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Xanthophyll cycle activity and photosynthesis of *Dunaliella tertiolecta* (Chlorophyceae) and *Thalassiosira weissflogii* (Bacillariophyceae) during fluctuating solar radiation

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Short-term ultraviolet (UV) radiation (280–400 nm) effects on xanthophyll cycle activity and photosynthesis were assessed during fluctuating irradiance (60- and 10-min cycles – saturating irradiance to near-zero irradiance) for the marine algae *Thalassiosira weissflogii* (Bacillariophyceae) and *Dunaliella tertiolecta* (Chlorophyceae). Laboratory cultures were cycled, as above, up and down the water column for 8 h under solar radiation, during which photosystem II (PSII) quantum yield in the light $[(F_{m'} - F_t)/F_{m'}]$ was monitored over 1-min intervals. In addition, pigment composition, xanthophyll de-epoxidation state and carbon assimilation were assessed during the fluctuating irradiance cycles. Although PSII quantum yield in the light of both species mirrored irradiance, the PSII response to irradiance fluctuations changed over time as PSII quantum yield was downregulated at midday. This coincided with maximal xanthophyll de-epoxidation that developed during the course of the day for both species. In contrast to the de-epoxidation levels, nonphotochemical quenching (NPQ) and PSII quantum yield in the light fluctuated with the irradiance dynamics at noon in both species. Maximal xanthophyll de-epoxidation and NPQ at noon was lower under photosynthetically active radiation (PAR) + UV than under PAR exposure for *T. weissflogii* during the 10-min cycle, whereas this was not found for the 60-min cycle and in *D. tertiolecta*. Synthesis of xanthophyll cycle pigments occurred in both species, and was faster for *D. tertiolecta* during PAR + UV than during PAR exposure. Carbon incorporation and on most occasions PSII quantum yield in the light were lower during UV exposure for both species, regardless of xanthophyll de-epoxidation state. UV effects on carbon assimilation were higher during 10-min than during 60-min irradiance fluctuation cycles. However, the 10-min irradiance fluctuation cycle appeared to enhance overall carbon assimilation in *D. tertiolecta* but depressed productivity of *T. weissflogii*, as compared with the 60-min cycles.

Key Words: Photosynthesis, Pigments, Xanthophyll cycle, UV radiation, Photoacclimation, Photoinhibition

INTRODUCTION

The radiation that algae experience in their environment is not only governed by daily and seasonal solar cycles and weather, but is also strongly dependent on physical processes in the water column. Wind-induced vertical mixing, upper mixed layer depth and attenuation characteristics of the water column modify the quantity and spectral composition of the radiation that algae experience (Denman & Gargett 1983; MacIntyre & Geider 1996; Neale *et al.* 2003). Vertical mixing below the euphotic zone can impose irradiance limitation, which requires expanded light-harvesting capacity to drive the photosynthetic electron transport chains. In contrast, a shallow upper mixed layer depth traps algae in the sunlit part of the water column. This can increase periodic excessive photosynthetically active radiation and ultraviolet (PAR, UV) exposure near the water surface that can overreduce the photosynthetic electron transport chain and inactivate photosystem II (PSII) reaction centers (photoinhibition, Osmond 1994).

During photoinhibition, linear photosynthesis is impaired and the reassembly of functional PSII reaction centers requires metabolically expensive degradation and synthesis of proteins that can take several hours (Aro *et al.* 1994). To minimize damage to PSII, algae deploy a suite of mechanisms that can adjust photosynthesis on short timescales (seconds to minutes). The enzymatic conversion of xanthophylls (the xanthophyll cycle) has been recognized as an important regulatory process (Demming-Adams 1990; Olaizola *et al.* 1994). De-epoxidation of xanthophyll pigments enhances energy dissipation in the light-harvesting antenna and reduces excitation pressure on PSII. Two main xanthophyll cycles are described for algae: the violaxanthin–antheraxanthin–zeaxanthin (VAZ) and the diadinoxanthin–diatoxanthin (Dd-Dt) cycle. Both cycles minimize PSII damage during excess light exposure or when the dark reactions are saturated. However, several differences

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have been noted with regard to the enzymatic de-epoxidation and epoxidation (Wilhelm *et al.* 2006). The de-epoxidation is triggered by acidification of the chloroplast lumen for both cycles, although de-epoxidation of diadinoxanthin requires less protonation than that of violaxanthin (Jakob *et al.* 1999). Furthermore, the proton gradient is a prerequisite for maintaining de-epoxidated xanthophylls for the VAZ cycle, whereas less coupling between these two was observed for the Dd-Dt cycle (Lavaud *et al.* 2004). Therefore, de-epoxidation of the VAZ cycle cannot be maintained in darkness. In contrast, epoxidation of diatoxanthin occurs only under weak light (Grouneva *et al.* 2009).

Apart from the xanthophyll cycle, state transitions (reversible phosphorylation-mediated antenna size reduction of PSII by redistribution of energy to PSI) can contribute to nonphotochemcial fluorescence quenching (Finazzi *et al.* 2001). In addition, damaged reaction centers are involved in quenching, whereas excess energy can also be dissipated by alternative electron cycling via PSII and PSI (Wagner *et al.* 2005; Lavaud *et al.* 2007). Light harvesting and excessive energy dissipation capacity can be adjusted on a timescale of hours to days by *de novo* pigment synthesis (Kana *et al.* 1997). Therefore, photosynthesis and photoprotection characteristics are strongly influenced by the radiation history of the algae (Falkowski and LaRoche 1991; van de Poll *et al.* 2005). However, the capacity for photoprotection and photoacclimation responses also differs between and among phytoplankton species. In diatoms the involvement of de-epoxidated xanthophylls in nonphotochemical quenching (NPQ) is stronger than in green algae, whereas the latter are able to perform state transitions (Wilhelm *et al.* 2006). Differences in the xanthophyll cycle capacity have been observed between diatom species (Dimier *et al.* 2007; Lavaud *et al.* 2007).

Variability in photoprotection and photoacclimation capacity can determine the ecological success of algal species under the contrasting water column conditions that govern the underwater light climate (Arrigo *et al.* 1999). However, algal physiological responses during fluctuating (fluence, spectral composition) radiation regimes are not well understood. Several approaches have been adopted to study photosynthesis during dynamic radiation conditions. Vertical mixing has been mimicked in the lab by imposing rapid changes in the light–dark period or by using electronically modulated light sources (Kroon *et al.* 1992; Ibelings *et al.* 1994; Lavaud *et al.* 2002; Havelková-Doušová *et al.* 2004; van de Poll *et al.* 2007). This allows the control of irradiance, but the spectral quality and irradiance levels diverge from those experienced in the water column. Other investigations have used rotation of layers of neutral density screens in combination with natural irradiance, or frequent changes in *in situ* incubation depth (Marra 1978; Barbieri *et al.* 2002). The mentioned investigations differed strongly in duration, overall irradiance levels, simulated mixing speed and analyzed parameters, making the results difficult to compare. The duration of vertical mixing cycles affects the duration and frequency of UV exposure in the water column (Neale *et al.* 1998). Interactions between photoprotection from excess PAR and UV radiation are not well understood. Recently, contrasting UV effects on the xanthophyll cycle of algae have been reported, ranging from stimulation to inhibition of de-epoxidation (Bischof *et al.* 2002; Mewes & Richter 2002; Sobrino *et al.* 2005).

