

SHORT-TERM AND LONG-TERM EFFECTS OF TEMPERATURE ON PHOTOSYNTHESIS IN THE DIATOM *THALASSIOSIRA PSEUDONANA* UNDER UVR EXPOSURES¹

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Temperature is expected to modify the effects of ultraviolet radiation (UVR) on photosynthesis by affecting the rate of repair. We studied the effect of short-term (1 h) and long-term (days) acclimation to temperature on UVR photoinhibition in the diatom *Thalassiosira pseudonana* Hasle et Heimdal. Photosynthesis was measured during 1 h exposures to varying irradiances of PAR and UVR + PAR at 15, 20, and 25°C, the latter corresponding to the upper temperature limit for optimal growth in *T. pseudonana*. The exposures allowed the estimation of photosynthesis–irradiance (P–E) curves and biological weighting functions (BWFs) for photoinhibition. For the growth conditions used, temperature did not affect photosynthesis under PAR. However, photoinhibition by UVR was highly affected by temperature. For cultures preacclimated to 20°C, the extent of UVR photoinhibition increased with decreasing temperature, from 63% inhibition of PAR-only photosynthesis at 25°C to 71% at 20°C and 85% at 15°C. These effects were slightly modified after several days of acclimation: UVR photoinhibition increased from 63% to 75% at 25°C and decreased from 85% to 80% at 15°C. Time courses of photochemical efficiency (Φ_{PSII}) under UVR + PAR were also fitted to a model of UVR photoinhibition, allowing the estimation of the rates of damage (k) and repair (r). The r/k values obtained for each temperature treatment verified the responses observed with the BWF ($R^2 = 0.94$). The results demonstrated the relevance of temperature in determining primary productivity under UVR exposures. However, the results suggested that temperature and UVR interact mainly over short (hours) rather than long (days) timescales.

Key index words: biological weighting functions; photoinhibition; photosynthesis–irradiance curves; pulse-amplitude-modulated fluorescence; repair; temperature; *Thalassiosira pseudonana*; UVR

Abbreviations: BWF, biological weighting function; ERC, exposure response curve; PAM, pulse ampli-

tude modulated; P–E curve, photosynthesis–irradiance curve; T, transmittance; UVR, ultraviolet radiation

Ultraviolet radiation (UVR) and temperature are two environmental factors that affect phytoplankton growth and photosynthesis in natural waters (Roos and Vincent 1998, Doyle et al. 2005). In general, UVR inhibits photosynthesis and growth, while a rise in temperature usually increases growth and photosynthesis as a result of the enhancement of enzymatic activity. Cellular enzymes are involved in repair processes, such as the reactivation, resynthesis, or repair of damaged molecules, and are therefore essential to counteract the damage produced by UVR exposures.

Average temperature in a habitat depends on location, but variation around the average occurs at several timescales. Fast fluctuations can occur as a result of atmospheric changes, tidal displacements of the thermocline, and diurnal variation in solar irradiance. In the long term, changes in temperature are related to seasonal variations, interannual variability, and progressive increases in surface temperature due to global warming (IPCC 2001). As a result, increases in solar radiation are not always accompanied by synchronous increases in temperature, and the interaction of both these environmental factors can be more deleterious for aquatic organisms than the individual effect of each. For instance, the seasonal increase in incident UVR occurs earlier in the year than the rise in temperature, creating seasonal periods of higher UVR:temperature ratios (winter and early spring) and lower UVR:temperature ratios (summer and fall), which can affect organism survival and viability in dissimilar ways (Williamson et al. 2002).

Many of the photosynthetic molecules in phytoplankton cells are susceptible to direct damage by UVR [reviewed in Vincent and Neale (2000)]. Moreover, UVR can also indirectly affect several targets in the cells through oxidative stress (Rijstenbil 2002). Recovery from these types of damage requires the enzymatic activity of chloroplast- and nuclear-encoded proteins to bring about degradation of defective complexes as well as synthesis and

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integration of new complexes (Sass et al. 1997, Neale et al. 1998a, Heraud and Beardall 2000). Because of the numerous sites of UVR damage and the variability in the light and experimental conditions utilized, the exact molecular mechanism of damage and repair induced by UVR in the cell has not been well defined. In general, exposure to UVR decreases the cellular capability to assimilate the energy absorbed through photochemical processes. This can create an imbalance of energy that affects PSII excitation pressure, as indicated by the relative reduction state of the photosystem. Concomitantly, changes in temperature are able to induce changes in excitation pressure through the enhancement or reduction of the metabolic activity and its consequent effect on energy utilization (Huner et al. 1998). These changes dissipate or augment the reduction state of the photosystem and are therefore able to interact with UVR effects modulating the severity of the photoinhibitory response. Over longer (growth) timescales, excitation pressure resulting from the combined effects of temperature change and UVR can induce acclimatory responses, such as changes in carotenoid:chl *a* ratio (Roos and Vincent 1998). This can further modulate UVR sensitivity by protecting against oxidative stress and excessive PSII excitation pressure (Ivanov et al. 2000, Miskiewicz et al. 2000, Sobrino et al. 2005b).

The ameliorating effects of increased temperature under UVR on phytoplankton have been demonstrated for growth in cyanobacteria and natural lake assemblages (Rae and Vincent 1998, Roos and Vincent 1998, Doyle et al. 2005). There are also reports showing the beneficial effects of increased temperature on the germination rate, cell number, maximum quantum yield (F_v/F_m), and DNA repair rates of macroalgae (Pakker et al. 2000a,b, Van de Poll et al. 2002, Hoffman et al. 2003). However, the duration of temperature acclimation varies between studies, from nil or a few hours to several days, and no study has evaluated the effects of temperature on the magnitude of UVR photoinhibition comparing short- and long-term timescales.

