Antioxidants, a radical solution?
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Chapter 7

Antioxidative responses of two marine microalgae during static and dynamic natural ultraviolet radiation exposure

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Abstract

High light acclimated cultures of the marine microalgae *Thalassiosira weissflogii* and *Dunaliella tertiolecta* were exposed to static and dynamic natural ultraviolet radiation (UVR), the latter by applying artificial shallow mixing. Antioxidant (superoxide dismutase; SOD, ascorbate peroxidase; APX, glutathione cycling) responses were followed for one week during static and dynamic natural UVR and compared with PAR (Photosynthetically Active Radiation) only controls. In addition, changes in light harvesting and photoprotective pigment pools were followed. The overall impact of UVR was assessed by monitoring growth as well as UVR sensitivity, measured as UVR-induced inhibition of carbon assimilation under an indoor solar simulator. We hypothesized that enhanced UVR resistance during prolonged UVR exposure could at least partly be explained by increased antioxidant activity.

Both species showed immediate antioxidant responses due to their transfer to the outdoor conditions. Furthermore, carbon assimilation and growth rates were reduced in both species in all outdoor treatments compared to initial conditions. *D. tertiolecta* was affected most by the outdoor exposures, with respect to antioxidant and pigment pools as well as growth rate and UVR sensitivity. Yet, in both species, static and dynamic UVR exposure did not alter antioxidant levels as compared with PAR only controls. In contrast, growth was most affected in the static UVR cultures. At the same time, the static UVR cultures showed significantly enhanced UVR resistance. We therefore conclude that antioxidants play a minor role in the reinforcement of natural UVR resistance in *T. weissflogii* and *D. tertiolecta*. 

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Introduction

Marine microalgae are subject to fluctuating irradiance conditions due to co-occurring changes in intensity of incoming irradiance (daily and seasonally), cloud coverage, and their changing position in the water column (Helbling et al. 1994, Lubin & Jensen 1995, Neale et al. 1998b, 2003). In a shallow upper mixed layer (UML; Neale et al. 2003) vertical mixing can mediate phytoplankton transport through a gradient of photosynthetic active radiation (PAR; 400-700 nm) ranging from saturating to excessive irradiance close to the water surface (Neale et al. 1998b, 2003). In contrast, ultraviolet radiation (UVR; 280-400 nm) is attenuated much faster and can be insignificantly low at the bottom of the UML by which shallow mixing has a stronger influence on UVR than on PAR exposure. As excess PAR and UVR can reduce carbon incorporation in different ways, irradiance-induced effects are subject to spectral quality and exposure duration. Also, UVR may inhibit repair pathways and therefore algae can benefit from periods with low UVR levels to recover from photodamage (Neale et al. 1998b). Therefore, the effect of UVR on photosynthesis in a shallow UML depends on the optical properties of water, and mixing depth and speed (Neale et al. 1998b, Helbling et al. 2003).

The detrimental action of excess irradiance including UVR is, among other things, mediated through enhanced reactive oxygen species (ROS; Mehler 1951, Asada et al. 1974) production. During excess irradiance exposure, light harvesting capacity can exceed photosynthetic capacity leading to overreduction of the photosystems and thereby to ROS generation (Gechev et al. 2006). ROS may damage DNA, phospholipids and proteins such as photosystem II reaction centers, and cause a decrease in photosynthetic efficiency or even viability loss (Van de Poll et al. 2005, Gechev & Hille 2005). Additionally, UVR is able to directly damage biological molecules (i.e. DNA, proteins), causing obstruction of metabolic pathways, therewith augmenting overreduction of the photosystems and production of ROS (Hideg & Vass 1996, Mackerness 2000, Bischof et al. 2003).

To prevent irradiance-induced formation of ROS, microalgae apply immediate regulatory mechanisms such as non photochemical quenching via xanthophyll cycling, and antioxidant action (Mewes & Richter 2002, Van de Poll et al. 2006, Janknegt et al. 2008). On a longer time scale photoacclimation occurs during which photosynthesis and its regulatory mechanisms are adjusted to the prevailing irradiance (Van de Poll et al. 2005, Falkowski & LaRoche 1991). When these systems are inadequate, overreduced photosystems initiate generation of the moderately dangerous superoxide (O$_2^-$) radicals. A second reduction step generates the mildly reactive hydrogen peroxide (H$_2$O$_2$) which, in the presence of O$_2^-$, can form the most destructive hydroxyl radical (HO$^*$; Kehrer 2000). To counteract ROS accumulation, oxygen radicals are scavenged by a network of antioxidants which is aimed to prevent HO$^*$ formation. In this network, O$_2^-$ is converted into H$_2$O$_2$ by superoxide dismutase (SOD; Gregory & Fridovich 1973a, b) and is subsequently scavenged by peroxidases like ascorbate peroxidase (APX; Shigeoka 2002, Asada 2006). Hereby, APX consumes ascorbic acid which is regenerated using glutathione as a reducing agent. Oxidized glutathione is reduced again by glutathione reductase (GR; De Kok & Stulen 1993, Noctor et al. 2002).

In contrast to constant irradiance conditions, the unpredictable nature of fluctuating light regimes makes it difficult for microalgae to adequately photoacclimate. Van de Poll et al. (2007) found a low irradiance type acclimation response in a diatom under a fluctuating
light regime with respect to pigment composition as compared with static conditions having an identical daily irradiance dose. As a result, cells could constantly be subjected to changing levels of (over) reduced photosystems and therewith to variable levels of ROS production.

Several laboratory studies have shown that microalgae adjust their antioxidant levels during photoacclimation to excess irradiance including UVR (Rijstenbil 2002, Bhargava et al. 2007, Janknegt et al. 2008). Yet, virtual nothing is known about antioxidant responses in marine microalgae under ambient UVR, let alone under more natural fluctuating irradiance regimes. In the present study we investigated antioxidant acclimation strategies of the diatom *Thalassiosira weissflogii* and the green phytoflagellate *Dunaliella tertiolecta* under static and dynamic natural UVR, the latter by applying artificial mixing. We chose a diatom and a phytoflagellate because representatives of these taxa have been demonstrated to fundamentally differ in dynamic irradiance responses as well as in excess irradiance vulnerability including UVR (Davidson & Marchant 1994, Van Leeuwe et al. 2005, Wagner et al. 2006). We assessed immediate (1 d), short term (3 d) and long term (7 d) responses during exposure to outdoor static UVR and simulated mixing regimes (60 min. and 10 min. cycle), using PAR only cultures as controls. Acclimation responses to static and dynamic conditions +/- UVR were followed for enzymatic and non-enzymatic antioxidants (SOD, APX, GR, glutathione levels). Also photoprotective and light harvesting pigment pools were measured. The overall impact of UVR was assessed by monitoring growth as well as UVR sensitivity, the latter measured as UVR-induced inhibition of carbon assimilation under an indoor solar simulator. We hypothesized that: I: antioxidant levels would be enhanced as a result of prolonged natural UVR exposures, compared with PAR only cultures; II: antioxidant levels would be lower in dynamic UVR as opposed to static UVR cultures; III: enhanced antioxidant activity could contribute to enhanced UVR resistance during prolonged natural UVR exposure.

