

## CARBON UPTAKE IN A MARINE DIATOM DURING ACUTE EXPOSURE TO ULTRAVIOLET B RADIATION: RELATIVE IMPORTANCE OF DAMAGE AND REPAIR<sup>1</sup>

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### ABSTRACT

Experiments on a marine diatom, *Thalassiosira pseudonana* (Hustedt) clone 3H, demonstrate that under moderate photon flux densities ( $75 \mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) of visible light the inhibition of photosynthesis by supplemental ultraviolet (UV) radiation (UV-B: 280–320 nm) is well described as a hyperbolic function of UV-B irradiance for time scales of 0.5–4 h. Results are consistent with predictions of a recently developed model of photosynthesis under the influence of UV and visible irradiance. Although net destruction of chlorophyll occurs during a 4-h exposure to UV-B, and the effect is a function of exposure, the principal effect of UV-B is a decrease in chlorophyll-specific photosynthetic rate. The dependence of photoinhibition on dosage rate, rather than cumulative dose, and the hyperbolic shape of the relationship are consistent with net photoinhibition being an equilibrium between damage and repair. The ratio of damage to repair is estimated by a mathematical analysis of the inhibition of photosynthesis during exposures to UV-B. A nitrate-limited culture was much more sensitive to UV-B than were the nutrient-replete cultures, but the kinetics of photoinhibition were similar. The analysis suggests that the nutrient-limited culture was more sensitive than the nutrient-replete cultures because repair or turnover of critical proteins associated with photosynthesis is inhibited. An inhibitor of chloroplast protein synthesis was used to suppress repair processes. Photoinhibition by UV-B was enhanced, and inhibition was a function of cumulative dose, as would be expected if damage were not countered by repair. The fundamental importance of repair processes should be considered in the design of field experiments and models of UV-B effects in the environment, especially in the context of vertical mixing. Repair processes must also be considered whenever biological weighting functions are developed.

**Key index words:** *Bacillariophyceae*; carbon uptake; photoinhibition; photosynthesis; repair processes; *Thalassiosira pseudonana*; UV-B

Global decreases in stratospheric ozone have been observed in both the northern (Blumthaler and Am-

bach 1990) and southern hemispheres (Frederick and Snell 1988). The anthropogenically induced decrease in stratospheric ozone permits an increase in the flux of biologically damaging mid-ultraviolet (UV-B; 290–320 nm) radiation reaching the biosphere (Solomon 1988, Hardy and Gucinski 1989, Smith 1989). Environmental ultraviolet radiation, particularly UV-B, is known to have deleterious effects on many biological processes (Worrest 1982) such as photosynthesis and growth in terrestrial plants (Tevini and Teramura 1989) and phytoplankton (Lorenzen 1979, Smith et al. 1980, Smith and Baker 1982, Jokiel and York 1984, Bühlmann et al. 1987), nitrate uptake in diatoms (Döhler and Biermann 1987), locomotion in protists (Häder and Häder 1988), growth and photosynthesis of zooxanthellae in culture (Jokiel and York 1982, Lesser and Shick 1989), and growth of macroalgae (Wood 1987). Both UV-A (320–400 nm) and UV-B wavelengths can penetrate to ecologically significant depths in seawater (Jerlov 1950, Smith and Baker 1979, Calkins and Thordardottir 1980, Wood 1987, Smith et al. 1992), with the absorption of UV radiation largely dependent on the concentration of chlorophyll and dissolved organic matter (Smith and Baker 1979, 1989). Recent measurements in the Antarctic, using an underwater spectroradiometer, detected UV-B radiation down to a depth of 70 m (Smith et al. 1992).

In a previous publication (Cullen and Lesser 1991), we considered the effects of dose and dosage rate on the inhibition of photosynthesis by UV-B radiation. Here, we analyze further the kinetics of carbon uptake and the variation in chlorophyll content during exposure to UV-B radiation, comparing results to predictions of a recently developed model (Cullen et al. 1992a) and seeking empirical evidence for the balance between damage and repair that is implied in the model. We present evidence indicating that the synthesis of proteins associated with photosynthesis is important in repairing damage to the photosynthetic apparatus (Kok 1956, Van Baalen 1968, Hirose and Miyachi 1983, Ohad et al. 1984, Samuelsson et al. 1985, Neale 1987), which is manifested by changes in photosynthetic performance. These processes complicate efforts to describe the biological weighting function for photoinhibition of photosynthesis by UV radiation. These repair processes are also likely to be important in the ocean, where the damage inflicted by UV-B radiation may be reversed during vertical transport away from near-surface UV-B exposure.

<sup>1</sup> Received 24 May 1993. Accepted 13 December 1993.

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## MATERIALS AND METHODS

Culture conditions and experimental procedures were described in detail by Cullen and Lesser (1991). Important points and additional procedures are presented here. A culture of the marine diatom *Thalassiosira pseudonana* (clone 3H) was obtained from the Provasoli-Guillard Culture Collection for Marine Phytoplankton and grown on a 12:12 h LD cycle at 20° C. The containers were polycarbonate, and the cultures were bubbled with acid-scrubbed air and stirred. Nutrient-replete semicontinuous cultures were kept in exponential phase using f/2 growth medium (Guillard 1975). Nitrate-limited continuous cultures were maintained at a growth rate of 0.3 d<sup>-1</sup> using f/2 growth medium with 50 μM nitrate. Illumination for all cultures was from Vita-Lite full-spectrum fluorescent lamps providing a quantum scalar irradiance of 75 μmol quanta·m<sup>-2</sup>·s<sup>-1</sup> (photosynthetically active radiation) as measured by a Biospherical Instruments QSL-100 4π sensor immersed in a water-filled culture vessel.

**Analyses.** The concentration of chlorophyll *a* was measured fluorometrically using a calibrated Turner Designs 10-005R fluorometer on triplicate samples of 1 mL collected on Whatman GF/F filters and extracted in 10 mL of 90% acetone and dimethylsulfoxide (6:4 vol/vol) at -4° C in the dark for at least 24 h. This solvent mixture yields results equivalent to those obtained using 90% acetone (cf. Stramski and Morel 1990).

The same fluorometer was used for discrete measurements of the fluorescence of chlorophyll *a* *in vivo*. Fluorescence was measured after at least 30 min acclimation in the dark and then again after exposure to 3 × 10<sup>-6</sup> M DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) (Vincent et al. 1980). The initial fluorescence reading (F) was made after 15 s in the fluorometer, then DCMU was added. Fluorescence in the presence of DCMU (F<sub>d</sub>) was recorded after 45 s in the fluorometer. Distilled water served as the blank. The cellular fluorescence capacity index was then calculated [(F<sub>d</sub> - F)/F<sub>d</sub>] as one measure of the physiological condition of the culture (Vincent et al. 1980).

Triplicate specimens of 50 mL were taken for determination of particulate carbon and nitrogen. These samples were filtered onto baked (450° C for 4 h) GF/F filters and stored in a desiccator. Specimens were frozen at -50° C and freeze-dried overnight immediately prior to use. Samples were combusted in a Control Equipment Corporation (Perkin Elmer) 240 XA elemental analyzer with an automatic sampler in an air-tight box to keep the samples dry. Acetanilide was used for a standard; prefiltered culture medium was passed through baked filters and used as blanks.