Here we investigated the response of algae with different xanthophyll cycles during dynamic solar radiation conditions with and without UV radiation (UVR). We hypothesized that differences in xanthophyll cycle capacity may affect productivity of the green alga *Dunaliella tertiolecta* (VAZ cycle) and the diatom *Thalassiosira weissflogii* (Dd-Dt cycle) under excess radiation conditions and during strongly fluctuating radiation conditions. Therefore, xanthophyll epoxidation state, chlorophyll fluorescence, and carbon assimilation were investigated during fluctuations imposed on solar radiation with and without UVR mimicking turbulent mixing in a shallow mixed layer.

METHODS

Experimental design

UVR effects on xanthophyll cycle activity and photosynthesis of cultivated *T. weissflogii* (Bacillariophyceae) and *D. tertiolecta* (Chlorophyceae) were studied during two fluctuating solar radiation regimes. The experiments were conducted outside the Estación de Fotobiología Playa Unión (EFPU), Patagonia, Argentina (43°S, 65°W), during January/February 2006. One-liter batch cultures of *T. weissflogii* and *D. tertiolecta* were grown in F-2 enriched autoclaved seawater in an illuminated culture chamber (Sanyo MLR 350) (12/12 h light/dark cycle) under 250 μmol photons m^{-2} s^{-1} of PAR at a temperature of 20°C. The cultures were grown semicontinuously, maintaining exponential growth by diluting regularly with fresh medium. Before the mixing experiments the cultures were diluted with fresh medium (to a concentration 92 and 82.4 μg chlorophyll *a* (Chl *a*) l^{-1} for *D. tertiolecta* and *T. weissflogii*, respectively) and transferred to the experimental vessels [UV-transparent poly(methylmethacrylate) (PMMA), 2-litre capacity, 13-cm height].

Vertical mixing was simulated by transporting the vessel with the algae up and down in a water-filled reservoir (max. depth: 1.1 m, diameter: 5 m) for 8 h under solar radiation. Dried sediment from the nearby Chubut river (which strongly affected turbidity) was used to enhance PAR and UV attenuation in the reservoir, which was regularly stirred. The goal was to reduce UVB radiation so that this could not be detected at the bottom. Two radiation conditions were created with different spectral composition: (1) PAR, by covering the vessel with film that removed wavelengths below 390 nm (Ultraphan, UV Opak Digefra, Germany, removing also 10% PAR), and (2) PAR + UV, without filter film. In addition, the effects of two different radiation fluctuation speeds were tested (10 and 60 min to complete one cycle from the surface down to 0.8 m and back to the surface). The experiments with both species, radiation treatments and fluctuation cycles were done on separate days from 30 January to 14 February 2006 (Table 1). During this period surface water temperature in the reservoir varied between 19 and 24°C.

Table 1. Radiation conditions and measurements. Mean calculated photosynthetically active radiation (PAR) (400–700 nm), ultraviolet radiation A (UVA; 315–400 nm) and UVB (280–315 nm) radiation (W m^{-2}) from 10:00 to 16:30 h during the PAR + UV and PAR treatments for 60- and 10-min mixing cycles with *Dunaliella tertiolecta* and *Thalassiosira weissflogii*. The radiation calculations were based on spectroradiometer measurements in air, combined with attenuation coefficients and mixing cycles. Note that for carbon incorporation PAR and PAR + UV conditions were tested at the same time.

Date (2006)	Species	Mixing cycle, condition	PAR[1] (W m^{-2})	UVA (W m^{-2})	UVB (W m^{-2})
30 Jan.	*Dunaliella tertiolecta*	60-min PAR+UV & ^{14}C	230	21.8	0.512
31 Jan.	*D. tertiolecta*	10-min PAR+UV & ^{14}C	235	22.6	0.556
1 Feb.	*D. tertiolecta*	60-min PAR	230	21.8	0.512
2 Feb.	*D. tertiolecta*	10-min PAR	218	20.9	0.512
6 Feb.	*D. tertiolecta*	60-min (2) PAR	218	20.5	0.470
7 Feb.	*D. tertiolecta*	60-min (2) PAR+UV	175	17.1	0.470
8 Feb.	*Thalassiosira weissflogii*	60-min PAR+UV	218	20.1	0.470
9 Feb.	*T. weissflogii*	60-min PAR & ^{14}C	209	18.8	0.470
13 Feb.	*T. weissflogii*	10-min PAR & ^{14}C	205	18.3	0.427
14 Feb.	*T. weissflogii*	10-min PAR+UV	184	16.7	0.385

Simulation of vertical mixing

The PMMA vessel was vertically transported in the reservoir by a custom-made mixing simulator that was positioned next to the water reservoir. A frequency-controlled DC motor (Maxon motor, Switzerland) was used to impose a sinusoidal transport rate on the vessel from the bottom to the surface, thereby simulating the radiation experienced by algae during circular (vertical) movement in the water column. The frequency was adjusted to create 10-min and 60-min cycles of the vessel, as shown in Fig. 1, thereby simulating the vertical movement of algae for conditions that have been reported for Patagonian coastal waters (Barbieri *et al.* 2002). Weights were added to the vessel to compensate the weight for the removal of pigment samples.

Chlorophyll fluorescence

The quantum yield of PSII $[(F_{m'} - F_t)/F_{m'}]$ of the algae in the 2-litre vessel was determined with a pulse amplitude-modulated fluorometer (Water PAM, Walz, Germany). A custom-made darkened flow-through measuring cuvette (quartz, 5 ml) was connected to a rotation pump that continuously pumped algae from and to the vessel via darkened 1.2-m silicon tubing. Because the time between sampling and the saturating light pulse was in the order of a few seconds, the quantum yield of PSII photochemistry in the light $[(F_{m'} - F_t)/F_{m'}]$ was determined (Maxwell & Johnson 2000). These fluorescence measurements were performed every 10 s during the vertical movement of the vessel. The measurements were not affected by pumping the algae from and to the cuvette; however, withdrawal of pigment samples did affect the measurements, and those data were excluded. NPQ was calculated as $(F_m - F_{m'})/F_{m'}$ (Bilger & Björkman 1990), where F_m is the maximal (dark-adapted) fluorescence after a saturating light pulse, recorded at the beginning of the experiments after the algae were adapted for 30 min to low light at the bottom of the reservoir.