**Materials and Methods**

**Culturing conditions**

*Dunaliella tertiolecta* (Dunal) Teodoresco (Chlorophyceae) and *Thalassiosira weissflogii* (Grunow) G. Fryxell et Hasle (Bacillariophyceae) were obtained from the Algal Culture Collection of Estación de Fotobiología Playa Unión. *T. weissflogii* and *D. tertiolecta* were grown at 250 μmol photons m⁻²·s⁻¹ photosynthetically active radiation (PAR; 12/12 h l/d cycle) without UVR in f/2 enriched autoclaved sea water at a salinity of 34.5 PSU in a culture cabinet (Sanyo MLR 350) at 20°C for several weeks prior to the experiments. Cultures were kept in the exponential growth phase by regular dilution with fresh medium.

**Experimental design.**

Three types of experiments were performed: Experiment 1 (60 minute mixing cycle: from 3rd till 10th of February, 2006) and Experiment 2 (10 minute mixing cycle: from 10th till 17th of February, 2006) lasted seven days; Experimental series 3 were one-day experiments (*D. tertiolecta* experiments on January 31st and February 16th, 2006; *T. weissflogii* experiments on February 15th and 17th). For Experiment 3, only 10 minute mixing cycles were applied.
During all experiments, cultures of *D. tertiolecta* and *T. weissflogii* were subjected to mixed PAR (mixed PAR), mixed PAR+UVR (mixed PUV), static PAR (static PAR) and static PAR+UVR (static PUV) solar radiation regimes. At the start of experiment 1 and 2 cultures of *D. tertiolecta* and *T. weissflogii* were transferred to UV transparent 2.2 L polymethylmethacrylate (PMMA) bottles, diluted with 500 mL fresh medium and simultaneously exposed to the four irradiance conditions (hence giving a total of 8 experimental bottles: two species, four irradiance conditions each). During experiment 1 and 2, sampling volume (see below) was replenished with fresh medium once a day. As a result, cultures were growing under semi continuous batch conditions. The one day exposures of experiment 3 (one species per experimental day) were executed in UV transparent 2.2 L PMMA bottles, with cultures diluted with 500 mL fresh medium.

### Irradiance conditions

The experiments were performed in an outside water filled basin (depth: 1.1 m, diameter: 5 m) to which sediment of the nearby Chubut river was added to enhance light attenuation. Sediment was resuspended twice a day during experimentation. Irradiance attenuation was checked three times a day, at three water depths: at 0.22, 0.44 and 0.8 m, and turbidity was adjusted when necessary. Bottles for the PAR only exposures were covered with 390 nm cut-off foil (Ultraphan, UV Opak Digefra, Germany, for transmission characteristics see Figueroa *et al*. 1997), while uncovered PMMA bottles transmitted the full solar spectrum (PUV). For each species, two bottles (PAR, PUV) were incubated 15 cm below the surface (Static) and two bottles (PAR, PUV) were moved through the water column between 15 and 80 cm depth by a home-made mixing simulator (dynamic). Note that static and dynamic irradiance conditions did not give identical daily PAR and UVR doses (see results, Table 1 and Discussion section). Sinusoidal vertical transport of the bottles was established by a DC motor (Maxon Motor, Switzerland). The speed of the motor and thus that of the bottles in the water was frequency controlled and adjusted to 60 min (Experiment 1) or 10 min (Experiment 2) mixing cycles.

### Sampling

Pre-experimental samples (Initial) were taken for GR (120 mL), glutathione (160 mL), APX (120 mL), SOD (120 mL), pigments (100 mL), UV-sensitivity (100 mL) and cell counts (2 mL) (see below). During Experiments 1 and 2 samples were taken daily at 7:30 am for GR, glutathione, pigments, UV-sensitivity, and cell counts. This sampling time was chosen, because in this way we could follow longer term acclimation patterns without the interference of short term regulatory responses, which might have occurred during the daily exposure cycle. On the 3rd and the 7th day, additional samples were taken for APX and SOD activity. During experiment 3 samples for APX, SOD and GR activity, and glutathione were obtained at 9:00 am. However, here we also sampled at 1:00 pm and 5:00 pm, to collect additional data during the first day of the outdoor exposure. Samples for SOD, APX, and GR activity and glutathione content were filtered over polycarbonate filters (Ø 47 mm, 2.0 µm pore size; Osmonics Inc., USA), frozen in liquid nitrogen and stored at -80°C. Samples for pigment analysis were immediately filtered over GF/F filters (Ø 25 mm) in a darkened room, immediately frozen in liquid nitrogen and stored at -80°C. Samples for cell
counts were fixed with formalin (0.1%, V/V) and stored at 4°C in darkness. Samples for the assessment of UV-sensitivity were processed immediately (see below).

**Measurement of UV sensitivity**

To determine UV-sensitivity, we performed carbon assimilation experiments using an indoor solar simulator. Samples were collected from all outdoor cultures, transferred to quartz tubes (50 mL, in duplicate), spiked with $^{14}$C sodium bicarbonate (3.75 μCi) and incubated under the solar simulator (Hönle, Sol 1200, Germany) for 1.5h in a water bath inside a temperature controlled chamber (20 °C). Half of the tubes was covered with 390 nm cut-off foil (receiving simulated PAR only) while the others remained uncovered (receiving simulated PUV). The lamp was covered with cellulose acetate film to avoid UV-C exposure. The applied radiation was 183, 69.9 and 1.7 W·m$^{-2}$ for PAR, UVAR (315 – 400 nm) and UVBR (280 – 315 nm), respectively. After exposure, samples were filtered on 25 mm GF/F filters (Whatman), stored in scintillation vials, and exposed to concentrated HCl fumes overnight to remove unbound $^{14}$C sodium bicarbonate. Radioactivity was quantified using liquid scintillation counting. $^{14}$C incorporation, corrected for dark controls, and normalized to chlorophyll $a$ concentration measured the beginning of the solar simulator experiment to provide carbon assimilation numbers. Chlorophyll $a$ concentration was determined by filtering 50 mL culture on 25 mm GF/F filters followed by extraction in (100%) methanol (Holm-Hansen & Riemann 1978). The samples were sonicated (20 min) and extracted for at least 2 hours in darkness at 4°C. After centrifugation (10 min, 3000 rpm), the Chl$a$ concentration of the supernatant was determined by measuring the fluorescence of the extract before and after acidification, using a calibrated Turner Designs TD700 fluorometer. UV sensitivity was determined by calculating the relative difference in carbon assimilation between PAR-only and PUV exposure, expressed as percentage UVR-inhibition.