**Carbon uptake during exposure to UV-B.** Samples were placed in open glass dishes that were exposed in an experimental incubator to uniform visible light from below and a range of UV-B fluxes from above. Air temperature was controlled at 20° C. Visible light at 75 μmol quanta·m<sup>-2</sup>·s<sup>-1</sup> was provided by Vita-Lite fluorescent lamps shone through UV-B opaque acrylic (cut-off, 385 nm) and a neutral-density screen. Measurements of photosynthesis vs. photosynthetically active radiation (PAR; not shown) indicate that this irradiance is within the light-limited portion of the photosynthesis-irradiance relation but exceeds the characteristic photon flux density (PFD) for the onset of light saturation, I<sub>k</sub> (=P<sub>k</sub>/α). Decreases in photosynthesis at this PFD can result from changes in P<sub>k</sub> (maximum photosynthetic rate) or α (maximum photosynthetic efficiency). UV radiation came from either two or four aged (200 h) fluorescent lamps (FS40 T12-UV-B, Bulbtronics Inc., Farmingdale, NY) suspended 60 or 50 cm, respectively, above the samples. Six different fluxes of UV radiation (2, 8, 14, 37, 72, and 100% of incident) were obtained by placing perforated nickel screens over the dishes. An acrylic plate, opaque to UV-B (cut-off, 385 nm), was placed over another dish, which served as a control. A rotating table was used to stir the samples continually during the experiment. Except where stated, the UV-B lamps were filtered with aged cellulose diacetate to attenuate shorter wavelengths not encountered in nature (Caldwell et al.

1986, Cullen and Lesser 1991). The spectral output of these lamps represents an unnatural radiation regime enriched in UV-B (see Fig. 1, Cullen and Lesser 1991) and without the higher UV-A and visible radiation that would be encountered in nature. Our approach here was mechanistic in nature in order to describe the kinetics of carbon uptake.

Photosynthesis was measured as the uptake of <sup>14</sup>C-bicarbonate. At the outset, a portion of the culture was harvested and inoculated with a solution of <sup>14</sup>C-bicarbonate to a final specific activity 0.7 μCi·mL<sup>-1</sup>. Aliquots of 25 mL were dispensed into the series of glass dishes, which were placed in the experimental incubator. Duplicate subsamples of 1 mL were taken after an initial period of 60 min, when only the visible lamps were on, then periodically over the next 240 min, when both the UV and visible lamps were illuminated. The subsamples were dispensed into scintillation vials, immediately poisoned with 50 μL borate-buffered formalin, then acidified with 0.25 mL 6 N HCl and shaken in a hood to expel inorganic <sup>14</sup>C. Subsamples (20 μL) from the original inoculation were placed in 4 mL fluor plus 0.2 mL phenethylamine to determine the amount of label added. It was determined in a pilot experiment that the amount of label or the number of cells in each dish did not change significantly in the open dishes during the experimental period.

Measurements of photosynthesis indicated that visible light from below was uniform: the variation in carbon uptake for the seven treatments during the first 60 min in visible light was consistent with the average deviation between duplicate subsamples during the course of the experiment. We concluded that during exposure to UV radiation differences between samples were attributable to UV-B rather than to differences in visible light. Some PAR came from the FS40 lamps, however. For the two experiments using two lamps, it was a minor component: about 4 μmol quanta·m<sup>-2</sup>·s<sup>-1</sup> at the 100% UV-B dish and at the control dish, proportionally less in the other treatments. For the experiment with four lamps at 50 cm, maximum PAR from the UV lamps was 11 μmol quanta·m<sup>-2</sup>·s<sup>-1</sup>. Thus, light-dependent carbon uptake was underestimated in the low-UV treatments because they received less PAR than the controls. For each experiment, a linear correction was made for this second-order effect; the measured carbon uptake was multiplied by the ratio of PAR in the control to PAR in the treatment.

Spectral irradiance (E(λ), mW·m<sup>-2</sup>·nm<sup>-1</sup>) from the UV lamps was measured using a calibrated diode-array spectroradiometer system (Cullen and Lesser 1991). Biologically effective fluence rate (E\*<sub>inh</sub>, dimensionless) for different treatments was calculated as ∑ E(λ) ε(λ) Δλ using a biological weighting function (ε(λ), λ = 286–390 nm, units of reciprocal mW·m<sup>-2</sup>) for the inhibition of photosynthesis in the diatom *Phaeodactylum tricoratum* (Cullen et al. 1992a). The bare lamps emitted radiation <286 nm, for which weightings were not determined. The comparison between bare and filtered lamps, however, was sufficiently interesting to warrant tentative estimation of weightings for those wavelengths. The extrapolated weightings were obtained from the generalized plant action spectrum (Caldwell 1971) as tabulated by Smith et al. (1980). Values for 275–285.8 nm were obtained at 0.2-nm intervals using linear interpolation and scaled to match E(λ) for *Phaeodactylum* at 286 nm. Weightings were assumed constant between 270 and 275 nm. For the analysis presented here, essentially identical results were obtained using extrapolation schemes based on Setlow's (1974) DNA action spectrum and an action spectrum for DNA damage in alfalfa seedlings (Quaite et al. 1992).

**Inhibition of repair.** To assess the effects of repair mechanisms on short-term carbon uptake, an experiment was carried out on a culture exposed to four FS40 UV lamps in the presence of the antibiotic streptomycin. Our definition of repair for these experiments is the difference in photosynthetic rate associated with exposure to streptomycin vs. cultures not exposed to streptomycin. The repair or synthesis of new proteins encoded in the

TABLE 1. Parameters measured prior to exposure to UV-B to assess the physiological status of the experimental cultures. All measurements were made just prior to the experiment. R2 = nutrient replete subsequently exposed to two UV lamps, L2 = nutrient limited subsequently exposed to two UV lamps, R4 = nutrient replete subsequently exposed to four UV lamps; CFC = cellular fluorescence capacity. Carbon uptake was measured for 1 h prior to exposure to UV-B radiation. C:N and C:chlorophyll ratio  $\mu\text{g}/\mu\text{g}$  ( $\pm$ SD).

Experiment	CFC	Chlorophyll [ $\mu\text{g L}^{-1}$ ( $\pm$ SD)]	Carbon uptake (mg C mg chloro- phyll <sup>-1</sup> h <sup>-1</sup> )	C:N ratio	C:chloro- phyll ratio
R2	0.61	91.17 $\pm$ 4.79	3.86	4.99 $\pm$ 0.84	37.1
L2	0.56	107.30 $\pm$ 5.05	3.18	8.95 $\pm$ 1.26	90.2
R4	0.63	91.50 $\pm$ 3.48	3.54	4.95 $\pm$ 0.64	36.8

chloroplast to replace proteins damaged by exposure to UV-B radiation is assumed to be the cause of the observed differences. The antibiotic streptomycin is an RNA translation inhibitor acting upon the *de novo* synthesis of chloroplast-encoded proteins, particularly the D1 protein, on the 30s subunit of the 70s ribosomes (Ohad et al. 1984, Samuelsson et al. 1985). Previous studies using inhibitors of cytoplasmic protein synthesis and transcription have demonstrated that the primary site of photoinhibitory repair is the chloroplast (Ohad et al. 1984, Samuelsson et al. 1985, Greer et al. 1986, Kyle 1987). It is possible that during these short-term experiments mitochondrial protein synthesis could also be affected. We would expect this to have a larger effect on growth than on net photosynthesis, although differences in cell number before and after the experiment were not significantly different (Lesser, unpubl. data). The experimental treatments were visible minus UV radiation, visible plus 14% UV radiation, and visible plus 100% UV radiation with and without streptomycin (250  $\mu\text{g mL}^{-1}$ ).

**Statistics.** A statistical analysis was applied to the chlorophyll samples, which fulfilled the condition of independency of sampling. All other measurements were pseudoreplicated (Hulbert 1984) due to limitations on the space required for independency of samples. A Model I ANOVA (StatView II, Brainpower Inc., Calabasas, CA) at a significance level of 0.05 was performed on the 0- and 4-h specimens of chlorophyll, with the fixed effect being the percentage of UV-B irradiance. No unequal variances were detected using the  $F_{\text{max}}$ -test (Sokal and Rohlf 1981). Where significant treatment effects occurred, a Student-Newman-Keuls multiple comparison test was applied at the 0.05 significance level to identify individual differences among treatments.

Additionally, a regression method was used to test the hypothesis that changes in chlorophyll *a* were unrelated to UV-B irradiance. Both chlorophyll and photosynthesis at the end of the experiments were normalized to time 0 values and analyzed. We tested the hypothesis that the slope is 1 (i.e. change in photosynthesis is due to the loss of chlorophyll: rejected) and used the estimated slope to see how much of the change in photosynthesis was due to changes in chlorophyll.