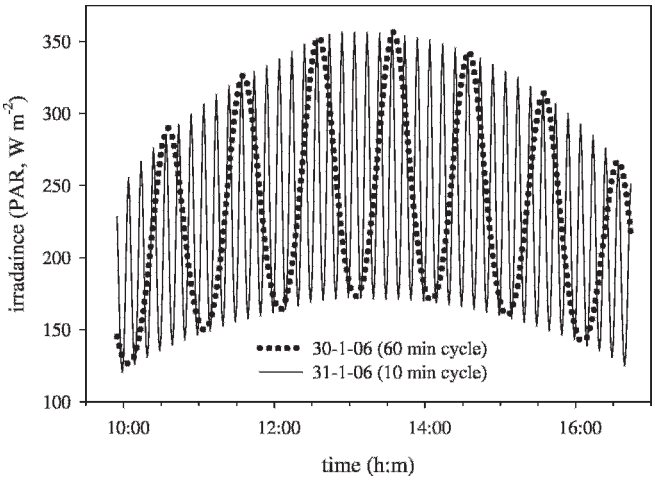

Fig. 1. PAR (400–700 nm) irradiance during the 10- and 60-min mixing cycles calculated from incident irradiance and PAR attenuation coefficients and mixing cycles on 30 January 2006 and 31 January 2006, respectively. Irradiance is shown from 10:00 to 16:30 h.

Pigments

Pigment samples (50 ml) were obtained with a syringe via the silicon tubing when the vessel was at the surface (maximal irradiance), at the bottom of the reservoir (minimal irradiance), and back at the surface. This sampling protocol was repeated every 30–60 min and allowed us to study the xanthophyll epoxidation state on a 5-min (10-min mixing cycle) and 30-min (60-min mixing cycle) timescale throughout the experimental time course. Samples were immediately filtered in a darkened room on 25-mm glass fiber filters (GF/F, Whatman) by mild vacuum (< 10 mm Hg), after which the filters were frozen and stored in liquid nitrogen. The entire procedure was finished within 2–3 min after sampling. After transport to the Netherlands in liquid nitrogen, the samples were freeze-dried and pigments were extracted in 90% acetone overnight in darkness at 4°C. Pigments in the extracts were resolved by high-performance liquid chromatography (Waters) with a C18 5-μm Delta Pack reversed-phase column (Milford, MA, USA) after Van Leeuwe *et al.* (2006). Pigment identification was done by retention time and diode array spectroscopy (Waters). Chlorophylls *a* and *b*, fucoxanthin, diadinoxanthin, diatoxanthin, zeaxanthin, antheraxanthin, violaxanthin, and lutein standards (DHI, Denmark) were used for quantification. Cellular pigment concentrations were calculated from cell counts (see below) and extraction volume.

Carbon incorporation

For each mixing cycle of a single species 36 quartz tubes (10 ml) were supplemented with radioisotope-labeled sodium bicarbonate (3.75 μCi). The tubes were mounted on a platform that was transported up and down in the reservoir by the vertical mixing simulator (Table 1). To discriminate between PAR and PAR + UV, half of the tubes were covered with 390-nm cutoff foil (Ultraphan Opak Digefra), whereas the others remained uncovered. Therefore, carbon incorporation under these two radiation conditions was determined on the same experimental day. Duplicate PAR- and PAR + UV- exposed tubes were removed every hour from the platform, when tubes were at the surface and filtered on 25-mm GF/F (Whatman). The filters were stored in scintillation vials and exposed to concentrated HCl fumes overnight to remove unbound labeled sodium bicarbonate. Radiocarbon incorporation was quantified using liquid scintillation counting. The carbon incorporation was corrected for dark controls that were covered with aluminium foil. Assimilation numbers were calculated by dividing the carbon incorporated during the hour interval of sampling (i.e., total carbon incorporated at the time of sampling minus the total carbon incorporated in the previous sampling) by Chl *a* concentration at the beginning of the experiment.

Chlorophyll concentrations (for carbon incorporation)

For Chl *a* concentration, a 50-ml sample was obtained from the cultures, filtered on 25-mm GF/F, and extracted in methanol (Holm-Hansen & Riemann 1978). The samples were sonicated for 20 min and extracted for at least 2 h in darkness at 4°C. After centrifugation (10 min at 1750 × *g*), the Chl *a* concentration of the supernatant was determined using a fluorometer (Turner Designs, TD700). The fluorescence of the methanolic extract was measured before and after acidification, and the Chl *a* concentration was calculated from these readings. The fluorometer was routinely calibrated using pure Chl *a* from *Anacystis nidulans* (Sigma C6144). Absolute values of Chl *a* were obtained with a Hewlett Packard diode array spectrophotometer (Hewlett Packard model HP 8453 E) using a 5-cm path length cuvette; the Chl *a* concentration was calculated using the equation of Porra (2002).

Irradiance

PAR (400–700 nm), UVA (315–400 nm) and UVB (280–315 nm) were continuously recorded with a broadband filter ELDONET radiometer (Real Time Computers Inc., Germany) permanently located on the roof of the EFPU as the average of 1-min intervals. The ELDONET instrument was calibrated at the factory against a 1000-W quartz halogen lamp operated with a highly stabilized power supply (SL 1000 W, Powertronic Lab. 710 D). The absolute calibration was controlled in an intercomparison of several spectroradiometers and the ELDONET instrument (Hader *et al.* 2007). Attenuation of UV and PAR in the water reservoir was determined with a diode array spectrophotometer (HR 2000CG-UV-NIR, Ocean Optics, Duneclin, USA) with a 10-m fiber optics and cosine diffuser. The radiation that was experienced by the vessel was calculated from incident radiation, attenuation coefficients in the reservoir, and the depth of the vessel during the mixing cycles. This allowed us to estimate the radiation that the algae experienced during transport in the reservoir (Table 1).

Cell counts

Duplicate formalin-fixed (0.1%, V/V) samples (2 ml) were obtained at the start and end of each mixing experiment and stored at 4°C in darkness. Cell counts were performed on a Coulter MCL flow cytometer (Beckmann-Coulter) as described in van de Poll *et al.* (2007).

Analytical considerations and statistics

The quantum yield of PSII in the light, carbon incorporation, and pigment composition for 10- and 60-min mixing cycles and for PAR- and PAR + UV-exposed *D. tertiolecta* and *T. weissflogii* were obtained on different experimental days. Because of this, there were slight differences in the irradiance conditions, and this caused variability in the maximal quantum yield of PSII and pigment composition. In addition, other factors influenced the measurements in the morning (9:00–12:00 h). Shading by the side of the reservoir affected irradiance when the vessel resided near the bottom until ~ 10:00 h. Incidental problems with the PAM caused two ~ 30-min gaps in the data set of PAR-exposed *D. tertiolecta* (60-min cycle). A malfunction in the mixing device caused a 40-min irregularity in the mixing cycle of PAR- + UV-exposed *D. tertiolecta* (60-min cycle). Considering all factors above, most PSII quantum yield comparisons concentrated on data obtained between 12:00 and 15:00 h. Differences between groups of data were tested for significance by a single-factor ANOVA.