**Cell counts and growth rates**

Cell counts were performed on a Coulter MCL flow cytometer (Beckmann-Coulter) as described in Van de Poll et al. (2007). For estimation of increase in cell density, cell counts were corrected for the dilution factor. Growth rates were determined by measuring the slope of the natural log (Ln) of cell numbers as a function of time. Growth rates of the initial cultures were determined by measuring the cell concentrations over four successive days, prior to experimentation.

**SOD activity**

SOD analyses were done using the riboflavin/Nitro Blue Tetrazolium (RF/NBT) method modified for algal material, as described in Janknegt et al. (2007). Filters were disrupted by sonication in 0.75 mL protein extraction buffer X (50 mM KH$_2$PO$_4$, pH7.8; 0.1 mM EDTA; 0.1% Triton X-100; 2% PVP and complete protease inhibitor cocktail). After spinning down cell debris, supernatants were transferred to a clean 1.5 mL Eppendorf tube. SOD activity was defined as the amount of sample required to achieve 50% inhibition of the NBT reduction ($V_{50}$). NBT reduction was measured (in duplicate) in 4 reaction mixtures containing 0 (blank, no inhibition), 12.5, 25, and 50 μL extract. Absorption was measured at 560 nm on a Cary 3E UV/vis double beam spectrophotometer (Varian, Middelburg, The
Responses during static and dynamic UVR exposure

Netherlands) equipped with a temperature controlled cell attached to a water bath. \( V_{50} \) was calculated by regression using the linear part of a natural semi-log curve. The specific activity (S.A.) of SOD was calculated according to \( 1 / (V_{50} \times \text{protein}) \).

**APX and GR activity**

Supernatants for both APX and GR activity assays were prepared and protein concentrations were determined as described for the SOD activity assay (see above). APX activity was determined spectrophotometrically by measuring the consumption of its substrate, ascorbic acid, over a 3 min. interval, in a 3 mL quartz cuvette containing 2.4 mM buffer A (50 mM KH\(_2\)PO\(_4\), pH7; 0.1 mM EDTA), 150 µL, 10 mM ascorbic acid (Merck, Darmstadt Germany), 400 µL cell extract and 30 µL, 20 mM H\(_2\)O\(_2\) (Merck, Darmstadt Germany). Before H\(_2\)O\(_2\) was added, the reaction mixture was incubated for 5 min. at 20°C. The rate of ascorbate consumption was measured at 290 nm on a Cary 3E UV/VIS double beam spectrophotometer. Specific APX activity (U/mg prot) was calculated according to: \( (-\text{slope} \times \text{volume}_{\text{tot}} / \varepsilon \times \text{volume}_{\text{extr}}) \) / [protein] of which \( \varepsilon = 2.8 \text{ mM}^{-1} \cdot \text{cm}^{-1} \). Determination of GR activity was based on its catalytic property to convert oxidized glutathione (GSSG) into its reduced form (GSH) thereby consuming NADPH as a reducing agent. The NADPH consumption was measured spectrophotometrically by determining its decrease over a 3 min. interval in a 3 mL quartz cuvette containing 1 mL buffer A; 150 µL, 10 mM GSSG (Merck, Darmstadt Germany), 300 µL cell extract and 50 µL, 2.5 mM NADPH (Merck, Darmstadt Germany). Before adding NADPH, the reaction mixture was incubated for 5 min in a cryostat at the appropriate temperature. Specific activity of GR (in U/mg prot) was calculated by: \( (-\text{slope} \times \text{volume}_{\text{tot}} / \varepsilon \times \text{volume}_{\text{extr}}) / [\text{protein}] \) of which \( \varepsilon = 6.22 \text{ mM}^{-1} \cdot \text{cm}^{-1} \).

**Glutathione content**

Filters were suspended in 0.4 mL glutathione extraction buffer containing 50 mM sulfosalicylic acid, 1 mM EDTA, and 0.15% ascorbic acid hereafter cells were disrupted by sonication (30 s, at A=30) at 4°C. Extracts were centrifuged for 25 min (20,000 x g) at 4°C and supernatants were transferred to a clean 1.5 mL Eppendorf tube. The total amount of glutathione (TAG) was determined using the SIGMA Glutathione Assay Kit (CS0260; Sigma, Saint Louis, MI, USA); all chemicals were purchased from Sigma unless indicated otherwise. The assay was performed in 96 wells microtiter plates (Cayman chemical) containing 150 µL working mixture 1 (100 mM KH\(_2\)PO\(_4\), pH7; 100 mM EDTA, GR, 0.170 U/mL; 0.043 mg/mL 5,5'-dithiobis-2-nitrobenzoic acid), and 10 µL cell extract. After 5 minutes of incubation at room temperature, 50 µL of a 0.16 mg/mL NADPH solution was added. 5-thio-2-nitrobenzoic acid (TNB) production was then followed spectrophotometrically for 10 min at 405 nm using a Victor\(^{m}\) 1420 multilabel counter plate reader (PerkinElmer, Waltham, MA, USA) reading at 1 minute intervals. To determine the glutathione content in 10 µL algal extract, a reference line was included based on 0.5, 0.25, 0.125, 0.0625 and 0.0312 nmoles reduced glutathione (GSH, Sigma, Saint Louis, MI, USA) in 10 µL sample. The TNB production rate was calculated for 1 n mole of glutathione by extrapolation. The TAG (in nmol) in 1 mL algal sample was calculated according to: \( \text{slope}_{\text{sample}} \times \text{dilution factor original sample} / \text{slope}_{\text{nmol GSH}} \times 0.01 \text{ mL sample} \). The amount of GSH was measured by the same principle as TAG but without adding GR and NADPH by which only GSH reacts with DTNB. This assay was performed in 96 wells microtiter
plates containing 100 µL working mixture 2 (500 mM KH₂PO₄, pH7; 500 mM EDTA; 0.065 mg/mL DTNB), and 100 µL cell extract. After 10 min of incubation at RT the total production of TNB was determined spectrophotometrically at 405 nm. A reference line was included based on 2, 1, 0.5, 0.25, and 0.125 nmoles GSH in 100 µL sample. The amount of GSH in the reaction mixture was calculated in accordance to the reference line. The amount of GSH (in nmol) in 1 mL algal extract was calculated according to: GSH in reaction mixture x dilution factor original sample / 0.1 mL sample. The glutathione redox status (GRS) was expressed in sulphur atoms and calculated according to: GSH / (GSH + 0.5 GSSG) of which GSSG = TAG – GSH.

