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Acclimation to a dynamic irradiance regime changes excessive irradiance sensitivity of *Emiliania huxleyi* and *Thalassiosira weissflogii*

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Abstract

Effects of fluctuating irradiance regimes on excessive photosynthetically active radiation (PAR) and ultraviolet (UV) radiation sensitivity were assessed for *Emiliania huxleyi* (Lohman) and *Thalassiosira weissflogii* (Grunow) Fryxell and Hasle. Cultures acclimated to low irradiance were subjected to two irradiance regimes of equal daily dose: dynamic irradiance simulating vertical mixing within the water column and constant irradiance. For each regime two irradiance levels were studied. Growth was monitored for 3 d, after which pigment composition was determined. Next, excessive PAR and UV sensitivity was measured by studying viability loss during 4-h exposure to simulated surface irradiance (SSI). Furthermore, the effects of inhibition of D1 reaction center protein turnover were investigated by incubating samples with lincomycin prior to exposure. Dynamic irradiance reduced growth rates of both species as compared to constant irradiance. Pools of light-harvesting pigments increased in dynamic irradiance, whereas the protective pigment pools decreased compared to constant irradiance. Excessive irradiance sensitivity was enhanced in cells grown in fluctuating irradiance. Furthermore, viability loss was most pronounced in UV treatments combined with lincomycin. *E. huxleyi* was more sensitive to excessive irradiance than *T. weissflogii*, which coincided with a lower ratio between protective and light-harvesting pigments in the former species. Irradiance modulation by deep vertical mixing influences growth, pigment composition, and excessive PAR and UV sensitivity within days.

Marine phytoplankton experiences irradiance oscillations due to seasonal, diurnal, and weather-related variability. Superimposed on this, phytoplankton cells are subjected to strong irradiance fluctuations as a result of transport through the irradiance gradient in the water column by wind-induced vertical mixing. Close to the water surface, irradiance can exceed photosynthetic requirements and cause photoinhibition and viability loss in algae (van de Poll et al. 2006; Llabrés and Agustí 2006). Photoinhibition occurs when the photosynthetic electron transport chain becomes over-reduced. Initially, photoinhibition is associated with a decrease in functional PSII reaction centers, which reduces photosynthetic efficiency. Progressive reduction in functional PSII limits the reducing power of antioxidant metabolism, which leads to uncontrolled reactive oxygen formation and viability loss (van de Poll et al. 2006). Photoinhibition is reversible, but it requires synthesis of PSII components such as the D1 reaction center protein (Hazzard et al. 1997). These effects are primarily caused by photosynthetically active radiation (PAR: 400–700 nm) and ultraviolet-A radiation (UVA: 315–400 nm), which are most abundant in sunlight and penetrate deep into the water column. Ultraviolet-B radiation (UVB: 280–315 nm) attenuates faster and therefore only contributes to these processes close to the surface (Cullen et al. 1992). Consequently, the process of vertical mixing differentially modulates the dynamics of PAR and UV exposure due to wavelength-dependent attenuation (Neale et al. 1998). Because excessive irradiance exposure is restricted by vertical mixing and by the diurnal cycle, algae spend much of their time in low irradiance, which requires efficient light harvesting. Algae adjust photosynthetic light-harvesting and photoprotection capacity to changing irradiance conditions by varying pigment composition, PSII reaction center abundance, and Calvin cycle activity (MacIntyre and Geider 1996; Moore et al. 2006). Light-harvesting pigments absorb light energy and transfer this to the photosystems, thereby driving the photosynthetic electron transport chains. Xanthophyll pigments fulfill a dual function: epoxidized equivalents assist in light harvesting, whereas de-epoxidized equivalents dissipate excessive irradiance in the form of heat (Olaizola et al. 1994). Enzymatic xanthophyll conversion (the xanthophyll cycle) responds within seconds to irradiance changes and is crucial in preventing photoinhibition and viability loss during excessive irradiance, including UV (Moisan et al. 1998; van de Poll et al. 2005, 2006). Therefore, conditions influencing the absorption and dissipation of irradiance affect protection against UV and excessive PAR and modulate sensitivity to photoinhibition and viability loss (Litchman and Neale 2005; van de Poll et al. 2005). Although photoacclimation responses share a strong degree of similarity, recent studies indicate that species-specific (genotypic) differences in pigment composition modify photosynthesis and survival in high irradiance. Consequently, differences in photoacclimation potential may determine which species have a competitive advantage under fluctuating irradiance conditions (Van Leeuwe et al. 2005; Moore et al. 2006). This study was aimed at resolving dynamic irradiance–mediated pigment changes and their impact on excessive irradiance sensitivity. Therefore, we compared photoacclimation, growth, and excessive irradiance sensitivity of dynamic (experienced during vertical mixing) and constant irradiance (normal light : dark cycle, as provided in the lab) regimes on cells of the diatom *Thalassiosira weissflogii* and the prymnesiophyte *Emiliania huxleyi.*

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Table 1. Irradiance conditions (photosynthetically active radiation [PAR], ultraviolet [UV] radiation) during the cultivation experiments (LL is low irradiance; LDI, HDI are low and high dynamic irradiance; LCI, HCI are low and high constant irradiance) and during excessive irradiance exposure (SSI: simulated surface irradiance).