Assessment of UVR effects on aquatic organisms depends on numerous factors, such as water transparency and organism sensitivity (Neale 2001), and is mainly related to the spectral composition of the incoming radiation. Biological weighting functions (BWFs) quantify the effectiveness (or "weight") of UVR in causing some biological effect in relation to wavelength [reviewed in Cullen and Neale (1997) and Neale (2000)]. They allow the comparison between biological responses to different spectral UVR conditions and can be utilized to predict responses if applied using an appropriate primary-productivity model. Recent publications have combined the BWFs for the inhibition of phytoplankton photosynthesis with primary-productivity models to assess photosynthesis in the presence of artificial or solar UVR. They showed 10-fold variations in the

sensitivity of phytoplankton photosynthesis to UVR measured by the BWFs, and decreases of 16%–30% in primary productivity of lakes, estuarine, and Antarctic waters (Neale et al. 1998b,c, 2001, Neale 2001, Hiriart-Baer and Smith 2004, Litchman and Neale 2005). The BWF is one part of a photoinhibitory term in a model [called the BWF/P–E (photosynthesis–irradiance) model] that also takes into account the kinetics of damage and repair (Cullen and Lesser 1991, Neale 2000). Damage and repair rates can be estimated from exposure response curves (ERCs) under UVR exposure using a pulse-amplitude-modulated (PAM) fluorometer (Neale et al. 1998a, Heraud and Beardall 2000, Litchman et al. 2002, Sobrino et al. 2005a). For a wide range of phytoplankton cultures and natural assemblages, responses are consistent with repair being proportional to UVR damage until reaching a steady state (i.e., repair balances damage). However, variations include cases of negligible repair (Neale et al. 1998b), the presence of thresholds (Sobrino et al. 2005a), or a steady state not being reached during exposure (Hiriart-Baer and Smith 2004).

Several studies have addressed the importance of temperature and UVR in the prediction of aquatic ecosystem response to future scenarios of global climate change (Williamson et al. 2002, MacFadyen et al. 2004, Sanders et al. 2005). The objective of this study was to assess to what extent changes in phytoplankton sensitivity to UVR, related to fast changes in temperature, remain after temperature acclimation. For that purpose, we chose a widely distributed diatom, *Thalassiosira pseudonana*, as a model species. Its growth temperature ranges from 4°C to 25°C, and it has been described as being relatively less sensitive to UVR than other species in estuaries (Banaszak and Neale 2001, Litchman and Neale 2005). In addition, the relevance of *T. pseudonana* as a model species for physioecological studies has increased since the recent publication of its complete genome (Armbrust et al. 2004). The present study shows the variations in the spectral and temporal response of *T. pseudonana* photosynthesis by using BWFs, and analysis of the rates of damage and repair, respectively, and compares the response to short-term and long-term changes in temperature for photosynthesis under PAR and UVR exposures.

MATERIALS AND METHODS

Culture growth conditions. *Thalassiosira pseudonana* culture was provided by the Provasoli-Guillard Center for Culture of Marine Phytoplankton (CCMP1335, strain 3H). The growth medium was filtered seawater from the Gulf Stream with salinity adjusted to 15‰ and enriched with f/2 nutrients (Guillard and Ryther 1962). *Thalassiosira pseudonana* was grown with constant aeration in semicontinuous cultures diluted every 2 d. Cultures were illuminated with 75 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ PAR irradiance (16.3 $\text{W} \cdot \text{m}^{-2}$ applying a 4.6 $\mu\text{mol photons} \cdot \text{J}^{-1}$ conversion factor) provided by cool-white fluorescent lamps under a 16:8 light:dark (L:D) photoperiod. Irradiance was measured inside a flask filled with filtered seawater using a 4 π

irradiance sensor QSL-2101 (Biospherical Instruments Inc., San Diego, CA, USA).

The study consisted of two sets of experiments to determine the effect of short-term versus long-term changes in temperature on photosynthesis sensitivity to UVR. The temperature limits selected for this study correspond to temperatures characteristic of the spring (15°C) and summer (25°C) in the Chesapeake Bay (Edgewater, MD, USA) region. *Thalassiosira pseudonana* forms extensive blooms during these seasons in the subestuaries that drain into the bay and typically disappears during winter and fall and during high summer temperatures (S. Hedrick, personal communication). Average temperature for the whole year in the Rhode River, a subestuary of the Chesapeake Bay, varies from 0°C in winter to 29°C in summer. In the first set of experiments, the cultures were grown at 15, 20, and 25°C during at least 1 week (>15 generations) to promote acclimation. Subsequently, the cultures were exposed to PAR or PAR + UVR at growth temperature to measure photosynthesis in terms of ¹⁴C uptake or effective quantum yield of PSII (Φ_{PSII}). In the second set of experiments, the cultures acclimated to 20°C were exposed to PAR or PAR + UVR at 15°C or 25°C to study the effect of a fast change in temperature on photosynthesis. In these experiments, the samples were acclimated to the selected temperature in the dark for 10 min previous to exposure. All the exposures were carried out during the exponential growth phase (day 2) under nutrient-replete conditions.

Cellular density and chlorophyll concentration. Culture cell density was counted using a Neubauer hemacytometer (Hausser Scientific, Horsham, PA, USA). The growth rate (μ , d⁻¹) was calculated as the slope of $\ln N(t)$ versus time during exponential growth phase, where $N(t)$ is the cell concentration on day t after standardized initial density of 0.75×10^6 cell · mL⁻¹ for cultures grown at 15°C and 20°C, and 0.3×10^6 cell · mL⁻¹ for cultures grown at 25°C.

The Q_{10} values were calculated as follows:

$$\ln Q_{10} = \frac{10(\ln V_2 - \ln V_1)}{(T_2 - T_1)} \quad (1)$$

where V_1 and V_2 are the growth rates at temperatures T_1 and T_2 , respectively.

Chlorophyll concentration was measured in aliquots concentrated on glass fiber filters (Whatman GF/F, Maidstone, UK) extracted with acetone 90%, and maintained in darkness at least overnight at 4°C. After centrifugation, absorption of the supernatant was measured spectrophotometrically at the appropriate wavelengths according to the equations of Jeffrey and Humphrey (1975).

Photosynthetic responses to PAR. Photosynthesis-irradiance curves for PAR-only exposure were obtained in a "photosynthetron" incubator (laboratory constructed) using a modification of the protocol described by Lewis and Smith (1983). The temperature-regulated incubator uses a halogen lamp to assess the photosynthetic response to PAR ($n = 24$) as the conversion of inorganic $H^{14}CO_3^-$ (approximately $0.7 \mu Ci \cdot mL^{-1}$) into organic compounds during a 1 h incubation. Photosynthetic parameters in PAR, P_s^B and E_s , were estimated using nonlinear regression fitting of the equation:

$$P^B = P_s^B \tanh\left(\frac{E_{PAR}}{E_s}\right) \quad (2)$$

where P^B is photosynthesis normalized to chl *a* content ($g C \cdot g chl^{-1} \cdot h^{-1}$), P_s^B is the maximal rate of photosynthesis, E_s is the saturation irradiance for PAR, and E_{PAR} is the PAR irradiance ($W \cdot m^{-2}$).

