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Photoacclimation modulates excessive photosynthetically active and ultraviolet radiation effects in a temperate and an Antarctic marine diatom

Willem H. van de Poll,[1] *Anne-Carlijn Alderkamp, Paul J. Janknegt, Jan Roggeveld and Anita G. J. Buma*

Department of Marine Biology, Center for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

Abstract

The influence of photoacclimation on the effects of excessive photosynthetically active (PAR; 400–700 nm) and ultraviolet (UVR; 280–400 nm) radiation was assessed for the marine diatoms *Thalassiosira weissflogii* (Grunow) Fryxell and Hasle and *Thalassiosira antarctica* (Comber). Low and high PAR acclimated cultures were subjected to simulated surface irradiance (SSI) that mimicked irradiance around noon, including UVR. PSII efficiency, xanthophyll conversion, superoxide dismutase (SOD) activity, carbohydrate buildup, and lipid peroxidation were investigated after 30 min SSI and during 120 min recovery in low irradiance. Furthermore, viability loss was measured during 4 h SSI. Prior to SSI, the diadino-diatoxanthin pool was increased in high irradiance acclimated cells, compared with cells grown under low irradiance. Thirty-minutes SSI caused a pronounced decline in PSII efficiency. This coincided with de-epoxidation of diadinoxanthin in high irradiance acclimated cells, which was completely reversed during recovery in low irradiance. De-epoxidation was lower for low irradiance acclimated cells, whereas PSII efficiency and carbohydrate buildup were lower during the recovery phase. Furthermore, clear UVR effects on PSII efficiency were observed in low irradiance but not in high irradiance acclimated cells. Although 30 min SSI did not increase cellular SOD activity and lipid peroxidation, prolonged (4 h) SSI caused viability loss in low irradiance acclimated cells, which was enhanced by UVR. Therefore, PAR and UVR-induced PSII inactivation and viability loss were reduced by high irradiance–mediated changes in light harvesting and the xanthophyll pigments. In addition to photoacclimation-modulated differences, minor sensitivity differences were found between species.

Season, diurnal cycle, weather, and optical properties of particles, detritus, and sediment shape the incident irradiance experienced by phytoplankton (Kirk 1994). Furthermore, deep vertical mixing of the water column imposes strong irradiance oscillations (Denman and Gargett 1983; Neale et al. 2003). This reduces the daily irradiance dose, although phytoplankton occasionally experience periods of excessive irradiance when residing near the surface. These continuous alterations between low and excessive irradiance require regulation and acclimation of light harvesting, photosynthesis, and photoprotection. In response to irradiance limitation, algae maximize light harvesting and photosynthetic efficiency, whereas Calvin cycle activity increases at the expense of light harvesting pigments during saturating irradiance (Falkowski and LaRoche 1991). However, rapid irradiance fluctuations impose challenges to the photoregulation and photoacclimation processes because transition from low to excessive irradiance can overreduce photosynthetic electron transport and initiate formation of reactive oxygen species (ROS). ROS are potentially harmful for all biomolecules and can initiate chain reactions that destroy membranes (Hideg and Vass 1996). Yet initial ROS effects are primarily observed in the chloroplasts causing the inactivation of PSII reaction centers. Protection against excessive irradiance involves down regulation of PSII efficiency to regulate excitation energy for photosynthesis. Short-term adjustment of light harvesting is achieved by thermal dissipation of excessive energy due to de-epoxidation of xanthophylls. The xanthophyll cycle of algae comprises enzymatic conversion of carotenoids such as violaxanthin to antheraxanthin and zeaxanthin in green algae, and diadinoxanthin to diatoxanthin in diatoms (Demming-Adams 1990; Olaizola et al. 1994; Moisan et al. 1998). It is generally accepted that de-epoxidation and epoxidation of xanthophylls is controlled by the pH of the lumen (Lavaud et al. 2002). Furthermore, photo-induced PSII damage can be compensated by increased turnover of the D1 reaction center binding protein (Kim et al. 1993). In addition, ROS accumulation can be prevented by antioxidants such as superoxide dismutase (SOD), ascorbate peroxidase, and glutathione metabolism in the chloroplasts (Miyake and Asada 2003). Overall, sensitivity to excessive irradiance is strongly influenced by photoacclimation and nutrient availability because these conditions influence cellular pigment composition and protein turnover rates (Geider et al. 1993; Shelly et al. 2003; van de Poll et al. 2005). Interspecific differences in photoacclimation potential have been reported between a diatom and a green flagellate (Van Leeuwe et al. 2005). Also, differences in PSI and cytochrome b_6f content between the marine diatoms *Thalassiosia weissflogii* and *Thalassiosia oceanea* were suggested to initiate changes in sensitivity to fluctuating irradiance (Strzepec and Harrison 2004).

Current knowledge on the interactive effects between ultraviolet radiation (UVR) and excessive photosynthetically active radiation (PAR) is still scattered. In addition to excessive PAR (400–700 nm), UVBR (280–315 nm) and UVAR (315–400 nm) cause PSII inactivation that can reduce carbon accumulation under field and laboratory

[1] Corresponding author (W.H.van.de.Poll@RUG.nl).

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conditions (Cullen et al. 1992; Bracher and Wiencke 2000; Barbieri et al. 2002). Furthermore, UVBR is suggested to affect xanthophyll conversion and therefore may influence sensitivity to high irradiance (Bischof et al. 2002; Mewes and Richter 2002). However, acclimation to UVR exposure by accumulation of UVR absorbing compounds and increased D1 turnover are also reported (Helbling et al. 1996; Hazzard et al. 1997). Confusion about the UVBR contribution to overall high irradiance effects are partly due to differences in spectral irradiance quality and quantity of UVR sources. Unbalanced UVR and PAR can exaggerate UVR effects because important mechanisms involved in prevention and repair of UVR-induced damage use or are activated by PAR. Estimates of UVBR-induced productivity losses in the field vary from not significant up to 10%, depending on exposure scenarios (Smith et al. 1992; Neale et al. 1998*c*; Arrigo et al. 2003). Because vertical mixing combined with the diurnal cycle limits the period of potential UVR exposure, effects of prolonged UVBR on phytoplankton have little ecological relevance in a dynamic irradiance environment. Nevertheless, short exposure to surface irradiance can have a pronounced effect on viability, whereas reduced photosynthetic efficiency may linger on for some time after exposure (van de Poll et al. 2005). Other variability sources are phytoplankton species composition and the complex interactions with their physical environment. UVR effects may be species-specific and can be influenced by photoacclimation, nutrient availability, and temperature (Neale et al. 1998*b*; Shelly et al. 2003; van de Poll et al. 2005).

