amount of cellular pigments, the ratio of different pigments, or both. The light energy absorbed by pigments is either used in photosynthesis, re-emitted as fluorescence or dissipated as heat (Falkowski & Raven 1997). In stressful high light conditions, if phytoplankton cells are unable to utilize the high level of energy absorbed by pigments, the excess energy will cause damage to intracellular materials or metabolic processes, e.g. destruction of chloroplast membranes or inactivation of enzymes. Some carotenoids can serve as photoprotection against such photoinhibition by quenching the excess energy (Vincent et al. 1984; Cogdell & Frank 1987; Demmig-Adams 1990). The xanthophyll cycle is one of the photoprotective systems of carotenoids. This cycle can dissipate the excess light energy by mutual transformations between epoxy-containing xanthophylls (oxy-derivatives of carotenes) and epoxy-free xanthophylls (Hager 1975). In higher plants and chlorophytes, this cycle contains the 3 components of violaxanthin, antheraxanthin and zeaxanthin (Hager 1975), and the formation of zeaxanthin helps in the process of dissipation of excess energy (Demmig et al. 1987). The conversions are mediated by a reversible light epoxidizing enzyme in the thylakoid membranes that uses a pH gradient across the lumen membrane (Gilmore & Yamamoto 1993). The xanthophyll pigments, diadinoxanthin and diatoxanthin, found in chromophyte algae such a diatoms, dinoflagellates and prymnesiophytes have recently been reported to possess a similar photoprotective system to the xanthophyll cycles of higher plants and chlorophytes (Demers et al. 1991; Arsalane et al. 1994; Olaizola & Yamamoto 1994; Moisan et al. 1998). When the algae are ex-

Corresponding author: Tetsuichi Fujiki; e-mail, thujiki@edu.t.soka.ac.jp

posed to stressful high irradiance, some diadinoxanthin is de-epoxidized to diatoxanthin within 5 min. Light energy absorbed by xanthophyll cycle pigments is not transferred to the reaction centers for photosynthesis, but is dissipated by mutual transformations between diadinoxanthin and diatoxanthin. What is not well understood at present is how the xanthophyll cycle pigments contribute to cellular light absorption. It is imperative to study quantitatively the photoprotection process by diadinoxanthin and diatoxanthin.

The spectral reconstruction technique for phytoplankton absorption can provide information on the relative contribution of individual pigments to total in vivo pigment absorption (Bidigare et al. 1989; Hoepffner & Sathyendranath 1991; Babin et al. 1996). In this study, we have attempted to address this question by examining the contribution of absorption by diadinoxanthin plus diatoxanthin to total in vivo pigment absorption for diatoms grown under high light conditions using the spectral reconstruction technique (assuming that all diadinoxanthin and diatoxanthin is related to the xanthophyll cycle).

Materials and Methods

Cultures

Cultures of the diatoms *Thalassiosira weissflogii* [Grunow] G. Fryxell & Hasle (NEPCC741), *Chaetoceros gracilis* Schutt (NEPCC645) and *Phaeodactylum tricornutum* Bohlin (CCMP1327) were grown semicontinuously in f/2 medium (Guillard & Ryther 1962) at 25°C. *T. weissflogii* and *C. gracilis* were obtained from The North East Pacific Culture Collection (NEPCC) at the University of British Columbia, Canada. *P. tricornutum* was obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP), Bigelow Laboratory for Marine Sciences, Maine, USA. Cultures were exposed under cool fluorescent lights ranging from 90 to 750 μmol $m^{-2}s^{-1}$ on a 12:12 h light/dark cycle. Growth irradiance, I_0, was measured with a scalar quantum sensor (QSL-100, Biospherical Instruments Inc.). The relative spectral quantum irradiance $E'(\lambda)$ was measured with a spectroradiometer (MSR-7000, OPTO Research Corporation). Cell densities of the three species were determined using a microscope and a hematocytometer. Specific growth rate (μ) was calculated over the exponential growth phase. The cells grown in light/dark cycles were harvested at the end of the photoperiod to compare photoadaptation responses due to differences in growth irradiance.