RESULTS

Irradiance

High incident PAR and UVR were measured throughout the experimental period (maximal 354 W m^{-2} PAR = 1650 μmol photons m^{-2} s^{-1}, 58 W m^{-2} UVA and 1.8 W m^{-2} UVB). Mean attenuation coefficients (K_d) in the reservoir for PAR, UVA and UVB irradiances were 0.91, 2.74 and 3.95, respectively (n = 3). This resulted in a 52, 89 and 96% reduction of surface PAR, UVA and UVB irradiance at 0.8-m depth. Radiation conditions on the different experimental days were compared by calculating the mean PAR, UVA and UVB radiation for the mixed algae from 10:00 to 16:30 h. Vertical transport of the experimental vessel in the reservoir reduced the mean PAR, UVA and UVB radiation to an estimated 71, 44, and 35% of the ambient values over the same time interval, respectively. Irradiance fluctuation cycles of 60 and 10 min introduced a minimal difference (~ 1%) in mean radiation. Cloud cover influenced the radiation conditions, particularly on 2, 7, and 14 February, causing a 25% difference between the highest and lowest mean radiation between 10:00 to 16:30 h (Table 1).

Table 2. Photosystem II (PSII) quantum yield and pigment composition of *Dunaliella tertiolecta* and *Thalassiosira weissflogii* before and during 10- and 60-min mixing cycles under photosynthetically active radiation (PAR) and PAR + ultraviolet (UV) exposure. Maximal quantum yield of PSII $[(F_m - F_0)/F_m]$ was measured after 30-min adaptation to low light at the bottom of the reservoir (start). Mean PSII quantum yield in the light $(F_{m'} - F_t)/F_{m'}$ ($n = 180$) and the mean (relative) slope of PSII quantum yield vs irradiance between 12:00 and 15:00 h are also shown. The latter was calculated from a linear fit between PSII quantum yield in the light and irradiance for the time interval when the vessel was going up, and the same was done when the vessel was going down in the reservoir. The mean and standard deviation are shown for 6 and 36 replicate linear fits for the 60- and 10-min cycles, respectively. For pigment composition, xanthophyll cycle activity [de-epoxidation state (DES)] between 12:00 and 15:00 h is shown as the mean percentage of diatoxanthin (*T. weissflogii*) and anthera + zeaxanthin (*D. tertiolecta*) of the xanthophyll cycle pool. Mean and standard deviation are shown for 6–13 replicates. The rate of change in the ratio between protective and light-harvesting pigments (ratio P/L) is shown as the slope obtained from a linear fit of this ratio over time for samples collected during the first 240 min of the experiment.

	PSII quantum yield		PSII/irradiance	Pigments	
	Start	Mean (12–15 h)	Mean slope (12–15 h)	DES[1] (12–15 h)	Ratio(P/L) rate, first 4 h
Dunaliella tertiolecta					
60-min PAR + UV	0.551	0.411 (0.06)	$0.77\ (0.10)\cdot10^{-3}$	83.3 (4)	$0.40\cdot10^{-3}$
60-min PAR + UV (2)	0.605	0.391 (0.06)	$0.45\ (0.20)\cdot10^{-3}$	87.1 (2)	$0.40\cdot10^{-3}$
60-min PAR	0.518	0.370 (0.03)	$0.35\ (0.05)\cdot10^{-3}$	90.5 (2)	$0.25\cdot10^{-3}$
60-min PAR (2)	0.535	0.281 (0.01)	$0.19\ (0.04)\cdot10^{-3}$	90.9 (1)	$0.11\cdot10^{-3}$
10-min PAR + UV	0.537	0.349 (0.03)	$0.42\ (0.04)\cdot10^{-3}$	88.3 (1)	$0.35\cdot10^{-3}$
10-min PAR	0.527	0.430 (0.03)	$0.16\ (0.09)\cdot10^{-3}$	89.9 (2)	$0.19\cdot10^{-3}$
Thalassiosira weissflogii					
60-min PAR + UV	0.356	0.142 (0.02)	$0.30\ (0.20)\cdot10^{-3}$	83.0 (4)	$0.30\cdot10^{-3}$
60-min PAR	0.559	0.246 (0.03)	$0.23\ (0.05)\cdot10^{-3}$	88.0 (4)	$0.30\cdot10^{-3}$
10-min PAR + UV	0.605	0.274 (0.03)	$0.43\ (0.10)\cdot10^{-3}$	70.5 (3)	$0.40\cdot10^{-3}$
10-min PAR	0.601	0.319 (0.02)	$0.38\ (0.20)\cdot10^{-3}$	87.0 (2)	$0.30\cdot10^{-3}$

Quantum yield of PSII

The mean maximal quantum yield of PSII $[(F_m - F_0)/F_m]$ after 30-min adaptation to low light at the bottom of the reservoir was 0.550 ± 0.03 and 0.538 ± 0.126 for *D. tertiolecta* and *T. weissflogii* cultures, respectively at the start of solar radiation exposure (Table 2). The PSII quantum yield in the light $[(F_{m'} - F_t)/F_{m'}]$ of both species fluctuated with the irradiance changes imposed by the vertical transport: reduced efficiency was found near the surface, whereas it increased at the bottom. Superimposed on this pattern we observed variations that were caused by the solar cycle, and occasionally by cloud cover. For both species, the mean PSII quantum yield in the light decreased significantly around noon (12:00–15:00 h, Table 2). An example of a complete daily cycle for *T. weissflogii* is shown in Fig. 2; similar patterns were observed for *D. tertiolecta* and for both mixing cycles (data not shown). Between 12:00 and 15:00 h the mean PSII quantum yield in the light of PAR- + UV-exposed *T. weissflogii* (0.208 ± 0.071) was significantly lower than that of only PAR (0.282 ± 0.043) exposure (one-way ANOVA, $P < 0.05$, $n = 362$), for both irradiance fluctuation cycles (Fig. 3). For *D. tertiolecta*, mean PSII quantum yield in the light between 12:00 and 15:00 h was significantly lower during PAR + UV than that during PAR alone for the 10-min fluctuation cycle (0.326 ± 0.04 vs 0.401 ± 0.06), but higher for the 60-min fluctuation cycle (0.430 ± 0.03 vs 0.352 ± 0.03) (Fig. 3). On average, the PSII quantum yield in the light of *D. tertiolecta* between 12:00 and 15:00 h was reduced to 68 ± 9% of the initial values, whereas this was 45 ± 5% of the initial value for *T. weissflogii* when considering both mixing cycles. Therefore, the mean PSII quantum yield in the light between 12:00 and 15:00 h was significantly lower in *T. weissflogii* than in *D. tertiolecta* (Table 2). After 15:00 h PSII quantum yield in the light of both species showed an upward trend, except