Photosynthetic response to UVR. Similar to the photosynthetron incubations, photosynthetic response to UVR was assessed

using ¹⁴C incorporation in a special polychromatic incubator, the "photoinhibetron" (Cullen et al. 1992, Cullen and Neale 1997, Neale and Fritz 2001). The incubator uses a 2500 W xenon lamp (Solar simulator lamp, Schoeffel Instrument Corp., Westwood, NJ, USA), which, after appropriate filtration, provides PAR, UVA, and UVB in similar proportions as solar irradiance. The beam passes through an array of eight long-pass filters (nominal 50% transmittance [T] at 280, 295, 305, 320, 335, 350, 370, and 395 nm), which are combined with neutral density screens to produce 10 irradiances for each filter for a total of 80 treatments of varying spectral composition and irradiance. Additional cellulose acetate film (<1% T at less than 288 nm) was also used with the 320, 305, 295, and 280 nm long-pass filters to eliminate exposure to very short wavelengths. The cellulose acetate was positioned after the long-pass filter in the optical path to minimize solarization. The long-pass filters and the cellulose acetate allow the transmission of wavelengths longer than the cutoff value (i.e., the wavelength of 50% T), while the neutral density screens reduce irradiance. The light treatments are directed to 1.8 cm diameter, flat-bottom quartz cuvettes that are mounted within a temperature-regulated block and filled with 1 mL of culture for exposure. Spectral irradiance was measured with a scanning monochromator (SPG 300 Acton Research, Acton, MA, USA) with a fiber optic and photomultiplier tube as previously described (Neale and Fritz 2001).

Model of UVR response: BWF_{Threshold}/P-E model (BWF_T/P-E model). The model is based on the PAR-only P-E curve equation, with the addition of a term that represents inhibition of photosynthesis by UVR and PAR and includes the BWF (eq. 3). The inhibition term relates the effect of UVR to "weighted" irradiance, E_{inh}^* [i.e., spectral irradiance scaled for biological effect or "weight" (eq. 4)], and accounts for the kinetics of UVR damage and repair during exposure (Neale 2000). The BWF_T/P-E model describes photosynthesis when repair completely compensates for damage, until damage exceeds repair (Sobrinho et al. 2005a). Above this threshold, photosynthesis declines according to the following equation:

$$P^B = P_s^B \tanh\left(\frac{E_{PAR}}{E_s}\right) \min\left(1, \frac{1}{E_{inh}^*}\right) \quad (3)$$

$$E_{inh}^* = \sum_{\lambda=280}^{400} \varepsilon(\lambda)E(\lambda)\Delta\lambda + \varepsilon_{PAR}E_{PAR} \quad (4)$$

where min denotes the minimum function, E_{inh}^* is a dimensionless index for the biologically effective or weighted irradiance, and $\varepsilon(\lambda)$ is biological weight [$(mW \cdot m^{-2})^{-1}$] at wavelength λ (nm). $E(\lambda)$ is spectral irradiance ($mW \cdot m^{-2} \cdot nm^{-1}$) at λ , and $\Delta\lambda$ is the wavelength resolution, 1 nm. Inhibition by PAR is included using a single, broadband weight, ε_{PAR} [$(W \cdot m^{-2})^{-1}$], for total PAR irradiance (Cullen et al. 1992). The BWFs were estimated from the measured rates of photosynthesis using nonlinear regression and principal component analysis of spectral irradiance, similar to that previously described (Cullen et al. 1992, Cullen and Neale 1997, Neale 2000). The BWFs were calculated for each temperature treatment ($n = 2-3$), and only two components were necessary to explain more than 99% of spectral variance. The BWFs shown in the manuscript correspond to the average BWF for each temperature treatment, with the standard error of the mean (SEM) for each wavelength calculated from individual error estimates by propagation of errors (Bevington 1969).

Estimation of the rates of damage and repair. The rates of damage (k , min⁻¹) and repair (r , min⁻¹) under UVR exposure at the different temperatures were estimated from the time course of PSII quantum yield. Our approach used fluorescence yield as measured using a PAM fluorometer, Diving PAM/B (Walz, Effeltrich, Germany) with blue LED (470 nm) excitation.

Exactly 1.3 mL of culture was placed in a quartz cuvette, mounted in a temperature-regulated holder, and side-illuminated using a small xenon lamp (150 W) filtered through selected filters. Emission and excitation irradiance was transmitted between the culture and the PAM through a fiber optic inserted in the top of the cuvette. The data are expressed as the quantum yield of PSII electron transport, $\Phi_{\text{PSII}} = (F_m' - F_s)/F_m'$, which has been correlated with variations in the quantum yield of photosynthesis (Genty et al. 1989). The variable F_s is the steady-state yield of in vivo chl fluorescence of phytoplankton, and F_m' is the maximum yield of fluorescence obtained from an illuminated sample after a saturating light pulse (400 ms pulse duration) was applied every 30 s. For each temperature treatment ($n = 2-3$) there were two different light exposures: (1) only PAR and (2) PAR + UVR. In each case, a dark-adapted sample was maintained without actinic illumination for 5 min to measure the maximum quantum yield ($F_v/F_m = [F_m - F_0]/F_m$) and was followed by periods (3-5 min) of increasing PAR irradiances (Schott GG 395 long-pass filter), avoiding PAR inhibition and promoting acclimation. When the maximal irradiance of 470 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (102 $\text{W} \cdot \text{m}^{-2}$) was reached, the 395 nm long-pass filter was maintained during at least 45 min for the PAR-only treatment. In the PAR + UVR treatment, the 395 nm long-pass filter was maintained for 15 min to be subsequently replaced by the 320 nm long-pass filter (Schott WG 320), resulting in the same level of PAR with added UVR. After 45 min under UVR, the 320 nm long-pass filter was replaced with the 395 nm long-pass filter for the study of recovery under PAR for 30 min. Treatment spectral irradiance was measured as described previously for the photoinhibitor. In order to measure responses exclusively attributed to the UVR effect, the Φ_{PSII} during the UVR + PAR treatment was normalized to the Φ_{PSII} after an equivalent period of PAR-only exposure. More details about the characteristics of this procedure are described in Sobrino et al. (2005a).

The UVR-dependent decrease in the quantum yield was used to fit a first-order function of exposure time:

$$P = \frac{r}{k} + \left(\frac{k-r}{k} \right) e^{-kt} \quad (5)$$

where P is Φ_{PSII} , t is time, and k and r are the damage and repair rates, respectively (Sobrino et al. 2005a). The steady-state rate of photosynthesis under UVR exposure should therefore be proportional to r/k .