**Pigment**

Pigments were analyzed as described by Van Leeuwe et al. (2006). In short, filters were handled under dim light, freeze-dried (48 h) after which pigments were extracted in 4 mL 90% cold acetone (v/v, 48 h, 5°C). Pigments were resolved using HPLC (Waters 2690 separation module, 996 photodiode array detector) with a C₁₈ 5 μm DeltaPak reversed-phase column (Milford, MA, USA) and identified by retention time and diode array spectroscopy. Quantification was done using standard dilutions of Chlorophyll a, Chlorophyll b, fucoxanthin, diadinoxanthin, diatoxanthin, violaxanthin, antheraxanthin and zeaxanthin. Cellular pigment concentrations were calculated from cell counts and extraction volume.

**Irradiance**

Irradiance (PAR, UVR) was continuously recorded as the average of one min intervals using a broadband ELDONET radiometer (Real Time Computers Inc.) located on the roof. Attenuation of UVR and PAR in the reservoir was determined with a USB diode array spectrophotometer (HR 2000CG-UV-NIR, Ocean Optics, Dunedin, USA) with a 4 m fiber optics and cosine diffuser. Attenuation coefficients were used to estimate the irradiance for the cultures during mixing and static solar radiation regimes. Irradiance measurements were done three times a day during experimental days at three water depths: 0.22, 0.44 and 0.8 m. By considering mixing depth and mixing speed, we estimated the irradiance that the mixed cultures experienced.

**Data analysis and statistics.**

Differences in irradiance conditions were tested using a paired t-test. Differences between *D. tertiolecta* and *T. weissflogii* were tested by performing a paired t-test. Since no significant differences were found between Experiments 1 and 2, as well as some experimental days, we distinguished immediate (data collected during one day, experiment 3), short term (using the average of data from day 1-3, with experiments 1 and 2 pooled) and long term (using the average of data from day 4-7, with experiments 1 and 2 pooled) responses. Intraspecific differences between response periods (immediate, short and long term) and irradiance treatments (static PUV, static PAR, mixed PUV, mixed PAR) for SOD activity, APX activity, GR activity, GRS, total glutathione content, pigment composition and growth, were tested by performing a multivariate ANOVA. When significant differences were found, an LSD post-hoc test was applied to specify these differences.
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Results

Irradiance conditions
During the entire experimental period, the average daily outdoor radiation dose fluctuated between 4900 and 8200 kJ·m\(^{-2}\) PAR, 460 and 960 kJ·m\(^{-2}\) UVAR and 10 and 22 kJ·m\(^{-2}\) UVBR (Table 1). Attenuation coefficients (K\(_d\)) in the experimental basin were 0.91 (PAR), 2.74 (UVAR) and 3.95 (UVBR) so that the irradiance intensities at 0.15 m were reduced to 87, 66% and 55% respectively. At 0.8 m, irradiance intensities were reduced to 48, 11 and 4%, respectively, compared to surface irradiance. Regardless of the mixing speed, cultures subjected to the simulated mixing regime received on average 79% (PAR), 50% (UVAR) and 36% (UVBR) of the daily radiation dose compared with the static cultures. When comparing experiments 1 and 2, the average daily radiation doses between the two short term and the two long term periods did not differ significantly (data not shown). Therefore, the corresponding cultures were exposed to comparable radiation conditions during both experiments. This similarity was reflected in the response parameters of both experiments (UV sensitivity; SOD, GR and APX activity; cellular glutathione content and the glutathione redox potential) which showed highly corresponding trends in time. Therefore, average values of the two experiments were pooled.

Table 1: Calculated daily average outdoor doses (KJ·m\(^{-2}\)) experienced by the *Dunaliella tertiolecta* and *Thalassiosira weissflogii* PUV cultures for photosynthetically active radiation (PAR), ultraviolet A radiation (UVAR: 315 – 400 nm) and ultraviolet B radiation (UVBR: 280 – 315 nm). Immediate: day 1 between sunrise and 5:00pm. Short term: averaged over first 3 days of the outdoor exposures. Long term: averaged over days 4 – 7 of the outdoor exposures.

<table>
<thead>
<tr>
<th>Response time</th>
<th>Condition</th>
<th>PAR</th>
<th>UVAR</th>
<th>UVBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-culturing</td>
<td>Indoors</td>
<td>2200</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td><em>D. tertiolecta</em> Static</td>
<td>8163</td>
<td>958</td>
<td>22</td>
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<tr>
<td></td>
<td><em>D. tertiolecta</em> Mixed</td>
<td>4897</td>
<td>460</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td><em>T. weissflogii</em> Static</td>
<td>6264</td>
<td>673</td>
<td>15</td>
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<tr>
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<td>5632</td>
<td>514</td>
<td>11</td>
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<tr>
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<td><em>D. tertiolecta</em> Static</td>
<td>7226</td>
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<td>18</td>
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<tr>
<td></td>
<td><em>T. weissflogii</em> Static</td>
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<td>555</td>
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<tr>
<td>Short term</td>
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<td></td>
<td>Mixed</td>
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<td>Long term</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td></td>
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</tr>
</tbody>
</table>

Growth
Before their transfer to the experimental basin, the growth rate of *D. tertiolecta* was higher than that of *T. weissflogii* (Table 2). During the first three days (short term) of exposure to outdoor irradiance mean growth rates of *D. tertiolecta* cultures remained at a similar level compared to initial values after which they all decreased significantly (p<0.05) by 37-66% (Table 2, 4). Except for the mixed PAR cultures growth rates of *T. weissflogii* were already reduced significantly (p<0.05) during the first three days (short term) by 14-26% and did not change significantly thereafter (Table 2, 4). Growth rates of both species were
significantly (p<0.05) affected by UVR (Table 2, 4). Eventually, *D. tertiolecta* was significantly (p<0.05) more affected by long term (7 d) outdoor irradiance exposure than *T. weissflogii*.