Pigment concentrations

Triplicate samples were filtered onto Whatman GF/F glass fiber filters and immediately immersed in 90% acetone. Extraction of pigments was conducted for 24 h in the dark at 4°C after grinding and sonicating the cells harvested by filtration. Subsamples were analyzed on an HPLC (System Gold, Beckman) using a solvent gradient system similar to that described by Head & Horne (1993). Integrated peak areas were quantified with external standards obtained from the International Agency for ^{14}C Determination.

Particulate carbon contents

Triplicate samples were filtered onto precombusted Whatman GF/F filters, dried at 60°C for 24 h, and analyzed with an elemental analyzer (FISON Instrument NA1500). Acetanilide was used as an external standard.

Light absorption

Phytoplankton absorption spectra were measured with the glass fiber filter technique (e.g. Trüper & Yentsch 1967; Mitchell & Kiefer 1988; Roesler et al. 1989). Triplicate samples were collected on Whatman GF/F filters at <50 mmHg. Optical density spectra were recorded from 400 to 750 nm with a Beckman DU640 spectrometer using a wet Whatman GF/F filter as a blank. Optical density spectra were converted to absorption coefficients $a_p(\lambda)$ by subtracting the optical density at 750 nm from all wavelengths, dividing by the geometrical path length (ratio of filtered volume to the filtered clearance area of the filter) and adjusted for path length amplification. The path length amplification factor described by Cleveland & Weidemann (1993) was employed for all absorption spectra. Pigments were then extracted from filtered samples using cold methanol (Kishino et al. 1985), and the absorption of the remaining particles was measured (defined as the nonpigment absorption coefficient, $a_d(\lambda)$). The phytoplankton absorption coefficient, $a_{ph}(\lambda)$, was calculated as the difference between $a_p(\lambda)$ and $a_d(\lambda)$. Finally, $a_{ph}(\lambda)$ was converted to the Chl-*a*-specific absorption coefficient, $a^*(\lambda)$, by dividing by the Chl-*a* concentration.

Spectral reconstruction of absorption coefficients

Absorption spectra of pure pigment standards were wavelength-shifted to match their in vivo absorption peaks and shoulders using the approach described by Bidigare et al. (1990). Then, absorption spectra of the 6 pigments were scaled to their respective weight-specific absorption coefficients to give in vivo weight-specific absorption coefficients (Fig. 1). Reconstructed absorption coefficients were estimated as the product of the volume-based pigment concentrations and in vivo weight-specific absorption coefficients. The spectral reconstruction technique for phytoplankton absorption does not take package effect into consideration. Moreover, there are also some problems regarding spectral shape and molarity when compared with in vivo absorption (Bidigare et al. 1989; Hoepffner & Sathyendranath 1991; Moisan & Mitchell 1999). However, if the spectral reconstruction technique is used in combination with the glass fiber filter technique described above, one can obtain the contribution of absorption by individual pigments to total in vivo pigment absorption and this overcomes these problems

(Babin et al. 1996).

The relative contribution of absorption by diadinoxanthin plus diatoxanthin to total in vivo pigment absorption for wavelengths from 400 to 700 nm (PAR), c_{DD+DT}, was calculated by the following equation,

$$c_{DD+DT}=\frac{\int_{400}^{700} a^*(\lambda)[a_{DD+DT}(\lambda)/a_{TP}(\lambda)]E_0(\lambda)d\lambda}{\int_{400}^{700} a^*(\lambda)E_0(\lambda)d\lambda} \quad (1)$$

where $a_{DD+DT}(\lambda)$ is the reconstructed absorption coefficient of diadinoxanthin plus diatoxanthin, $a_{TP}(\lambda)$ is the total reconstructed absorption coefficient of the six pigments found in marine diatoms (cf. Fig. 1). $E_0(\lambda)$ is the spectral quantum scalar irradiance. $E_0(\lambda)$ was determined by the following equation,

$$E_0(\lambda)=\frac{I_0E'(\lambda)}{\int_{400}^{700} E'(\lambda)d\lambda} \quad (2)$$

where $E'(\lambda)$ is the relative spectral quantum irradiance measured with a spectroradiometer.

