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Increased sensitivity to ultraviolet radiation in nitrogen-limited dinoflagellates: Photoprotection and repair

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Abstract

Nitrogen (N) limitation significantly increased the sensitivity of photosynthesis to inhibition by ultraviolet radiation (UV) in two estuarine dinoflagellates, *Akashiwo sanguinea* (= *Gymnodinium sanguineum*) and *Gymnodinium* (= *Gyrodinium*) cf. *instriatum*. Biological weighting functions (BWFs) and the kinetics of photosynthetic response to UV indicated that the main mechanism for the increase in sensitivity was less efficient repair. A decrease in cell size and in the concentration of the photoprotective mycosporine-like amino acids also elevated sensitivity. The BWFs predict that increased UV-B due to ozone depletion would cause a more than 1.5-fold greater additional inhibition of N-limited compared to nutrient-sufficient dinoflagellates. The BWFs of the N-limited cultures are similar to those measured for natural assemblages of phytoplankton in the Chesapeake Bay under low N availability.

Solar ultraviolet radiation (UV) (290–400 nm) is a significant stressor in aquatic environments. Its adverse effects on phytoplankton, the major primary producers in most aquatic ecosystems, are well documented: UV inhibits photosynthesis (Lorenzen 1979; Cullen et al. 1992) and growth (Ekelund 1990), and damages DNA (Karentz et al. 1991). Climate change and ozone depletion modify the intensity of UV stress (Pienitz and Vincent 2000), but predicting the effects of changing UV exposure is complicated by variable phytoplankton sensitivity (Neale et al. 1998*b*) resulting from differences in community composition or physiological state (Karentz et al. 1994), such as nutritional status.

Nitrogen (N) limitation can potentially increase phytoplankton susceptibility to UV by constraining defenses against UV as well as the ability to counteract photodamage. Phytoplankton repair UV-induced damage using several mechanisms, many of which involve N-requiring enzymes and/or protein cofactors (Roy 2000). Other enzymes are important in detoxifying UV-induced reactive oxygen species (Lesser and Shick 1989). Nitrogen is also required for UV-screening compounds such as mycosporine-like amino acids (MAAs). Another mechanism that protects photosynthetic electron transport is the dissipation of excess reductant through nonassimilatory reduction of NO_3^- (Lomas and Glibert 1999). In addition, N limitation can lead to a cell size reduction (Doucette and Harrison 1990), which decreases the pathlength for UV attenuation in the cell and thus increases UV-induced damage (Garcia-Pichel 1994).

The importance of N availability to many mechanisms of defense and repair of UV effects is noteworthy considering how widespread N limitation is in marine and fresh waters (Falkowski and Raven 1997). However, the consequences of N limitation for the magnitude and spectral dependence of UV effects on primary productivity are largely unknown. Photosynthesis was more inhibited by UV in N-limited compared to N-sufficient cultures of the diatom *Thalassiosira pseudonana* (Lesser et al. 1994). During UV exposures of 30 min to 4 h, cultures attained a steady-state rate of photosynthesis consistent with a balance between damage and repair processes. The steady-state rate was lower in N-limited cultures, indicating a shift in the balance toward greater damage, but the specific mechanisms shifting the balance between damage and repair could not be resolved. Moreover, information is needed on the spectral and temporal dependence of UV effects in N-limited phytoplankton to evaluate impacts on aquatic productivity.

Quantitative assessments of the effect of UV in the aquatic environment require a set of weights (a biological weighting function or BWF) that specify the biological effectiveness of UV radiation as a function of wavelength. A detailed BWF is particularly important for assessing the effect of enhanced UV-B (290–320 nm for solar UV) due to ozone depletion (Cullen et al. 1992; Neale and Kieber 2000). Because phytoplankton photosynthesis reflects the simultaneous, interacting effects of multiple UV and photosynthetically available radiation (PAR, 400–700 nm) wavebands, polychromatic exposures are used to estimate BWFs for UV inhibition of photosynthesis (Cullen et al. 1992). Comparing BWFs for phytoplankton that differ in their sensitivity to UV can give insight into the effectiveness of specific UV defense strategies. For example, the accumulation of MAAs has been related to decreased sensitivity of photosynthesis to the UV band absorbed by MAAs (320–360 nm) (Neale et al. 1998*a*).

UV impacts on aquatic productivity also depend on the kinetics of damage and recovery. Increased damage hastens inhibition, whereas decreased repair delays the attainment of steady-state and prolongs the recovery period (Neale 2000). Therefore, N limitation could accelerate, slow down, or have no net effect on inhibition kinetics depending on the relative contribution of increased damage versus decreased repair to

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increased UV sensitivity. Lesser et al. (1994) observed that steady-state photosynthesis (^{14}C assimilation) was attained during UV exposure even in N-limited cultures; however, the temporal resolution of the study was insufficient to determine whether the rate of inhibition was different between N-limited and N-sufficient cultures. A better temporal resolution of the kinetics of inhibition and recovery of photosynthesis is now possible using active fluorometry (Neale et al. 1998*a;* Heraud and Beardall 2000).