Table 2: Growth rates of *Dunaliella tertiolecta* and *Thalassiosira weissflogii*. Initial: acclimated to 250 µmol photons m⁻² s⁻¹ PAR, Short term: exposed for 3 days (1-3 d) to outdoor irradiance conditions. Long term: days 4-7 of outdoor irradiance. In brackets standard error of the linear regression of log transformed cell numbers.

<table>
<thead>
<tr>
<th></th>
<th><em>D. tertiolecta</em></th>
<th><em>T. weissflogii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Short term</td>
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<tr>
<td></td>
<td>0.53 (0.040)</td>
<td>0.57 (0.088)</td>
</tr>
<tr>
<td>Mixed PAR</td>
<td>0.57 (0.075)</td>
<td>0.22 (0.052)</td>
</tr>
<tr>
<td>Static PAR</td>
<td>0.57 (0.062)</td>
<td>0.33 (0.229)</td>
</tr>
<tr>
<td>Static PUV</td>
<td>0.57 (0.085)</td>
<td>0.18 (0.094)</td>
</tr>
</tbody>
</table>

**UVR sensitivity**

During the entire experimental period, *D. tertiolecta* showed significantly (p<0.05) higher assimilation numbers than *T. weissflogii* when exposed to PAR under the solar simulator (Figs. 1a, b). However, *D. tertiolecta* showed a significantly (p<0.05) larger reduction in carbon assimilation on the short and long term than the corresponding cultures of *T. weissflogii*. (Figs. 1a, b; Table 4). After long term (7 d) outdoor exposure, assimilation numbers of all cultures of both species had decreased significantly (p<0.005) compared to pre-experimental values (Table 4). Although both species showed lowest assimilation numbers for the static PUV cultures, there were no significant differences between the 4 outdoor irradiance conditions for both species. *D. tertiolecta* was significantly (p<0.05) more UV sensitive (expressed as percentage UV-inhibition) than *T. weissflogii* (Figs. 1c, d; Table 4). In addition, UVR sensitivity of the static PUV cultures of both *T. weissflogii* and *D. tertiolecta* decreased significantly (p<0.01) over time, becoming significantly (p<0.0001) lower than the other (PUV mixed, PAR static and mixed) cultures. UV sensitivity decreased for both species following mixed PAR > static PAR > mixed PUV > static PUV.

**Superoxide dismutase and ascorbic peroxidase**

Species specific differences were observed in antioxidant enzyme activity and responses. When acclimated to pre-experimental irradiance (250 µmol·m⁻²·s⁻¹), SOD activity of *D. tertiolecta* (103 U/mg protein, sd=3.0) was significantly (p<0.05) higher than *T. weissflogii* (68 U/mg protein, sd=10.6; Table 3). APX activity of *D. tertiolecta* (0.89 U/mg protein, sd=0.2) was significantly (p<0.05) lower than that of *T. weissflogii* (3.6 U/mg protein, sd=0.6; Table 3). During the first day of the outdoor exposure, no significant differences were found between samples taken during the daily cycle (9.00 am, 1.00 pm and 17.00 pm.) and therefore these data were pooled. Also, there were no significant differences in SOD and APX activities between the four radiation conditions during short and long term exposure. Therefore, these data were pooled as well. *T. weissflogii* showed no significant responses in SOD and APX activity regardless of radiation condition or exposure duration.
Responses during static and dynamic UVR exposure (Table 3, 4). In contrast, *D. tertiolecta* showed a slight but significantly (*p*<0.05) enhanced SOD activity after three days which remained on this level until day 7 (Table 3, 4).

![Assimilation number (from solar simulator PAR condition only) and UVR-induced inhibition of 14C incorporation of *Thalassiosira weissflogii* a), c) and *Dunaliella tertiolecta* b), d). Short term: cultures exposed up to 3 days. Long term: cultures exposed to outdoor irradiance from day 4 to day 7. Bars show mean values of three (Short term) or four (Initial and Long term) replicates, error bars represent standard deviations. * differs significantly from the Initial value (*p*<0.05) a: differs significantly from the previous exposure period (*p*<0.05). b: differs significantly from the other irradiance conditions within the same exposure period (*p*<0.05).

**Table 3:** Superoxide dismutase and ascorbate peroxidase activity (U/mg protein) of *Dunaliella tertiolecta* and *Thalassiosira weissflogii*. Initial: acclimated to 250 µmol photons m⁻² s⁻¹ PAR. Immediate: cultures exposed for one day (from sunrise till 5:00 pm) to outdoor irradiance conditions. Short term: exposed for 3 days (1-3 d) to outdoor irradiance conditions. Long term: days 4-7 of outdoor irradiance. Immediate APX responses of *D. tertiolecta* (See ⇒) showed significant differences between the four treatments and are indicated in the last two columns (treatment and Immed. APX). Hereby, S. PAR = static PAR culture, S. PUV = static PUV culture, M. PAR = mixed PAR culture and M. PUV = mixed PUV culture. In brackets standard deviation; Initial, Short term and Long term, *n*=4; Immediate APX (except for *D. tertiolecta*) *n*=12; Immediate SOD, *n*=6; Immediate APX activity responses of each of the four *D. tertiolecta* treatments, *n*=3. n/a = not available. * = differs significantly from initial value. * = differs significantly from other treatments.

<table>
<thead>
<tr>
<th></th>
<th><em>T. weissflogii</em></th>
<th><em>D. tertiolecta</em></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SOD</td>
<td>APX</td>
</tr>
<tr>
<td>Initial</td>
<td>67.9 (10.6)</td>
<td>3.6 (0.60)</td>
</tr>
<tr>
<td>Immediate</td>
<td>n/a</td>
<td>4.4 (1.20)</td>
</tr>
<tr>
<td>Short term</td>
<td>66.9 (0.8)</td>
<td>3.9 (0.29)</td>
</tr>
<tr>
<td>Long term</td>
<td>64.1 (9.9)</td>
<td>4.4 (0.38)</td>
</tr>
</tbody>
</table>
Regarding APX activity, only static cultures of *D. tertiolecta* showed an immediate (1 d) significant (p<0.05) decrease by which they were significantly (p<0.05) lower than the cultures exposed to a mixed irradiance regime. After 3 days (3 d) their APX activities had returned to initial values and remained on this level until day 7 (Table 3, 4).