In this paper we investigated the interaction between photoacclimation and excessive UVR and PAR effects by exposing high and low irradiance acclimated *Thalassiosira weissflogii* and *Thalassiosira antarctica* to irradiance that mimicked conditions near the water surface at 12:00 h. These marine diatoms belong to the same genus and are commonly observed in the phytoplankton community of temperate and polar waters. By comparing these species, we examined potential differences in photoacclimation and excessive irradiance responses in species growing under contrasting temperature conditions. Photoacclimation and excessive irradiance effects were studied by monitoring pigment composition and PSII efficiency after exposure and during subsequent recovery in low irradiance. Furthermore, buildup of carbohydrates, the common energy and carbon storage products, was analyzed to investigate effects on Calvin cycle activity. In addition, SOD activity was used as an indicator for antioxidant capacity, whereas cellular thiobarbituric acid reactive substances (TBARS) were used as a measure for ROS-induced lipid peroxidation. Finally, photo-induced viability loss was used to demonstrate differences in excessive irradiance sensitivity.

Methods

Experimental setup—T. antarctica and *T. weissflogii* were acclimated to low and high irradiance for 3 weeks and subsequently exposed to 30 min simulated surface irradiance (SSI). Glass filters were used to create three SSI treatments with different spectral composition, including UV. After SSI exposure the algae were placed for up to 180 min in low irradiance (no UV) for recovery. PSII efficiency and pigment composition were investigated before and after SSI exposure and during recovery in low irradiance, whereas carbohydrate buildup and SOD activity were determined before exposure and after the recovery period. TBARS and viability were measured before and directly after SSI. Additional experiments were performed to demonstrate effects of prolonged (up to 4 h) SSI exposure on viability. All measurements were performed in duplicate (i.e., algae were obtained from two separate cultures).

Cultivation conditions—T. antarctica (Comber, strain CCMP 982) and *T. weissflogii* (Grunow) Fryxell and Hasle (strain CCMP 1049) were cultivated under low (8–9 μmol photons m^{-2} s^{-1}) and high (290 μmol photons m^{-2} s^{-1}) irradiance from Biolux (Osram) fluorescent lamps under a 16 : 8 light : dark (LD) cycle in f/2 enriched (Guillard and Ryther 1962) autoclaved seawater (salinity 35). Irradiance was measured in air with a QSL-100 (Biospherical Instruments) with a spherical sensor. The algae were grown at least 3 weeks in these irradiance conditions, and the medium was changed each week. Experiments were performed at 4.5°C and 18°C for *T. antarctica* and *T. weissflogii*, respectively. These temperatures support high growth rates of both species and approximate summer temperatures for Antarctic and temperate waters, respectively.

SSI exposure—Prior to SSI exposure, low and high irradiance acclimated *T. antarctica* and *T. weissflogii* were diluted to 200×10^3 and 40×10^3 cells mL^{-1}, respectively, to create transparent suspensions with comparable properties (measured for PAR in suspension with a QSL-100). For PSII efficiency, pigment, carbohydrate, SOD, and TBARS measurement duplicate quartz vessels with each 150 mL of cell suspension were prepared. These were irradiated from below by a 250 W MHN-TD power tone (Philips) and two UVA 340 (Q-panel) lamps. WG 305, WG 335, and GG 385 glass filters (Schott) were used to create treatments consisting of PAR + UVAR + UVBR, PAR + UVAR, and PAR, respectively (Fig. 1; Table 1). Note that spectral irradiance of these treatments does not follow CIE (International Commission on Illumination) definitions. The irradiance conditions were comparable with surface values at noon on a sunny day at mid latitudes, and therefore were defined as SSI. For comparison, solar irradiance was measured at 13:00 h on 17 June 2005 in Haren, The Netherlands, 53°10′N, 6°30′E (Fig. 1; Table 1). Irradiance was measured with a MACAM SR9910 double monochromator scanning spectroradiometer (Macam Photometrics) and a spherical sensor and was the same as in van de Poll et al. (2005). Quartz vessels were cooled by a temperature-controlled water bath (4.5°C and 18°C for experiments with *T. antarctica*. and *T. weissflogii*, respectively) during SSI exposure.

Fluorescence(PSII efficiency after 5-min dark adaptation, $F_v : F_m$*)*—Samples (20 mL, PAR, PAR + UVAR, and

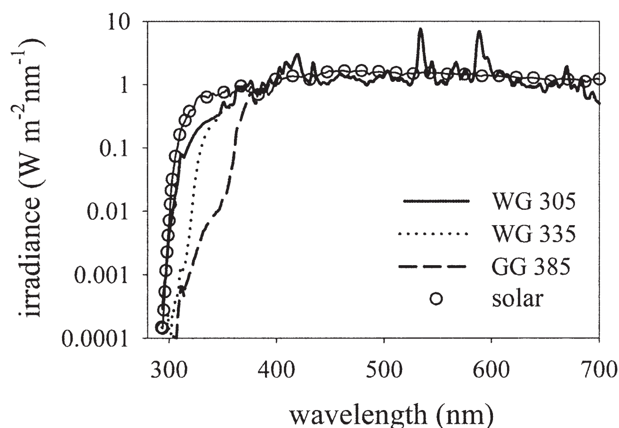

Fig. 1. Spectral irradiance of PAR + UVAR + UVBR (WG 305), PAR + UVAR (WG 335), and PAR (GG 385) treatments during simulated surface irradiance. For comparison, a solar spectrum (13:00 h, 17 June 2005, The Netherlands) is shown.

PAR + UVAR + UVBR) for fluorescence measurements were obtained from the duplicate quartz vessels directly after SSI exposure and after 30, 60, and 120 min recovery under low irradiance (9 μmol photons m^{-2} s^{-1}). Measurements were as in van de Poll et al. (2005). In short, algae were filtered on GF/F (Whatman, 10 mm) and placed in a cooled cuvette. Variable fluorescence was determined after 5 min dark acclimation with a PAM 2000 pulse amplitude modulated fluorometer (Walz). Samples from low and high irradiance acclimated cultures (no SSI exposure) served as control.