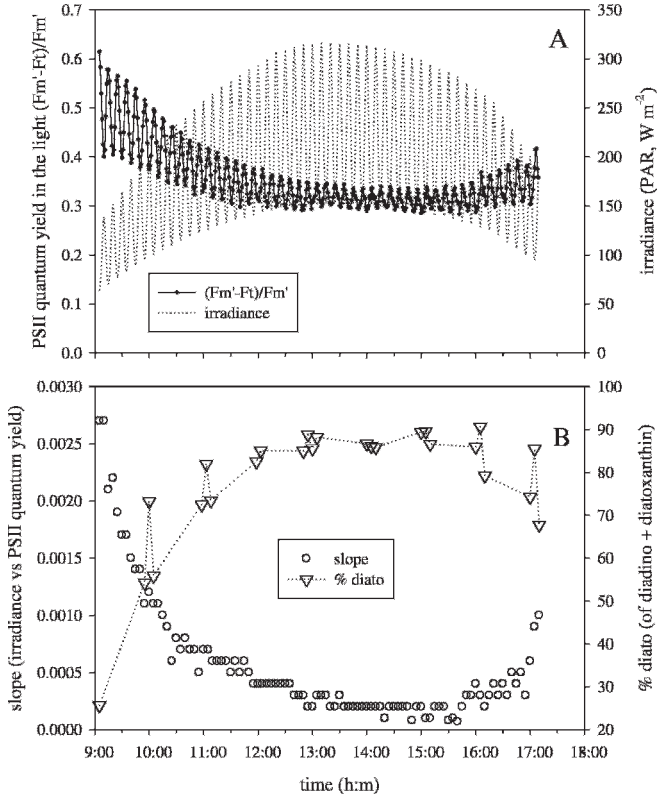

Fig. 2. Changes in PSII quantum yield in the light $[(F_{m'} - F_t)/F_{m'}]$ and calculated PAR irradiance over time of PAR-exposed *Thalassiosira weissflogii* during a 10-min mixing cycle (A). The second graph (B) shows the (relative) PSII efficiency response to irradiance fluctuations expressed as the slope of a linear fit of irradiance and PSII quantum yield over 5-min intervals (up and down), together with xanthophyll cycle activity for this species during a 10-min mixing cycle under PAR exposure. The xanthophyll cycle activity is shown as the percentage of diatoxanthin of the xanthophyll cycle pool.

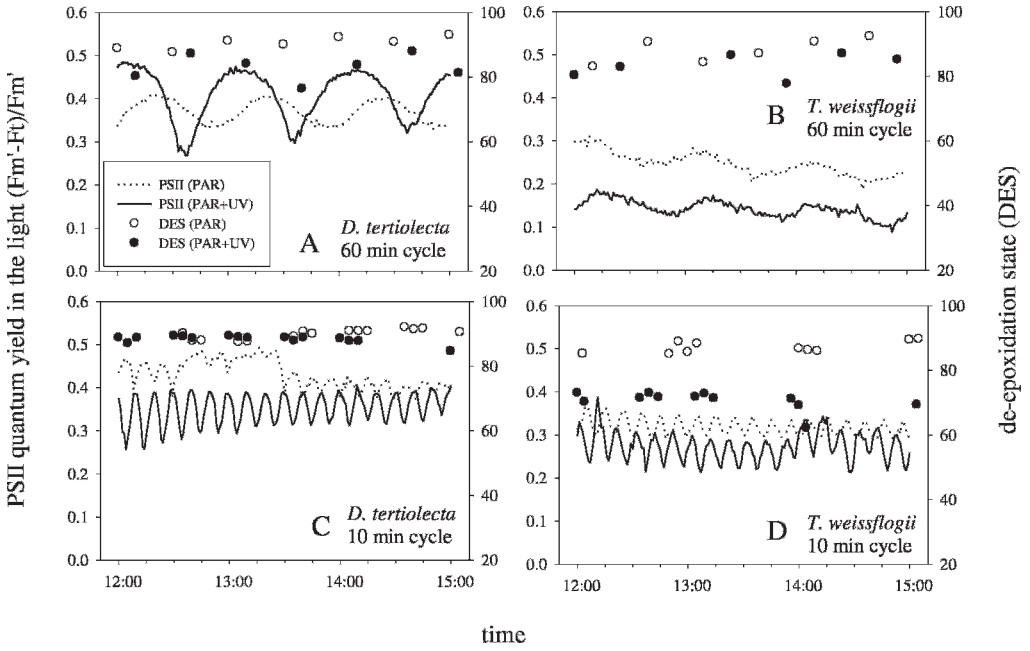

Fig. 3. PSII quantum yield in the light [$(F_{m'} - F_t)/F_{m'}$, line, dots] and de-epoxidation state of the xanthophyll cycle pigments (DES, circles) of PAR and PAR- + UV-exposed *Dunaliella tertiolecta* (A, C) and *Thalassiosira weissflogii* (B, D) during the 10- (C, D) and 60- (A, B) min mixing cycle from 12:00 to 15:00 h. PSII quantum yield was measured at 10-s intervals. The DES of xanthophyll pigments is shown as the percentage of anthera + zeaxanthin or diatoxanthin of the xanthophyll cycle pool.

for *T. weissflogii* during the 60-min irradiance fluctuation cycle (results not shown).

The induction of NPQ followed the fluctuations in irradiance, with maximal NPQ values observed at noon (results not shown). Mean NPQ between 12:00 and 15:00 h during PAR + UV was lower than that during PAR alone for the 10-min cycle, whereas this was not found for the 60-min cycle for both species (Fig. 4).

The light-adapted PSII quantum yield response to irradiance changes was further investigated by regression analysis between PSII quantum yield (1-min average) and PAR irradiance data (1-min average) that were calculated for the respective mixing treatments. This allowed us to compare the PSII response during PAR and PAR + UV. This relationship changed with time for all species and conditions. Therefore, these parameters were fitted for a half fluctuation cycle (5 and 30 min for 10- and 60-min fluctuation cycles, respectively). Because no difference between down- (positive slope) and upward (negative slope) transport was found, the relative slope was used. The relative slope of the relationship between PSII quantum yield in the light and irradiance decreased toward noon for all experiments (example *T. weissflogii* in Fig. 2B). For the period with highest (UV) irradiance (12:00–15:00 h) the slopes were compared with a one-way ANOVA. For *D. tertiolecta* and *T. weissflogii* it was found that between 12:00 and 15:00 h the PAR + UV treatments showed significantly higher slopes than during the PAR treatments (Table 2). For *D. tertiolecta* the mean slopes were $0.50 \pm 0.2 \times 10^{-3}$ [yield $(W\ m^{-2})^{-1}$] and $0.20 \pm 0.1 \times 10^{-3}$ for PAR + UV and PAR, respectively ($n = 53$). For *T. weissflogii* these values were $0.40 \pm 0.18 \times 10^{-3}$ and $0.25 \pm 0.10 \times 10^{-3}$ for PAR + UV and PAR, respectively ($n = 46$). This showed that the light-adapted PSII quantum yield response was stronger during experiments with PAR + UV than during those with PAR alone.