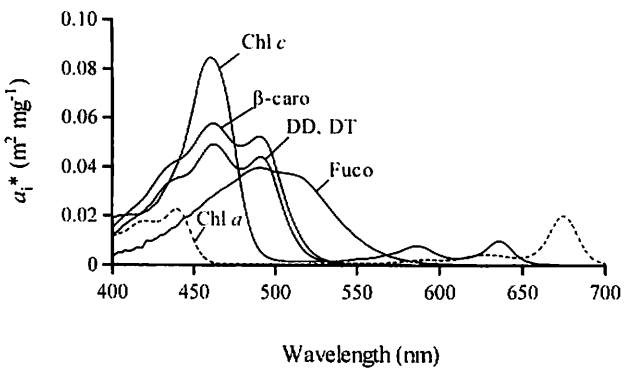

Fig. 1. In vivo weight-specific absorption coefficients (a_i^*) for the major pigments found in marine diatoms using the approach described by Bidigare et al. (1990). Chl *a*=chlorophyll *a*; Chl *c*=chlorophyll *c*; Fuco=fucoxanthin; β-caro=β-carotene; DD=diadinoxanthin; DT=diatoxanthin. The mean absorption coefficient of DD and DT was used because the weight-specific absorption coefficients of DD and DT were similar.

Results

Specific growth rate, μ, ranged from 0.64 to 0.98 d^{-1} for *P. tricornutum*, 0.85 to 1.35 d^{-1} for *C. gracilis* and 0.66 to 1.14 d^{-1} for *T. weissflogii* (Table 1). The optimal irradiance providing the maximum specific growth rate differed among the 3 species. Cellular carbon contents (C_{cell}) ranged from 7.3 to 10.3 pg cell^{-1} for *P. tricornutum*, 20.1 to 26.6 pg cell^{-1} for *C. gracilis* and 122 to 155 pg cell^{-1} for *T. weissflogii* (Table 1). The C_{cell} of *P. tricornutum* was inversely related to irradiance ($p<0.05$). The C_{cell} of the other two species did not show such a relationship. Cellular Chl-*a* content (Chl a_{cell}) of all 3 species decreased exponentially with increasing irradiance ($p<0.01$) (Table 1). For all three species, the ratio of cellular Chl-*a* content to carbon content (Chl *a* : *C*) decreased exponentially with increasing irradiance ($p<0.05$) (Table 1).

For all 3 species, Chl *c*, fucoxanthin, diadinoxanthin, diatoxanthin and β-carotene were measured as accessory pigments. All three species showed no change in the pigment ratios of Chl *c*, fucoxanthin and β-carotene to Chl *a* in response to irradiance, except for the ratio of Chl *c* to Chl *a* which decreased with increasing irradiance for *T. weissflogii* ($p<0.05$) (Table 2). The ratios of diadinoxanthin plus diatoxanthin to Chl *a* of all species increased with increasing irradiance ($p<0.05$). For example, this ratio for *P. tricornutum* was 5.5 times higher in the cells grown under 750 μmol m^{-2} s^{-1} compared to the cells grown under

Table 1. Variations in growth rate (μ), cellular carbon content (C_{cell}) and Chl-*a* content (Chl a_{cell}) and the ratio of cellular Chl-*a* content to carbon content (Chl *a* : *C*) for 3 species of diatom grown under 6 irradiances. μ in d^{-1}, C_{cell} and Chl a_{cell} in pg cell^{-1}.

Species	Variables	Irradiance (μmol m^{-2} s^{-1})					
		90	190	330	450	600	750
Phaeodactylum tricornutum	μ	0.67	0.64	0.98	0.87	0.90	0.90
	C_{cell}	10.3	10.0	8.78	8.10	8.74	7.27
	Chl a_{cell}	0.36	0.30	0.22	0.21	0.17	0.14
	Chl *a* : *C*	0.035	0.030	0.025	0.026	0.020	0.019
Chaetoceros gracilis	μ	0.85	0.97	0.91	1.35	1.28	1.27
	C_{cell}	21.3	20.1	26.4	25.3	26.6	25.5
	Chl a_{cell}	0.98	0.67	0.65	0.63	0.49	0.36
	Chl *a* : *C*	0.046	0.033	0.025	0.025	0.018	0.014
Thalassiosira weissflogii	μ	0.66	1.13	1.10	1.10	1.14	1.04
	C_{cell}	138	122	146	155	143	139
	Chl a_{cell}	4.13	3.60	3.28	3.08	2.87	2.79
	Chl *a* : *C*	0.030	0.029	0.022	0.020	0.020	0.020