Pigments—Cell suspensions in duplicate quartz vessels were sampled for pigment composition (30 mL, PAR, PAR + UVAR, and PAR + UVAR + UVBR) before; after 30 min SSI exposure; and after 30, 60, and 120 min recovery in low irradiance (9 μmol photons m^{-2} s^{-1}). Samples were filtered on 25 mm GF/F (Whatman), immediately frozen in liquid nitrogen, and stored at -80°C. Filters were freeze dried (48 h) and extracted in 90% acetone (48 h). Pigments were separated and quantified on a high performance liquid chromatography (HPLC) system (Waters 2690 separation module, 996 photodiode array detector, and a C_{18} 5 μm DeltaPak reversed-phase column) according to Kraay et al. (1992).

Water-extractable carbohydrates—To investigate SSI effects on carbohydrate buildup in low irradiance, samples were exposed to 30 min SSI (PAR, PAR + UVAR, and PAR + UVAR + UVBR) followed by 3 h low irradiance (9 μmol photons m^{-2} s^{-1}), whereas 3 h low irradiance alone (no SSI) served as control. Samples for initial cellular carbohydrate content were obtained from the cultures before SSI exposure. Carbohydrate samples (150 mL) were filtered on 25 mm precombusted (4 h, 450°C) GF/F filters (25 mm; Whatman), frozen in liquid nitrogen and stored at -20°C. Particulate water-extractable carbohydrates were extracted in MilliQ (1.5 mL) for 30 min at 80°C, followed by centrifugation (10,000 $\times$ g, 15 min). Carbohydrates were quantified in the supernatant by the TPTZ method

Table 1. Irradiance (W m^{-2}) for the PAR + UVAR + UVBR (WG 305), PAR + UVAR (WG 335), and PAR (GG 385) treatments during simulated surface irradiance. For comparison, solar irradiance is shown for 17 June 2005, recorded in Haren, The Netherlands (53°10′N, 6°30′E), at 13:00 h on a sunny day.

	PAR (400–700 nm)	UVAR (315–400 nm)	UVBR (280–315 nm)
WG 305	448	50.9	0.608
WG 335	440	47	0.012
GG 385	450	25.7	0.005
Solar	418	62	1.89

described by Myklestad et al. (1997) with D(+)-glucose as reference.

Flow cytometry and viability—Twenty-milliliter suspension of low and high irradiance acclimated cells were exposed to 4 h SSI (PAR + UVAR + UVBR and PAR) in duplicate quartz vessels. Samples (1 mL) for viability staining were obtained after 30, 60, 120, 180, and 240 min SSI exposure for low irradiance acclimated cells (PAR + UVAR + UVBR and PAR) and only at 240 min for high irradiance acclimated cells (PAR + UVAR + UVBR). Samples were incubated (45 min, darkness) with 10 μL SYTOX Green (Molecular Probes, 100 times diluted in MilliQ) prior to flow cytometry. Cell counts and viability measurements were performed with a Coulter XL-MCL flow cytometer (Beckman Coulter) as in van de Poll et al. (2005).

SOD activity—Cell suspensions in quartz vessels were exposed to 30 min SSI (PAR, PAR + UVAR, and PAR + UVAR + UVBR), followed by 3 h low irradiance (9 μmol photons m^{-2} s^{-1}). Samples (150 mL) were filtered on 25 mm polycarbonate filters (10 and 2 μm pore size for *T. weissflogii* and *T. antarctica*, respectively; Osmonics), frozen in liquid nitrogen and stored at -80°C. Proteins were extracted after mild sonification in extraction buffer (0.5 mol L^{-1} KH_2PO_4, 0.01 mol L^{-1} EDTA, 0.1% Triton X-100, 2% PVP, complete protease inhibitor, Roche), followed by 20 min centrifugation (20,000 $\times$ g) and quantified according to Bradford (1976). SOD activity was quantified in fresh extracts using the Riboflavin/NitroBlue Tetrazolium assay described by Gechev et al. (2003). Nitro blue tetrazolium (NBT) reduction was measured after 15 min irradiance (56 μmol photons m^{-2} s^{-1}) for five different protein concentrations at 560 nm on a Cary 3E UV/vis double beam spectrophotometer (Varian). SOD activity was defined as the sample amount required for 50% inhibition (Beauchamp and Fridovich 1971), which was calculated by regression on the linear part of the natural log transformed curve. Specific SOD activity (SA) was expressed as activity per milligram protein (U : mg protein).

Thiobarbituric acid reactive substances (TBARS)—Cellular TBARS were used as an indicator of ROS-induced lipid peroxidation. After 30 min SSI (PAR, PAR + UVAR, and PAR + UVAR + UVBR) exposure, TBARS samples