Dinoflagellates are an important component of many estuarine and coastal food webs, especially under stratified, N-limited conditions, and often cause harmful algal blooms that affect many coastal ecosystems (Anderson et al. 1998). Previous work showed that defense against UV in nutrient-replete cultures of the dinoflagellate *Akashiwo sanguinea* (= *Gymnodinium sanguineum*) (Daugbjerg et al. 2000) involves both photoprotective and repair processes (Neale et al. 1998*a*). We examined how N limitation affects UV sensitivity of two common red-tide dinoflagellates, *A. sanguinea* and *Gymnodinium* (= *Gyrodinium*) cf. *instriatum* (Daugbjerg et al. 2000). In this study, we grew both species under nutrient-replete and N-limited conditions and then determined the BWFs for UV inhibition of photosynthesis for cultures grown under both conditions. The kinetics of inhibition and recovery were defined using active fluorometry measurements of the quantum yield of photosystem II (PSII) before, during, and after the exposure to UV (Neale et al. 1998*a*). The photoprotective potential was also estimated using cellular biooptical models of self-shading (Garcia-Pichel 1994).

Materials and methods

Cultures and growth conditions—Clones of the red-tide dinoflagellates *Akashiwo sanguinea* (Hirasaka) G. Hansen et Moestrup and *Gymnodinium* cf. *instriatum* Freudenthal et Lee isolated by D. Wayne Coats from the Rhode River, a subestuary of the Chesapeake Bay, were grown in monocultures under nutrient-replete and N-limited conditions. The nutrient-replete medium was a standard ''f/2'' enrichment (Guillard 1975) of filtered Gulf Stream seawater diluted to 15‰ salinity. The N-limited medium was the same f/2 enriched diluted seawater but with a reduced NO_3^- content (5 μM or 25 μM vs. 883 μM of the standard f/2). All cultures were grown at an irradiance of 76 W m^{-2} provided by cool-white fluorescent lights with no UV on a 14 : 10 h light : dark cycle at 25°C. Algae were first grown in the nutrient-replete f/2 medium in batch regime for 1 week and then aliquots of the exponentially growing cultures were inoculated into media with different nitrate concentrations. After a week of batch growth, the daily dilutions of cultures started. The dilution rates were adjusted so that cell density remained more or less constant during the experiment. The BWF experiments were performed after at least 1 week of semicontinuous regime, except for the BWFs of nutrient-replete *G.* cf. *instriatum,* for which batch cultures were used.

Biological weighting functions—The dependence of photosynthesis on PAR and inhibition by UV radiation was measured as the uptake of $H^{14}CO_3^-$ under 72 spectral treatments (eight UV cutoffs and nine intensities) during a 1-h exposure to a Xe (solar simulator) lamp with irradiance and spectral composition of exposures similar to previous experiments with *A. sanguinea* (Neale et al. 1998*a*). BWFs were determined from the measured rates of photosynthesis using principal component analysis of spectral irradiance and nonlinear regression as previously described (Cullen et al. 1992). The data were fit to the following equation:

$$P^B = P_S^B(1 - e^{-E_{\mathrm{PAR}}/E_S})\frac{1}{1 + E^*_{\mathrm{inh}}} \quad (1)$$

where P^B is the rate of photosynthesis normalized to Chl *a* content (mg C mg Chl a^{-1} h^{-1}), P_s^B is the saturated rate of photosynthesis in the absence of photoinhibition, and E_s is a saturation parameter for PAR irradiance (E_{PAR}, W m^{-2}). E^*_{inh} is a dimensionless inhibition index defined as follows:

$$E^*_{\mathrm{inh}} = \sum_{\lambda=280}^{400} \epsilon(\lambda)E(\lambda)\Delta\lambda \quad (2)$$

where $\epsilon(\lambda)$ is the biological weight (reciprocal mW m^{-2}) at wavelength λ (nm) and $E(\lambda)$ is the spectral irradiance at λ (mW m^{-2} nm^{-1}). BWFs were estimated for each experiment, and the mean BWF for each N treatment was calculated, with confidence limits for the mean derived from individual error estimates of $\epsilon(\lambda)$ by propagation of errors (Bevington 1969).

Kinetics of inhibition and recovery—The quantum yield of PSII photochemistry was measured with a PAM 101US fluorometer (Walz) as previously described (Neale et al. 1998*a*). The maximum quantum yield (F_v/F_m) was measured in the dark, and the quantum yield during illumination ($\Delta F/F'_m$) was measured at 30 s intervals during PAR and PAR + UV exposure as described previously (Neale et al. 1998*a*). Briefly, algae were acclimated to a PAR-only (140 W m^{-2}) illumination, then the inhibition time course was monitored after supplementation with moderate UV. The recovery was monitored after removal of UV and simultaneous lowering of PAR to 60 W m^{-2}. The rates of inhibition and recovery were determined by fitting a first-order exponential equation to the time series: $a_1 + a_2\exp(-r_{\mathrm{inh}}t)$ for inhibition and $a_1 - a_2\exp(-r_{\mathrm{rec}}t)$ for recovery where a_1 is the asymptotically approached equilibrium quantum yield, a_2 is the change in quantum yield over the time course of inhibition or recovery, t is time (min), and r_{inh} and r_{rec} are the rate constants (min^{-1}) of inhibition and recovery, respectively.