Table 2. Ratios (w : w) of various accessory pigments to cellular Chl-*a* for 3 diatom species grown under different irradiances. Chl *c*= chlorophyll *c*; Fuco=fucoxanthin; β-caro=β-carotene; DD+DT=diadinoxanthin+diatoxanthin.

Species	Pigments	Irradiance (μmol m^{-2} s^{-1})					
		90	190	330	450	600	750
Phaeodactylum tricornutum	Chl *c*	0.139	0.150	0.162	0.159	0.145	0.165
	Fuco	0.617	0.714	0.814	0.722	0.649	0.944
	β-caro	0.007	0.007	0.007	0.006	0.006	0.007
	DD+DT	0.077	0.116	0.223	0.277	0.287	0.424
Chaetoceros gracilis	Chl *c*	0.171	0.143	0.125	0.148	0.152	0.102
	Fuco	0.512	0.469	0.454	0.388	0.369	0.422
	β-caro	0.031	0.036	0.037	0.040	0.042	0.035
	DD+DT	0.096	0.138	0.191	0.222	0.257	0.279
Thalassiosira weissflogii	Chl *c*	0.088	0.079	0.073	0.064	0.060	0.066
	Fuco	0.413	0.439	0.452	0.321	0.295	0.410
	β-caro	0.026	0.030	0.032	0.045	0.045	0.036
	DD+DT	0.206	0.278	0.300	0.293	0.325	0.543

90 μmol m^{-2} s^{-1}. This was primarily due to the increase in the ratio of diatoxanthin to Chl *a* with increasing irradiance (Fig. 2).

The mean Chl-*a*-specific absorption coefficient from 400 to 700 nm (PAR), $\bar{a}^*$, increased with increasing irradiance ($p<0.01$) (Table 3). Maximum variation in $\bar{a}^*$ was observed for *C. gracilis*, where $\bar{a}^*$ varied about 1.6 times. The characteristic absorption peaks by accessory pigments occurred around 440, 462, 490, 636 and 675 nm. The absorption peaks relating to diadinoxanthin plus diatoxanthin were located at 440, 462 and 490 nm (cf. Fig. 1). The contribution of absorption by diadinoxanthin plus diatoxanthin to total in vivo pigment absorption ($a^*_{(DD+DT)/TP}(\lambda)$) was determined by the ratio of the reconstructed absorption coefficient of diadinoxanthin plus diatoxanthin ($a_{DD-DT}(\lambda)$) to total reconstructed absorption coefficient ($a_{TP}(\lambda)$) (Fig. 3). The contribution of absorption by diadinoxanthin plus diatoxanthin to total in vivo pigment absorption at 440, 462 and 490 nm increased logarithmically with increasing irradiance ($p<0.05$) (Table 3). For example, the maximum contribution of absorption by diadinoxanthin plus diatoxanthin to total in vivo pigment absorption was 57% at 462 nm in *T. weissflogii* grown under 750 μmol m^{-2} s^{-1}.

The c_{DD+DT} calculated from equation 1 increased logarithmically with increasing irradiance ($p<0.01$) (Fig. 4). As the growth irradiance varied from 90 to 750 μmol m^{-2} s^{-1}, the c_{DD-DT} increased 3.8 times ranging from 4.5 to 17% for *P. tricornutum*, 3.3 times ranging from 5.8 to 19% for *C. gracilis*, and 2.3 times ranging from 13 to 30% for *T. weissflogii*.