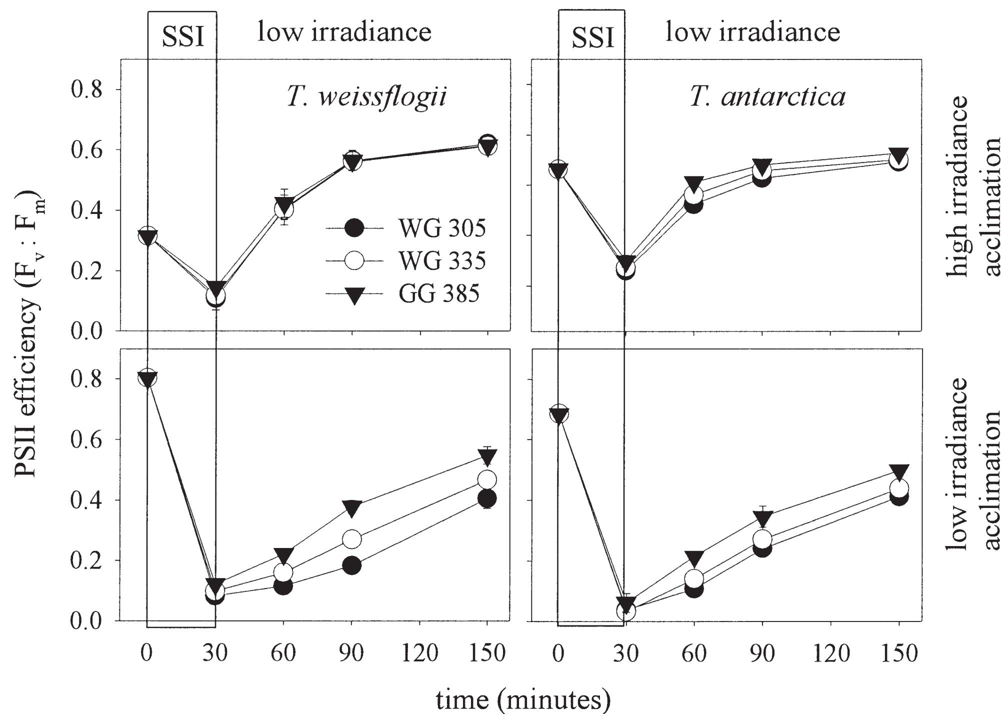

Fig. 2. PSII efficiency ($F_v : F_m$) dynamics during PAR + UVAR + UVBR (WG 305), PAR + UVAR (WG 335), and PAR (GG 385) treatments and recovery in low irradiance for low and high irradiance acclimated *T. weissflogii* and *T. antarctica*. Mean and standard deviation are shown for two replicates.

(150 mL) were filtered on polycarbonate filters (10 and 2 μm pore size for *T. weissflogii* and *T. antarctica*, respectively); frozen in liquid nitrogen; and stored at −80°C. Analyses were done after Heath and Packer (1968). In short, filters were resuspended in 0.8 mL MilliQ and 0.8 mL thiobarbituric acid (0.5% w/v, Sigma) in 20% trichloroacetic acid (TCA). The mixture was heated for 30 min at 90°C and put on ice. After removal of the filter debris, the supernatant was centrifuged (10 min 20,000 × g). TBARS were detected by subtracting nonspecific turbidity at 600 nm from TBARS maximal absorption at 532 nm, as measured on a Cary 3E double beam UV/vis spectrophotometer. The extraction solvent was used as a blank.

Statistics—Differences between groups were tested for significance with a type 1 single factor analysis of variance (ANOVA) and were considered significant at $p < 0.05$. When differences were not significant, data were pooled and tested at the next level.

Results

Fluorescence: PSII efficiency after 5 min dark adaptation—Initial $F_v : F_m$ values were different between species and between photoacclimation conditions. Lower $F_v : F_m$ was measured in high irradiance acclimated cells than in low irradiance acclimated cells. On average, high irradiance acclimated *T. antarctica* showed a higher $F_v : F_m$ than *T. weissflogii* (0.46 ± 0.001 versus 0.31 ± 0.006, $n = 2$). In contrast, $F_v : F_m$ of low irradiance acclimated *T. weissflogii* was higher than that of *T. antarctica* (0.8 ± 0.003 vs. 0.69 ± 0.004, $n = 2$).

SSI exposure caused a strong reduction of $F_v : F_m$, which was more pronounced in low irradiance acclimated cells (*T. weissflogii*, 0.10 ± 0.02; *T. antarctica*, 0.043 ± 0.02, $n = 6$) than in high irradiance acclimated cells (*T. weissflogii*, 0.124 ± 0.03; *T. antarctica*, 0.077 ± 0.02; Fig. 2). Differences between spectral irradiance treatments were only significant for low irradiance acclimated *T. weissflogii*; where PAR + UVAR + UVBR caused the highest reduction ($F_v : F_m$, 0.08±0.01, $n = 2$) followed by PAR + UVAR and PAR ($F_v : F_m$, 0.1 ± 0.001, 0.12 ± 0.01, $n = 2$).

Pronounced differences between species, photoacclimation conditions, and SSI irradiance conditions were observed during recovery in low irradiance. $F_v : F_m$ recovery was faster for high irradiance acclimated cells than for low irradiance acclimated cells. For high irradiance acclimated cells, the $F_v : F_m$ increase over time was significantly faster in *T. weissflogii* than in *T. antarctica*. For the former, $F_v : F_m$ after 2 h low of irradiance was two times higher than before SSI exposure. UVR effects during recovery were not found in high irradiance acclimated *T. weissflogii*, whereas slightly lower values were found for PAR + UVAR and PAR + UVAR + UVBR exposed *T. antarctica*, compared with PAR exposed samples. Pronounced UV effects were observed during recovery of low irradiance acclimated cells. Two hours recovery resulted in

Table 2. Cellular pigment composition (chlorophyll c_2, fucoxanthin, diadinoxanthin, diatoxanthin, chlorophyll *a*, and β carotene in picogram per cell) of high and low irradiance acclimated *T. antarctica* and *T. weissflogii*. Mean and standard deviation are shown for 24 measurements.

Species	*T. antarctica*		*T. weissflogii*	
Irradiance	High	Low	High	Low
Chlorophyll c_2	0.027 (0.002)	0.052 (0.015)	0.15 (0.014)	0.46 (0.078)
Fucoxanthin	0.076 (0.005)	0.19 (0.032)	0.67 (0.05)	1.90 (0.265)
Diadinoxanthin	0.091 (0.002)	0.033 (0.002)	0.40 (0.03)	0.35 (0.028)
Diatoxanthin	0.014 (0.003)	0.004 (0.003)	0.460 (0.11)	0.016 (0.018)
Chlorophyll *a*	0.15 (0.013)	0.19 (0.069)	1.83 (0.18)	4.68 (0.89)
β carotene	0.019 (0.001)	0.014 (0.003)	0.13 (0.013)	0.18 (0.029)

lower $F_v : F_m$ for PAR + UVA + UVB and PAR + UVA treated cells compared with the PAR-only treatment. For low irradiance acclimated cells, UVR effects were more pronounced in *T. weissflogii* than in *T. antarctica* (i.e., $F_v : F_m$ differences between PAR- and UVR-exposed samples were smaller in the latter).

Pigment composition—In both species, chlorophyll c_2, fucoxanthin, diadinoxanthin, diatoxanthin, chlorophyll *a*, and β carotene were identified. Cellular pigment content, pigment composition, and SSI-induced pigment changes were different for low and high irradiance acclimated *T. antarctica* and *T. weissflogii* (Table 2). Apart from the epoxidation state of diadinoxanthin, no significant pigment changes were found after SSI and during recovery in low irradiance when compared with initial values, showing that de novo synthesis or degradation of pigments was not significant for the investigated time interval. Therefore, all pigment data were pooled for the calculation of cellular pigment content (Table 2).