Condition	PAR (400–700 nm) (μmol m^{-2} s^{-1})	UVA (315–400 nm) (W m^{-2})	UVB (280–315 nm) (W m^{-2})
LL	9	0	0
HDI	1,350–20	0	0
LDI	770–11	0	0
HCI	260	0	0
LCI	148	0	0
SSI-PAR	1,250	25.7	0.005
SSI-PAR + UV	1,260	51	0.6

Method

Precultivation—Triplicate batch cultures of *Emiliania huxleyi* Lohman (strain L, isolated from Oslo fjord by Paasche) and *Thalassiosira weissflogii* (Grunow) Fryxell and Hasle (strain CCMP 1049) were grown in f-2–enriched (Guillard and Ryther, 1962) autoclaved seawater (salinity 35) in a temperature-controlled cabinet (17°C) in low irradiance (9 μmol m^{-2} s^{-1}, provided by Osram biolux lamps) during a 16 : 8 h light : dark (LD) cycle. The cultures were regularly diluted in new medium for 3 weeks and served as starting material for the dynamic and constant irradiance treatments.

Dynamic and constant irradiance treatments—Triplicate Erlenmeyer flasks with 100-mL f-2 medium were inoculated with low irradiance–acclimated cells and grown for 3 d under dynamic and constant irradiance (*see* below). These irradiance shifts were meant to investigate short-term (days) photoacclimation changes, since full acclimation takes more time. Both irradiance treatments were carried out in a homemade UV and PAR transmissive water bath that was maintained at 17°C by a cryostat. The water bath was placed inside a U-shaped lamp setup containing 12 fluorescent lamps (six biolux and six skywhite lamps, Osram) equipped with reflectors (Doublelux) and connected to dimmers (Osram). The dimmers were computer-controlled by LabVIEW (National Instruments) software. This allowed irradiance fluctuations between 20 and 1,350 μmol m^{-2} s^{-1} without changing spectral quality (Table 1; Fig. 1), as measured with a QSL-100 (Biospherical Instruments) and a MACAM SR 9910 double-monochromator scanning spectroradiometer (Macam Photometrics) in air. In the dynamic irradiance treatment, algae were cultivated under electronically modulated irradiance, with mixing speed (one cycle in 4 h), mixing depth (80 m), and attenuation (Kd = 0.5 for PAR) superimposed on the diurnal cycle (16 : 8 h LD). A scenario was chosen where algae reside near the water surface around noon and experience five complete cycles during the light period (Fig. 1). In the constant irradiance treatment, the mean daily irradiance for the dynamic irradiance treatment (260 μmol m^{-2} s^{-1}) was provided as a block function (on–off) during a 16 : 8 h LD cycle (Fig. 1). Two irradiance levels were created for dynamic irradiance (high dynamic irradiance [HDI]: 100%; low dynamic irradiance [LDI]: 57%) and constant irradiance (high constant irradiance [HCI]: 100%; low constant irradiance [LCI]: 57%) treatments by covering part of the setup with a neutral-density screen (Table 1).

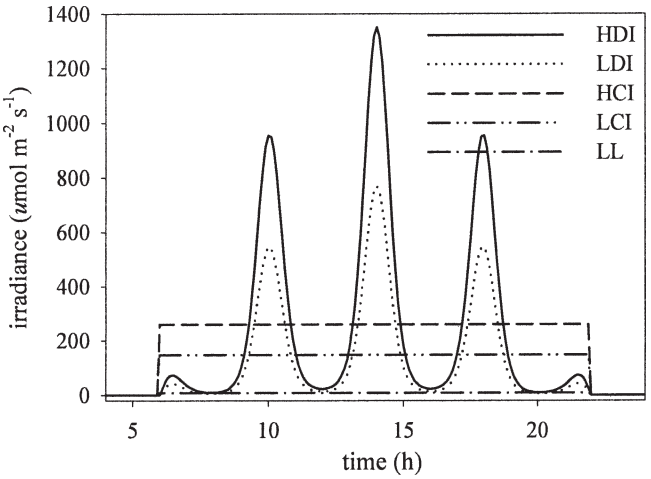

Fig. 1. Irradiance (μmol m^{-2} s^{-1}) during the low irradiance (LL), low and high dynamic irradiance (LDI, HDI), and low and high constant irradiance (LCI, HCI) treatments.

Growth rate and flow cytometry—Samples (1 mL) for cell counts were obtained each day, and cell concentrations were determined on a Coulter Epics MXL flow cytometer (Beckman Coulter). Growth rates (divisions d^{-1}) were calculated by linear regression on natural log–transformed cell numbers for the three replicates.

Pigment composition—Samples (30–75 mL) for pigment composition (three replicates) were obtained during precultivation in low irradiance (9 μmol m^{-2} s^{-1}) and after 3 d of dynamic and constant irradiance. The samples were filtered on 25-mm GF/F filters (Whatman) by mild vacuum, immediately frozen in liquid nitrogen, and stored at –80°C. Analyses were after Van Leeuwe et al. (2006). In short, filters were freeze-dried (48 h) followed by immediate extraction of pigments in 4-mL 90% cold acetone (v/v, 48 h, 4°C). Detection of pigments was carried out using an high pressure liquid chromatography (HPLC) (Waters 2690 separation module, 996 photodiode array detector) equipped with a C_{18} 5-μm DeltaPak reversed-phase column (Waters). Peak identification was done by retention time and diode array spectroscopy. Pigments were quantified using standard dilutions of chlorophyll *a* (Chl *a*), c_2, c_3, fucoxanthin, 19′-hexanoyloxyfucoxanthin, diadinoxanthin, diatoxanthin, and β carotene. Cell counts (flow cytometer) were used to calculate cellular pigment concentrations.