RESULTS

Higher temperatures significantly increased the growth rate in *T. pseudonana* cultures (one-way analysis of variance [ANOVA], $P = 0.006$), with Q_{10} values close to 1.4 over the entire temperature range (Table 1). In contrast, cellular chl values and the photosynthetic parameters estimated from the P-E curves were similar across different growth temperatures (cf. Tables 1 and 2).

TABLE 1. Growth parameters (mean \pm SEM) of *Thalassiosira pseudonana* cultures grown at 15, 20, and 25°C ($n = 3-4$).

Growth parameters	Temperature		
	15°C	20°C	25°C
Growth rate (d^{-1})	0.77 \pm 0.01	0.88 \pm 0.04	1.04 \pm 0.05
Chl <i>a</i> ($\mu\text{g} \cdot \text{mL}^{-1}$)	1.43 \pm 0.25	1.97 \pm 0.17	1.30 \pm 0.30
Cellular chl <i>a</i> ($\text{pg} \cdot \text{cell}^{-1}$)	0.44 \pm 0.07	0.45 \pm 0.05	0.44 \pm 0.03

Exposure of *T. pseudonana* to UVR in the photoinhibitor allowed the estimation of BWFs for the inhibition of photosynthesis at the different temperatures. The predicted P^B values using the BWF_T/P-E model (eq. 3) were in good agreement with the observed rates of photosynthesis, showing a mean $R^2 = 0.97$. Based on the weighting factor, $\varepsilon(\lambda)$, the BWF_T/P-E model also allowed for the calculation of the effect of irradiance at every wavelength (λ) on the ^{14}C assimilation, with higher values of $\varepsilon(\lambda)$ indicating higher sensitivity to UVR. For all the treatments, $\varepsilon(\lambda)$ increased as λ decreased from 400 nm to 290 nm, similar to previous BWFs for inhibition of photosynthesis (Neale et al. 1998b, Litchman and Neale 2005; Figs. 1 and 2). The BWFs also showed that cultures grown at 20°C and exposed at 15°C were more sensitive than those exposed at the growth temperature (i.e., 20°C) over the entire UVR spectrum. In contrast, exposure of these cultures at 25°C decreased the sensitivity to UVR (Figs. 1 and 3). These results showed that sensitivity to UVR is related to temperature, as expected if net photodamage is dependent on repair processes. However, acclimation to 15°C for at least 1 week decreased the sensitivity to UVR compared with the nonacclimated cultures (Figs. 2a and 3), while acclimation to 25°C increased sensitivity, as shown by the higher $\varepsilon(\lambda)$ from 325 nm to 400 nm (Figs. 2b and 3). In both cases, acclimation to the shifted temperature did not significantly affect $\varepsilon(\lambda)$ below 300 nm. In order to compare changes in sensitivity among temperature treatments, $\lambda = 340$ nm was selected as a wavelength with high effectiveness for photoinhibition under solar exposures (Fig. 3). While the pattern of variation between temperature treatments in mean $\varepsilon(340)$ is similar to that described above for each set of replicate cultures, there was variation in the absolute weight between sets. The standard deviation of $\varepsilon(340)$ between experiments is therefore significantly higher than the SEM (shown in Figs. 1 and 2), although treatment differences are still significant (two-way ANOVA, $P = 0.003$).

The results, applying a summer midday solar spectrum for the Chesapeake Bay (Edgewater, MD, USA) and the estimated BWFs in the primary-productivity model (Eq. 3), showed that *T. pseudonana* cultures were very sensitive to incident UVR. In the cultures preacclimated to 20°C, the extent of UVR photoinhibition increased with decreasing temperature, from 63% inhibition of PAR-only photosynthesis at 25°C to 71% inhibition at 20°C (the growth temperature) and 85% inhibition at 15°C. These effects were slightly modified after several days of acclimation to the temperature of subsequent UVR exposure. The extent of UVR photoinhibition increased from 63% to 75% at 25°C and decreased from 85% to 80% at 15°C.

Average maximum Φ_{PSII} showed values of 0.604 \pm 0.018 (mean \pm SD) and did not exhibit significant differences among temperature treatments.

TABLE 2. Photosynthetic parameters (mean \pm SEM) of *Thalassiosira pseudonana* cultures estimated from photosynthesis-irradiance curves obtained during 1 h incubation in the photosyntheticron ($n = 2-3$).

Photosynthetic parameters	Temperature				
	G15°C-E15°C	G20°C-E15°C	G20°C-E20°C	G20°C-E25°C	G25°C-E25°C
P_s^B	2.13 ± 0.23	2.48 ± 0.03	2.38 ± 0.03	3.27 ± 0.57	2.46 ± 0.36
E_s	122.1 ± 4.7	126.5 ± 3.5	161.2 ± 23.6	177.1 ± 25.2	153.3 ± 13.8
R^2	1.00	1.00	0.99	1.00	1.00

P_s^B ($\text{g C} \cdot \text{g chl}^{-1} \cdot \text{h}^{-1}$) is the maximum photosynthetic rate, and E_s ($\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) is the light-saturation index. The temperatures in the column header correspond to growth temperature (G) and exposure temperature (E).

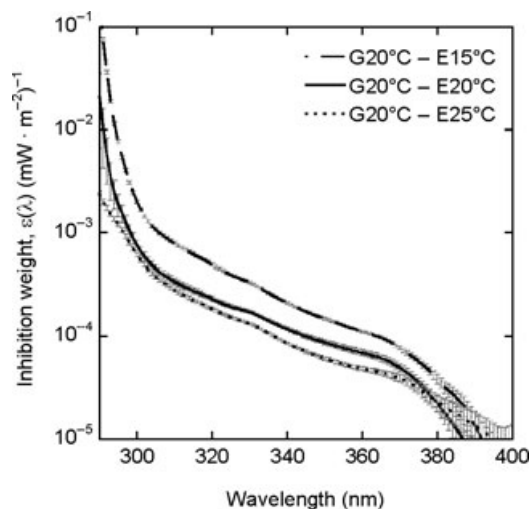


FIG. 1. Biological weighting functions (BWFs) for the inhibition of photosynthesis by ultraviolet radiation [$\varepsilon(\lambda)$, ($\text{mW} \cdot \text{m}^{-2}$) $^{-1}$] of *Thalassiosira pseudonana* grown at 20°C and exposed during 1 h at 15°C, 20°C, and 25°C. Symbol key indicates growth temperature (G) and exposure temperature (E). Values are the average BWF ($n = 2-3$) for each temperature treatment, with the SEM for each wavelength calculated from error estimates of individual BWFs.