High and low irradiance acclimated cells of *T. antarctica* contained on average 0.38 ± 0.03 and 0.47 ± 0.11 pg pigment cell^{-1}, whereas this was 3.65 ± 0.3 and 7.58 ± 1.3 pg for *T. weissflogii* (n = 24). Growth in low irradiance caused a 231% and 286% increase in fucoxanthin content of *T. antarctica* and *T. weissflogii*, respectively, relative to growth in high irradiance. Chlorophyll *a* content of *T. antarctica was* not significantly different between high and low irradiance, in contrast to that of *T. weissflogii*, which was 256% higher in low irradiance compared with high irradiance acclimated cells. Differences in the diadino-diatoxanthin pool relative to other pigments were found between species and photoacclimation conditions (Figs. 3 and 4). Diadino and diatoxanthin comprised 16% and 4%, respectively, of the total pigment pool of high and low irradiance acclimated *T. antarctica*. This was significantly lower for high and low irradiance acclimated *T. weissflogii* (13.7% and 2.5%, respectively). On average, 13.5% was de-epoxidated (diatoxantin) before SSI exposure in high and low irradiance acclimated *T. antarctica*, whereas this was 53.3% and 4.2% for high and low irradiance acclimated *T. weissflogii*, respectively (Fig. 4). Thirty-minutes SSI caused a significant increase in de-epoxidation of diadinoxanthin for all conditions (Fig. 4). Because significant UV-induced differences were not observed, data of PAR, PAR + UVAR, and PAR + UVAR + UVBR treatments during and after SSI were pooled. Photoacclimation to high and low irradiance resulted in differences in de-epoxidation (of diadinoxanthin) after SSI and epoxidation (of diatoxanthin) during recovery in low irradiance. Significantly more diadinoxanthin was de-epoxidized in high irradiance acclimated cells (67% and 82%) than low irradiance acclimated cells (47% and 27%) of *T. antarctica* and *T. weissflogii*, respectively. Most diatoxanthin of high irradiance acclimated cells was converted to diadinoxanthin after 30 min recovery in low irradiance. Epoxidation of diatoxanthin during recovery in low irradiance was slower in low irradiance acclimated cells, whereas changes in epoxidation state were more pronounced for *T. antarctica* than for *T. weissflogii*.

Water extractable carbohydrates—The initial cellular carbohydrate concentration (before SSI exposure, 4 h after the onset of the light period) varied among species and photoacclimation state (Fig. 5). *T. weissflogii* contained on average nine times more carbohydrate than *T. antarctica*, whereas carbohydrate content of high irradiance acclimated cells was twice that of low irradiance acclimated cells. Changes in cellular carbohydrate of low and high irradiance acclimated cells were different after SSI followed by 3 h low irradiance and 3 h low irradiance alone. Because no significant UV effects were found for low and high

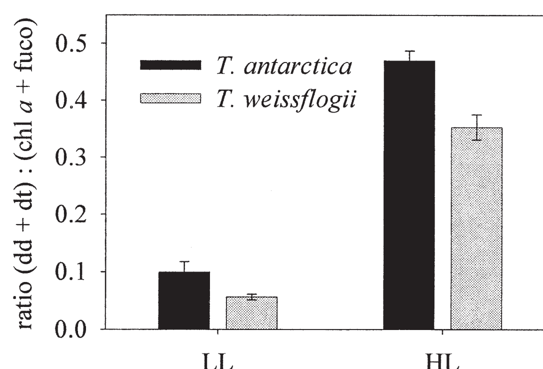

Fig. 3. Ratio between the xanthophyll pool (diadinoxanthin, diatoxanthin) relative to light harvesting pigments (chlorophyll *a* and fucoxanthin) for low and high irradiance acclimated *T. antarctica* and *T. weissflogii*. Mean and standard deviation are shown for 24 measurements.

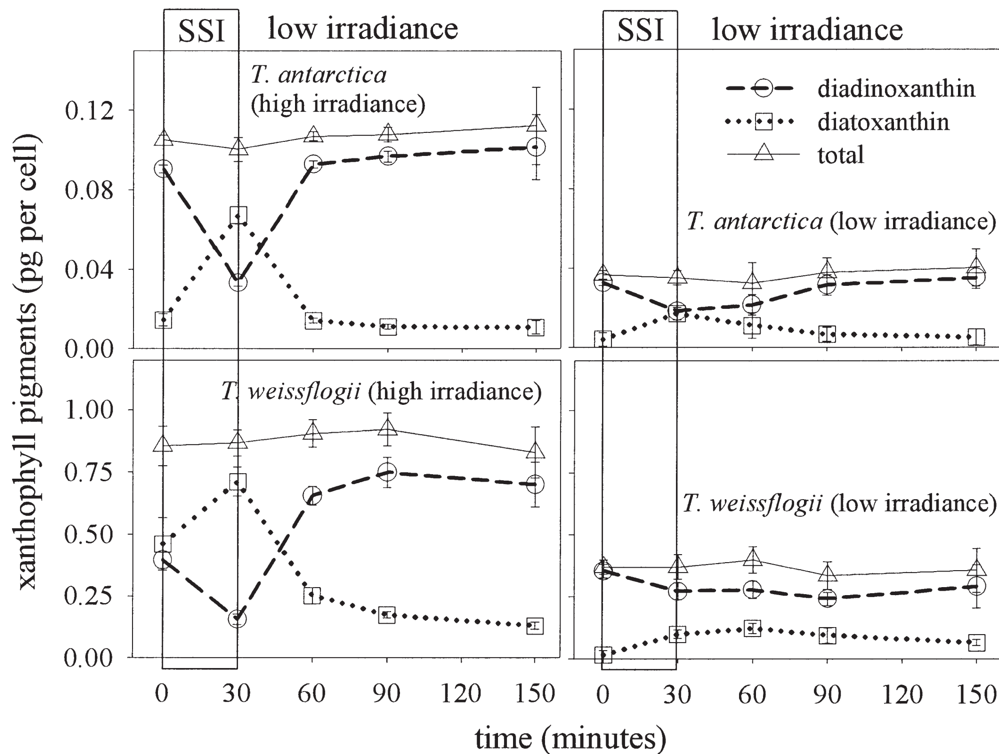

Fig. 4. Changes in diadinoxanthin, diatoxanthin, and total xanthophylls in picograms per cell during simulated surface irradiance (SSI) and recovery in low irradiance of low and high irradiance acclimated *T. antarctica* and *T. weissflogii*. Mean and standard deviation are shown for six measurements (data for PAR + UVAR + UVBR, PAR + UVAR, and PAR treatments pooled) and four control samples.