Excessive PAR and UV sensitivity—Sensitivity of low irradiance–grown algae and those grown under the

dynamic and constant irradiance treatments were assessed during 4-h exposure to strong irradiance (simulated surface irradiance, SSI). This treatment imitates irradiance near the water surface around noon and is therefore composed of PAR, UVA, and UVB radiation (Table 1; for setup, lamps, and spectra, *see* van de Poll et al. 2005, 2006). Spectral irradiance measurements were performed using a MACAM SR 9910 double-monochromator scanning spectroradiometer (Macam Photometrics). Prior to exposure, algae were diluted in 20-mL seawater in quartz vessels (3×10^5 and 2×10^3 cells mL^{-1} for *E. huxleyi* and *T. weissflogii*, respectively). GG 385 and WG 305 glass filters (Schott) were used to create PAR and PAR + UV treatments. In both treatments, one sample was treated with 0.6 $\times 10^{-3}$ mol L^{-1} (final concentration) lincomycin, (Sigma, 60×10^{-3} mol L^{-1} freshly prepared in 96% EtOH). Lincomycin inhibits transcription of chloroplast-encoded proteins such as the D1 reaction center protein, which is a crucial component of PSII at a high turnover rate. Samples were obtained over 1-h intervals during the SSI viability assay (*see* below). Excessive irradiance sensitivity of the algae was determined 4.5 h after the start of the photoperiod for all irradiance conditions.

Discrimination between viable and nonviable cells—Viability was assessed by flow cytometry after SYTOX staining. A 1-mL sample was incubated for 30 min in darkness with 10-μL SYTOX (Molecular probes) solution that was 100 times diluted in MiliQ. SYTOX is a deoxyribonucleic acid (DNA) stain that cannot pass intact cell membranes. The presence of compromised membranes in nonviable cells is exploited in this and other viability assays (Veldhuis et al. 2001; Llabrés and Agustí 2006) because it enables SYTOX to enter the cells and stain DNA. This typically enhances green fluorescence by two orders of magnitude, allowing clear separation of viable and nonviable cells. The method relies on the presence of DNA, if cellular DNA is degraded, nonviable cells (debris) will not test positive after SYTOX staining. For each data point, at least 30×10^3 individual cells were analyzed with the flow cytometer.

Statistics—Differences among groups were tested for significance with a single-factor analysis of variance (ANOVA) and a least significant difference (LSD) post hoc test using Statistical Package for Social Sciences (SPSS) software. Viability loss after 4 h SSI was correlated with pigment composition prior to exposure (mean ratio between protective and light-harvesting pigments) in SPSS.

Results

Growth rates—During precultivation in low irradiance, growth of *E. huxleyi* was significantly higher than growth of *T. weissflogii* (Table 2). Growth rates of *E. huxleyi* and *T. weissflogii* in dynamic irradiance were significantly lower than those in constant irradiance (one-way ANOVA). In the HDI treatment, cell numbers of *E. huxleyi* decreased on average by 75% after the first 24 h, i.e., growth was negative. By exception, this experiment was continued for 6 d to grow sufficient biomass, and a mean growth rate of 0.59 d^{-1} was calculated over the entire period. Mortality of *T. weissflogii* cells was not observed. For this species, growth rates in dynamic irradiance were significantly lower than those in constant irradiance, but differences between low and high irradiance conditions were not significant, i.e., HDI = LDI and LCI = HCI.

Table 2. Growth rates (divisions d^{-1}), cell characteristics (forward, side scatter, indicators for cell volume), and total cellular pigment content of *Emiliania huxleyi* and *Thalassiosira weissflogii*. Growth was determined over a period of 3 d, except for where noted by asterisk (*, details in text), whereas the other measurements were performed on the last day. Mean and standard deviation are shown for three replicates obtained from low irradiance (LL); low and high dynamic irradiance (LDI, HDI); and low and high constant irradiance (LCI, HCI).

	Growth (d^{-1})	Forward scatter (a.u.)	Side scatter (a.u.)	Pigments (pg $cell^{-1}$)
E. huxleyi				
LL	0.32(0.02)	31.7(0.82)	4.2(0.09)	0.70(0.03)
LDI	0.81(0.03)	38.4(1.1)	5.2(0.42)	0.78(0.06)
LCI	0.89(0.03)	45.6(0.21)	9.2(0.06)	0.53(0.09)
HDI	0.59(0.04)*	38.2(0.06)	5.4(0)	0.51(0.01)
HCI	0.76(0.06)	48.3(1.1)	10.4(0.7)	0.48(0.06)
T. weissflogii				
LL	0.20(0.007)	172.5(2.7)	29.5(1.2)	21.2(4.1)
LDI	0.66(0.01)	205.7(0.21)	39.8(1.8)	12.1(0.49)
LCI	0.80(0.02)	213.3(4.0)	39.2(2.7)	8.09(0.11)
HDI	0.71(0.03)	206.2(1.4)	40.1(0.8)	10.3(1.44)
HCI	0.85(0.06)	205.1(3.4)	39.7(1.4)	6.73(0.67)

Mean forward and side scatter (flow cytometry) of both species increased significantly after 3 d of cultivation in constant and dynamic irradiance compared to cultivation in low irradiance (Table 2). For *T. weissflogii*, differences between cells grown in dynamic and constant irradiance were not significant, and forward and side scatter were 120% and 134% of those grown in low irradiance. Scatter of *E. huxleyi* increased significantly during cultivation in constant irradiance (forward scatter: 147%, side scatter: 236% of low irradiance cells) compared to cultivation in dynamic irradiance (forward scatter: 121%, side scatter: 128% of low irradiance cells).