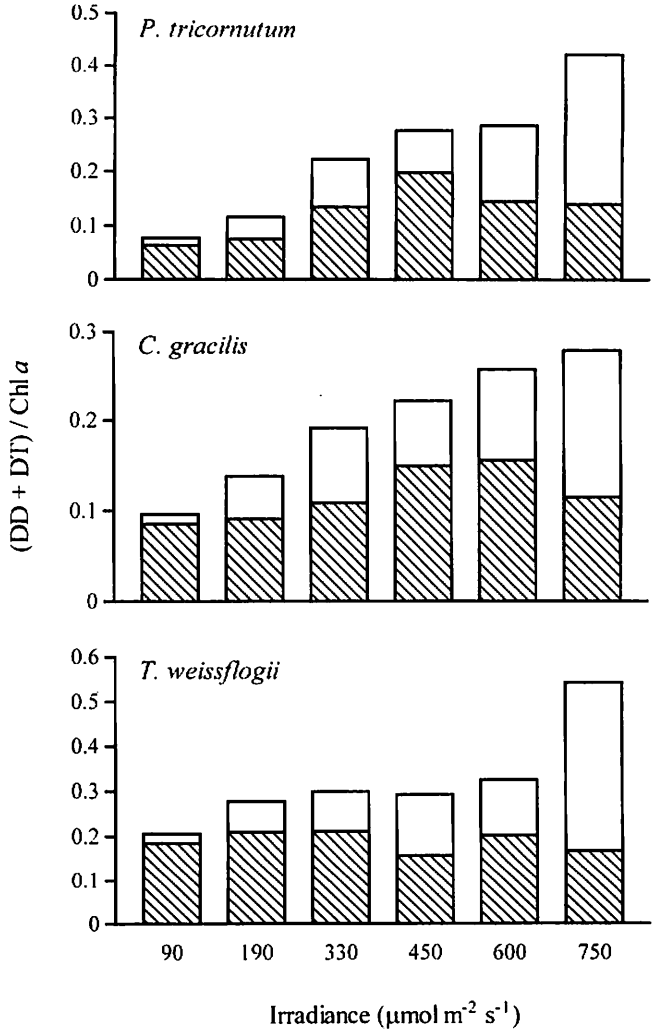

Fig. 2. Ratios (w : w) of cellular diadinoxanthin (DD; hatched) plus diatoxanthin (DT; open) to cellular Chl *a* for 3 diatom species grown under 6 irradiances ranging from 90 to 750 μmol m^{-2} s^{-1}.

Discussion

A marked decrease in μ due to photoinhibition was not observed for the 3 species studied, and for the range of irradiances used in the present study (Table 1). This result may

Table 3. Variations in the mean specific absorption coefficient ($\bar{a}^*$) and the contribution of absorption by diadinoxanthin plus diatoxanthin to total in vivo pigment absorption ($a^*_{(DD+DT)/TP}$) at 440, 462 and 490 nm. $\bar{a}^*$ in m^2 [mgChl a]$^{-1}$.

Species	Wavelength (nm)	Irradiance (μmol m^{-2} s^{-1})					
		90	190	330	450	600	750
Phaeodactylum tricornutum	400–700 [$\bar{a}^*$]	0.014	0.016	0.017	0.018	0.019	0.021
	440	0.066	0.092	0.156	0.193	0.206	0.251
	462	0.110	0.144	0.225	0.281	0.308	0.334
	490	0.114	0.146	0.224	0.287	0.316	0.323
Chaetoceros gracilis	400–700 [$\bar{a}^*$]	0.008	0.009	0.010	0.011	0.012	0.013
	440	0.080	0.115	0.156	0.177	0.200	0.220
	462	0.131	0.193	0.264	0.291	0.323	0.370
	490	0.154	0.218	0.285	0.342	0.384	0.385
Thalassiosira weissflogii	400–700 [$\bar{a}^*$]	0.009	0.010	0.011	0.011	0.011	0.012
	440	0.177	0.224	0.237	0.243	0.267	0.365
	462	0.321	0.387	0.406	0.446	0.486	0.570
	490	0.327	0.381	0.392	0.449	0.492	0.557