## RESULTS

Measurements made just prior to each experimental exposure to UV-B radiation were typical for nutrient-replete (semicontinuous culture) and nutrient-limited (continuous culture) growth (Table 1). The results for these cultures are similar to published values for *Thalassiosira pseudonana* (clone 3H) (Cullen et al. 1992b) and other species of microalgae grown under similar conditions (Falkowski et al. 1985, Sakshaug et al. 1989, Thompson et al. 1989).

TABLE 2. Biologically weighted UV dose ( $\text{mW}\cdot\text{m}^{-2}$ , 275–400 nm) and unweighted UV-B dose ( $\text{mW}\cdot\text{m}^{-2}$ , 290–320 nm) for the cellulose-acetate-filtered FS40 lamps. The weightings tabulated by Smith et al. (1980) were used. Weightings for intermediate wavelengths were calculated by linear interpolation. R2 = nutrient replete with two UV bulbs, L2 = nutrient limited with two UV bulbs, R4 = nutrient replete with four UV bulbs.

Weighting function	Experiment	UV irradiance ( $\text{mW}\cdot\text{m}^{-2}$ )
DNA	R2, L2	7.7
Plant	R2, L2	31.15
Photoinhibition	R2, L2	223.07
Unweighted UV-B (290–320 nm)	R2, L2	344.38
DNA	R4	67.11
Plant	R4	189.58
Photoinhibition	R4	1391.26
Unweighted UV-B (290–320 nm)	R4	2148.76

**Biologically effective dose during experiments.** Unnaturally enhanced UV-B treatments (Table 2) were used in these experiments so that the action of UV-B on the kinetics of photoinhibition could be observed specifically. In our original analysis (Cullen and Lesser 1991), no attempt was made to relate experimental UV-B exposures to nature. The ratio of damage to repair (RDR) analysis of the results suggested, however, that cellulose-acetate filters reduced the effectiveness of unfiltered FS40 lamps by a factor of  $10.0 \pm 2.04$  (SE). This could not be distinguished from differences predicted by either DNA (Setlow 1974) or generalized plant (Caldwell 1971) spectra. Subsequently, we used a broad range of irradiance treatments to estimate a spectral biological weighting function for the inhibition of photosynthesis in the marine diatom *Phaeodactylum tricornutum* grown in batch culture under the same conditions as for the experiments described here (Cullen et al. 1992a). Because the function is in absolute units (reciprocal  $\text{mW}\cdot\text{m}^{-2}$ ), it can be used to estimate biologically effective fluence rate ( $E^*_{\text{inh}}$ ) and reduction of photosynthesis [ $1/(1 + E^*_{\text{inh}})$ ; cf. Cullen et al. 1992a] for our UV-B treatments using acetate-filtered lamps. The relative differences in biologically effective dose between bare FS40 lamps and those filtered with cellulose acetate were calculated using the weightings for Setlow's (1974) DNA spectrum, Caldwell's generalized plant spectrum (Caldwell 1971), and the Jones and Kok (1966) chloroplast photoinhibition from Smith et al. (1980), normalized to 1.0 at 300 nm and considered constant for 270–275 nm. A difference between bare and filtered lamps of 16.6, 10.4, and 4.6 was calculated using the DNA, plant, and photoinhibition function, respectively. Using extrapolated weightings for wavelengths <286 nm emitted by bare lamps (this accounted for 28% of biologically effective irradiance) calculated *Phaeodactylum* weightings predicted a difference of 11.1 between bare and filtered lamps using the biological weighting function presented by Cullen et al. (1992a) for *Phaeodactylum* (286–390 nm). An "empirical" difference of  $10.0 \pm 2.0$  was calculated by nonlinear

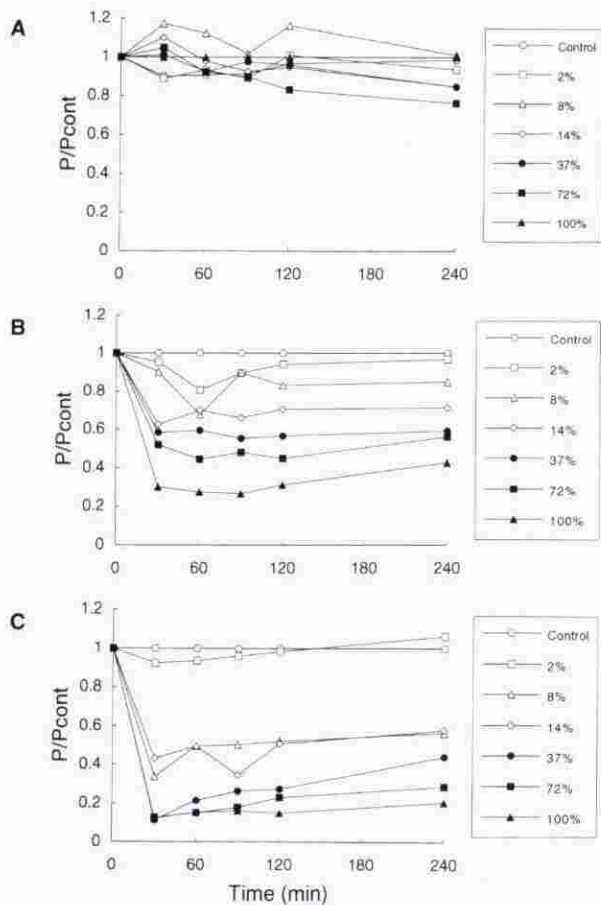


FIG. 1. Kinetics of cumulative carbon uptake ( $\mu\text{g C}\cdot\text{L}^{-1}$ ) from the three experimental cultures. Photoinhibition of photosynthesis for A) nutrient replete exposed to two UV-B bulbs, B) nutrient limited exposed to two UV-B bulbs, and C) nutrient replete exposed to four UV-B bulbs. Treatments are the percentage of UV-B to which subsamples of the culture were exposed.

regression (Cullen and Lesser 1991:fig. 4), and an "RDR" difference of  $9.0 \pm 1.0$  was calculated. Errors are standard errors. Relative inhibitions [i.e. reduction of photosynthesis ( $1/1 + E^*_{inh}$ )] for bare and filtered lamps of 57 and 10%, respectively, were calculated using the *Phaeodactylum* weightings. The relative difference compares well to the empirical observations, but the magnitudes of inhibition, about 75 and 25%, were somewhat less than those observed after 240 min.

**Photoinhibition and changes in chlorophyll content.** When cultures of *Thalassiosira pseudonana* were treated for 4 h to UV-B, photoinhibition [defined as  $(P_{control} - P)/P_{control}$ ] ranged from about 25 to more than 80%, depending on irradiance treatment and nutritional status (Fig. 1). In all cases where photoinhibition could be discerned, the relationship between photosynthesis ( $P/P_{control}$ ) and relative dosage rate was a hyperbolic function. In principle, these results could be due to net destruction of chlo-

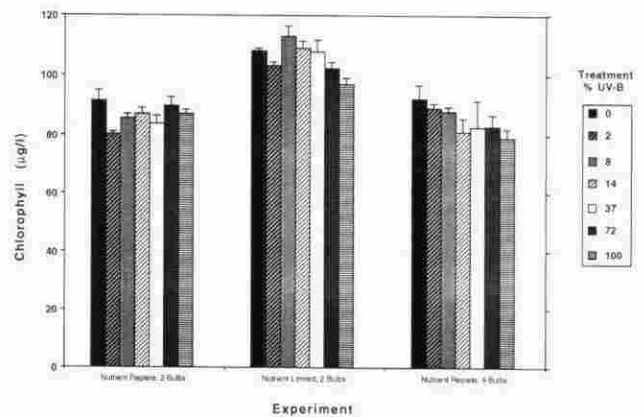


FIG. 2. Chlorophyll *a* concentration ( $\mu\text{g}\cdot\text{L}^{-1}$ ) from the three experimental cultures: nutrient replete exposed to two UV-B bulbs, nutrient limited exposed to two UV-B bulbs, and nutrient replete exposed to four UV-B bulbs. Treatments are the percentage of UV-B to which subsamples of the culture were exposed. Measurements were taken after a 4-h exposure period.