Pigments

In *D. tertiolecta*, Chl *a* and Chl *b* were the main light-harvesting pigments, combined with lutein, violaxanthin, antheraxanthin, and zeaxanthin. For *T. weissflogii*, Chl *a* and fucoxanthin were the main light-harvesting pigments and diadinoxanthin and diatoxanthin the xanthophyll cycle pigments. Chl *c*1+ Chl *c*2 and β-carotene were also detected in this species but not quantified. Cellular pigment concentrations were calculated at the start and after 8 h of ambient exposure (Table 3). Data from PAR and PAR + UV, and data from 10- and 60-min irradiance fluctuation cycles were pooled for both species because pigment composition was not significantly different after 8 h of solar radiation treatments, giving 12 and 8 replicates for *D. tertiolecta* and *T. weissflogii*, respectively. For both species significant changes in pigment composition were found after 8 h of ambient treatments. Significant increases were observed in cellular xanthophyll pools (*D. tertiolecta*: viola + anthera + zeaxanthin; *T. weissflogii*: diadino + diatoxanthin, Table 3). On average, the cellular VAZ pool in *D. tertiolecta* doubled, whereas the Dd-Dt pool of *T. weissflogii* increased by 27%, compared with the initial concentrations. Cellular lutein also significantly increased by 60% over the 8-h period for *D. tertiolecta*, whereas Chl *a* and Chl *b* increased significantly by 49 and 34%, respectively. For *T. weissflogii* cellular Chl *a* did not change significantly over time, but fucoxanthin decreased significantly by 20%.

For both species the ratio between protective (*D. tertiolecta*: VAZ; *T. weissflogii*: Dd-Dt) and light-harvesting pigments (*D. tertiolecta*: Chl *a*, Chl *b*, *T. weissflogii*: Chl *a*,

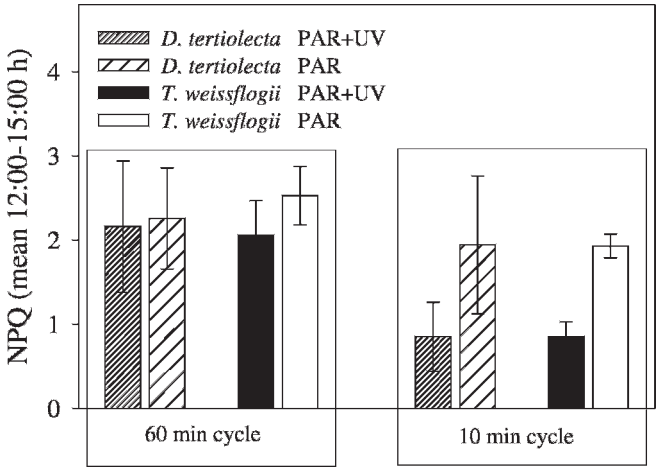

Fig. 4. Mean nonphotochemical quenching (NPQ) between 12:00 and 15:00 h for PAR and PAR- + UV-exposed *Dunaliella tertiolecta* and *Thalassiosira weissflogii* during the 60- and 10-min mixing cycles.

Table 3. Mean cellular pigment composition (pg cell^{-1}) of *Dunaliella tertiolecta* and *Thalassiosira weissflogii* after precultivation under high irradiance and after 8 h of simulated mixing. Data from different mixing cycles and irradiance conditions were pooled. Asterisks indicate when differences in pigment composition before and after the mixing treatments were significant.

	Initial	8-h mixing
D. tertiolecta (n = 12)		
Chlorophyll *a*	0.92 (0.24)	1.38 (0.29)*
Chlorophyll *b*	0.36 (0.10)	0.49 (0.09)*
Lutein	0.15 (0.03)	0.24 (0.04)*
Violaxanthin	0.06 (0.03)	0.04 (0.02)*
Antheraxanthin	0.03 (0.01)	0.06 (0.02)*
Zeaxanthin	0.04 (0.03)	0.18 (0.09)*
T. weissflogii (n = 8)		
Chlorophyll *a*	2.06 (0.15)	2.03 (0.27)
Chlorophyll *c*	0.39 (0.04)	0.29 (0.05)*
Fucoxanthin	0.60 (0.05)	0.50 (0.05)*
Diadinoxanthin	0.50 (0.04)	0.21 (0.14)*
Diatoxanthin	0.16 (0.03)	0.62 (0.20)*

fucoxanthin) was calculated to follow the rate of change in these pigments over time. The ratios significantly increased during the first 4 h of solar radiation exposure for both species, to level off or decrease during afternoon (data not shown). The mean ratios between 12:00 and 15:00 h were slightly but not significantly higher for UV-exposed samples (7–10% higher) as compared with PAR-exposed samples. The ratios between protective and light-harvesting pigments were fitted by linear regression for the first 240 min of the experiments, and the slopes were used as a measure for protective pigment synthesis. For *D. tertiolecta* the slopes of UV-exposed samples were significantly higher as compared with PAR-exposed samples, whereas no effect of mixing cycle was found (Table 2). For *T. weissflogii* there were no significant differences between radiation and mixing treatments.

The initial cellular xanthophyll cycle pool of *D. tertiolecta* (0.13 pg cell^{-1}) was smaller than that of *T. weissflogii* (0.66 pg cell^{-1}) (Table 3). De-epoxidation state of xanthopylls was calculated as the percentage of anthera + zeaxanthin or diatoxanthin of the total xanthophyll cycle pool. De-epoxidation at the start of the experiment of *D. tertiolecta* (51 ± 20% anthera and zeaxanthin) was higher and showed more variation than that of *T. weissflogii* (25 ± 2% diatoxanthin). De-epoxidation increased during the morning (9:00–12:00 h) for both species (example *T. weissflogii* Fig. 2) and reached maximal values between 12:00 and 15:00 h (Fig. 3). No significant differences were found between the mixing conditions for both species.

Mean de-epoxidation between 12:00 and 15:00 h was higher under PAR than PAR + UV exposure during the 10-min cycle of *T. weissflogii*, whereas this was less evident during the 60-min cycle (Fig. 3, Table 2). For *D. tertiolecta*, de-epoxidation values between PAR and PAR + UV exposure were not significant.

Carbon incorporation

Significant differences in assimilation numbers were found between species, irradiance conditions, and between irradiance fluctuation cycles. For *D. tertiolecta* the assimilation numbers were higher during the 10-min cycle (Fig. 5C) than during the 60-min fluctuation cycle experiments (Fig. 5A). In contrast, lower carbon incorporation rates were found during the 10-min cycle (Fig. 5D) for *T. weissflogii*, as compared with the 60-min fluctuation cycle (Fig. 5B). Assimilation numbers for *D. tertiolecta* and *T. weissflogii* were similar during the 60-min mixing cycles (Fig. 5A, B). For both mixing cycles clear UV effects were found on carbon incorporation. On average the UV-mediated reduction in carbon incorporation between 11:25 and 15:25 h was more pronounced during 10-min (57 ± 0.6% inhibition) than during 60-min fluctuation cycles (34 ± 8.7% inhibition).