After reaching the maximal irradiance of $470 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ($102 \text{ W} \cdot \text{m}^{-2}$), PAR exposures produced a flat time course in Φ_{PSII} in most cases. However, variations from a constant value (average maximum variation \pm SD = 0.038 ± 0.015) were sometimes observed (Fig. 4a), and a PAR correction was applied to the UVR time series to assess the UVR response exclusively (see Materials and Methods). Exposure of *T. pseudonana* to UVR resulted in an exponential decrease in Φ_{PSII} with time, similar to that observed in other phytoplanktonic species with an initial linear decrease, followed by a transitional phase and a final steady state in which damage was balanced by repair (Fig. 4b). Relative yields also recovered to values close to those at the beginning of the UVR exposure during a subsequent PAR-only exposure (Fig. 4b). However, unlike time courses measured for other species, shortly after steady state was attained in some of the samples, Φ_{PSII} began to increase progressively (Fig. 4b). The increase of Φ_{PSII} values under UVR exposures is interpreted as a rapid acclimation to UVR that has

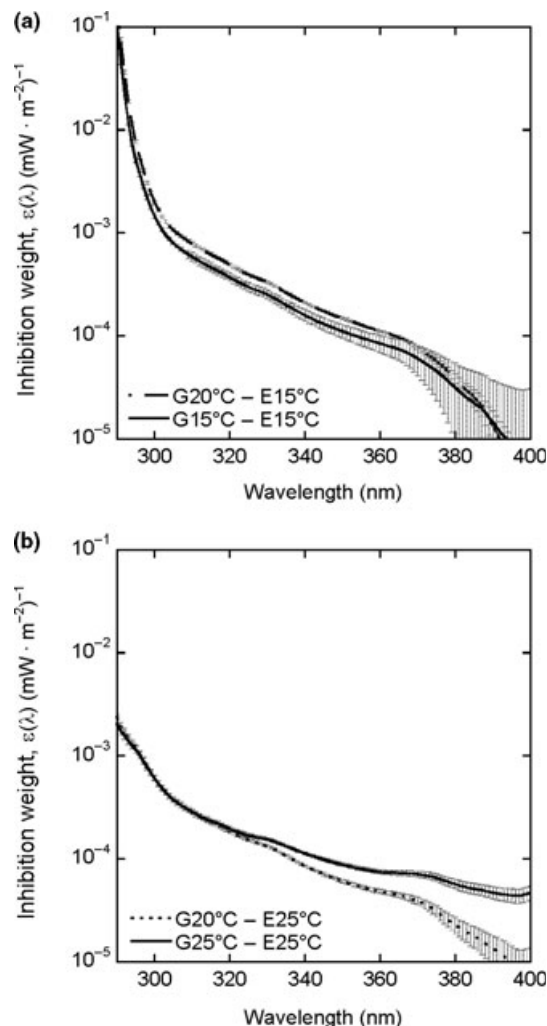


FIG. 2. Biological weighting functions (BWFs) for the inhibition of photosynthesis by ultraviolet radiation [$\varepsilon(\lambda)$, ($\text{mW} \cdot \text{m}^{-2}$) $^{-1}$] of *Thalassiosira pseudonana* after short- and long-term acclimation to (a) 15°C and (b) 25°C. Symbol key indicates growth temperature (G) and exposure temperature (E). Values are the average BWF ($n = 2-3$) for each temperature treatment, with the SEM for each wavelength calculated from error estimates of individual BWFs.

not been previously described in *T. pseudonana* or other species. The presence of acclimation complicates the estimation of damage and repair rates, and so this portion of the time course was omitted from the analysis.

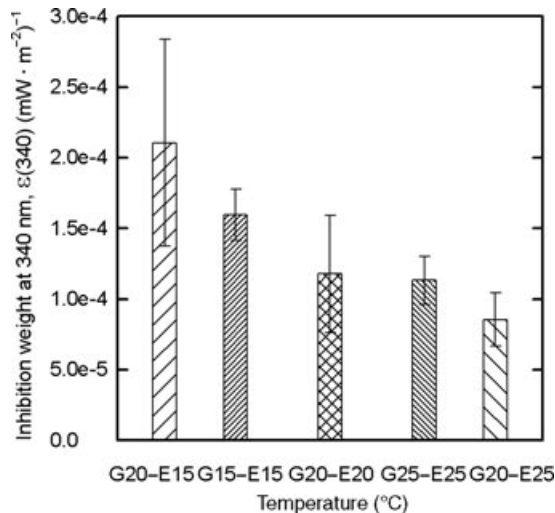


FIG. 3. Weighting factors at 340 nm [$\text{mean} \pm \text{SD}$, $(\text{mW} \cdot \text{m}^{-2})^{-1}$, $n = 2-3$] for the inhibition of photosynthesis of *Thalassiosira pseudonana* exposed to ultraviolet radiation under different temperature treatments. The SD shows the variability between replicate cultures. The labels of the x-axis indicate growth temperature (G) and exposure temperature (E).

In accordance with the results obtained from the BWFs, the decrease in Φ_{PSII} was larger in cultures exposed to a colder temperature than the growth temperature, and smaller when cultures were exposed to a warmer temperature. In addition, differences were smaller after acclimation to the selected temperature (Fig. 5). Rates of damage and repair estimated from the average decrease in Φ_{PSII} for each temperature treatment demonstrated that for our experimental conditions changes in temperature affected both damage and repair (Table 3). For cultures acclimated to 20°C, short-term temperature increases elevated the repair rates from 0.047 min^{-1} to 0.086 min^{-1} , while lower temperatures decreased repair rates to 0.036 min^{-1} (Table 3). Rates of damage were similar for the cultures grown at 20°C and exposed to UVR at 15°C and 20°C, but increased to 0.105 min^{-1} in the cultures exposed at 25°C (Table 3). Acclimation to temperature decreased the rates of damage and repair at 25°C but increased the rates in the cultures grown at 15°C (Table 3). Analysis of the r/k ratio as a good indicator of the sensitivity to UVR under the different temperature treatments showed that exposure to UVR using the 150 W Xe lamp decreased Φ_{PSII} to 67.1% of the PAR-only value for cells acclimated to 20°C (Table 3). In addition, comparisons with the PAR-only cultures for each temperature treatment demonstrated that fast changes in temperature from 20°C to 15°C and from 20°C to 25°C changed the Φ_{PSII} under UVR to 42.4% and 81.9%, respectively. After acclimation, Φ_{PSII} changed to 47.5% and 71.4% for the cultures at 15°C and 25°C, respectively (Table 3).