rophyll as well as to reductions of photosynthetic rate normalized to chlorophyll. All experiments showed fairly small but significant (ANOVA,  $P < 0.05$ ) effects of UV-B radiation on chlorophyll *a* concentration after 4 h of exposure (Figs. 2, 3). Our regression analysis rejected the hypothesis that changes in photosynthesis are due to losses of chlorophyll for both experiments using only two bulbs (ANOVA,  $P > 0.05$ ), while the experiment exposing cultures to four bulbs showed a significant relationship between chlorophyll loss and photosynthesis (ANOVA,  $P = 0.007$ ). Using the slope from the generated regression equation, we could explain only 20% of the decrease in photosynthesis by a decrease in chlorophyll *a* content. We conclude that decreases

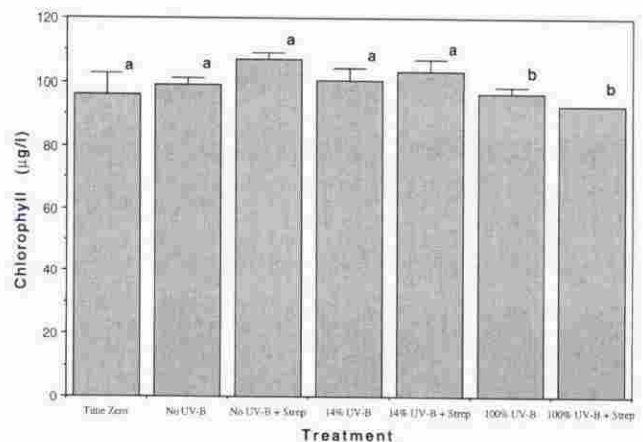


FIG. 3. Chlorophyll *a* concentration ( $\mu\text{g}\cdot\text{L}^{-1}$ ) from the nutrient replete culture exposed to four UV-B bulbs plus the antibiotic streptomycin ( $250 \mu\text{g}\cdot\text{mL}^{-1}$ ). Treatments are the percentage of UV-B to which subsamples of the culture were exposed. Measurements were taken after a 4-h exposure period. Superscripts denote groupings of means not significantly different from one another at  $P \leq 0.05$ .

in chlorophyll concentrations explain very little of the observed photoinhibition on the time scales of these experiments, even in our four-bulb experiment where the magnitude of photoinhibition is higher than predicted. We recognize that it is possible that the four-bulb experiment exhibited a higher percentage of photoinhibition than expected as a result of changes in culture conditions during the experiment.

**Damage and repair.** The dependence of photoinhibition on UV-B dosage rate, rather than dose (Cullen and Lesser 1991), indicated that photoinhibition had to be a balance between damage by UV-B and repair from that damage. The relative role of damage and repair rates was studied by statistical analysis of the relationship between UV-B dose rate and inhibition of photosynthesis. We hypothesized that the rate of carbon assimilation during UV-B exposure is determined both by the rate of irradiance-caused damage and by the rate of repair due to ongoing repair processes. A similar approach has been used to analyze visible light photoinhibition (Kok 1956, Neale 1987). In the simplest approach, the rate of change of carbon assimilation is described by the differential equation

$$dP/dt = -\phi_i \cdot \sigma \cdot I \cdot P + K_r \cdot (P_0 - P), \quad (1)$$

where  $P$  is the rate of photosynthesis,  $P_0$  is the initial rate before UV-B treatment,  $\phi_i \cdot \sigma$  is the apparent quantum yield of inactivation, i.e. the product of the absolute quantum yield ( $\phi_i$ ) and the absorption cross-section for UV-B ( $\sigma$ ),  $I$  is the dose rate of UV-B, and  $K_r$  is a constant related to the repair rate of the damaged components (see Neale 1987 for further discussion). After extended (>90 min) exposure to UV-B,  $P$  would reach a steady state resulting from an equilibrium between the net rate of damage and repair as described by the following equation:

$$\begin{aligned} P/P_0 &= 1/(1 + E^*_{inh}) \\ E^*_{inh} &= \phi_i \cdot \sigma \cdot I/K_r \end{aligned} \quad (2)$$

where  $E^*_{inh}$  is a nondimensional measure of the sensitivity of phytoplankton photosynthesis to UV-B damage. Equation 2 predicts that the steady-state rate of photosynthesis under UV-B exposure will have a hyperbolic relationship with the relative dose rate. Previous results have already suggested that a hyperbolic relationship of photosynthesis with dose rate does indeed apply to acute exposures of 0.5–4 h (Cullen and Lesser 1991). The relationship implies that the RDR, i.e.  $\phi_i \cdot \sigma / K_r$ , determines the sensitivity of photosynthesis to UV inhibition.

The RDR was estimated for the present experimental cultures by fitting equation 2 using nonlinear regression of  $P/P_{cont}$  on relative dose (Table 3). Normalized cumulative photosynthesis ( $P/P_{cont}$ ) will be close to steady-state  $P/P_0$  over sufficiently long sampling intervals (>90 min). The analysis provided a good fit for the response of photosynthetic rate to

TABLE 3. Results of a nonlinear regression analysis of normalized cumulative carbon uptake ( $P/P_{cont}$ ) after 90–240 min of UV-B exposure fitted to equation 2 with relative dose rate (=1 for maximum dose in any one experiment) as the independent variable. Analysis was performed using a quasi-Newton method (SHAZAM statistical program) to obtain an estimate of the RDR. Only data at 240 min were used for the experiment with nutrient-replete culture and two UV bulbs.

Experiment	Estimated RDR	SE	N	R <sup>2</sup>
R2	0.306	0.049	6	0.81
L2	2.16	0.199	18	0.97
R4	5.76	1.32	18	0.90
RB2	2.75	0.217	7	0.98

relative dose, especially for the nutrient-limited culture and the nutrient-replete culture exposed to four lamps (Table 3). A single RDR described 90–97% of the variation of  $P/P_{cont}$  after 90–240 min of UV exposure. The equation was less successful in explaining the variation of the nutrient-replete culture with two lamps, where a good fit could only be obtained at  $t = 240$  min and only 81% of the variation was explained. In the latter case, the relative decrease was small compared to sampling variation.

Because the maximum dose rate was set equal to 1, the estimated RDR can be interpreted as the ratio between the specific rate of damage and repair at the full dose rate in each experiment. For the nutrient-replete culture, the results suggest that the damage rate was about 31% of the repair rate under two UV bulbs (Fig. 4). The RDR differs by a factor of 7 between nutrient-replete and nutrient-limited cultures under the same type of UV exposure, which could be due to either a lower repair rate or higher damage rate. Protein synthesis is required for repair processes counteracting PAR photoinhibition (Ohad et al. 1984). Less is known about the role of protein synthesis in UV photoinhibition, but a lower rate of repair is likely to be the cause of the higher RDR under nutrient limitation (see further discussion later).