Other physiological parameters—Cultures were sampled every day or every other day, and cell densities were determined using a Sedgwick-Rafter counting chamber. Growth rates were calculated as the slopes of the natural logarithms of cell densities versus time, adjusted for a dilution rate. Cell dimensions were measured under the microscope with an ocular micrometer, and cell volumes were calculated using the formula for an approximating geometric figure (prolate ellipsoid). Also, the effective diameter of a sphere of equivalent volume was calculated to characterize potential photoprotective properties (Garcia-Pichel 1994). Chlorophyll *a* (Chl *a*) concentration and particulate absorbance were measured as described previously (Neale et al. 1998*a*). The con-

Table 1. Physiological parameters (mean ± SE) of N-limited and nutrient-replete cultures of *Akashiwo sanguinea* and *Gymnodinium* cf. *instriatum*. P_S^B is the saturated rate of photosynthesis in the absence of photoinhibition, and E_s is a light saturation constant, as estimated from the fitting of the BWF/P-I model (Eq. 1). The coefficient of determination (R^2) for the overall fit is given (mean for each culture and condition). Within each species, treatments were compared using a one-way ANOVA. Values that are significantly different ($P < 0.05$) are indicated by asterisks.

	A. sanguinea			*G.* cf. *instriatum*	
Parameter	Nutrient-replete	25 μM NO_3^-	5 μM NO_3^-	Nutrient-replete	5 μM NO_3^-
Growth rate, day^{-1}	0.25 ± 0.02	0.15* ± 0.013	0.11* ± 0.012	0.35 ± 0.023	0.09* ± 0.011
Cell volume, 10^4 μm^3	4.46 ± 0.37	1.53* ± 0.11	1.39* ± 0.10	3.19 ± 0.2	2.39* ± 0.27
Effective diameter, μm	44.0	30.8*	29.8*	39.4	35.7
Chl *a*, pg $cell^{-1}$	25.0 ± 2.0	14.2* ± 1.52	8.5** ± 1.0	31.3 ± 2.2	8.06* ± 0.23
P_S^B, g C g Chl a^{-1} h^{-1}	4.57 ± 0.73	4.66 ± 0.75	3.63 ± 0.36	4.31 ± 0.77	1.71 ± 0.21
E_s, W m^{-2}	77.41 ± 16.49	95.19 ± 19.31	68.51 ± 8.75	58.84 ± 12.11	59.77 ± 12.27
R^2	0.90	0.90	0.91	0.89	0.90

centrations of MAAs in *A. sanguinea* were determined by reverse-phase, isocratic, high performance liquid chromatography (HPLC) as described previously (Neale et al. 1998*a*).

Biooptical calculations—We calculated the sunscreen factor, *S*, and the dose modification factor, DMF (=1 − *S*) (Garcia-Pichel 1994), for nutrient-replete and the 25μM nitrate cultures (*A. sanguinea* only) relative to the most N-limited cultures from the particulate absorbances at 1 nm intervals, assuming the background efficiency for self-shading to be equal to that of the most N-limited culture. For *A. sanguinea*, *S* and DMF were also calculated using the average cellular MAA concentration in each N treatment and assuming the average absorbance of MAAs to be 2.7×10^{-2} L g^{-1} μm^{-1} (Garcia-Pichel 1994). The resulting inhibition index, E^*_{inh}, was then calculated with the spectral irradiance multiplied by the appropriate DMF.

We also estimated the relative contribution of cell size to photoprotection by comparing the E^*_{inh} of the N-limited cells with the E^*_{inh} of the cells with the same low MAA (or absorbance) as in N-limited cells but with the size equal to nutrient-replete cells.

Predicted photosynthesis under different UV conditions—We calculated the effects of different UV exposures on photosynthesis of N-limited and nutrient-sufficient cultures of the two species. Clear-sky spectral irradiances were calculated using a radiative transfer model (Ruggaber et al. 1994) for 40°N at summer solstice, under different ozone conditions (concentration in Dobson Units [100 DU = 1 mm O_3 at STP]). The BWF-based estimates of photosynthesis were determined from Eqs. 1 and 2.

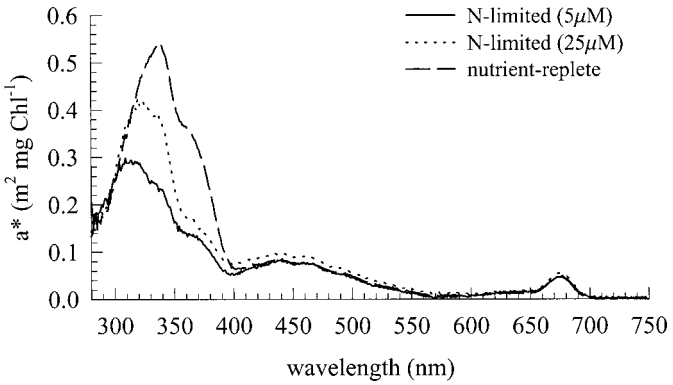

Fig. 1. Chlorophyll-normalized spectral absorbances of nutrient-replete (average of two cultures) and nitrogen-limited cultures [25 μM NO_3^- (average of three cultures) and 5 μM NO_3^- (average of six cultures)] of *A. sanguinea*.

Results

Physiological parameters—N limitation significantly changed major physiological parameters of *A. sanguinea* and *G.* cf. *instriatum*. The growth rates, cell volumes, and cellular chlorophyll concentration of N-limited cultures of both species declined significantly under N limitation (Table 1). The largest decline in growth rates and cell volumes occurred between replete and 25 μM cultures, with little additional change at 5 μM. In contrast, chlorophyll content decreased over the full range of N availability.