**Glutathione and glutathione reductase**

After acclimation to 250 µmol·m⁻²·s⁻¹ GR activity of *D. tertiolecta* (0.19 U/mg protein; sd=0.014) was significantly (p<0.05) higher than that of *T. weissflogii* (0.052 U/mg protein, sd=0.006; Figs. 2a, b). Both species contained comparable amounts of cellular glutathione (Figs. 2c, d; 18.1 nmol/10⁶ cells, sd=2.1 and 15.9 nmol/10⁶ cells, sd=1.6 respectively) and a similar glutathione redox status (GRS = GSH/GSH + 0.5 GSSG; Figs. 2e, f; 0.21, sd=0.024 and 0.20, sd=0.014 respectively). During the first day of the outdoor exposure, no significant differences were found during the daily cycle, and therefore these data were pooled. When exposed to outdoor radiation GR activity of *T. weissflogii* showed no response regardless of irradiance condition or duration of exposure (Fig. 2a; Table 4). Static *D. tertiolecta* cultures immediately (1 d) increased their GR activity reaching significantly (p<0.05) higher values than those of the mixed cultures. (Fig. 2b). Subsequently, both mixed cultures increased their GR activity significantly (p<0.005) to similar enzyme activities as the static cultures (3 d) response. During the following four days (7 d) all four cultures decreased their GR activity significantly (p<0.05) to initial values (Fig. 2b; Table 4).

Regardless of the outdoor irradiance conditions, all *T. weissflogii* cultures immediately (1 d) responded significantly (p<0.0005) by doubling their glutathione content (Fig. 2c). This was accompanied by a significant (p<0.01) decrease in their GRS (Fig. 2e). Throughout the following three days (short term), both parameters returned to their initial values and remained on this level for the rest of the experiments (7 d; Table 4). *D. tertiolecta* responded by immediately increasing the cellular amount of glutathione of both static cultures significantly (p<0.05) by which they were significantly (p<0.05) higher than both mixed cultures (Fig. 2d). Throughout time, the cellular glutathione content of all cultures dropped significantly (p<0.01) to comparable levels which were significantly (p<0.005) lower than the initial values (Table 4). During the first day outside, the GRS of all *D. tertiolecta* cultures decreased significantly (p<0.005) by which the static cultures had dropped to a significantly (p<0.05) lower level than the mixed cultures (Fig. 2f). During the short term response, the GRS of both static cultures increased again to similar values as the mixed cultures after which they remained on this same level, significantly lower than initial value (Fig. 2f; Table 4).

**Pigments**

*T. weissflogii* contained Chlorophyll a (Chl.a) and fucoxanthin (Fuco) as major photoharvesting pigments and the xanthophyll cycle pigments diatoxanthin and diadinoxanthin (DT/DD cycle). *D. tertiolecta* contained Chl.a and Chlorophyll b (Chl.b) as major light harvesting pigments and the xanthophyll cycle pigments violaxanthin, antheraxanthin and zeaxanthin (VAZ cycle). Before their transfer outdoors, *T. weissflogii* had a significantly (p<0.05) lower content of light harvesting pigments than *D. tertiolecta* (Figs. 3c, d; 1.91 pg/cell, sd= 0.16 and 2.57 pg/cell, sd=0.11, respectively) and significantly
Figure 2: Antioxidant responses of Dunaliella tertiolecta and Thalassiosira weissflogii. a), b) Glutathione reductase (GR) activity. c), d) Cellular amount of glutathione and e), f) glutathione redox status (GRS) Immediate: cultures exposed for one day (from sunrise till 5:00 pm) to outdoor irradiance conditions. Short term: cultures exposed for 3 days to outdoor irradiance conditions. Long term: cultures exposed to outdoor irradiance during the 4 subsequent days. Bars show mean values of three (Immediate and Short term) or four (Initial and Long term) replicates, error bars represent standard deviations. * differs significantly from the Initial value (p<0.05) a: differs significantly from the previous exposure period (p<0.05). b: differs significantly from the other irradiance conditions within the same exposure period (p<0.05).
(p<0.05) more protective pigments (Figs. 3e, f; 0.44 pg/cell, sd= 0.08 and 0.27 pg/cell, sd=0.009 respectively). As a result, the ratio of protective over harvesting pigments (prot/harv ratio; Figs. 3a, b) of *T. weissflogii* (0.26, sd=0.01) was significantly (p<0.0005) higher than that of *D. tertiolecta* (0.10, sd=0.001). At the end of the long term period (7 d),

![Diagram](image_url)

*Figure 3: Pigment dynamics of *Thalassiosira weissflogii* and *Dunaliella tertiolecta*. a), b): Photoprotective over harvesting ratio. c), d): cell quota of harvesting and e), f): photoprotective pigments. Short term: cultures exposed for 3 days to outdoor irradiance conditions. Long term: cultures exposed to outdoor irradiance during the 4 subsequent days. Bars show mean values of three (Short term) or four (Initial and Long term) replicates, error bars represent standard deviations. * differs significantly from the Initial value (p<0.05) a: differs significantly from the previous exposure period (p<0.05). b: differs significantly from the other irradiance conditions within the same exposure period (p<0.05).*
PAR-only exposed *T. weissflogii* had significantly (p<0.05) lower amounts of light harvesting and protective pigments as compared with PUV exposed cells (Figs. 3c, e; Table 4). However, the prot/harv ratios did not differ significantly between PAR and PUV cultures, and between short and long term exposure periods (Fig. 3a). During the first three days (short term) of outdoor exposure, all cultures of *D. tertiolecta*, significantly (p<0.05) decreased their cellular amount of harvesting and protective pigments with PUV exposed cultures containing significantly (p<0.05) less pigments than PAR-only exposed cultures (Figs. 3d, f). As a result of the long term (7 d.) exposure, PAR-only exposed cultures showed significant (p<0.05) decreases in their cellular pigment contents, reaching similar values as cultures subjected to PUV. As a result, prot/harv ratio of *D. tertiolecta* increased significantly (p<0.01; Fig. 3b; Table 4). Yet, no significant differences between the four irradiance conditions were found.