Cell counts

Cell concentrations at the start of the experiments were on average 1.0×10^5 and 0.4×10^5 cells ml^{-1} for *D. tertiolecta* and *T. weissflogii*, respectively. After 8 h of simulated mixing, cell concentration increased on average to 118(12) and 125(8)% of the initial concentrations for *D. tertiolecta* and *T. weissflogii*, respectively. Treatment effects due to mixing cycle or irradiance conditions were not observed.

DISCUSSION

Phytoplankton responses to excessive PAR and UV radiation depend on numerous factors such as light and nutrient history (Litchman *et al.* 2002; Litchman & Neale 2005; van de Poll *et al.* 2005). High irradiance precultivation typically enhances the xanthophyll cycle relative to the light-harvesting pigment pool (Buma *et al.* 2006). Because *D. tertiolecta* and *T. weissflogii* were precultivated under high radiation, a strong involvement of the xanthophyll cycle in photosynthetic downregulation was expected. High radiation acclimation resulted in a relatively small antenna size for both species. For example, reported cellular Chl *a*

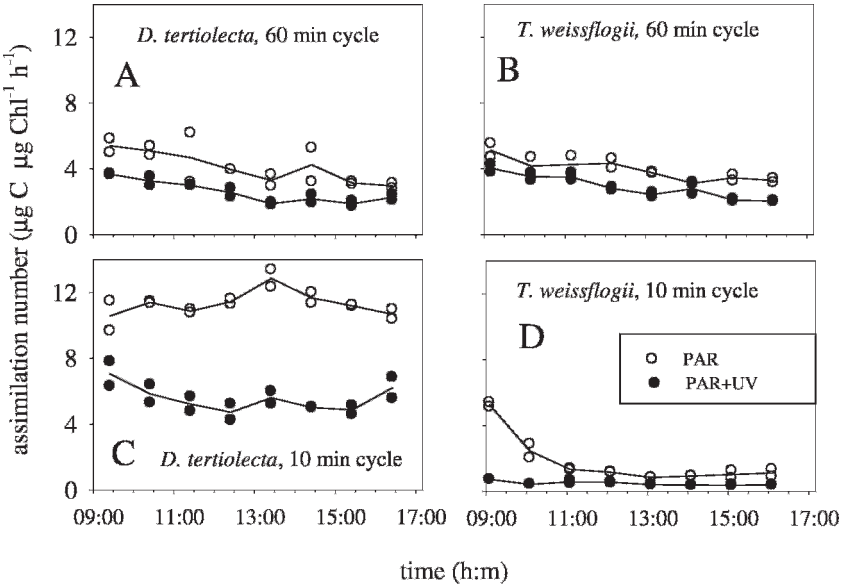

Fig. 5. Carbon incorporation over time for PAR- and PAR- + UV-exposed *Dunaliella tertiolecta* (A, C) and *Thalassiosira weissflogii* (B, D) during the 60- (A, B) and 10- (C, D) min mixing cycle from 9:00 to 17:00 h. Assimilation number was normalized to chlorophyll *a* concentrations at the start of the incubation and were corrected for dark controls. The results for two duplicate incubations (symbols) are shown as well as the median (line).

concentrations of low-light-acclimated *T. weissflogii* were twofold higher than those observed under the growth conditions used for this experiment (van de Poll *et al.* 2006). The relatively low maximal quantum yield of PSII that was found after 30-min adaptation to low light together with incomplete epoxidation of the xanthophyll cycle pigments at the beginning of the experiment showed that a longer dark adaptation period was required to obtain a true maximal quantum yield for both species. This influenced the NPQ calculations, i.e. NPQ was probably higher than reported. During the fluctuating radiation treatments we measured the PSII quantum yield in the light. The NPQ that was induced during high radiation exposure can be caused by damage to PSII components (photoinhibition) and by photoprotective protective processes such as the xanthophyll cycle. The relative importance of these components can be estimated from recovery kinetics in low light because PSII damage is reversed on a longer timescale than the protective processes. However, because of the complexity of our radiation regimes and the overall high radiation levels it was difficult to estimate the contribution of protective processes and PSII damage from the fluorescence measurements.

We observed a buildup of de-epoxidized xanthophyll cycle pigments during the morning, with maximal de-epoxidation values between 12:00 and 15:00 h. This coincided with decreasing light-adapted PSII quantum yield and PSII quantum yield responses to irradiance fluctuations. Therefore, the xanthophyll cycle was involved in the induction of NPQ in both species. Furthermore, xanthophyll synthesis increased the xanthophyll cycle pool of both species within hours after transfer to solar radiation. The xanthophyll synthesis rates were in agreement with those found for phytoplankton assemblages and diatoms, and demonstrated that algae can adjust this pool on a timescale of hours (Claustre *et al.* 1994; Moline 1998; Lohr & Wilhelm 1999). Xanthophyll synthesis was independent of the duration of the mixing cycles and therefore can be considered as a general response to high radiation exposure.

UV-exposed *D. tertiolecta* showed faster xanthophyll synthesis than during PAR exposure, indicating that the former condition required more energy dissipation. This could be due to higher energy levels under PAR + UV than under PAR. In addition, UV radiation can inactivate Calvin cycle enzymes and subsequently reduce the Calvin cycle electron sink, which further increases energy dissipation demand (Bischof *et al.* 2002). Xanthophyll synthesis may have been particularly evident in *D. tertiolecta* because of the small initial xanthophyll cycle pool. In contrast, the initial xanthophyll cycle pool relative to Chl *a* of *T. weissflogii* was twofold higher than that of *D. tertiolecta*, which agrees with the idea that diatoms rely more on energy dissipation from xanthophyll cycling during excess radiation conditions (Lavaud *et al.* 2003). It has been found that dynamic irradiance increases both light-harvesting and protective pigment pools compared with constant irradiance (Havelková-Doušová *et al.* 2004; van de Poll *et al.* 2007). In the current experiments an increase in both pigment pools was found for *D. tertiolecta*, but for *T. weissflogii* we mainly observed synthesis of xanthophyll cycle pigments after the shift to dynamic solar radiation.