Particulate absorbances and mycosporine-like amino acids—N limitation decreased chlorophyll-normalized absorbance of *A. sanguinea* (Fig. 1) and *G.* cf. *instriatum* (data not shown) in the UV region (290–400 nm), compared to nutrient-sufficient cultures. At 330 nm, the absorbances of the 5 μM nitrate cultures of both species decreased about twofold compared to their nutrient-replete cultures. At 370 nm, the relative decrease was even greater: 2.6-fold in *A. sanguinea* and 4.3-fold in *G. cf. instriatum*. As was found previously for these dinoflagellates, high absorbance in the UV region is mainly due to the presence of photoprotective compounds, MAAs, which are induced by high PAR in the absence of UV under nutrient-replete conditions (Neale et al. 1998*a*). The decrease in particulate absorbance in the UV region with N limitation corresponded to a dramatic decrease in cellular MAA concentrations, more than sixfold and threefold decreases in the 5 μM and 25 μM nitrate cultures of *A. sanguinea*, respectively (Fig. 2). The decrease in MAAs under N limitation was greater than the decrease in cellular chlorophyll concentration (about threefold and twofold decreases in Chl *a* in the 5 μM and 25 μM nitrate cultures, respectively).

Not all MAAs declined equally. Mycosporine-glycine de-

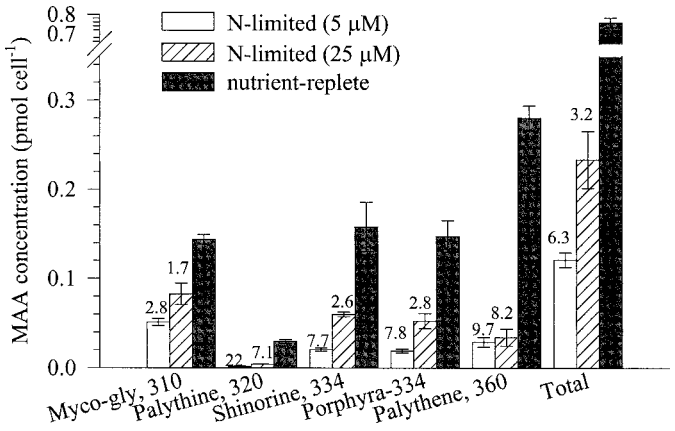

Fig. 2. Cellular concentrations of mycosporine-like amino acids (pmol cell^{-1}) in N-replete (average of two cultures) and N-limited cultures [25 μM (average of three cultures) and 5 μM NO_3^- (average of five cultures)] of *A. sanguinea.* Numbers next to the MAA names are wavelengths of their absorbance peaks. Numbers above the bars are times decrease in a given MAA compared to nutrient-replete culture.

clined the least (Fig. 2, Myco-gly). This MAA is the least N rich among all MAAs found in these dinoflagellates, containing one atom of N (all other MAAs found in this dinoflagellate have two atoms of N, Dunlap and Shick 1998) and offers protection in the most harmful part of the spectrum (absorbance peak 310 nm). The increase in the relative concentration of the MAA with the shortest wavelength absorbance peak agreed well with the observed shift in the absorbance peak to shorter wavelengths and an increase in the 310/370 nm ratio with increased N limitation (Fig. 1).

Biological weighting functions—The BWF/P-I model provided a good fit to measured photosynthesis as a function of UV exposure for all growth conditions (R^2, Table 1). The changes in physiological parameters due to N limitation were accompanied by a significant increase in the sensitivity to UV in both species as determined by the BWF comparison (Fig. 3a,b). The overall sensitivity was the highest under the most N-limited conditions (Fig. 3a). Sensitivity increased across the whole UV spectrum and, outside of the 320–360 nm band for *A. sanguinea,* the sensitivity of the 25 μM and 5 μM cultures was about the same. The greatest change in sensitivity for *A. sanguinea,* however, was in the spectral region of highest absorption by MAAs (320–360 nm, cf. Fig. 1). Within this spectral region, there also was divergence between the BWFs of the 25 μM and 5 μM cultures although the difference was not statistically significant. The increase in sensitivity in *A. sanguinea* is spectrally correlated with a decrease in cellular absorbance, with the greatest difference in absorbance and largest proportional increase in sensitivity both occurring for UV around 340 nm wavelength (Fig. 3c). The BWFs for nutrient-replete and 5 μM cultures of *G.* cf. *instriatum* were similar to the BWFs of *A. sanguinea* except that the region of low sensitivity around 340 nm prominent in the nutrient-replete *A. sanguinea* culture was absent from the *G.* cf. *instriatum* BWF.