**Inhibition of repair.** The relative roles of repair and damage in the responses of *Thalassiosira pseudonana* to UV-B were further examined in experiments with streptomycin, an inhibitor of chloroplast protein synthesis. If repair was dependent on chloroplast protein synthesis, and if secondary effects of streptomycin were insignificant over the course of the experiment, then inhibition in the presence of streptomycin would describe the damage function, whereas inhibition in the absence of streptomycin would describe the balance between damage and repair. Consistent with this expectation, the addition of streptomycin to algal samples during exposure to UV-B radiation increased the degree of photoinhibition when compared to the same treatment without streptomycin (Fig. 5). There was also an effect of streptomycin on the control treatment without UV-B radiation: a slight inhibition of photosynthesis that was less than 20% after 2 h of ex-

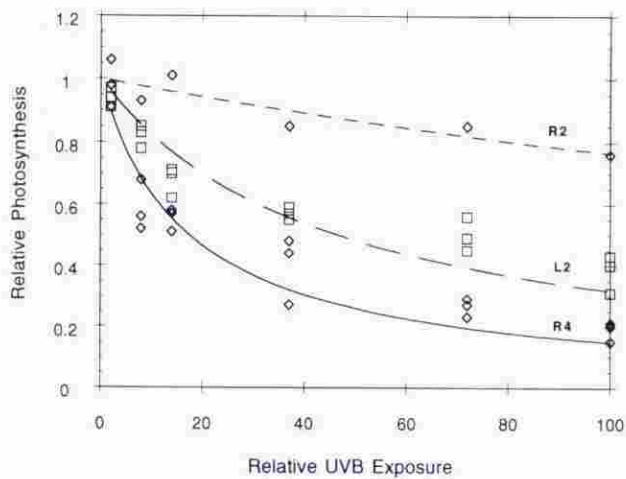


FIG. 4. Relationship between the asymptotic rate of cumulative carbon uptake and dose rate of UV-B exposure for experimental cultures of *Thalassiosira pseudonana*. The experiments were nutrient replete exposed to two UV-B bulbs (R2), nutrient limited exposed to two UV-B bulbs (L2), and nutrient replete exposed to four UV-B bulbs (R4). Data for cumulative uptake over a minimum of 90 min to a maximum of 240 min is shown in relation to relative UV-B dose rate (100 = no screen). For R2 only the 240-min data are shown. Rates were normalized to the average control (UV-B = 0) rate for each sampling time. Lines show best fit curve obtained by nonlinear regression for the RDR model (equation 2).

posure. This is not unexpected. Streptomycin should affect all treatments where it is included; however, the kinetics are different because the rate of replacement of key photosynthetic proteins, such as the 32-kDa D1 protein of photosystem II (PSII) in chloroplasts, in cells not exposed to UV radiation would presumably be decreased. What is significant here is that when streptomycin is added to cells exposed to UV-B, photoinhibition is observed immediately, and during the entire course of the experiment, suggesting that a significant amount of

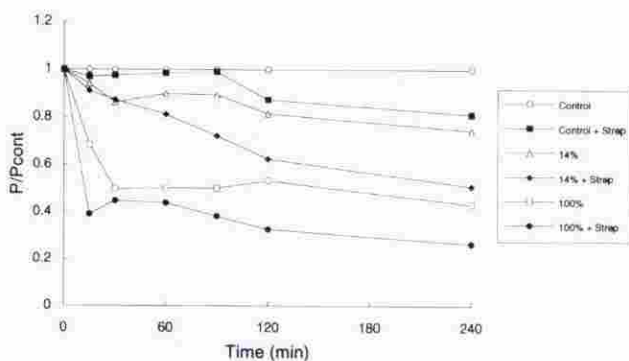


FIG. 5. Kinetics of cumulative carbon uptake ( $\mu\text{g C}\cdot\text{L}^{-1}$ ) and repair from the experimental culture (nutrient replete exposed to four UV-B bulbs) treated with the antibiotic streptomycin during the course of the experiment presented as photoinhibition of photosynthesis for cumulative carbon uptake.

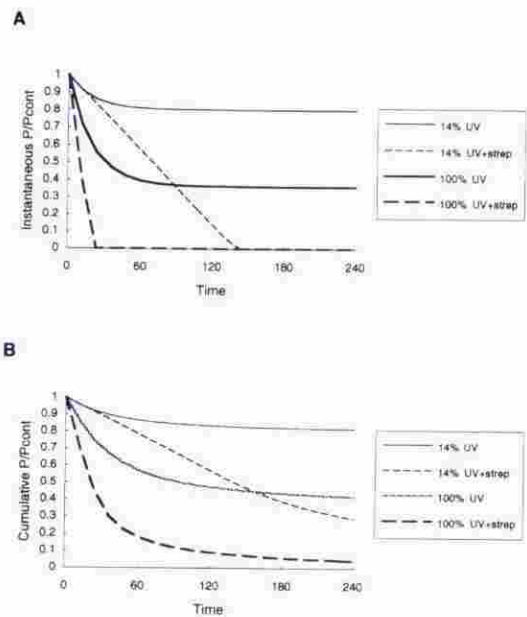


FIG. 6. The influence of repair processes on the kinetics of photoinhibition. Solid lines correspond to inhibition as a balance between damage and repair (equation 1); dashed lines show inhibition as a function of dose, as might occur during treatment with streptomycin, the inhibitor of chloroplast protein synthesis (no repair function). Heavier lines show predictions for 100% exposure to constant UV-B irradiance, with RDR = 1.75; lighter lines correspond to 14% exposure, RDR = 0.25. Parameters were chosen to correspond to results in Figure 4, with damage rates varying by a factor of 7. A) Predicted instantaneous rates of photosynthesis, relative to a control exposed to no UV-B. Untreated samples approach a balance between damage and repair according to equation 2. In samples with no repair, photosynthetic rate declines linearly to 0. B) Cumulative uptake, relative to a control exposed to no UV-B. For samples treated with no repair, the cumulative uptake, relative to control, declines linearly until the instantaneous rate reaches 0 and then declines asymptotically to 0. These predicted kinetics are consistent with measurements on samples treated with streptomycin (Fig. 5).

damage to important photosynthetic proteins is taking place from the onset of exposure.

The results of Figure 5 can be interpreted by comparison with a simple kinetic model of photoinhibition with or without a repair function. If we integrate equation 1  $[(P/P_0 = R/(D + R) + (D/D + R))\exp(-(D + R)T)]$ , where  $R$  = repair,  $D$  = damage, and  $T$  = time] for the instantaneous rates of photosynthesis (Fig. 6A), inhibition of photosynthesis follows exponential kinetics when repair processes are functioning with an asymptotic rate as described by equation 2. In contrast, photosynthesis declines linearly to 0 when repair mechanisms are inhibited (plus streptomycin). In the latter case, we predict that reciprocity would apply and instantaneous photosynthetic rate would be a linear function of cumulative dose. If we integrate equation 1  $[P/P_{\text{cont}} = R/(D + R) + (D/T \cdot (D + R)^2)(1 - \exp(-(D + R)T))]$  and look at cumulative photosynthesis, however (Fig. 6B), asymptotic behavior is predicted whether re-

pair is functioning is not. When repair processes are inhibited, cumulative uptake relative to the control declines linearly at first, then asymptotically to zero after the instantaneous rate reaches zero (Fig. 6B). When repair processes are active, however, cumulative photosynthesis relative to the control approaches an asymptote early and is subsequently nearly constant. Our results for the streptomycin experiment (Fig. 5) compare closely with changes in kinetics of cumulative photosynthesis expected upon inhibition of repair processes (Fig. 6B).

#### DISCUSSION

*Changes of carbon uptake and chlorophyll during exposure to UV-B radiation.* Our previous analysis of these experiments showed that the photoinhibition of photosynthesis by UV-B is better described as a function of dosage rate rather than dose (i.e. reciprocity fails) for *Thalassiosira pseudonana*. Cumulative carbon uptake, normalized to a control with no UV-B exposure, reaches an equilibrium between damage and repair 30–60 min after exposure to UV-B. The kinetics of photoinhibition for nutrient-replete and nutrient-limited cultures are the same and similar to the kinetics of photoinhibition by visible radiation (Kok 1956, Powles 1984, Neale 1987). The initial analysis did not explore the nonlinearity in the relationship between exposure and response; however, this nonlinearity is fundamentally important, not only to model development, but also in understanding the kinetics of photoinhibition. The relationship between photoinhibition and UV-B irradiance is hyperbolic because increases in UV-B irradiance cannot be fully compensated for by the repair capabilities of the alga. The relative balance between repair and damage processes can be expressed as the RDR. Our experiments show that the RDR provides a nondimensional basis for comparing the relative sensitivity of phytoplankton to UV irradiance. The RDR varies as both a function of irradiance and nutrient regimen. The RDR and hence  $E^*_{inh}$  will also depend on the spectral composition of the UV irradiance regime. A new model of spectrally dependent photoinhibition has been constructed under the assumption that inhibition depends on a weighted dose rate ( $E^*_{inh}$ ) and that the spectral dependence of  $E^*_{inh}$  can be defined from a biological weighting function (Cullen et al. 1992a). This model was successful in predicting the photosynthesis of a diatom and a dinoflagellate under a wide range of UV fluxes and UV/PAR ratios (Cullen et al. 1992a). Further, the biological weighting function for the marine diatom described by Cullen et al. (1992a) did a fairly good job of predicting photoinhibition for *T. pseudonana* under our unnatural irradiance treatments in the experiments described here.