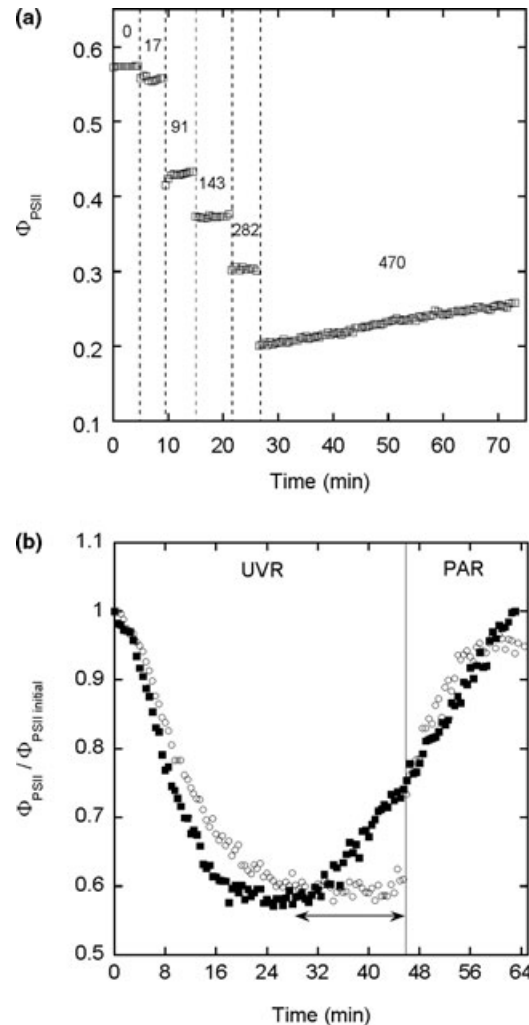


FIG. 4. Time course of PSII quantum yield (Φ_{PSII}) of *Thalassiosira pseudonana* under PAR (xenon lamp and 395 nm long-pass filter) and ultraviolet radiation (UVR) (xenon lamp and 320 nm long-pass filter) exposures. (a) Variations in Φ_{PSII} with increasing irradiance and during 45 min exposure to saturating PAR as observed for a culture grown at 25°C and exposed at 25°C. Times of progressive changes in light intensity from the darkness to the maximal irradiance of $470 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ($102 \text{ W} \cdot \text{m}^{-2}$) PAR in the pulse-amplitude-modulated cuvette obtained using different neutral density screens are indicated by vertical lines. The numbers in the graph correspond to the irradiance ($\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) used during each 3–5 min period. (b) Comparison between *T. pseudonana* (grown at 20°C-exposed at 15°C, ■) and *Nannochloropsis gaditana* (○) time courses of relative PSII quantum yield (Φ_{PSII}) under 45 min UVR and 30 min PAR exposures. Data for *N. gaditana* is from Sobrino et al. (2005a). The vertical line shows the end of the UVR and the start of the PAR exposure for the assessment of subsequent recovery. The horizontal arrow indicates the period of increase in Φ_{PSII} in *T. pseudonana* under UVR exposure attributed to an acclimation to UVR.

To quantitatively evaluate photosynthesis under UVR exposures predicted by both methods, PAM and ^{14}C incubations, we compared the r/k ratios and the potential photosynthetic rates ($1/E_{\text{inh}}^*$) estimated using the BWF $_7/P-E$ model, and the BWFs

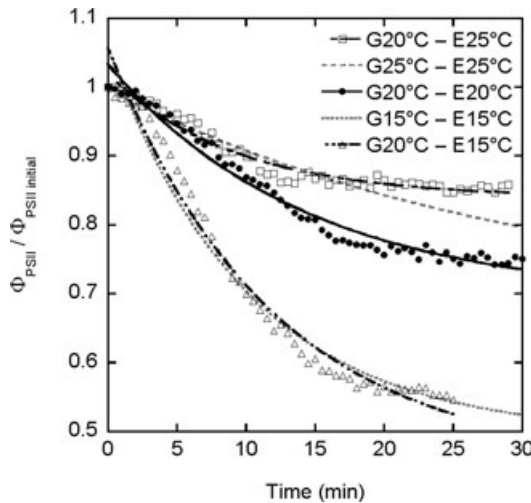


FIG. 5. Time course of average relative PSII quantum yield (Φ_{PSII} , $n = 2-3$) during 45 min of ultraviolet radiation exposure (xenon lamp and 320 nm long-pass filter) of *Thalassiosira pseudonana* under different temperature treatments. Curves for each temperature treatment were fit using nonlinear regression to equation 5 ($R^2 = 0.98-0.99$). The labels of the symbol key indicate growth temperature (G) and exposure temperature (E). Observed Φ_{PSII} values for G15°C-E15°C and G25°C-E25°C cultures are not shown for clarity. In those cases, only the nonlinear regression obtained from fitting the observed values is shown.

calculated for each temperature treatment (eq. 3). For this estimate, we utilized the spectra from the filtered 150 W Xe lamp, as used for the UVR exposures in the PAM cuvette. The relative differences among the different temperature treatments showed similar responses when calculated from both methods, with a regression of $1/E_{\text{inh}}^* = 1.08(r/k) + 13.3$ ($R^2 = 0.94$, $n = 5$; Fig. 6). The 95% confidence interval indicates that the regression is not significantly different from an equation with a slope of 1 and intercept 0 (i.e., $1/E_{\text{inh}}^* = r/k$; Fig. 6).