Pigment composition—Total cellular pigment content was lower in both species after 3 d of cultivation under all treatments as compared with that during precultivation in low irradiance, except for *E. huxleyi* grown in LDI (Table 2). The total pigment content per cell was significantly higher in dynamic irradiance compared to constant irradiance, except for HDI and HCI from *E. huxleyi* (Table 2). Clear differences in light-harvesting and protective pigments were found between dynamic and constant irradiance treatments: the latter treatments caused a more pronounced decrease in light-harvesting pigments and a more pronounced increase in protective pigments. This caused significant differences in the ratio between protective and light-harvesting pigments, which increased from

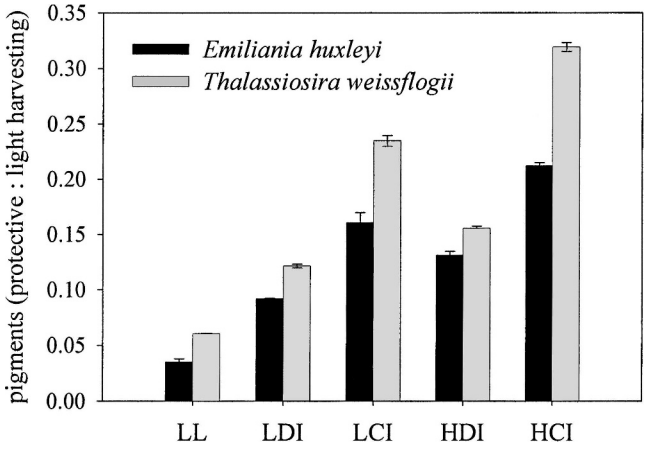

Fig. 2. Ratio between protective (diadinoxanthin, diatoxanthin) and light-harvesting (Chlorophyll *a*, c_1, c_2, c_3, phytylated chlorophyll c_2, fucoxanthin, 19′-butanoloyfucoxanthin, 19′-hexanoloyfucoxanthin) pigments in *Emiliania huxleyi* and *Thalassiosira weissflogii*. The algae were grown in low irradiance (LL), low and high dynamic irradiance (LDI, HDI), and low and high constant irradiance (LCI, HCI) treatments. Mean and standard deviation are shown for three replicates.

LDI, HDI, to LCI and HCI (Fig. 2). Species-specific changes were found in individual pigments, particularly in the xanthophylls diadinoxanthin and diatoxanthin (Table 3).

Protective pigments: Diadinoxanthin and diatoxanthin—The xanthophyll cycle pigments diatoxanthin and diadinoxanthin showed the most pronounced changes of all pigments. The cellular diadinoxanthin-diatoxanthin pool increased in all conditions compared to precultivation in low irradiance. Differences in xanthophyll pool size between high (HDI, HCI) and low (LDI, LCI) conditions were significant for both species (single-factor ANOVA). Mean increases were higher in constant irradiance (185% and 449 % of low irradiance in *T. weissflogii* and *E. huxleyi*, respectively) compared to dynamic irradiance (140% and 347% of low irradiance in *T. weissflogii* and *E. huxleyi*, respectively). Significant de-epoxidation was found in dynamic and constant irradiance treatments compared to that in low irradiance cultivation (not shown). De-epoxidation was significantly higher in *T. weissflogii* than in *E. huxleyi*. De-epoxidation during LDI and LCI was not significantly different at the time of sampling for both species, where diatoxanthin comprised on average 15% ± 4% (*E. huxleyi*) and 64% ± 7% (*T. weissflogii*) of the total xanthophyll cycle pool. De-epoxidation during HDI and HCI was significantly higher than that during LDI and LCI (*E. huxleyi*: 43% ± 18%, *T. weissflogii*: 81% ± 7% diatoxanthin) for both species. Note that pigment samples for the dynamic irradiance–grown cells were obtained during the second irradiance peak.

Light-harvesting pigments—Apart from 19′-hexanoyloxyfucoxanthin (*E. huxleyi*), all cellular light-harvesting pigments decreased in LDI, HDI, LCI, and HCI treatments compared to low irradiance cultivation, except for *E. huxleyi* grown in LDI (Table 3). Chl *a* decreased in *T. weissflogii* from LDI (56% of low irradiance), HDI, to LCI and HCI (28% of low irradiance). For *E. huxleyi*, changes in Chl *a* were not significant between low irradiance and LDI but decreased on average to 75% of low irradiance in HDI, LCI, and HCI. Fucoxanthin decreased from LDI (*T. weissflogii*: 50%) to HDI (45%), LCI (30%), and HCI (20% of low irradiance) in all treatments except for *E. huxleyi* grown in LDI. In *E. huxleyi*, a fucoxanthin-like pigment was found (presumed 19′-butanoyloxyfucoxanthin), which decreased in a similar pattern to around 40% of low irradiance in HCI. Cellular 19′-hexanoyloxyfucoxanthin increased significantly when grown in HCI and LCI (185% of low irradiance), whereas differences in dynamic irradi-

Table 3. Pigment composition (pg cell^{-1}) of *Emiliania huxleyi* and *Thalassiosira weissflogii* after growth in low irradiance (LL) and 3 d in low and high dynamic irradiance (LDI, HDI), and low and high constant irradiance (LCI, HCI). Mean and standard deviation are shown for three replicates.