The mechanism(s) explaining photoinhibition are not fully discernable from this study but potentially

include the observed decrease in chlorophyll, direct and indirect effects on the major CO<sub>2</sub> fixing enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), and changes in photochemistry. Additionally, direct effects on cellular DNA can affect cell division and survival in diatoms (Karentz et al. 1991a), although the activation and effectiveness of DNA repair mechanisms have been shown to be dependent on the flux of visible and UV-A radiation (Sutherland 1977), which was present during the experimental time period. The absorption of UV radiation by many cellular constituents (Quaite et al. 1992), especially the chloroplast, is likely to limit the direct effects on DNA within the time scale of these experiments.

Chlorophyll concentration in this study generally decreases upon exposure to UV-B radiation or UV-B radiation in the presence of streptomycin. Photo-bleaching of pigments associated with the photosynthetic apparatus has been observed in microalgae after exposure to high visible radiation (Belay and Fogg 1978, Young and Britton 1990) and UV radiation (Döhler 1986, Lesser and Shick 1989) and probably occurs by photooxidation during exposure to active forms of oxygen in the chloroplast (Asada and Takahashi 1987).

A direct effect of UV-B on the activity of Rubisco has been demonstrated in pea and soybean (Vu et al. 1984, Strid et al. 1990) and by our own data on light-limited continuous cultures of the dinoflagellate *Prorocentrum micans* (PRORO III), acclimated for 5 weeks with and without UV-B radiation (Lesser, unpubl. data). Rubisco is also sensitive to H<sub>2</sub>O<sub>2</sub>, which is produced in large quantities within the chloroplast during photooxidative stress (Asada and Takahashi 1987). Loss of Rubisco activity would primarily affect the maximum rate of photosynthesis but will also affect light-limited photosynthesis at irradiances greater than  $I_k$ . A recent study by Vosjan et al. (1990) demonstrated a decrease in adenosine triphosphate (ATP) content in natural planktonic assemblages. As ATP and NADPH<sub>2</sub> are consumed during carbon metabolism, reduced levels of ATP could decrease carbon fixation in phytoplankton depending on the length of exposure to UV-B and cellular ATP pool size. The PSII reaction center is also known to be a target for damage by UV-B radiation in isolated thylakoids (e.g. Jones and Kok 1966, Renger et al. 1989), but it is unknown whether or not it is an important target during exposure of intact plants (Neale et al. 1993).

*Nutrient limitation.* Nutrient-limited phytoplankton are more sensitive than their nutrient-replete counterparts to excess visible radiation (Kiefer 1973, Belay and Fogg 1978, Prézélin et al. 1986). The same enhanced sensitivity has been observed for UV-B radiation (Cullen and Lesser 1991, Figs. 3, 4). The analysis presented here suggests that increased sensitivity to UV-B in the nitrogen-limited culture was the consequence of diminished repair capabili-

ties. This may be because nitrogen limitation slows the turnover of critical proteins and protein-pigment complexes within the photosynthetic apparatus. This would increase the RDR for these cultures through a decrease in the specific rate of repair. We would also expect a decreased "package effect" and subsequent increase in the optical cross-section for nutrient-limited cultures (Herzig and Falkowski 1989, Dubinsky 1992), allowing an increase of UV radiation into the chloroplast stroma affecting enzymes associated with carbon fixation. This would increase the specific rate of damage, but probably not enough to explain the large differences in RDR associated with nutrient limitation. On a longer time scale, effects of UV-B on nitrogen assimilation pathways (Döhler 1986, Döhler and Biermann 1987) might influence photoinhibition by slowing repair processes.

*Inhibition of repair.* Our approach is not only to establish a role for repair mechanisms, as we have defined them for these experiments, but to examine the equilibrium between repair and damage as it is affected by different exposures of UV-B radiation. Our experiments show that the photoinhibition of cumulative carbon uptake in *Thalassiosira pseudonana* is more susceptible to UV-B in the presence of the antibiotic streptomycin and is consistent with strictly dose-dependent damage when repair functions are eliminated (Fig. 5). Previous studies have shown that suppressing synthesis of chloroplast proteins increases the rate of photoinhibition of photosynthesis by visible light (review, Prasil et al. 1992), but this is the first report of similar effects during UV-B inhibition. These previous studies suggested the critical role of the 32-kDa D1 protein that needs to be resynthesized as part of the reactivation of damaged PSII reaction centers. We do not know whether it is the synthesis of D1 or synthesis of other chloroplast proteins that is involved in repair processes counteracting UV-B exposure in these experiments, but note that UV-B-specific turnover of D1 does occur in higher plants (Greenberg et al. 1989).

*Importance of repair functions in aquatic systems.* The observed kinetics of carbon uptake demonstrate that repair processes involving protein synthesis are an integral factor in the net photoinhibitory effect. We have previously demonstrated that the effects of exposure to UV-B radiation are similar to excess visible radiation and that for *Thalassiosira pseudonana* inhibition of photosynthesis is a function of dosage rate for time scales of 0.5–4 h (Cullen and Lesser 1991). Vertical mixing in nature has been shown to mitigate the photoinhibitory effects of visible radiation (Marra 1978, Gallegos and Platt 1985) and is recognized as a complicating factor in studying the effect of exposure to UV-B radiation on primary productivity (Kullenberg 1982). Both repair mechanisms and vertical mixing will determine the temporal scale of inhibition of photosynthesis by UV-B

radiation. Repair mechanisms and the time dependence of photoinhibition also interfere with the dose-response relationship and complicate the determination of biological weighting functions (Cullen et al. 1992a).

The possible influence of repair mechanisms on the prediction of UV inhibition is illustrated by our kinetic model. When repair mechanisms are functioning, we predict that asymptotic rates will have a hyperbolic relationship with dose rate. Such a relationship was incorporated into a model of UV- and PAR-dependent photosynthesis and inhibition and successfully predicted photosynthesis over a wide range of UV irradiances and UV/PAR ratios (Cullen et al. 1992a). A dependence of UV inhibition on cumulative dose would be expected only if repair processes have an insignificant contribution during the exposure period. In contrast, recent analyses of the inhibition of Antarctic phytoplankton photosynthesis by solar UV have related effects to cumulative dose (i.e. weighted  $J \cdot m^{-2}$ ; Helbling et al. 1992, Smith et al. 1992). The present results suggest that it may be inappropriate to extrapolate these relationships beyond the exposure times in the original experiments, depending on the rates of repair processes. Further studies of repair processes in Antarctic phytoplankton are needed.