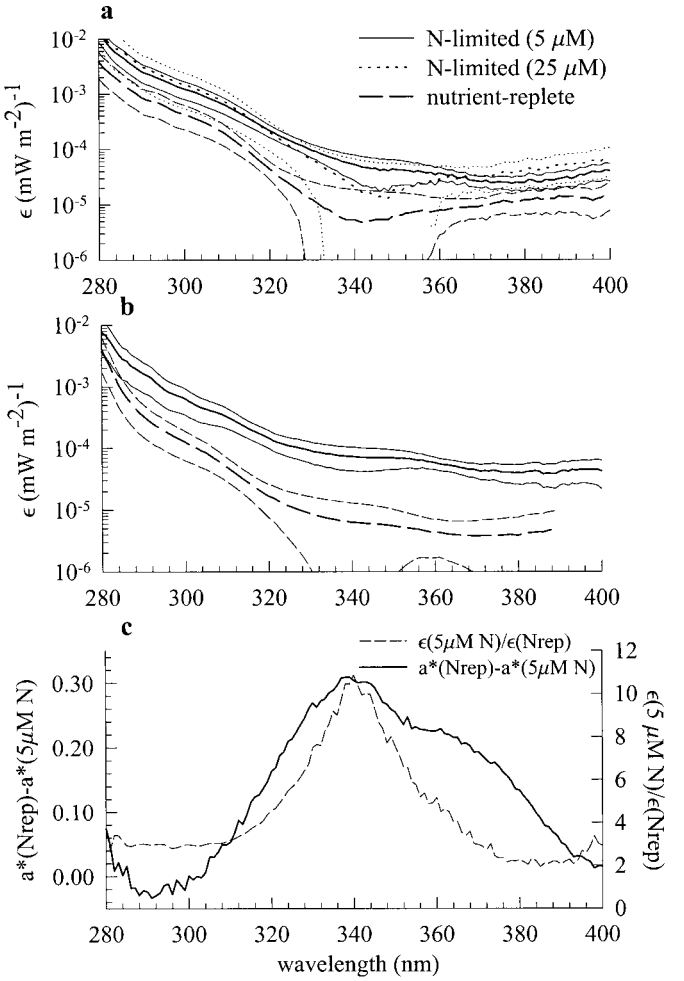

Fig. 3. Average biological weights (BWF) for photoinhibition of photosynthesis by UV ($\epsilon(\lambda)$, reciprocal mW m^{-2}) for nutrient-replete and N-limited cultures. The thick lines are the estimated averages and the thin lines are the 95% confidence intervals of the estimates. (a) *A. sanguinea,* nutrient-replete (n = 2), 25 μM NO_3^- (n = 3) and 5 μM NO_3^- (n = 6); (b) *G.* cf. *instriatum* nutrient-replete (dashed line, n = 3) and 5 μM NO_3^- (n = 2); and (c) the ratio of the BWFs of the 5 μM NO_3^- cultures to nutrient-replete cultures (*A. sanguinea)* compared to the difference between their average absorbances.

Kinetics of photoinhibition and recovery—The higher UV sensitivity of N-limited cultures was also reflected in the time course of photosynthesis during UV exposure. We used the quantum yield of PSII photochemistry during exposure to actinic light ($\Delta F/F_m'$) as an indicator of the overall quantum yield of photosynthesis. The quantum yield of the nutrient-sufficient culture was hardly affected by the moderate UV exposure, dropping only to 92–95% of the preexposure (PAR-only) level (Fig. 4). As expected from the increase in the BWF, N-limited *A. sanguinea* (5 μM NO_3^-) were much more sensitive, decreasing to 70–75% of the preexposure level (Fig. 4). Quantum yield in even the most N-limited culture (5 μM NO_3^-) approached steady-state after about 25–30 min from the beginning of UV exposure, indicating that damage was counteracted by ongoing repair (Lesser et al.

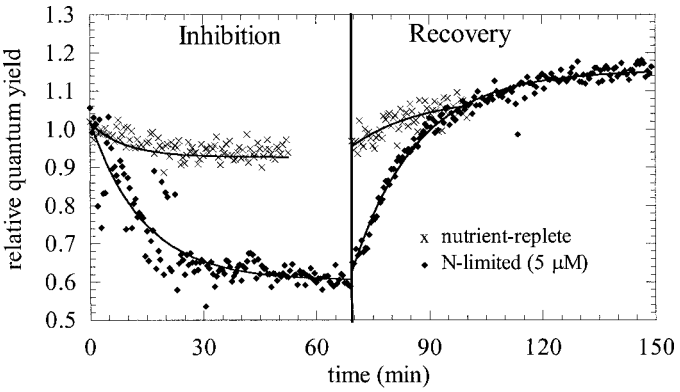

Fig. 4. Time course of the inhibition and recovery of the quantum yield for PSII photochemistry ($\Delta F/F'_m$) under UV exposure in nutrient-replete and 5 μM NO_3^- cultures of *A. sanguinea*. The lines are first-order kinetic curves fitted to the data (*see equations in Materials and Methods*). The quantum yields were normalized to the values at the beginning of the UV exposure.

1994; Neale et al. 1998*a*). The transition to steady-state was, however, slower in N-limited cultures (*t*-test, $P < 0.05$). The average rate of inhibition (r_{inh}) in *A. sanguinea* was 0.058 ± 0.012 min^{-1} ($n = 6$) in the 5 μM NO_3^- cultures versus 0.150 ± 0.026 min^{-1} ($n = 3$) in nutrient-replete cultures. This corresponds to an almost threefold increase in the time constant for inhibition, from 6.6 to 17.2 min. The rate of recovery (r_{rec}) also decreased from 0.120 ± 0.039 min^{-1} in N-replete cultures to 0.056 ± 0.011 min^{-1} in the 5 μM cultures, corresponding to time constants of 7.9 min and 17.9 min, respectively. The dark-adapted efficiencies of PSII (F_v/F_m) were similar in N-limited and nutrient-replete cultures (0.69 on average). In *G.* cf. *instriatum* the rate constants for inhibition and recovery also decreased under N-limited conditions, although the difference was not significant ($P > 0.05$). The inhibition rate constants were 0.15 ± 0.050 min^{-1} ($n = 3$) and 0.17 ± 0.052 min^{-1} ($n = 3$) for the 5 μM NO_3^- and nutrient-replete cultures, respectively. The rate constants of recovery were 0.096 ± 0.026 min^{-1} ($n = 3$) and 0.12 ± 0.021 min^{-1} ($n = 3$) for the 5 μM NO_3^- and nutrient-replete cultures, respectively.