irradiance acclimated cells, data of the (SSI) irradiance treatments (PAR, PAR + UVAR, and PAR + UVAR + UVBR) were pooled. Low irradiance acclimated cells increased cellular carbohydrate by on average 52% after 3 h low irradiance, whereas this was 19% for SSI plus 3 h low irradiance exposed cells. Apart from higher initial cellular carbohydrate, changes in high irradiance acclimated cells were different from low irradiance cells, whereas carbohydrate dynamics were species-specific. For *T. antarctica*, no significant carbohydrate increase was found after SSI plus 3 h low irradiance and 3 h low irradiance alone. In contrast, a 45% decrease in cellular carbohydrate after SSI plus 3 h low irradiance and 3 h low irradiance alone was found for *T. weissflogii*.

Viability—The number of nonviable cells before SSI was $<2\%$ for both high and low irradiance acclimated *T. weissflogii* and *T. antarctica*, with the exception of the high irradiance acclimated culture of *T. antarctica*, where 4% was nonviable (Fig. 6). Thirty-minutes SSI did not cause significant viability loss for any sample (results not shown). Prolonged SSI exposure revealed differences in photoinduced viability loss between species, photoacclimation state, and irradiance conditions during SSI. Increased viability loss was found during 4 h SSI for low irradiance acclimated cells, whereas this was minimal for high irradiance acclimated cells of both species. Low irradiance acclimated cells of *T. weissflogii* were more sensitive than those of *T. antarctica*. Extensive viability loss was observed within 3 h for the former. UVR (PAR + UVAR + UVBR treatment) caused increased viability loss over time compared with the PAR treatment in both species (i.e., viability loss was observed earlier during UV exposure).

SOD activity and TBARS—Calculated cellular protein content was different for *T. weissflogii* and *T. antarctica*, but did not change during SSI exposure and recovery. Cellular protein was on average 42.3 ± 3.2 pg cell^{-1} for *T. weissflogii* ($n = 20$), whereas no differences between low and high irradiance acclimated cells were found. For *T. antarctica* this was on average 5.28 ± 0.34 and 5.87 ± 0.17 pg protein for low and high irradiance acclimated cells, respectively ($n = 10$). Although cellular SOD was higher in *T. weissflogii* compared with *T. antarctica*, SOD activity between species was identical when normalized to protein (Table 3). SOD activity did not change after SSI, except in *T. antarctica*. Low irradiance acclimated cells of this species showed an SOD activity increase when exposed to 30 min SSI (PAR + UVAR) and 3 h low irradiance. Apart from this, no significant SOD activity changes were documented after SSI exposure and recovery.

Cellular TBARS content was higher for *T. weissflogii* than for *T. antarctica*, but were not influenced by photoacclimation in both species (Table 3). SSI exposure

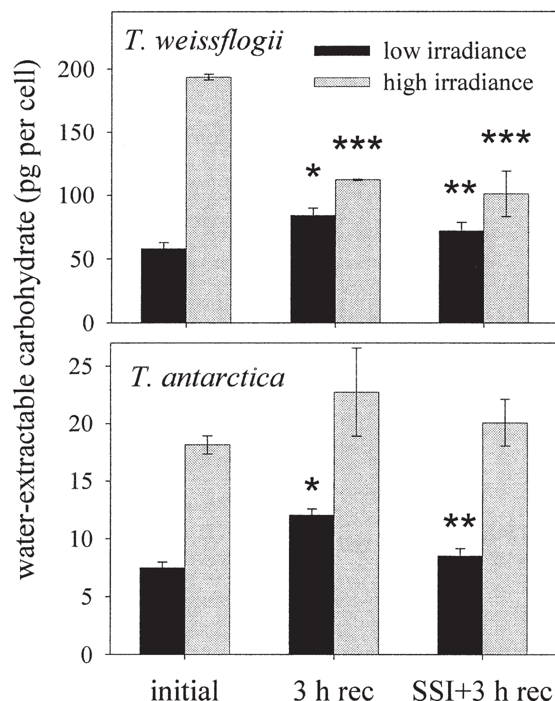

Fig. 5. Cellular water extractable carbohydrates for low and high irradiance acclimated *T. antarctica* and *T. weissflogii* before simulated surface irradiance (initial), after 3 h recovery in low irradiance (3 h rec), and after 30 min SSI and 3 h recovery (SSI + 3 h rec). Mean and standard deviation are shown for 6 measurements (data for PAR + UVAR + UVBR, PAR + UVAR, and PAR treatments pooled). Significant differences from initial values are indicated with asterisks.

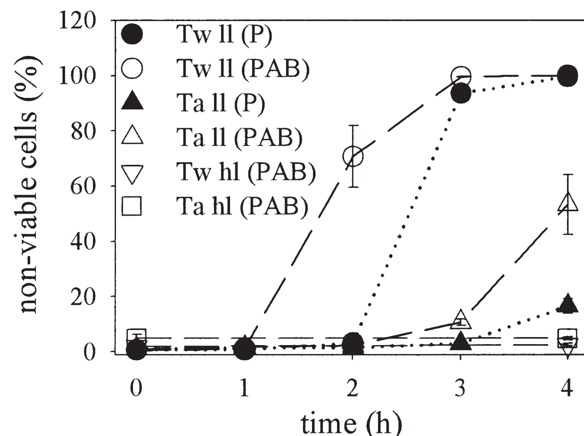

Fig. 6. Percentage of nonviable cells observed during PAR + UVAR + UVBR (PAB) and PAR (P) treatments of simulated surface irradiance for low and high irradiance acclimated *T. antarctica (Ta)* and *T. weissflogii (Tw)*. Mean and standard deviation are shown for the mean of two replicates, consisting of flow cytometry measurements on 30 × 10^3 cells each. Note that for high irradiance acclimated cells only results of PAR + UVAR + UVBR treatments are shown.

did not significantly affect cellular TBARS (Table 3, PAR, PAR + UVAR, PAR + UVAR + UVBR pooled).