**Table 4:** Long term (4-7 d) effects on cellular parameters for both species, expressed as percent change relative to initial conditions. * differs significantly from initial value.

<table>
<thead>
<tr>
<th></th>
<th><em>T. weissflogii</em></th>
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<th><em>D. tertiolecta</em></th>
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<tr>
<td></td>
<td>Mixed PAR</td>
<td>Mixed PUV</td>
<td>Static PAR</td>
<td>Static PUV</td>
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<td><strong>Ass no.</strong></td>
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<td>-39*</td>
<td>-54*</td>
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<td>-21*</td>
<td>-18</td>
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<tr>
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</table>

**Discussion**

Indoor mixing set-ups allow the accurate control of irradiance, but UVR/PAR ratios, the spectral quality within wavelength bands, as well as the irradiance levels fundamentally diverge from those occurring in the water column. Therefore we chose to perform our experiments under natural irradiance conditions, even though irradiance differences between experimental days were foreseen. Mixing speeds applied during our experiments (10 and 60 min.) are naturally occurring conditions in Patagonian coastal waters (Barbieri et al. 2002). During the 1 day experiments (experiment 3), weather conditions were rather variable. Yet, irradiance conditions during the two 7-day experiments (experiments 1 and 2) were highly stable allowing for the comparison between experimental treatments.

Previous investigations had shown that exposure of low irradiance acclimated cells to excess (artificial) sunlight can lead to significant viability loss (Van de poll et al. 2005, 2007). Therefore, in this study pre-cultivation was done at high irradiance (250 µmol
Chapter 7

photons m$^{-2}$ s$^{-1}$) to maximize photoprotection in order to guarantee survival after the transition to outdoor irradiance (Willemoës & Monas 1991, Mewes & Richter 2002, Buma et al. 2006). Due to this transfer, daily PAR doses increased 2-4 fold (Table 1), regardless of the irradiance treatments (static, mixed). In addition, both static and mixed cultures received on average more than 250 µmol photons m$^{-2}$ s$^{-1}$ between 07:45 and 19:15, even when mixed cultures were at the lower end of the mixing cycle. Therefore, our experimental irradiance conditions allowed comparison of excess irradiance effects without generating periods of irradiance limitation during the day. UVR intensities, on the other hand, were significantly lower at the bottom than at the surface of the mixing cycle. Therefore mixed cultures experienced periods of significantly low UVR irradiance as well as a lower daily UVR doses (Table 1). Thus, in the present experiments, antioxidant responses were compared for conditions of constant excess irradiance and shallow mixing, the latter characterized by fluctuating high to excess irradiance, without static and dynamic daily irradiance doses being identical (Table 1).

Intraspecific differences

Acclimation to the outdoor conditions did not lead to significantly different carbon assimilation numbers as determined in the solar simulator under PAR-only, when comparing the four irradiance treatments. This indicates that cultures that were acclimated to outdoor PUV did not exhibit a chronically obstructed photosynthetic machinery (MacIntyre et al. 1996) as compared with the cultures acclimated to outdoor PAR-only. Similarly, shallow mixing did not chronically affect photosynthetic performance as compared with the static cultures. In contrast, prolonged exposure to outdoor UVR (static as well as mixed cultures) strongly affected growth rates and UVR-sensitivity of both species. These results suggest that prolonged elevated excess irradiance induces increasing levels of photoprotection, therewith preventing photosystem damage and reduced carbon incorporation, however at the expense of growth, as found elsewhere (lesser 1996b, Zudaire & Roy 2001, Hernando et al. 2002). In support of this, static PUV cultures, that received the highest outdoor UVR-doses, were significantly more resistant to solar simulated UVR than the other three cultures. This indicates that UVR protection was effectively induced in static PUV cultures, however at strongly depressed growth rates. Mixed PUV cultures on the other hand exhibited somewhat higher growth rates, yet their UV resistance was only slightly higher than those of the PAR exposed cultures. In other words, the dynamic regimes increased growth rates, but decreased UVR resistance, possibly related with overall lower UVR levels as compared with the static PUV cultures (Table 1).

Strikingly, exposure to outdoor UVR or irradiance fluctuations did not induce distinct antioxidant responses between the four treatments in either T. weissflogii or D. tertiolecta on the longer term (3-7 d). This implies that prolonged outdoor excess PAR rather than fluctuating irradiance or outdoor UVR affected antioxidant levels, if at all (see below). Many studies have stressed the importance of xanthophyll cycling for quenching of excess irradiance energy (Young & Frank 1996, Niyogi 1999). Yet, protective over harvesting (prot/harv) ratios were not significantly different between irradiance treatments, despite the treatment-related changes in cellular pigment quantities. So, similar to the antioxidants, pigment ratios rather responded to the excess outdoor PAR than to additional UVR exposure or fluctuating irradiance. Therefore, irradiance induced differences in UV-
sensitivity or growth rates could not be explained by explicit antioxidant or pigment responses. Clearly, other mechanisms must have been responsible for the observed increase in UVR resistance in the static PUV cultures. These could involve upregulation of repair processes (Karentz et al. 1991b), increased protein turnover (Young & Frank 1996), production of UV-absorbing compounds (Karentz et al. 1991a, Hazzard et al. 1997, Laurion & Vincent 1998), *de novo* synthesis of D1 proteins (Ragni et al. 2008), increased level of lipid protecting α-tocopherol (Malanga & Puntarulo 1995, Malanga et al. 1997) or rerouting of metabolic pathways (Apel & Hirt 2004, Aguilera et al. 2002, Foyer & Noctor 2000).