	LL	LDI	LCI	HDI	HCI
E. huxleyi					
Chlorophyll ($c_{1,2}$)	0.068(0.004)	0.068(0.006)	0.042(0.008)	0.042(0.002)	0.035(0.004)
Chlorophyll c_3	0.064(0.004)	0.058(0.007)	0.038(0.007)	0.034(0.002)	0.032(0.003)
Phytylated Chl c_2	0.029(0.001)	0.035(0.003)	0.020(0.003)	0.020(0.001)	0.017(0.002)
Chlorophyll *a*	0.27(0.013)	0.31(0.024)	0.21(0.033)	0.20(0.006)	0.19(0.022)
Fucoxanthin	0.15(0.013)	0.15(0.013)	0.046(0.01)	0.073(0.004)	0.022(0.003)
19′-butafuco	0.053(0.003)	0.051(0.004)	0.028(0.005)	0.032(0.001)	0.022(0.003)
19′-hexafuco	0.041(0.007)	0.047(0.002)	0.078(0.012)	0.045(0.002)	0.073(0.009)
Diadinoxanthin + diatoxanthin	0.015(0.002)	0.055(0.004)	0.064(0.008)	0.051(0.001)	0.074(0.008)
β, ε carotene	0.008(0.001)	0.011(0.001)	0.009(0.001)	0.008(0.001)	0.009(0.001)
T. weissflogii					
Chlorophyll ($c_{1,2}$)	1.24(0.25)	0.566(0.02)	0.292(0.001)	0.449(0.06)	0.213(0.02)
Phytylated Chl c_2	0.067(0.02)	0.050(0.001)	0.022(0.001)	0.0415(0.005)	0.0256(0.004)
Chlorophyll *a*	13.8(2.67)	7.68(0.31)	4.83(0.086)	6.36(0.887)	3.83(0.377)
Fucoxanthin	4.87(0.89)	2.50(0.10)	1.41(0.02)	2.03(0.29)	1.03(0.10)
Diadinoxanthin + diatoxanthin	0.741(0.13)	0..988(0.05)	1.33(0.02)	1.090(0.16)	1.41(0.14)
β, ε carotene	0.45(0.10)	0.32(0.01)	0.21(0.013)	0.292(0.04)	0.22(0.023)

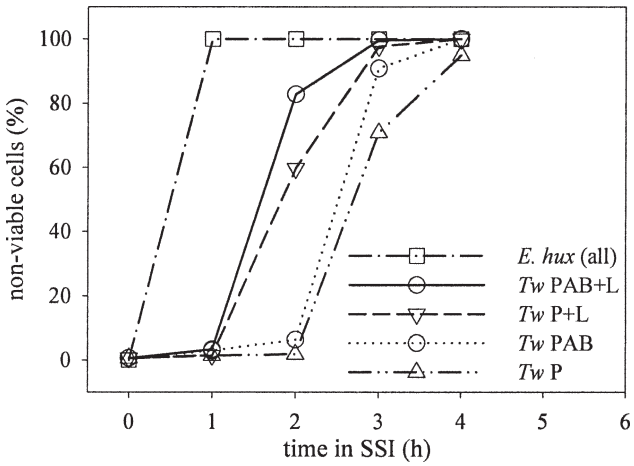

Fig. 3. Number of nonviable cells (% of total) of low irradiance–acclimated cells of *Emiliania huxleyi* and *Thalassiosira weissflogii* in excessive irradiance (SSI, simulated surface irradiance). During SSI, algae were exposed to PAR (P), PAR + UV (PAB), PAR + lincomycin (P + L), and PAR + UV + lincomycin (PAB + L). For each point in time, more than 30×10^3 cells were analyzed.

ance were not significant in this species. Chl c_1, c_2, phytylated Chl c_2 and Chl c_3 (*E. huxleyi* only) decreased in a similar way as fucoxanthin in both species. β carotene decreased to 65% and 45% of low irradiance in *T. weissflogii* when grown in dynamic and constant irradiance, respectively. For *E. huxleyi,* β carotene was not different as compared to low irradiance–acclimated cells.

Excessive PAR and UV sensitivity: Viability loss—Generally, *E. huxleyi* showed higher viability loss during the SSI treatment than *T. weissflogii* when precultivated in low, dynamic, and constant irradiance (Figs. 3, 4). For low irradiance–cultivated *E. huxleyi*, all cells were nonviable after 1 h of SSI exposure, regardless of UV or lincomycin addition. Although there were no replicates for the specific irradiance conditions, patterns in viability loss during excessive PAR and UV were similar for *E. huxleyi* and *T. weissflogii*. Low irradiance–acclimated cells were most vulnerable to photoinduced viability loss, whereas sensitivity decreased when algae were grown under dynamic and constant irradiance treatments. Constant irradiance–grown cells exhibited less viability loss than those cultivated in dynamic irradiance (Fig. 4). Viability loss over time was enhanced by UV radiation in both species. Differences between viability loss in PAR + UV treatments were small for LDI, HDI, and constant irradiance–grown *E. huxleyi* cells. There was a significant negative correlation between viability loss after 4-h PAR + UV treatment and the mean ratio between protective and light-harvesting pigments calculated for the samples prior to SSI exposure (R^2 = 0.94, n = 9, *T. weissflogii* HCI excluded; Fig. 5). Significant correlations were also found between pigment composition and viability loss after 4-h PAR + UV + lincomycin (R^2 = 0.83, n = 10) and PAR + lincomycin (R^2 = 0.7, n = 10) treatment, whereas no significant correlation was found for the data from the PAR treatment (R^2 = 0.5, n = 10) (results not shown). Lincomycin increased viability loss over time during excessive irradiance for all cultivation conditions in both species. The combined PAR + UV and lincomycin treatment caused the strongest viability loss for both species. The effect of lincomycin was more pronounced in *E. huxleyi* compared to *T. weissflogii* and followed the same pattern as excessive PAR and UV sensitivity without inhibitor, i.e., viability loss was lower in cells precultivated in constant irradiance (LCI, HCI). Viability loss in the presence of lincomycin was lowest in LCI- and HCI-cultivated *T. weissflogii*, where on average, 3% of the cells were nonviable with lincomycin after 4-h SSI compared to 1% without inhibitor. No lincomycin effect on viability loss was found after 4 h in darkness (results not shown).