DISCUSSION

The growth rates, based on cell density, increased with the temperature in *T. pseudonana* cultures. The Q_{10} values were lower than the theoretical value of 2 but agreed with values previously reported for *T. pseudonana* grown in a similar temperature range (Berges et al. 2002). It has been observed that Q_{10}

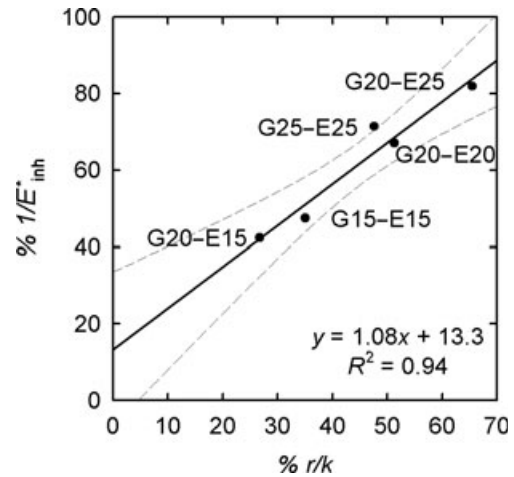


FIG. 6. Linear regression \pm 95% confidence interval of estimated relative photosynthesis of *Thalassiosira pseudonana* exposed to UVR using fluorescence yields [pulse-amplitude-modulated (PAM)] and ^{14}C assimilation (BWF_T/P-E model) for the different temperature treatments. The r/k ratios estimated from the time courses of relative PSII quantum yield and relative photosynthesis ($\frac{p^b}{p^a} = \frac{1}{E_{\text{inh}}^*}$), where E_{inh}^* is calculated using $\varepsilon(\lambda)$ and the $E(\lambda)$ reaching the PAM cuvette (eq. 4), are expressed as percentages of PAR-only values. The labels for each point indicate the growth temperature (G) and exposure temperature (E). r , repair rate; k , damage rate.

can vary from 1.2 to 3.1 in diatoms, depending on the degree of acclimation of the organisms and the range of temperature considered (Thompson et al. 1992, Smith et al. 1994, Berges et al. 2002). Berges et al. (2002) discuss the sources of variation in the Q_{10} values and also describes that in *T. pseudonana* cell C, chl *a* and the C:N ratio increased with increased temperature, concluding that C metabolism is more susceptible than N metabolism to changes in temperature (Lomas and Glibert 1999, Berges et al. 2002). In our study, *T. pseudonana* cultures showed no temperature-dependent changes in cellular chl or in the photosynthetic parameters estimated from P-E curves over 1 h PAR exposures. Discrepancies between growth and photosynthetic responses have been frequently observed (for review Davidson 1991). They are related to the fact that (gross) photosynthesis is not the only factor regulating growth. Other aspects of metabolism, such as the rate of dark respiration and release of organic

TABLE 3. Rates of damage (k , min^{-1}) and repair (r , min^{-1}) (mean \pm SEM) estimated from average Φ_{PSII} time courses ($n = 2-3$) of *Thalassiosira pseudonana* cultures exposed to UVR.

Rates of damage and repair	Temperature				
	G15°C-E15°C	G20°C-E15°C	G20°C-E20°C	G20°C-E25°C	G25°C-E25°C
r	0.047 \pm 0.004	0.036 \pm 0.004	0.047 \pm 0.004	0.086 \pm 0.008	0.025 \pm 0.004
k	0.099 \pm 0.006	0.085 \pm 0.007	0.070 \pm 0.006	0.105 \pm 0.009	0.035 \pm 0.005
% r/k	47.5 \pm 5.0	42.4 \pm 5.9	67.1 \pm 8.1	81.9 \pm 10.4	71.4 \pm 15.3
R^2	0.99	0.99	0.99	0.98	0.99

The SEM for % r/k was estimated by propagation of errors (Bevington 1969). The temperatures in the column header correspond to growth temperature (G) and exposure temperature (E).

carbon, can interact, affecting the final response. However, it is expected that following temperature acclimation realized rates of photosynthesis should exhibit a similar response to growth temperature as that of growth (Davidson 1991). We believe that in our study the lack of differences in the responses obtained for chl *a* and photosynthetic parameters is due to the acclimation of the cells to the low irradiance during growth (e.g., $75 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). This hypothesis is supported by Cullen (1990), who presents a model describing photosynthesis in *T. pseudonana* and shows that at a given temperature the adapted rate of photosynthesis normalized to chl is highly dependent on growth irradiance over a range from 20 to $2200 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. It is possible that differences in irradiance over the growth period due to self-shading enhanced the differences observed between the growth rates and the other parameters; that is, the chl *a* concentration and the photosynthetic parameters were always measured on day 2, whereas the calculated growth rate also represents growth in the early phase of the culture, which is less affected by self-shading. In addition, differences in the light transmission through the culture during the growth period produced by variations in chl concentrations can also be a source of the experimental variability observed in our study. This contention seems to be supported by the results shown in Figure 3, where the cultures with the highest chl concentration (e.g., cultures grown at 20°C) showed the largest variability in $\varepsilon(340)$.

Acclimation to low-light conditions also tends to increase sensitivity to UVR in *T. pseudonana* cultures and in Antarctic assemblages from deeply mixed water columns (Neale et al. 1998b, Litchman and Neale 2005). Predicted rates of photosynthesis as a fraction of P_s^B in the absence of UVR, using the estimated BWFs and an average summer midday spectra at the surface (Neale 2001), confirmed the high sensitivity of *T. pseudonana* grown at low PAR under no UVR. In the current study, a decrease of 71% of PAR-only photosynthesis was predicted for cultures at 20°C , comparable to the decrease predicted by Litchman and Neale (2005) (85% inhibition) for *T. pseudonana* cultures grown at $25 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Litchman and Neale (2005) showed that inhibition decreased to 26% for predicted midday rates of depth-integrated photosynthesis considering a 1.5 m water column and average spectral attenuation coefficient calculated for the Rhode River, a turbid subestuary of the Chesapeake Bay.

Predicted rates of photosynthesis in the presence of UVR using the fitted BWF_{T/P-E} model were in good agreement with those measured as ^{14}C assimilation for the different temperature treatments (average $R^2 = 0.97$). Unlike previous results from Litchman and Neale (2005), we used the BWF_{T/P-E} model instead of the BWF_{E/P-E} to predict photosynthesis in *T. pseudonana* (Sobrino et al. 2005a).

The differences in predictions from both models are very small, and the selection of one model rather than the other is mainly decided by the best-fit rule (i.e., the most appropriate model will be the one that gives higher R^2 and least bias in residuals). The repercussion of this small difference extends to the specific kinetics of damage and repair in the target organism (Neale 2000, Sobrino et al. 2005a). The BWF_{T/P-E} model applies to phytoplanktonic species with a constant rate of repair, while the BWF_{E/P-E} model is suitable for species in which repair rate during exposure is proportional to damage. An interesting characteristic of the BWF_{T/P-E} model developed from a constant repair rate is the presence of an explicit threshold for photosynthetic response to UVR. In the ERCs, repair fully counteracts damage until it exceeds repair; after this threshold, increased damage results in a net photoinhibitory response (Neale 2000, Sobrino et al. 2005a).