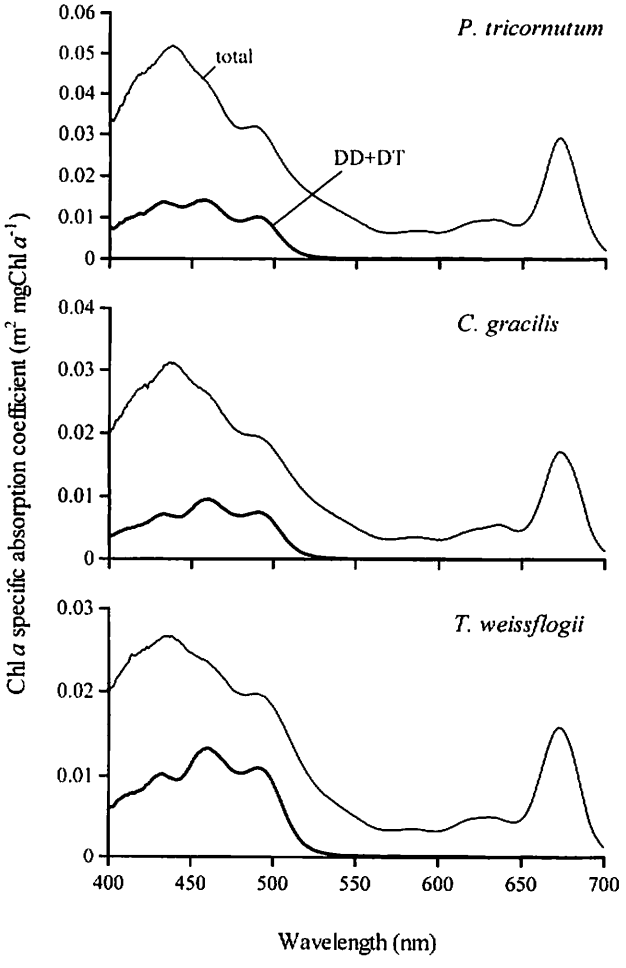

Fig. 3. Partitioning of $a^*(\lambda)$ between total pigments (thin line) and diadinoxanthin plus diatoxanthin (solid line) for three species of diatoms grown under an irradiance level of 750 μmol m^{-2} s^{-1}, as determined by the ratio of the reconstructed absorption coefficient of diadinoxanthin plus diatoxanthin to the total reconstructed absorption coefficient.

imply that the photoprotection system acted well against excess light energy for the range of irradiances provided. The pigment ratio (w : w) of diadinoxanthin plus diatoxanthin to Chl *a*, which showed the pool size of photoprotective pigments, increased linearly as the growth irradiance increased (Table 2). Sakshaug et al. (1991) showed that high-light (400 μmol m^{-2} s^{-1}) adapted cells of the diatoms *Thalassiosira nordenskioeldii* and *Chaetoceros furcellatus* contained up to 2 times more cellular diadinoxanthin plus diatoxanthin relative to Chl *a* than low-light (25 μmol m^{-2} s^{-1}) adapted cells. Using the freshwater diatom *Nitzschia palea*, Willemoës & Monas (1991) suggested that the conversion from diadinoxanthin to diatoxanthin took place only as growth irradiance was saturated because diatoxanthin was only found in the cultures grown at the saturated irradiance levels over 200 μmol m^{-2} s^{-1}. For all 3 species used in this study, however, diatoxanthin was found in cultures where the growth irradiance was not saturated (Table 1, Fig. 2).

As the growth irradiance varied from 90 to 750 μmol m^{-2} s^{-1}, the ratio (w : w) of diatoxanthin to diadinoxanthin plus diatoxanthin, which indicated the activity of the xanthophyll cycle, increased 4 times ranging from 17 to 67% for *P. tricornutum*, 5 times ranging from 11 to 59% for *C. gracilis*, and 7 times ranging from 10 to 69% for *T. weissflogii* respectively (Fig. 2). Cell size of the 3 species was determined from cellular carbon content, which indicated that *T. weissflogii* had the largest cell size, followed by *C. gracilis* and *P. tricornutum* (data not shown). The results could suggest that the activity of the xanthophyll cycle is species dependent and is related to cell size. Willemoës & Monas (1991) pointed out that unavailable diadinoxanthin for the xanthophyll cycle existed in the thylakoid membranes. The development of new methodology is required to quantify accurately the unavailable diadinoxanthin content, and to understand how this relates to total cellular di-