Discussion

These experiments present evidence that photoacclimation to dynamic irradiance modifies cellular pigment content and composition, cell volume (*E. huxleyi*), growth rates, and excessive irradiance sensitivity compared to photoacclimation to constant irradiance. Although our dynamic and constant irradiance treatments received the same daily irradiance dose (LDI = LCI; HDI = HCI), cells grown under constant irradiance were more high-light acclimated than those grown in dynamic irradiance. Consequently, growth, pigment composition, and excessive irradiance sensitivity were not proportional to daily cumulative exposure in both species, but were modified by temporal irradiance variability, thereby contradicting the view that microalgae acclimate to the average irradiance level. Differences between constant and dynamic irradiance cultivation were reflected in cellular pigment composition. Pigment composition of the dynamic irradiance regimes appears to be a trade-off between low and high irradiance acclimation, resulting in relatively high cellular content of protective (compared to low irradiance cultivation) and light-harvesting pigments (compared to LCI and HCI cultivation). This is consistent with results from phytoplankton assemblages from the Delaware bay, which have also shown characteristics of low and high irradiance acclimation (MacIntyre and Geider 1996). In addition, similar results have been obtained for green algae when comparing dynamic and constant irradiance regimes (Ibelings et al. 1994; Havelková-Doušová et al. 2004). On average, dynamic (LDI, HDI) irradiance–grown cells contained 33% more light-harvesting pigments than those grown in constant (LCI, HCI) irradiance. Surprisingly, constant irradiance–grown cells had on average 31% more xanthophyll cycle pigments than those from dynamic irradiance. This was unexpected because, in contrast to the dynamic irradiance treatments, there were no irradiance fluctuations during the light period that required protection. This effect may be caused by the long (16-h) duration of high, saturating irradiance. It has been found that short periods (up to 10 min) of strong irradiance can be utilized efficiently for photosynthesis, whereas prolonged exposure (longer than 10 min) causes a down regulation of photosynthesis (Marra 1978; MacIntyre and