*Conclusions.* We have not directly measured repair at the cellular or biomolecular level. Our initial approach has been to examine the kinetics of photoinhibition during exposure to UV-B radiation, during which we demonstrated an impairment of photosynthesis that was amplified by an inhibitor of chloroplast protein synthesis. This evidence supports, and is consistent with, the hypothesis that UV-B photoinhibition is a dynamic balance between damage and repair. Additionally, the shapes of our time-response curves were consistent with this postulated balance between damage and repair. Differences in repair capabilities (i.e. measured differences associated with inhibition of chloroplast protein synthesis and inferred differences associated with nitrogen limitation) are just as important as differences in weighted UV-B irradiance in determining the degree of photoinhibition. It is likely that factors such as temperature and irradiance history can significantly influence repair processes, and taxonomic differences may also be important. We should emphasize that these experiments of acute exposures tell us nothing about the effects of long-term exposure to UV-B radiation and the potential role of UV-B absorbing compounds, which might be produced under long-term exposures to UV radiation (Karentz et al. 1991b) in further mitigating the effects of UV-B radiation. Our preliminary results with chronic exposure of a marine dinoflagellate to UV-B radiation suggest that significant decreases of growth and photosynthetic rates occur even with the induction and accumulation of UV-B absorbing compounds (Lesser, unpubl. data).

These results have applicability in assessing the effects of increases in UV-B radiation on marine primary productivity by identifying those processes affected by UV-B radiation and the magnitude of those effects while accurately measuring the dose or dosage rate of UV-B radiation. We believe studies of this kind will enhance our ability to model the photoinhibition of photosynthesis in nature and help identify processes that might be exploited to assess UV-B-induced photoinhibition in the ocean.

This research was supported by grants from the National Science Foundation (DPP 881768 and DPP 9018441), the Office of Naval Research (N0014-89-J-1066, N0014-89-J-1239), NASA (NAGW-2072), and NSERC Canada. Bigelow Laboratory Contribution no. 93019.

- Asada, K. & Takahashi, M. 1987. Production and scavenging of active oxygen in photosynthesis. In Kyle, D. J., Osmond, C. B. & Arntzen, C. J. [Eds.] *Photoinhibition*. Elsevier, Amsterdam, pp. 228–87.
- Belay, A. & Fogg, G. E. 1978. Photoinhibition of photosynthesis in *Asterionella formosa* (Bacillariophyceae). *J. Phycol.* 14:341–7.
- Blumthaler, M. & Ambach, W. 1990. Indication of increasing solar ultraviolet-B radiation flux in alpine regions. *Science (Wash. D.C.)* 248:206–8.
- Bühlmann, B., Bossard, P. & Uehlinger, U. 1987. The influence of longwave ultraviolet radiation (u.v.-A) on the photosynthetic activity ( $^{14}\text{C}$ -assimilation) of phytoplankton. *J. Plankton Res.* 9:935–43.
- Caldwell, M. M. 1971. Solar ultraviolet radiation and the growth and development of higher plants. In Giese, A. C. [Ed.] *Photophysiology*. Academic Press, New York, pp. 131–77.
- Caldwell, M. M., Camp, L. B., Warner, C. W. & Flint, S. D. 1986. Action spectra and their key role in assessing biological consequences of solar UV-B radiation change. In Worrest, R. C. & Caldwell, M. M. [Eds.] *Stratospheric Ozone Reduction, Solar Ultraviolet Radiation and Plant Life*. Springer-Verlag, Berlin, pp. 87–111.
- Calkins, J. & Thordardottir, T. 1980. The ecological significance of solar UV radiation on aquatic organisms. *Nature (Lond.)* 283:583–6.
- Cullen, J. J. & Lesser, M. P. 1991. Inhibition of phytoplankton photosynthesis by ultraviolet radiation as a function of dose and dosage rate. *Mar. Biol. (Berl.)* 111:183–90.
- Cullen, J. J., Neale, P. J. & Lesser, M. P. 1992a. Biological weighting function for the inhibition of phytoplankton photosynthesis by ultraviolet radiation. *Science (Wash. D.C.)* 258:646–51.
- Cullen, J. J., Xang, X. & MacIntyre, H. L. 1992b. Nutrient limitation and marine photosynthesis. In Falkowski, P. G. & Woodhead, A. D. [Eds.] *Primary Productivity and Biogeochemical Cycles in the Sea*. Plenum Press, New York, pp. 69–88.
- Döhler, G. 1986. Impact of UV-B radiation on [ $^{15}\text{N}$ ] ammonia and [ $^{15}\text{N}$ ] nitrate uptake of *Ditylum brightwellii*. *Photochem. Photobiophys.* 11:115–21.
- Döhler, G. & Biermann, I. 1987. Effect of u.v.-B irradiance on the response of  $^{15}\text{N}$ -nitrate uptake of *Lauderia annulata* and *Synedra planctonica*. *J. Plankton Res.* 9:881–90.
- Dubinsky, Z. 1992. The functional and optical absorption cross-sections of phytoplankton photosynthesis. In Falkowski, P. G. & Woodhead, A. D. [Eds.] *Primary Productivity and Biogeochemical Cycles in the Sea*. Plenum Press, New York, pp. 31–45.
- Falkowski, P. G., Dubinsky, Z. & Wyman, K. 1985. Growth-irradiance relationships in phytoplankton. *Limnol. Oceanogr.* 30:311–21.
- Frederick, J. E. & Snell, H. E. 1988. Ultraviolet radiation levels during the Antarctic spring. *Science (Wash. D.C.)* 241:438–40.
- Gallegos, C. L. & Platt, T. 1985. Vertical advection of phytoplankton and productivity estimates: a dimensional analysis. *Mar. Ecol. Prog. Ser.* 26:125–34.
- Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates. In Smith, W. L. & Chanley, M. H. [Eds.] *Culture of Marine Invertebrate Animals*. Plenum Press, New York, pp. 29–60.
- Greenberg, B. M., Gaba, V., Canaani, O., Malkin, S., Mattoo, A. K. & Edelman, M. 1989. Separate photosensitizers mediate degradation of the 32 kDa photosystem II reaction center protein in the visible and UV spectral regions. *Proc. Natl. Acad. Sci. U.S.A.* 86:6617–20.
- Greer, D. H., Berry, J. A. & Björkman, O. 1986. Photoinhibition of photosynthesis in intact bean leaves: role of light and temperature, and requirement for chloroplast-protein synthesis during repair. *Planta (Berl.)* 168:253–60.
- Häder, D.-P. & Häder, M. 1988. Inhibition of motility and phototaxis in the green flagellate, *Euglena gracilis*, by UV-B radiation. *Arch. Microbiol.* 150:20–5.
- Hardy, J. & Gucinski, H. 1989. Stratospheric ozone depletion: implications for marine ecosystems. *Oceanogr. Mag.* 2:18–21.
- Helbling, E. W., Villafañe, V., Ferrario, M. & Holm-Hansen, O. 1992. Impact of natural ultraviolet radiation on rates of photosynthesis and on specific marine phytoplankton species. *Mar. Ecol. Prog. Ser.* 80:89–100.
- Herzig, R. & Falkowski, P. 1989. Nitrogen limitation in *Isochrysis galbana* (Haptophyceae). I. Photosynthetic energy conversion and growth efficiencies. *J. Phycol.* 25:462–71.
- Hirosawa, T. & Miyachi, M. 1983. Inactivation of Hill reaction by long-wavelength radiation (UV-A) and its photoreactivation by visible light in the cyanobacterium, *Anacystis nidulans*. *Arch. Microbiol.* 135:98–102.
- Hulbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* 54:187–211.
- Jerlov, N. G. 1950. Ultra-violet radiation in the sea. *Nature (Lond.)* 166:111–2.
- Jokiel, P. L. & York, R. H., Jr. 1982. Solar ultraviolet photobiology of the reef coral *Pocillopora damicornis* and symbiotic zooxanthellae. *Bull. Mar. Sci.* 32:301–15.
- 1984. Importance of ultraviolet radiation in photoinhibition of microalgal growth. *Limnol. Oceanogr.* 29:192–9.
- Jones, L. W. & Kok, B. 1966. Photoinhibition of chloroplast reactions. I. Kinetics and action spectra. *Plant Physiol.* 41:1037–43.
- Karentz, D., Cleaver, J. E. & Mitchell, D. L. 1991a. Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. *J. Phycol.* 27:326–41.
- Karentz, D., McEuen, F. S., Land, M. C. & Dunlap, W. C. 1991b. Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. *Mar. Biol. (Berl.)* 108:157–66.
- Kiefer, D. A. 1973. Chlorophyll *a* fluorescence in marine diatoms: responses of chloroplasts to light and nutrient stress. *Mar. Biol. (Berl.)* 23:39–46.
- Kok, B. 1956. On the inhibition of photosynthesis by intense light. *Biochim. Biophys. Acta* 21:234–44.
- Kullenberg, G. 1982. Note on the role of vertical mixing in relation to effects of UV radiation on the marine environment. In Calkins, J. [Ed.] *The Role of Solar UV Radiation on the Marine Ecosystems*. Plenum Press, New York, pp. 283–92.
- Kyle, D. J. 1987. The biochemical basis for photoinhibition of photosystem II. In Kyle, D. J., Osmond, C. B. & Arntzen, C. J. [Eds.] *Photoinhibition*. Elsevier, Amsterdam, pp. 197–226.
- Lesser, M. P. & Shick, J. M. 1989. Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of *Aiptasia pallida*: primary production, photoinhibition, and enzymic defenses against oxygen toxicity. *Mar. Biol. (Berl.)* 102:243–55.
- Lorenzen, C. J. 1979. Ultraviolet radiation and phytoplankton photosynthesis. *Limnol. Oceanogr.* 24:1117–20.
- Marra, J. 1978. Phytoplankton photosynthetic response to vertical movement in a mixed layer. *Mar. Biol. (Berl.)* 46:203–8.
- Neale, P. J. 1987. Algal photoinhibition and photosynthesis in