Discussion

Comparison with previous results—The absorbance peak and the MAA concentration in nutrient-replete cultures of *A. sanguinea* in this study were slightly lower than those reported in Neale et al. (1998*a*) for *A. sanguinea* grown under the same light and temperature conditions (absorbance peaks of 0.54 and 0.89 m^2 mg Chl^{-1} and the MAA concentrations of 0.76 pmol $cell^{-1}$ and 0.99 pmol $cell^{-1}$, for nutrient-replete cultures in this study and in Neale et al. (1998*a*), respectively). The difference may be due to different culturing regimes: daily dilutions in this study versus batch culturing in Neale et al. (1998*a*).

A decrease in cellular MAA concentration in *A. sanguinea* under N limitation (ca. sixfold in the most N-limited cultures) was similar in magnitude to a decrease in MAA concentration in cultures grown under low light (Neale et al. 1998*a*) (5.5-fold). In contrast to the low light conditions that caused an increase in sensitivity only in the region of MAA absorbance (Neale et al. 1998*a*), N limitation increased sensitivity of the *A. sanguinea* culture over the whole UV spectrum. The increase in sensitivity outside the MAA absorbance region is likely due to a decreased cell size and slower repair. The relative contribution of these three mechanisms to the change in sensitivity is discussed in more detail in the following section.

The sensitivity of the nutrient-replete *A. sanguineum* in this study was higher than the sensitivity of the nutrient-replete cultures in the study of Neale et al. (1998*a*). This agrees with the lower concentration of MAAs in this study and, again, could be due to differences in culturing regimes (semicontinuous versus batch cultures). The most N-limited culture of *A. sanguinea* was more sensitive than the low-light grown cultures in Neale et al. (1998*a*): the biological weights at 340 nm were 5.28×10^{-5} and 1.87×10^{-5} $(mW\ m^{-2})^{-1}$ respectively. This high sensitivity agrees with lower cellular MAA concentration (0.12 vs. 0.19 pmol $cell^{-1}$ in low-light cultures) and a slower repair in these cultures.

Effectiveness of photoprotection and repair—Comparison of the BWFs for nutrient-replete versus the most N-limited *A. sanguinea* cultures shows increased sensitivity over the full UV spectrum plus a spectrally specific component of increased sensitivity in the MAA absorbance range. This suggests that nutrient limitation led to a decrease in MAA photoprotection as well as to changes in more spectrally independent mechanisms such as defense and repair enzymes. To evaluate the relative importance of these different mechanisms in the context of solar exposure, we estimated the effect of an exposure to noon solar irradiance at summer solstice under typical ozone conditions of 300 DU. Under these conditions, biologically effective exposure, E^*_{inh} (Eq. 2), is about 4 times higher in the N-limited cultures compared to the N-replete cultures. The change in E^*_{inh} due to decreased MAA concentration and cell size (photoprotection) relative to the most N-limited culture can be estimated using a cellular biooptical model of self-shading (Garcia-Pichel 1994; Neale et al. 1998*a*). The relative sunscreen factor, *S*, and the dose modification factor, DMF, were calculated directly from the cellular diameter and MAA concentration (Garcia-Pichel 1994). This calculation suggested that the combined effect of less screening by MAAs and smaller cell size doubles E^*_{inh} for the most N-limited culture relative to the N-replete culture. While this is a significant increase, it is still substantially less than the observed increase in E^*_{inh} (four times) in N-limited versus N-replete cultures. An alternate approach, estimating the DMF from the particulate absorbances, also results in a predicted increase in E^*_{inh} due to MAA screening (1.8 times) that is smaller than the observed increase. Again, the calculated increase includes the effect of decreased cell size, which accounts for about 30% of the overall change in exposure.

One measure of the relative contribution of spectrally independent processes is the ratio between the N-limited and N-replete BWFs outside of the 320–360 nm region. This ratio varies between two to three times higher sensitivity in the N-limited (5 μM) versus nutrient-replete cultures (Fig.

3c). Decreases in cell size would be expected to increase average cellular exposure to UV, and thus sensitivity, across the spectrum. However, optical calculations suggested that the specific effect of size is small (about 1.2 times increase). Therefore, changes in defense and repair mechanisms probably account for most of the spectrally independent shift in sensitivity. Although reduced defense and repair both increase sensitivity, each mechanism is expected to have contrasting effects on the kinetics of inhibition and recovery. This is illustrated by the predictions of a simple kinetic model in which the overall impairment of photosynthesis (P) by UV from a reference level, P_{initial}, is described as

$$dP/dt = -kP + r(P_{\text{initial}} - P) \quad (3)$$

where k is the rate of damage and r is the rate of repair. For constant irradiance (constant k), Eq. 3 can be solved to give the relative change in photosynthesis as an exponential approach to an asymptotic rate:

$$\frac{P}{P_{\text{initial}}} = \frac{r}{k + r} + \frac{k}{k + r}e^{-(k+r)t} \quad (4)$$