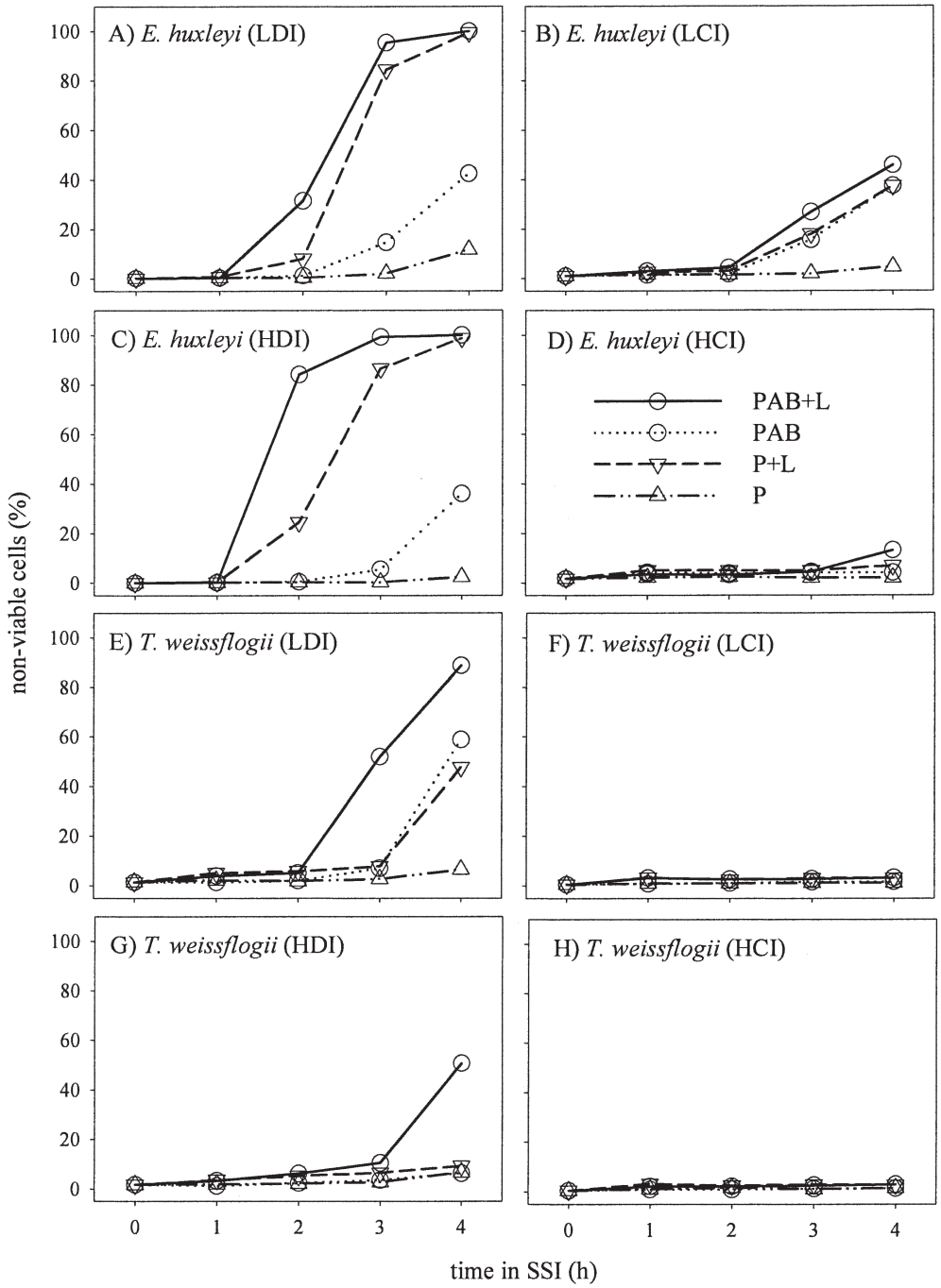

Fig. 4. Number of nonviable cells (% of total) of low and high dynamic irradiance (LDI, HDI), and low and high constant irradiance (LCI, HCI) treatments of (A–D) *Emiliania huxleyi* and (E–H) *Thalassiosira weissflogii* in excessive irradiance (SSI, simulated surface irradiance). During SSI, algae were exposed to PAR (P), PAR + UV (PAB), PAR + lincomycin (P + L), and PAR + UV + lincomycin (PAB + L). For each point in time more than 30×10^3 cells were analyzed.

Geider, 1996). Presumably, energy dissipation by xanthophyll de-epoxidation (the xanthophyll cycle) plays a role in this process, as was indicated by the increased diadinoxanthin-diatoxanthin pool and diatoxanthin concentration in cells grown in constant irradiance (LCI, HCI). We investigated pigment and excessive irradiance sensitivity 3 d after transfer from low irradiance to study if short-term changes in irradiance and irradiance dynamics influenced excessive irradiance sensitivity. Therefore, the data do not necessarily reflect the steady-state values of long-term acclimation to the LCI, HCI, LDI, and HDI treatments. Nevertheless, the HCI pigment ratios of *T. weissflogii* agree with those reported previously for several weeks of cultivation under similar conditions (van de Poll et al.

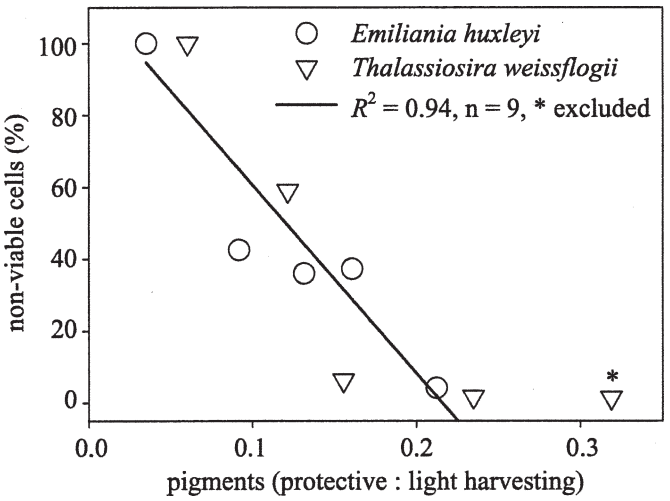

Fig. 5. Relationship between the number of nonviable cells after 4-h excessive irradiance (SSI PAR + UV) and the mean ratio between protective and light-harvesting pigments of *Thalassiosira weissflogii* and *Emiliania huxleyi* prior to exposure (* indicates *T. weissflogii* HCI was excluded from the analysis). Note that correlations for SSI PAR + UV + lincomycin, SSI PAR + lincomycin, and SSI PAR are not shown.

2006). An acclimation period of several days is an ecologically relevant time scale because irradiance changes on a short temporal scale in nature, and long periods (weeks) of constant irradiance do not exist. Because the irradiance that algae experience is controlled by the depth of the upper mixed layer and the transport rate through the irradiance gradient in the water column, irregular irradiance conditions can occur in situ. Therefore, the observed differences between constant and dynamic irradiance may be enhanced or reduced, depending on the extent and timescale of irradiance oscillations. In the present study, deep vertical mixing (80 m) was simulated combined with a high transport rate (80 m in 2 h), which resulted in strong fluctuations between limiting and excessive irradiance. This may be a realistic scenario for open oceans at mid-latitude, although simplified: variation in cloud cover, surface reflection, *Kd*, and wind speed can occur on a short temporal scales and lead to more complex fluctuation patterns.