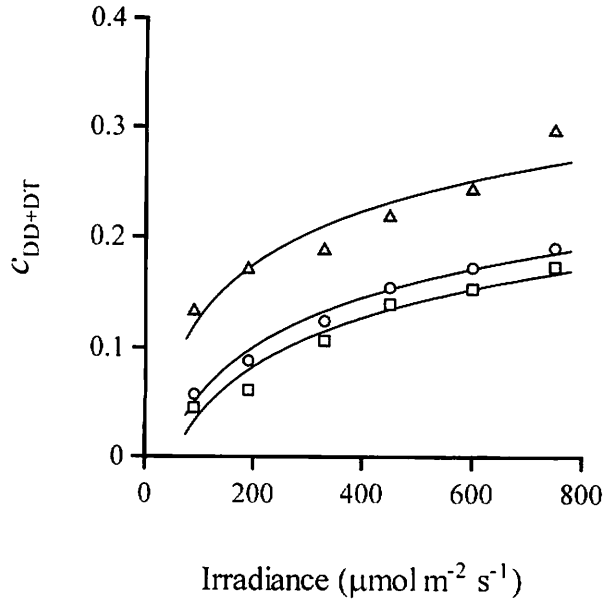

Fig. 4. The c_{DD+DT} of *Phaeodactylum tricornutum* (□), *Chaetoceros gracilis* (○) and *Thalassiosira weissflogii* (△) grown under 6 irradiances ranging from 90 to 750 μmol m^{-2} s^{-1}.

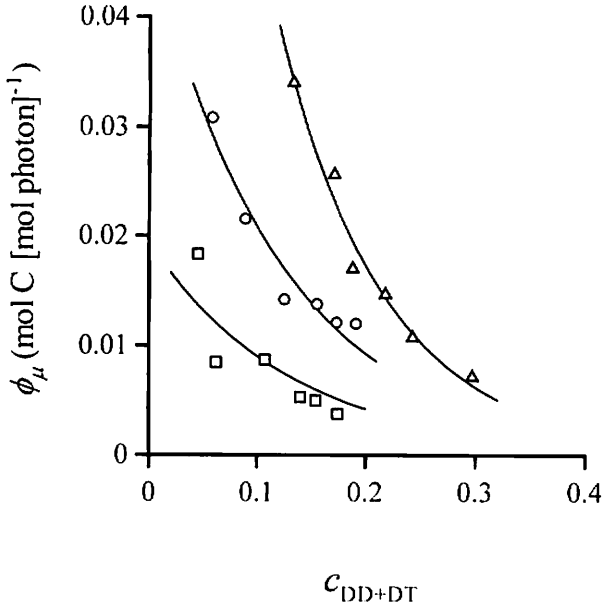

Fig. 5. Changes in ϕ_μ associated with c_{DD+DT} for *Phaeodactylum tricornutum* (□), *Chaetoceros gracilis* (○) and *Thalassiosira weissflogii* (△) grown under 6 irradiances ranging from 90 to 750 μmol m^{-2} s^{-1}.

adinoxanthin.

The ratios of Chl *c* to Chl *a* and fucoxanthin to Chl *a* varied little with growth irradiance, but species-specific differences were observed (Table 2). Both Chl *c* and fucoxanthin are able to transfer absorbed light energy to Chl *a* (Friedman & Alberte 1984; Owens & Wold 1986). β-carotene is thought to play an important photoprotective role in chlorophytes (Loeblich 1982). The ratio of β-carotene to Chl *a* remained under 0.05 for all species, irrespective of growth irradiance. Brown (1988) observed that β-carotene was preferentially found in pigment-protein complexes of photosynthetic reaction centers compared to antenna pigment-protein complexes for 5 species of diatoms including, *P. tricornutum*, *C. gracilis* and *Thalassiosira* sp.. These results may suggest that diadinoxanthin and diatoxanthin play a more significant photoprotective role than β-carotene in diatoms.