Discussion

We investigated effects of simulated surface irradiance in high and low irradiance acclimated *T. weissflogii* and *T. antarctica*. This provided insight in the relationship between pigment composition and PSII protection during excessive irradiance in marine diatoms. The diadino-diatoxanthin pool size, which is influenced by photoacclimation state, appears decisive in preventing PSII damage. Furthermore, the experiments indicate that PSII damage can cause photo-induced viability loss during prolonged exposure, despite the reversible nature of PSII inactivation. The occurrence of this phenomenon strongly depends on photoacclimation state, whereas minor sensitivity differences were observed between the diatom species.

Acclimation to high and low irradiance resulted in cells with different pigmentation and photosynthetic properties, consistent with other documented photoacclimation responses (Willemöes and Monas 1991; Falkowski and LaRoche 1991; van de Poll et al. 2005). Low irradiance limited growth in both species, as was shown by the low cellular carbohydrate pool compared with high irradiance acclimated cells. In response to irradiance limitation, cells enlarge light-harvesting capacity by increasing cellular fucoxanthin, chlorophyll *a* (*T. weissflogii*), and PSII efficiency. A high efficiency to exploit low irradiance was demonstrated by significant buildup of water extractable carbohydrates over a 3-h period. In addition, the cellular diadino-diatoxanthin pool was lower in low irradiance than high irradiance acclimated cells. In contrast to low irradiance, high irradiance saturated photosynthesis. Therefore, Calvin cycle activity probably increased at the expense of light harvesting pigments such as fucoxanthin and chlorophyll *a*. This reduces the ability to exploit low irradiance, as was shown by a lack of carbohydrate buildup (Fig. 5). However, high irradiance acclimated cells maintained a much larger diadino-diatoxanthin pool than low irradiance acclimated cells, which together with reduced light harvesting pigments caused a fourfold increased ratio between cellular xanthophyll and light-harvesting pigments.

PSII efficiency was strongly reduced by 30 min SSI, irrespective of UVR exposure. However, the nature of PSII down regulation was different for high and low irradiance acclimated cells as was shown by differences in recovery dynamics in low irradiance. Reduced recovery suggests severe SSI-induced PSII damage in low irradiance acclimated cells, whereas this was lower for high irradiance acclimated cells. Furthermore, clear UVR effects were observed in low irradiance acclimated cells, whereas these were nearly absent for high irradiance acclimated cells. Nevertheless, a small UVR effect on PSII efficiency was found for high irradiance acclimated *T. antarctica*. Presumably, PSII repair processes were slower in *T. antarctica* at 4.5°C, whereas repair compensated UVR effects at 18°C in *T. weissflogii*. Carbohydrate buildup in low irradiance acclimated cells was lower after SSI exposure compared with non-SSI exposed cells. The reduced capacity to utilize low irradiance provides additional evidence for PSII damage, although no UVR effects were found in the

Table 3. Superoxide dismutase activity (specific SOD activity, normalized to protein) and cellular TBARS for low and high irradiance acclimated *T. weissflogii* and *T. antarctica*. For SOD activity, mean and standard deviation are shown for initial values ($n = 4$) and after 30 min SSI exposure plus 3 h recovery in low irradiance ($n = 6$, PAR + UVAR + UVBR, PAR + UVAR, and PAR values pooled, except for *). For low irradiance acclimated *T. antarctica*, SOD activity of PAR + UVAR exposed samples increased significantly to 298 ± 43 ($n = 2$, results not shown). TBARS were measured directly after 30 min SSI exposure; initial values $n = 2$; SSI exposed samples $n = 6$ (PAR + UVAR + UVBR, PAR + UVAR, and PAR pooled).

	SOD activity (protein^{-1})		TBARS ($\times 10^{-7}$ mol L^{-1} cell^{-1})	
	Initial	SSI (30 min+3 h rec)	Initial	SSI (30 min)
Thalassiosira weissflogii				
Low irradiance	121.4 (9.6)	106.0 (28)	0.54 (0.58)	0.57 (0.59)
High irradiance	93.5 (17.5)	92.0 (8.8)	0.56 (1.84)	0.74 (0.53)
Thalassiosira antarctica				
Low irradiance	118.6 (8.9)	143.1 (38.0)*	1.16 (0.18)	1.39 (0.12)
High irradiance	128.8 (36.5)	120.6 (19.6)	1.24 (0.15)	1.35 (0.15)

carbohydrate data. In contrast, no SSI exposure effect was found in carbohydrate dynamics of high irradiance acclimated cells. Photoacclimation modulated variation in PSII sensitivity coincided with differences in de-epoxidation of diadinoxanthin: In addition to the enlarged diadino-diatoxanthin pool, de-epoxidation to diatoxanthin was more pronounced during SSI for high irradiance acclimated cells. The nature of decreased xanthophyll cycle dynamics in low irradiance acclimated cells is unclear but may be related to the activity of de-epoxidase enzymes. Furthermore, epoxidation of diatoxanthin during recovery in low irradiance was faster in high than in low irradiance acclimated cells, and this process preceded recovery of PSII efficiency. De-epoxidation of xanthophylls has been linked to increased non-photochemical quenching by facilitating thermal dissipation of excessive energy (Demming-Adams 1990; Olaizola et al. 1994). Down regulation of photosynthesis via this process reduces excitation pressure on PSII and thus minimizes damage to reaction center proteins, which agrees with our results. Furthermore, our data indicate that a large diadino-diatoxanthin pool protects against UVR-induced PSII damage, whereas UVR irradiance effects on epoxidation and de-epoxidation of xanthophylls were not found. Consequently, acclimation to high irradiance increases xanthophyll cycle pool and activity, which allows maintenance of active PSII in excessive PAR and UVR irradiance with a minimal requirement of energy-costly PSII repair mechanisms. Thus, photoacclimation state also determines the degree of UVR inhibition of photosynthesis in *T. weissflogii* and *T. antarctica*, as was also suggested for natural phytoplankton assemblages from the Southern Ocean (Neale et al. 1998*b*).