The physiological changes caused by dynamic irradiance influenced excessive irradiance sensitivity of both species when compared to constant irradiance cultivation. These results agree with previous experiments where algae with a lower ratio of protective relative to light-harvesting pigments were more sensitive to excessive irradiance (van de Poll et al. 2005, 2006). Furthermore, the earlier studies revealed that viability loss was preceded by PSII inactivation (photoinhibition). In the present study, viability loss in excessive irradiance increased when cells were treated with the D1 synthesis inhibitor lincomycin, showing that irradiance sensitivity increases when turnover of damaged PSII reaction centers is blocked. However, inhibitor studies should be treated with caution since unintended side effects cannot be excluded. Nevertheless, the lincomycin experiments suggest that PSII damage increased during UV exposure, which in turn increased viability loss. This is supported by the observation that D1 turnover was significantly increased by UV exposure compared to PAR alone in the diatom *Chaetoceros gracilis* (Hazzard et al. 1997). Photoacclimation to constant irradiance (LCI, HCI) strongly reduced viability loss in the presence of UV and lincomycin for both species. This suggests that PSII reaction centers of constant irradiance–acclimated cells were better protected against overexcitation by prolonged excessive irradiance. Increased excessive irradiance protection can be accomplished by a reduction in light-harvesting pigments and the increase in protective xanthophyll cycle pigments (van de Poll et al. 2005, 2006). Therefore, the reduced irradiance absorption and increased xanthophyll cycle capacity provide an explanation for the high survival rates of constant irradiance–grown algae in excessive irradiance in the presence of lincomycin. Previously, it has been shown that inhibition of PSII efficiency in the presence of lincomycin is low in tobacco plants with an active xanthophyll cycle, whereas xanthophyll conversion–deficient mutants show pronounced photoinhibition in the presence of this inhibitor after 90 min excessive irradiance (Sun et al. 2001). Furthermore, it should be noted that low excessive irradiance–induced viability loss does not imply that lincomycin exerts no effect on photosynthesis. Biological weighting functions for microalgae cultivated in constant, saturating irradiance show a lower UV sensitivity for UV-induced photoinhibition than algal assemblages obtained from the field (Banaszak and Neale 2001). Our data demonstrate that photoacclimation-mediated differences in excessive irradiance sensitivity occur on a time scale of days for both species. Consequently, day to day variation in excessive PAR and UV sensitivity can be expected in the field. This agrees with the considerable temporal variability in UV effects in natural phytoplankton reported in the literature (Banaszak and Neale 2001). Vertical mixing–mediated changes in irradiance exposure can intensify or moderate excessive irradiance effects on growth, photoinhibition, and viability loss. Excessive PAR and UV exposure in the upper part of the water column is reduced by vertical mixing compared to nonmixed conditions (Neale et al. 1998). Nevertheless, our results imply that photoacclimation to deep vertical mixing increases sensitivity to occasional UV exposure. This agrees with results obtained from Southern Ocean phytoplankton, which showed increased UV sensitivity at increased mixed-layer depth (Helbling et al. 1996). Paradoxically, UV effects in natural phytoplankton may increase despite decreased UV exposure as a result of vertical mixing–mediated photoacclimation to lower irradiances.

The results show that *E. huxleyi* was more sensitive to excessive irradiance than *T. weissflogii* when cultivated over a range of irradiance conditions. Genotypic variation in excessive irradiance sensitivity may be determined by differences in the ratio between protective and light-harvesting pigments, which was consistently lower in *E. huxleyi*. This agrees with differences in excessive irradiance sensitivity between diatom species (van de Poll et al. 2006). Furthermore, the presence of species-specific differences in

pigment composition for *E. huxleyi* and *T. weissflogii* indicate fixed differences in organization of light-harvesting and photoprotection. Apart from the additional presence of Chl c_1, c_2, c_3, the former species also contains different fucoxanthin derivatives. In this respect, it is interesting that cellular 19′-hexanoyloxyfucoxanthin increased in constant but not in dynamic irradiance. High irradiance–mediated 19′-hexanoyloxyfucoxanthin increases have been observed previously in *E. huxleyi* and *Phaeocystis antarctica*, but the physiological function remains speculative (Van Leeuwe and Stefels 1998; Stolte et al. 2000). Several studies have indicated that *E. huxleyi* is susceptible to UV-induced productivity loss (Buma et al. 2000). Nevertheless, numerous field studies have shown that *E. huxleyi* competes successfully in high irradiance environments in a stratified water column (Nanninga and Tyrrell 1996). Our experiments show that excessive irradiance sensitivity of *E. huxleyi* is particularly evident in cells acclimated to low irradiance (LL) and dynamic irradiance (LDI, HDI), i.e., conditions that occur during deep vertical mixing. Sensitivity differences between *E. huxleyi* and *T. weissflogii* were lower when grown in high constant irradiance (HCI) as experienced during stratification of the upper mixed layer. Furthermore, considerable strain-specific differences in pigment composition are reported for *E. huxleyi* cultivated under similar conditions, with respect to absolute and relative pigment levels, such as fucoxanthin and 19′-hexanoyloxyfucoxanthin (Stolte et al. 2000). It is currently unknown to what extent excessive irradiance ecotypes occur in these species. Recently, the existence of different ecotypes for *Prochlorococcus* was reported (Moore and Chisholm 1999). In our experiments, *E. huxleyi* outgrew *T. weissflogii* under low irradiance, suggesting that this species is fundamentally better adapted to low irradiance than the diatom. Dynamic irradiance decreased growth rates and cell volume (*E. huxleyi*) compared to constant irradiance. Possibly, the dynamic irradiance treatment requires increased allocation of resources to light harvesting, which constrain growth rate. It is expected that climate change mediates a rise in seawater temperature and enhances freshwater input due to ice melting and precipitation. This increases thermal and salt stratification and reduces mixed layer depth, which increases light availability for phytoplankton but also exposure to UV and excessive PAR because the algae reside closer to the surface (Schmittner 2005). Elsewhere, changing weather patterns will increase mean wind speeds, thereby on average increasing the depth and speed of vertical mixing. The present study shows that microalgae do not adjust to mean irradiance levels but are influenced by irradiance fluctuations. Changes occur on a temporal scale of days and can account for considerable differences in UV sensitivity. On this time scale, photoacclimation can mediate an eightfold change in the ratio between light-harvesting and xanthophyll pigments. Finally, we found genotypic differences in excessive irradiance sensitivity between *E. huxleyi* and *T. weissflogii* that appear to be linked to differences in pigment composition. Therefore, both genotypic and phenotypic differences can affect excessive PAR and UV sensitivity of phytoplankton.

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