Chl *a* specific absorption coefficient, $a^*(\lambda)$, is used widely as the fundamental index of light absorption by phytoplankton pigments (Morel & Bricaud 1981; Sosik & Mitchell 1994; Stuart et al. 1998). The $a^*(\lambda)$ varies due to changes in package effect and the relative proportion of Chl *a* and accessory pigments (Sathyendranath et al. 1987; Bricaud et al. 1988). The package effect describes the decreased absorption of pigments in a cell compared to the absorption potential for the same amount of pigment in solution (Duysens 1956; Kirk 1976, 1994; Geider & Osborne 1987). An increase in package effect occurs as cell size increases or the cellular pigment concentration increases (Kirk 1976; Morel & Bricaud 1981; Sosik & Mitchell 1994). In this study, the increase in $\bar{a}^*$ with increasing irradiance may result from the combined increase in the contribution of absorption by diadinoxanthin plus diatoxanthin to total in vivo pigment absorption as well as a decrease in the package effect due to a decrease in cellular pigment concentration (Table 3). Increase in the contribution of absorption by diadinoxanthin plus diatoxanthin to total in vivo pigment absorption with increasing growth irradiance was most apparent between 450 and 500 nm, where the contribution of Chl-*a* absorption was minimal (Table 3).

As the light energy absorbed by diadinoxanthin and diatoxanthin is not transferred to the photosynthetic reaction centers, the increase in c_{DD+DT} would reduce the amount of energy available for carbon fixation. To determine the molar ratio of carbon fixed for growth to the light energy absorbed by pigments, the quantum yield for growth ϕ_μ was calculated according to the model of Kiefer & Mitchell (1983) for each species,

$$\phi_\mu = \frac{\mu}{\text{Chl } a : C \int_{400}^{700} a^*(\lambda) E_0(\lambda) d\lambda} \quad (3)$$

where μ is the specific growth rate and Chl *a* : *C* is the ratio of cellular Chl-*a* content to carbon content. As the growth irradiance varied from 90 to 750 μmol m^{-2} s^{-1}, the ϕ_μ decreased from 0.018 to 0.0038 mol C (mol photon)$^{-1}$ for *P. tricornutum*, 0.031 to 0.012 mol C (mol photon)$^{-1}$ for *C. gracilis*, and 0.034 to 0.0072 mol C (mol photon)$^{-1}$ for *T. weissflogii*. For the range of irradiances studied, the minimum ϕ_μ were always observed at the highest irradiance of 750 μmol m^{-2} s^{-1}. The ϕ_μ of all 3 species decreased logarithmically with increasing c_{DD+DT} ($p<0.05$) (Fig. 5). The relationships were species dependent. Variations in the energy transfer efficiency, the turnover time of reaction cen-

ters, and the ratio of pigment in photosystem I to photosystem 2 could all contribute to the change in ϕ_μ (Kolber et al. 1988; Sosik & Mitchell 1991; Babin et al. 1996). These may be some of the reasons why the relationship between c_{DD+DT} and ϕ_μ was species dependent. The relationship between c_{DD+DT} and ϕ_μ suggests that the increase in the absorption by diadinoxanthin plus diatoxanthin due to photoprotection is closely related to the decrease in ϕ_μ at high irradiances.

Conclusion

In this study, the spectral reconstruction technique was utilized to quantify absorption by the xanthophyll cycle pigments in relation to total in vivo pigment absorption.

The diatoms *T. weissflogii*, *C. gracilis* and *P. tricornutum* demonstrated a two-component xanthophyll cycle between diadinoxanthin and diatoxanthin at non-saturating growth irradiances. The contribution of absorption by diadinoxanthin plus diatoxanthin to total in vivo pigment absorption ($a^*_{(DD+DT)/TP}(\lambda)$) was significant at high irradiances because the pigment ratio of diadinoxanthin plus diatoxanthin to Chl *a* increased substantially with increasing irradiance. The highest contribution of absorption by diadinoxanthin plus diatoxanthin to total in vivo pigment absorption was observed between 450 and 500 nm, where the contribution of Chl-*a* absorption was minimal. This increase in absorption by diadinoxanthin plus diatoxanthin contributed to a decline in the quantum yield for growth, and may help to maintain growth rates under high light conditions.

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