In contrast to pigmentation and PSII efficiency, SOD activity was not affected by 30-min SSI exposure in low and high irradiance acclimated *T. weissflogii*. Yet UVAR exposure appeared to increase SOD activity in low irradiance acclimated *T. antarctica*, whereas this was not found after PAR + UVAR + UVBR and PAR. UVR effects on SOD activity have been reported previously in algae and plants, although they lack consistency (Lesser 1996*a*,*b*; Hazzard et al. 1997). Possibly, SOD activity changes on a longer timescale than that of our experiment (3 h). However, 3 weeks of photoacclimation did not result in significantly different SOD activity in both species, suggesting that SSI effects on PSII were not related with SOD activity differences. Furthermore, TBARS, an indicator for ROS-induced lipid peroxidation, were not increased in both species after 30 min SSI. This indicated that cellular scavenging or antioxidant capacity was sufficient to prevent substantial ROS mediated membrane damage.

Overall, our data suggest that excessive irradiance primarily affects PSII. In addition to pigment composition, turnover of reaction center components such as the D1 protein are modulated by irradiance (Kim et al. 1993). Features of low irradiance acclimation, like increased light harvesting pigments, slow D1 turnover, and small xanthophyll pool size, can cause accumulation of damaged, inactivated PSII complexes during excessive irradiance. PSII inactivation can be regarded as a short-term protection mechanism, because this interrupts noncyclic electron transport and thus reduces ROS formation (Chow et al. 2002). Furthermore, cells typically harbor excessive PSII capacity, so that inactivation can be compensated and has little short-term effects (Kaňa et al. 2002). However, continuous decline of active PSII complexes decreases noncyclic electron transport mediated production of reductants. In addition to the Calvin cycle, these reductants are used to regenerate oxidized equivalents of ascorbate and glutathione. We hypothesize that limited reductant supply eventually reduces cellular antioxidant capacity, which in turn leads to irreversible ROS-induced damage and viability loss. Therefore, PSII inactivation of low irradiance acclimated cells can develop within hours of surface irradiance exposure to viability loss. Furthermore, this model implies that interaction of excessive irradiance with stressors that increase PSII damage enhance photo-induced viability loss during prolonged excessive irradiance exposure. Indeed, UVR (PAR + UVAR + UVBR) exposure significantly increased viability loss of low irradiance acclimated cells over time, compared with PAR exposure alone. In addition, the suggested scheme provides an

explanation for the reported variability in UVBR effects, since many stressors affect PSII or pigment content and composition. Therefore, the occurrence of UVR effects is modulated by photoacclimation state, as demonstrated here, and by nutrient availability as was demonstrated in a previous study (van de Poll et al. 2005). Although this was not specifically tested, the PSII data of UVBR exposed cells indicated a small UVBR effect on viability loss in our experiments. Previously, this was not found for *Chaetoceros brevis* in similar experiments (van de Poll et al. 2005). Possibly, this effect remained undetected due to our 1-h sampling scheme. Although solar irradiance can mediate viability loss in low irradiance acclimated *C. brevis*, the extent of photo-induced viability loss in the field is unknown. Apart from photoacclimation state, this will be determined by the time that algae experience excessive irradiance and the time course of the photoacclimation response to irradiance changes.

Although *T. weissflogii* and *T. antarctica* were different with respect to cell size and cultivation temperature, responses to SSI were markedly similar for low and high irradiance acclimated cells. Therefore, differences in SSI sensitivity were primarily caused by photoacclimation, and not by species-specific differences. Nevertheless, species-specific photoacclimation characteristics were observed in *T. weissflogii* and *T. antarctica*. For the former, a consistently lower diadino-diatoxanthin pool relative to light harvesting pigments was found during low and high irradiance acclimation, compared with *T. antarctica*. For low irradiance acclimated *T. weissflogii*, the small diadino-diatoxanthin pool size corresponded with reduced de-epoxidation during surface irradiance when compared with low irradiance acclimated *T. antarctica*. Furthermore, low irradiance acclimated *T. weissflogii* displayed increased sensitivity to photo-induced viability loss during prolonged SSI exposure. This suggests that small species-specific differences in pigment composition affect photo-induced viability loss. Although speculative, our results suggest that the xanthophyll : light harvesting pigment ratio is a sensitive indicator of excessive irradiance sensitivity in marine microalgae. The presence of mycosporine-like amino acids may protect PSII against UVR-induced damage (Neale et al. 1998*a*; Litchman et al. 2002) and therefore also influence photo-induced viability loss. Absorption peaks in methanol extracts indicating the presence of UVR absorbing compounds were not observed in high and low irradiance acclimated *T. weissflogii* and *T. antarctica* before SSI exposure (results not shown), whereas this was not investigated during and after SSI. The ability to produce mycosporine-like amino acids is species-specific and strongly influenced by photoacclimation and spectral irradiance composition (Karentz et al. 1991; Helbling et al. 1996).

It is not known to what degree pigment composition was influenced by cultivation temperature in these experiments. *T. weissflogii* almost doubled in cellular pigment content in low irradiance, in contrast to *T. antarctica*. Possibly, a higher temperature facilitates larger fluctuations in cellular pigment content. The (de-)epoxidation of diadinoxanthin during SSI in *T. antarctica* was not affected by the low temperature, although turnover of damaged PSII may be lower. Furthermore, carbohydrate dynamics caused by utilization of storage sugars may be temperature-dependent. The large decrease in cellular carbohydrates during recovery of high irradiance acclimated *T. weissflogii* may explain the higher PSII recovery rate after SSI exposure compared with *T. antarctica*. However, *T. weissflogii* has a reduced capacity to grow under high irradiance, compared with *T. antarctica*, as was shown by high de-epoxidation of diadinoxanthin and the low PSII efficiency during cultivation in high irradiance. Therefore, the reduced ability of *T. weissflogii* to acclimate to high irradiance may provide an alternative explanation for the large difference in cellular pigment content between low and high irradiance acclimated cells. In addition, the results show that under the investigated conditions, low irradiance acclimated *T. antarctica* is better protected against excessive irradiance than *T. weissflogii*. We conclude that although minor species-specific differences were observed, excessive irradiance sensitivity is primarily determined by intraspecific photoacclimation responses.

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