Between 12:00 and 15:00 h xanthophyll de-epoxidation was lower under PAR + UV than under PAR in *T. weissflogii* during the 10-min irradiance fluctuation cycle. For the 60-min cycle and for *D. tertiolecta* this effect was not evident, in contrast to the UV effects on carbon assimilation. It should be noted that the PAR and PAR + UV carbon incorporation experiments were performed on the same day, whereas pigment and PSII quantum yield experiments were performed on different days. Therefore, radiation differences due to cloud cover may have obscured UV effects on light-adapted PSII quantum yield and pigment responses. UV effects on the xanthophyll epoxida-

tion state have been reported previously for diatoms, dinoflagellates, and green macroalgae, although numerous studies observed no such effect (Evens *et al.* 2001; Bischof *et al.* 2002; Mewes & Richter 2002;). Reduced de-epoxidation has been attributed to UV-mediated membrane damage, which could lead to a reduced proton gradient. Therefore, the results could imply that UV-exposed *T. weissflogii* cells had a reduced capacity to dissipate absorbed energy via the xanthophyll cycle. However, this contrasts with studies such as that of Sobrino *et al.* (2005) that reported stronger de-epoxidation under UV in *Nanochloropsis graditana* when exposed for 1 h to a solar simulator. Apart form *T. weissflogii* under a 10-min cycle, UV effects on xanthophyll de-epoxidation were modest when considering the high UV exposure (maximal 58 W m^{-2} UVA and 1.8 W m^{-2} UVB). Furthermore, increased synthesis of the epoxidated xanthophyll cycle pigments under UV exposure may provide the impression of reduced de-epoxidation activity (van de Poll & Buma 2009). It was found previously that xanthophyll cycle pigments are only partly available for de-epoxidation (Moisan *et al.* 1998). Possibly, the newly synthesized xanthophyll cycle pigments are not converted but have an alternative function such as direct antioxidant properties (Baroli *et al.* 2003).

From 12:00 and 15:00 h no relationship was found between xanthophyll cycle activity, NPQ of Chl *a* fluorescence, and light-adapted PSII quantum yield dynamics. Minor epoxidation activity occurred during the mixing-mediated irradiance fluctuations, probably because fluctuations between 350 and 150 W m^{-2} (1622 and 699 μmol photons m^{-2} s^{-1}) PAR were too high to trigger significant epoxidation activity. Furthermore, the epoxidation reaction of xanthophyll cycle pigments and the subsequent relaxation of energy dissipation takes more time than the de-epoxidation reaction (Willemoës & Monas 1991; Moisan *et al.* 1998). In contrast to xanthophyll de-epoxidation, NPQ and the PSII quantum yield $[(F_{m'} - F_t)/F_{m'}]$ were influenced by short-term irradiance fluctuations in both species during this time interval. This makes it evident that the fluorescence parameters were also regulated by other mechanisms that operate on shorter timescales (seconds) than xanthophyll epoxidation reactions alone. Although diatoxanthin formation is required for the induction of NPQ in diatoms, Grouneva *et al.* (2008) recently described a fast proton gradient-induced NPQ component and a slower xanthophyll conversion-dependent component for the diatom *Cyclotella meneghiniana* (Grouneva *et al.* 2008). Our results indicated that this fast component is also present in *T. weissflogii*. The relatively small xanthophyll cycle pool in high-radiation-acclimated *D. tertiolecta* suggested a more important regulatory role for other mechanisms. Green algae can use state transitions as a major regulatory mechanism during high irradiance. *Chlamydomonas reinhardtii* can transfer up to 80% of absorbed energy from PSII to PSI via this process (Delsome *et al.* 1996; Finazzi *et al.* 2001). Also, alternative electron cycling around PSII and PSI is believed to contribute to the reduction of harmful effects on PSII during excess radiation in diatoms and green algae (Wagner *et al.* 2005; Lavaud *et al.* 2007).

The timescale of irradiance fluctuations appeared to influence the inhibition of carbon assimilation during UV exposure. Although exposed to comparable UV doses, irradiance fluctuations with a 10-min cycle caused a more pronounced UV effect (on average 57% inhibition) than a 60-min cycle (on average 34% inhibition) from 12:00 and 15:00 h. Furthermore, PSII quantum yield in the light and NPQ between 12:00 and 15:00 h were lower during PAR + UV exposure for the 10-min cycle in both species. The additional UV-mediated reduction in carbon assimilation under rapidly fluctuating light may be partly related to a reduced ability to regulate excess energy dissipation, thereby enhancing the accumulation of photodamaged PSII reaction centers. Previous research has indicated that a rapidly fluctuating radiation environment enhanced the effects of UV radiation (Helbling *et al.* 1994; Köhler *et al.* 2001; Neale *et al.* 1998). Regardless of the underlying mechanism, UV exposure increased the light-adapted PSII quantum yield responses to irradiance changes, and caused significantly lower carbon assimilation numbers compared with PAR exposure in both species. Thus, the xanthophyll cycle activity of these high-irradiance-acclimated algae did not prevent UV-mediated inhibition of carbon incorporation.

Despite the differences in pigments, pigment dynamics, and NPQ mechanisms between *D. tertiolecta* and *T. weissflogii*, we observed no clear differences in UV sensitivity of carbon assimilation. Therefore, UV sensitivity appeared to be independent of the xanthophyll cycle type or other regulatory mechanisms. However, overall carbon incorporation by *D. tertiolecta* during the 10-min cycle was a factor of 4 higher than that of *T. weissflogii*, whereas this difference was not evident during the 60-min cycle. This suggested that *D. tertiolecta* can exploit a highly dynamic irradiance environment. In contrast to *D. tertiolecta*, ^{14}C carbon incorporation in *T. weissflogii* was a factor of 2 lower during the 10-min mixing cycle than during the 60-min mixing cycle, suggesting that this species preferred more stable irradiance conditions. In general, our observations on the xanthophyll pool size and carbon fixation during strong radiation fluctuations agree with the hypothesis that diatoms prefer stable radiation environments that can be observed during stratification of the water column (Arrigo *et al.* 1999). It should be noted that the dynamic irradiance treatments by no means mimic actual vertical mixing in the water column. In contrast to the circular paths that were simulated, vertical transport rates are probably not constant in the field and may change over the day. Therefore, the variations in radiation experienced by algae are probably more complex than our radiation treatments.

In conclusion, we examined photosynthetic and pigment responses of two contrasting phytoplankton species that were acclimated to high light. Our results show that high-irradiance-acclimated *D. tertiolecta* performed better in a highly dynamic irradiance environment over a 8-h time course than *T. weissflogii* when regarding carbon assimilation. The current experiments were performed under strong radiation and therefore focussed on excess radiation responses of both species. Despite differences in the xanthophyll cycle pigments and xanthophyll cycle pool size, xanthophyll de-epoxidation seemed to be an important downregulation mechanism in both species. However, UV-mediated reduction in carbon assimilation rates were observed regardless of xanthophyll cycle activity and

differences in xanthophyll cycle pools. Furthermore, UV radiation effects increased during rapid irradiance dynamics in *D. tertiolecta* and *T. weissflogii*. The effects of the timescale of irradiance fluctuations on carbon assimilation under PAR and PAR + UV exposure require further investigation.

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