The BWFs for the inhibition of photosynthesis estimated for the different temperature treatments showed higher UVR sensitivity in cultures exposed to colder temperatures, and vice versa at warmer temperatures, after short-term changes in temperature. After acclimation to the shifted temperature, cultures still showed a similar pattern; however, differences were smaller than those observed in the short-term response. In fact, long-term acclimation to temperature decreased photoinhibition at 15°C , but slightly increased photoinhibition at 25°C when compared with the cultures at 20°C for a summer midday spectrum. The unexpected response after acclimation to 25°C may be explained by the proximity to the upper limit of the optimal temperature range for *T. pseudonana* (4°C – 25°C). At this temperature, some aspects of UVR defense and/or repair may already be under thermal stress, even though growth at 25°C was not detrimental for cell division or photosynthesis under PAR. In addition, acclimation to a selected temperature did not produce significant changes in $\varepsilon(\lambda)$ below 300 nm, the region of the solar spectra mainly affected by changes in the ozone-layer depletion. It is likely that changes in repair induced by the temperature acclimation were not large enough to counteract the damage produced by these highly energetic wavelengths.

Exposure to UVR resulted in an exponential decrease in Φ_{PSII} with time, approaching an asymptote as expected under moderately damaging light conditions in species with an active repair system (Heraud and Beardall 2000, Litchman et al. 2002, Sobrino et al. 2005a). The decrease in Φ_{PSII} under photoinhibitory UVR exposures was followed by an interesting increase in the Φ_{PSII} of *T. pseudonana*. This appears to be an initial phase of acclimation to UVR. Acclimation to UVR has been previously described for maximum Φ_{PSII} in macroalga after long-term exposures (days) to UVR (Van de Poll et al.

2002). In contrast, the acclimation to UVR in our study was observed after approximately 30 min UVR exposure, and it was more active in the cultures grown at 20°C and exposed at 15°C. The acclimation to UVR could be proportional to the degree of excitation pressure in the cell, resulting in stronger responses at low temperatures. Exposure to relatively high irradiance in the PAM cuvette compared with that during growth, plus the strong dependence of acclimation on growth irradiance of *T. pseudonana*, might have activated the UVR acclimation. So as to select the Φ_{PSII} values that were exclusively affected by photoinhibition, the acclimation portion of the curve was omitted for the estimation of the rates of damage (k) and repair (r) in *T. pseudonana* exposed to the different temperatures. However, it is also possible that acclimation was already affecting Φ_{PSII} before there was a net increase in the yields, resulting in higher estimates of the steady-state rates (i.e., r/k). The values for r and k were similar to those obtained for *Nannochloropsis gaditana* Lubián, a very sensitive picoplanktonic marine species, for similar light and temperature conditions. *Nannochloropsis gaditana* showed rates of $k = 0.068 \text{ min}^{-1}$ and $r = 0.048 \text{ min}^{-1}$ in cultures grown at 20°C, significantly higher than those reported for more resistant species, such as *Nannochloris atomus* Butcher ($k = 0.034 \text{ min}^{-1}$, $r = 0.022 \text{ min}^{-1}$) or *Dunaliella salina* (Dunal) Teodoresco ($k = 0.005 \text{ min}^{-1}$, $r = 0.005 \text{ min}^{-1}$), for similar UVR exposures (Heraud and Beardall 2000, Sobrino et al. 2005a). In the short term, the r/k ratio increased with the temperature mainly because of an increase in the rates of repair for similar rates of damage. Exposure to 25°C also significantly increased the rate of damage, probably due to similar reasons that produced more inhibition in the cultures acclimated to 25°C, as previously explained. In contrast, temperature acclimation changed both r and k and decreased the differences observed after short-term changes in temperature. The results also showed that there was an inverse relationship between temperature and the rates of damage and repair (i.e., higher temperatures had lower rates). Both our results and previous evidence demonstrate that variations in sensitivity to UVR, either due to acclimation to external conditions or species differences (i.e., *Thalassiosira* vs. *Dunaliella*) (Heraud and Beardall 2000, Sobrino et al. 2005a), occur because of changes in the rates of both damage and repair. Comparison of PAM fluorescence and ^{14}C incorporation, using the r/k ratio and predicted photosynthesis from the BWF₇/P-E model, showed good agreement. The results showed a linear relationship between both measurements, demonstrating the strong coupling between electron transport and carbon fixation under the different temperature treatments.

In an effort to estimate the amount of depth-integrated phytoplankton carbon fixation in the oceans, Behrenfeld and Falkowski (1997) encoun-

tered limitations in constraining photosynthetic parameters because of an unexplained variance in estimated P_s^B and disparities between functions describing temperature dependence. Differences in temperature sensitivity among primary productivity models can lead to dramatic differences in the prediction of the global warming responses (Sarmiento et al. 2004). Also, Behrenfeld and Falkowski (1997) noted that the improvement of productivity algorithms was dependent not on better mathematical formulation or finer detail in physics of light attenuation and absorption but on improvement in our understanding of phytoplankton ecology and photobiology. Our study shows that variation in UVR inhibition is an important source of temperature effects on phytoplankton photosynthesis because of the temperature dependence of repair. This temperature response was clearly evident in our measurements even though growth under low-light conditions obscured temperature effects on other PAR-related photosynthetic parameters. In addition, acclimation to temperature did not suppress this effect entirely, although interactions between multiple environmental factors can always limit the manifestation of temperature effects on phytoplankton photoinhibition by UVR (Doyle et al. 2005). Other factors, such as the optimal temperature range for UVR sensitivity and the capacity for temperature acclimation in each species, can also be relevant to assessing the interactive effects of UVR and temperature in the long term.

In conclusion, this study demonstrates that primary productivity in phytoplankton exposed to UVR is highly dependent on temperature. Higher temperatures decrease the sensitivity to UVR, and the opposite occurs with lower temperatures. The effect is mainly related to short-term changes in temperature but also remains to a smaller degree after long-term acclimation. The study also shows that the short-term changes in temperature affect sensitivity to UVR mainly because of changes in the rates of repair for similar rates of damage. However, this response is observed only during the short-term changes in temperature. More complex processes mediate long-term responses. As a result, acclimation to a selected temperature changed both damage and repair rate constants.

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