(Lesser et al. 1994; Heraud and Beardall 2000). According to this model, a decrease in the steady-state ($r/[k + r]$) is caused by an increased rate of damage (k) and/or a decreased rate of repair (r). Equation 4 has the same general form as the exponential equations used to fit the time course of quantum yield (Fig. 4), in particular, r_{inh} can be identified as $k + r$. According to the model, therefore, an increase in damage (k) as caused by less effective photoprotection and defense will increase r_{inh}, while a decrease in repair (r) will decrease r_{inh}. Our observation that r_{inh} was substantially slower for N-limited cultures of *A. sanguinea* suggests that decreased repair capacity is the dominant cause of the increased sensitivity. Indeed, the ratio of r_{inh} between N-replete and N-limited cultures is 2.6, which is in the same range as the spectrally independent increase in sensitivity calculated from the BWFs. The rate of recovery after UV exposure was also faster in N-replete cultures, though the difference was less, only about 2.3-fold. However, the recovery time courses may also reflect the transition kinetics in PSII quantum yield resulting from the decrease in PAR from 140 to 60 W m^{-2}, e.g., due to the relaxation of nonphotochemical quenching (Ting and Owens 1994). For *G.* cf. *instriatum*, r_{inh} increased slightly (though not significantly) in N-limited cultures, which suggests that decreased repair capacity is as important, or more important, as increased damage in increasing sensitivity to UV for this species as well.

Changes in both the spectral dependence (BWF) and the temporal dependence of UV responses indicate that decreased repair contributes at least as much as increased damage to greater sensitivity of N-limited cultures. However, there are several limitations to this analysis. The rate of damage depends also on the absorption cross-section of the photochemically active molecules (chromophores) involved in the damage (Lesser et al. 1994). It is not known how this cross-section changes in N-limited cultures, primarily because the identity of the chromophore(s) involved in damage to the photosynthetic apparatus is unknown, though there are several candidates (Vincent and Neale 2000). Photoprotection by MAAs could also exceed the predictions of the biooptical model if there was a nonhomogenous distribution in the cell, targeted around sensitive organelles, as has been previously suggested (Neale et al. 1998*a*).

A proportionately greater decrease in MAA concentration compared to chlorophyll under N limitation suggests a preferential allocation of the limiting resource (N) to the essential photosynthetic pigments. Moreover, mycosporine-glycine, the only MAA known to date with antioxidant properties and with a peak absorbance at the shortest, most harmful wavelength (Dunlap and Yamamoto 1995), decreased the least. This may indicate an optimization of the resource allocation even within one class of compounds (MAAs).

The shifts in UV sensitivity that occurred in these dinoflagellates may also occur in other large-celled phytoplankton species. Decreased growth rates and cellular Chl *a* concentration are common responses to N limitation in phytoplankton (Osborne and Geider 1986). A cell size decrease under N limitation has also been reported previously for dinoflagellates (Doucette and Harrison 1990) and other microalgae (Riegman et al. 2000). A decrease in UV-absorbance was also observed in N-depleted compared to N-replete cultures of the diatom, *Rhizosolenia formosa* (Richardson et al. 1996). These common physiological responses to N limitation can cause a significant increase in the sensitivity of photosynthesis to UV.

N limitation increased the UV sensitivity of these relatively large-celled species through several mechanisms (a decrease in repair, in the concentration of photoprotective compounds, and in cell size). Small-celled species (cell radius < 10 μm) may not exhibit all of these responses because small cells do not have significant amounts of MAAs or other photoprotective compounds (Garcia-Pichel 1994) and a cell size decrease may not be pronounced, due to a physiological limit on the lowest cell size (Raven 1998). In small-celled species, the main mechanism for increased sensitivity under N limitation may be a decline in repair enzymes.

Predicted photosynthesis under different UV conditions—The observed shifts in the BWFs due to N limitation strongly affect predicted inhibition of photosynthesis by solar UV. Based on the E^*_{inh} calculated for surface irradiance at summer solstice (as in the previous section) and Eq. 1, the predicted photosynthetic rate of the most N-limited *A. sanguinea* (5 μM NO_3^-) is 41% of the rate of nutrient-sufficient *A. sanguinea* (Table 2). Similarly, a N-limited culture of *G.* cf. *instriatum* would have a photosynthetic rate that is 32% of the nutrient-replete culture (Table 2).

The inhibition of photosynthesis by UV for N-limited dinoflagellates is further enhanced under elevated solar UV-B as occurs due to ozone depletion. Under low N availability (5 μM NO_3^-), the relative additional inhibition in both dinoflagellates due to ozone depletion (50% ozone decrease) would be about 1.5 times greater than under N-replete conditions (Table 2). Moreover, under moderate ozone depletion (33% ozone decrease) the difference in the relative additional inhibition between nutrient-replete and N-limited cultures would be even greater (Table 2).

The increased sensitivity to ozone depletion is mainly due

Table 2. Predicted effects of solar UV exposure (midday, surface, summer solstice at 40°N) on photosynthesis in nutrient-replete and N-limited cultures of *A. sanguinea* and *G.* cf. *instriatum.* Numbers are proportions of the maximum rates of photosynthesis for a given culture in the absence of UV. Values in parentheses are percent increased inhibition due to O_3 depletion, compared to inhibition under normal ozone conditions (300 DU): $(P_{300} - P_{low})/P_{300} \cdot 100\%$, where P_{300} is the photosynthetic rate (proportion of the maximum) under 300 DU and P_{low} is the photosynthetic rate under low ozone conditions (200 or 150 DU).