**Interspecific differences.**

Cell size or surface to volume (S/V) ratio may partly determine excess irradiance or UVR responses in marine microalgae (Karentz et al. 1991b, Laurion & Vincent 1998, Helbling et al. 2001a). However, in our study, *T. weissflogii* and *D. tertiolecta* had a similar cell volume (367 and 368 µm^3^, respectively) and S/V ratio’s (0.82 and 0.69 respectively) and therefore cell size characteristics had a minimal impact on the observed responses. The phytoflagellate *D. tertiolecta* was found to be more sensitive to outdoor excess irradiance including UVR than the diatom *T. weissflogii* when considering carbon assimilation (Fig. 1b; Table 4), UVR resistance (Fig. 1d; Table 4) and growth rate (Table 2, 4). It must be noted that growth rates of *D. tertiolecta* during pre culturing (250 µmol photons·m^{-2}·s^{-1}) and the short term (3 d) outdoor exposures were maintained at a high level of 0.53 – 0.57 d^{-1} before the strong reduction (4-7 d, Table 2) occurred.. Other authors also found rapid acclimation of *D. tertiolecta* to high PAR levels (360-1000 µmol photons·m^{-2}·s^{-1}), with respect to growth rate (Havelková-Doušová et al. 2004, Kristin et al. 2007). Havelková-Doušová and coworkers (2004) showed increased growth (up to 0.61 d^{-1}) with increasing PAR dose during a one week period. However growth was slightly reduced at the highest total irradiance dose, being lower than our outdoor conditions (max. of 1750 µmol photons·µm^{-2}·s^{-1}). This could indicate that prolonged exposure to PAR intensities exceeding 1000 µmol photons·m^{-2}·s^{-1} leads to growth inhibition in *D. tertiolecta*. In contrast, growth rates of *T. weissflogii* decreased immediately but to a lesser extent as compared with *D. tertiolecta*. Also growth rates of *T. weissflogii* were not further reduced after 3 days.

During pre-culturing, *D. tertiolecta* showed higher SOD and GR activity compared with the diatom. Considering that both species showed similar values for GRS and cellular glutathione content, this indicated that glutathione consumption by *D. tertiolecta* was much higher than by *T. weissflogii*. This implied enhanced levels of ROS in *D. tertiolecta* compared to *T. weissflogii* (27, 55-56). APX activity, on the other hand, was nearly four times higher in *T. weissflogii* than in *D. tertiolecta*. Yet, because microalgae use various pathways to remove hydrogen peroxide (catalase, glutathione peroxidase, non-enzymatic antioxidants, diffusion), these results do not imply that *T. weissflogii* experienced enhanced hydrogen peroxide production.

Immediate antioxidant responses during the first day after their transfer indicated enhanced levels of oxidative stress in both species. On a longer term, *D. tertiolecta* increased its SOD and GR activity, decreased its GRS and progressively reduced its glutathione quota throughout the experimental period in contrast to *T. weissflogii*. As SOD
is a $O_2^{*−}$ scavenger and glutathione plays an important role in $H_2O_2$ detoxification (Alscher 1989, De Kok & Stulen 1993), observed changes suggest prolonged and increased levels of ROS production during short (3 d) and long (7 d) term exposure periods. The stoichiometrically inverse relation between GR and GRS has been found before in plants and macroalgae (Karpinski et al. 1997, Shiu & Lee 2005) and was ascribed to a putative regulatory role of glutathione in controlling (antioxidant) gene expression (Wingate et al. 1988, Georgiou 2002, Noctor et al. 2002). T. weissflogii, on the other hand, did neither significantly change its glutathione parameters, nor its levels of SOD and APX activity during short (3 d) and long (7 d) exposure to outdoor irradiance. These responses show that antioxidants are especially important directly after an irradiance shift. As they did not correspond with the differences in growth rates or reduced photosynthetic activity under the solar simulator, antioxidant responses were not found to explain interspecific differences in irradiance sensitivity in our study.

Similar to the antioxidants, T. weissflogii did neither show differences in its prot/harv pigment ratio during short (3 d) and long (7 d) term exposure to outdoor irradiance conditions. The prot/harv pigment ratio of D. tertiolecta, on the other hand, increased gradually to similar values as T. weissflogii during exposure to outdoor irradiance. Yet, this increase was not achieved by increasing the cellular amount of protective pigments but by dramatically decreasing the cellular amount of light harvesting and protective pigments, on average 3.5 and 2 fold, respectively. So, despite its increased prot/harv ratio and antioxidant responses, D. tertiolecta still showed the largest adverse responses to the outdoor irradiance exposure.

It must be emphasized that photoprotection properties, as described above, might potentially vary during the day as a result of photoregulation processes. In accordance, other studies described diurnal variation in antioxidant and pigment levels as a response to increased irradiance (Dupont et al. 2004, Janknegt et al. 2008). Yet, our study focused on photoacclimation responses (variation in photoprotective pools) and not on diurnal photoregulation. So, to avoid influence of photoregulation, samples were collected before algal cultures were exposed to (excess) solar irradiance.

Our results imply that both species might use different photoprotection strategies during acclimation to increased irradiance conditions. It has been suggested that taxon specific photoprotection strategies depend on the deployment of additional energy dissipation mechanisms (Meyer et al. 2000, Van Leeuwe et al. 2005, Wagner et al. 2006). In contrast to diatoms, green flagellates depend less on their xanthophyll cycle but contain supplementary quenching mechanisms such as rapid redistribution of energy via state transitions and cyclic PSI electron transport. Antioxidant and pigment responses indicate that cyclic PSI electron transport could play a role in energy dissipation in D. tertiolecta, since circulation of electrons around PSI inevitably leads to formation of superoxide radicals (Ishida et al. 1997, Apel & Hirt 2004, Kristin et al. 2007). Consequently, D. tertiolecta showed high SOD activities during pre-culturing conditions (compared to T. weissflogii) which increased during outdoor exposure conditions. Furthermore, in contrast to T. weissflogii, D. tertiolecta only started to increase its prot/harv ratio when exposed to outdoor irradiance conditions indicating that this species uses additional quenching mechanisms besides its xanthophyll cycle. So, it seems the pre-experimental irradiance level had already induced maximal photoprotection potential in T. weissflogii whereas D. tertiolecta was only moderately protected against outdoor irradiance conditions. As a

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consequence, *T. weissflogii* could handle a long (7 d) exposure period of outdoor irradiance relatively well at the expense of growth whereas *D. tertiolecta* could grow relatively well during a short (3 d) exposure period at the expense of effective long (7 d) term protection mechanisms. In support of this, others also found that diatoms are more resistant than flagellates to high outdoor irradiance including UVR (Helbling *et al.* 1994, Buma *et al.* 2001, Villafañe *et al.* 2004).

We conclude first of all that simulated shallow mixing may offer protection against harmful UVR, but may increase UVR vulnerability, independent of mixing speed. Secondly, the green phytoflagellate *D. tertiolecta* was more sensitive to excess irradiance including UVR than *T. weissflogii* as a result of insufficient photoprotection strategies. Thirdly, antioxidants were primarily deployed as an initial response after the irradiance shift to excess outdoor PAR. Finally, antioxidant and pigment responses could not explain the observed differences in growth and UV sensitivity in both species.