	Ozone	Nutrient-replete	25 μM NO_3^-	5 μM NO_3^-
A. sanguinea	300 DU	0.51	0.21	0.21
	200 DU	0.47 (7.8%)	0.18 (14%)	0.18 (14%)
	150 DU	0.44 (13.7%)	0.17 (19%)	0.17 (19%)
G. cf. *instriatum*	300 DU	0.66	—	0.21
	200 DU	0.63 (4.5%)	—	0.19 (9.5%)
	150 DU	0.60 (9%)	—	0.18 (14%)

to the increase in sensitivity to UV-B. However, N depletion increased sensitivity across the UV part of the spectrum and, thus, any other environmental change that increases UV will be amplified under conditions of N depletion. For example, a decrease in chromophoric dissolved organic matter (CDOM) could also increase UV penetration and cause a greater inhibition of photosynthesis in N-limited phytoplankton, particularly in estuarine and coastal waters, which presently have relatively high concentrations of CDOM.

Behrenfeld et al. (1994) found that the growth rate of a N-limited diatom was affected less by UV than the growth rate of a nutrient-replete culture because the N limitation effect exceeded the potential effect of UV. Under their experimental conditions, nitrogen availability was very low (<1 μM nitrate). While UV inhibition of photosynthesis may have been significant in their nitrogen-limited cultures, daily production was apparently still sufficient to support as much growth as in the UV excluded cultures. They concluded that growth rate may not be a good indicator of UV stress under nutrient limitation. In our study, UV inhibited the photosynthesis of N-limited cultures more than of the nutrient-replete cultures. Consequently, photosynthesis may be a more sensitive indicator of UV stress under N limitation, although lower rates of photosynthesis do not always result in lower growth rates.

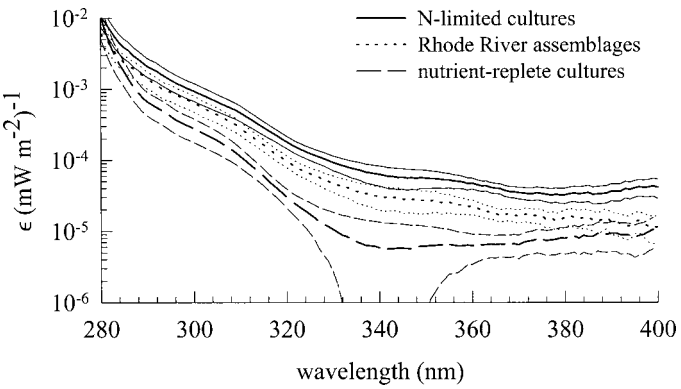

Fig. 5. Average biological weighting functions for inhibition of photosynthesis of the 5 μM NO_3^- cultures of *A. sanguinea* and *G.* cf. *instriatum,* their nutrient-replete cultures and the average BWF for the Chesapeake Bay (Rhode River) dinoflagellate-dominated summer assemblages (1998–1999, $n = 4$) shown in thick lines. The corresponding 95% confidence intervals are shown in thin lines.

Comparison with the UV sensitivity of natural communities—Low N availability may be one of the reasons why natural dinoflagellate communities generally are more sensitive than cultures grown under nutrient-replete conditions (Neale and Kieber 2000). The UV sensitivity of these two dinoflagellates under N-limited conditions was higher than the average sensitivity of nutrient-replete cultures and approached the average sensitivity of natural phytoplankton surveyed to date (Neale and Kieber 2000), including dinoflagellate-dominated summer assemblages in the Chesapeake Bay under low N availability (Banaszak and Neale 2001; Litchman and Neale unpubl. data, Fig. 5). The concentrations of nitrate in the Rhode River, a subestuary of the Chesapeake Bay, when dinoflagellates are present are similar to or even lower than the concentrations used in our study (Gallegos 1992). The spectral shape of the BWFs of the N-limited cultures is also closer to the shape of the summer dinoflagellate-dominated assemblages than the shape of the nutrient-sufficient dinoflagellate cultures (Fig. 5).

The extent to which sensitivity to UV is elevated for phytoplankton in environments other than the Chesapeake Bay is unknown, and more work is needed with N-limited natural assemblages. Variations in sensitivity also occur due to species composition (Xiong et al. 1996, Litchman and Neale unpubl. data). Moreover, N availability, temperature, and average growth irradiance exercise a combined influence on sensitivity to UV by determining the overall excitation pressure on the photosynthetic apparatus (Ivanov et al. 2000). Under conditions of chronic exposure, as occurs in stably stratified, optically clear oligotrophic environments, excitation pressure may induce UV defenses and a lowering of sensitivity despite resource limitation. On the other hand, acquisition of N for photoprotection and repair by UV-exposed assemblages may be further affected by direct UV inhibition of N uptake (Behrenfeld et al. 1995) and decreased motility of flagellates (Häder and Häder 1991). The latter effect could impair N acquisition occurring during diel migration to the nutricline. Low N availability may increase phytoplankton sensitivity to UV further, thus potentially creating a positive feedback between N limitation and the UV

sensitivity. A consideration of the numerous, and possibly counteracting, factors affecting UV responses will be needed before applying our conclusions to different N-limited environments. Nevertheless, the present results show that the sensitivity of nutrient-replete cultures is not an appropriate basis for assessing the impact of UV on the productivity of natural assemblages in N-limited environments. These environments are globally distributed and include both marine and freshwater ecosystems (Falkowski and Raven 1997). Thus, a better understanding of how N limitation modifies UV sensitivity in these diverse environments will significantly advance our ability to assess UV effects on global primary productivity.

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