- the aquatic environment. In Kyle, D. J., Osmond, C. B. & Arntzen, C. J. [Eds.] *Photoinhibition*. Elsevier, Amsterdam, pp. 35–65.
- Neale, P. J., Cullen, J. J., Lesser, M. P. & Melis, A. 1993. Physiological bases for detecting and predicting photoinhibition of aquatic photosynthesis by PAR and UV radiation. In Yamamoto, H. & Smith, C. [Eds.] *Photosynthetic Responses to the Environment*. American Society of Plant Physiologists, pp. 60–77.
- Ohad, I., Kyle, D. J. & Arntzen, C. J. 1984. Membrane protein damage and repair: removal and replacement of inactivated 32-kilodalton polypeptides in chloroplast membranes. *J. Cell Biol.* 99:481–85.
- Powles, S. B. 1984. Photoinhibition of photosynthesis induced by visible light. *Annu. Rev. Plant Physiol.* 35:15–44.
- Prasil, O., Adir, N. & Ohad, I. 1992. Dynamics of photosystem II: mechanism of photoinhibition and repair processes. In Barber, J. [Ed.] *The Photosystems: Structure, Function and Molecular Biology*. Elsevier, Amsterdam, pp. 295–348.
- Prézelin, B. B., Samuelsson, G. & Matlick, H. A. 1986. Photosystem II photoinhibition and altered kinetics of photosynthesis during nutrient-dependent high-light photoadaptation in *Gonyaulax polyedra*. *Mar. Biol. (Berl.)* 93:1–12.
- Quate, F. E., Sutherland, B. M. & Sutherland, J. C. 1992. Action spectrum for DNA damage in alfalfa lowers predicted impact of ozone depletion. *Nature (Lond.)* 358:576–8.
- Renger, G., Völker, M., Eckert, H. J., Fromme, R., Hohm-Veit, S. & Gräber, P. 1989. On the mechanism of photosystem II deterioration by UV-B irradiation. *Photochem. Photobiol.* 49:97–105.
- Sakshaug, E., Andresen, K. & Kiefer, D. A. 1989. A steady state description of growth and light absorption in the marine planktonic diatom *Skeletonema costatum*. *Limnol. Oceanogr.* 34:198–205.
- Samuelsson, G., Lönneborg, A., Rosenqvist, E., Gustafson, P. & Öquist, G. 1985. Photoinhibition and reactivation of photosynthesis in the cyanobacterium *Anacystis nidulans*. *Plant Physiol.* 79:992–5.
- Setlow, R. B. 1974. The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. *Proc. Natl. Acad. Sci. U.S.A.* 71:3363–6.
- Smith, R. C. 1989. Ozone, middle ultraviolet radiation and the aquatic environment. *Photochem. Photobiol.* 50:459–68.
- Smith, R. C. & Baker, K. S. 1979. Penetration of UV-B and biologically effective dose-rates in natural waters. *Photochem. Photobiol.* 29:311–23.
- 1982. Assessment of the influence of enhanced UV-B on marine primary productivity. In Calkins, J. [Ed.] *The Role of Solar Ultraviolet Radiation in Marine Ecosystems*. Plenum Press, New York, pp. 509–37.
- 1989. Stratospheric ozone, middle ultraviolet radiation and phytoplankton productivity. *Oceanogr. Mag.* 2:4–10.
- Smith, R. C., Baker, K. S., Holm-Hansen, O. & Olson, R. S. 1980. Photoinhibition of photosynthesis in natural waters. *Photochem. Photobiol.* 31:585–92.
- Smith, R. C., Prézelin, B. B., Baker, K. S., Bidigare, R. R., Boucher, N. P., Coley, T., Karentz, D., MacIntyre, S., Matlick, H. A., Menzies, D., Ondrusek, M., Wan, Z. & Waters, K. J. 1992. Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. *Science (Wash. D.C.)* 255:952–9.
- Sokal, R. R. & Rohlf, F. J. 1981. *Biometry*, 2nd ed. W. H. Freeman and Co., San Francisco, 859 pp.
- Solomon, S. 1988. The mystery of the Antarctic ozone hole. *Rev. Geophys.* 26:131–48.
- Stramski, D. & Morel, A. 1990. Optical properties of photosynthetic picoplankton in different physiological states as affected by growth irradiance. *Deep-Sea Res.* 37:245–66.
- Strid, A., Chow, W. S. & Anderson, J. M. 1990. Effects of supplementary ultraviolet-B radiation on photosynthesis in *Pisum sativum*. *Biochim. Biophys. Acta* 1020:260–8.
- Sutherland, B. M. 1977. Symposium on molecular mechanisms in photoreactivation. *Photochem. Photobiol.* 25:413–4.
- Tevini, M. & Teramura, A. H. 1989. UV-B effects on terrestrial plants. *Photochem. Photobiol.* 50:479–87.
- Thompson, P. A., Levasseur, M. E. & Harrison, P. J. 1989. Light-limited growth on ammonium vs. nitrate: what is the advantage for marine phytoplankton? *Limnol. Oceanogr.* 34:1014–24.
- Van Baalen, C. 1968. The effects of ultraviolet irradiation on a caecoid blue-green alga: survival, photosynthesis, and photoreactivation. *Plant Physiol.* 43:1689–95.
- Vincent, W. F. 1980. Mechanisms of rapid photosynthetic adaptation in natural phytoplankton communities. II. Changes in photochemical capacity as measured by DCMU-induced chlorophyll fluorescence. *J. Phycol.* 20:201–11.
- Vosjan, J. H., Döhler, G. & Nieuwland, G. 1990. Effect of UV-B irradiance on the ATP content of microorganisms of the Weddell Sea (Antarctica). *Netherlands J. Sea Res.* 25:391–3.
- Vu, C. V., Allen, L. H., Jr. & Garrard, L. A. 1984. Effects of enhanced UV-B radiation (280–320 nm) on ribulose-1,5-bisphosphate carboxylase in pea and soybean. *Env. Exp. Bot.* 24:131–43.
- Wood, W. F. 1987. Effect of solar ultra-violet radiation on the kelp *Ecklonia radiata*. *Mar. Biol. (Berl.)* 96:143–50.
- Worrest, R. C. 1982. Review of literature concerning the impact of UV-B radiation upon marine organisms. In Calkins, J. [Ed.] *The Role of Solar Ultraviolet Radiation in Marine Ecosystems*. Plenum Press, New York, pp. 429–57.
- Young, A. & Britton, G. 1990. Photobleaching in the unicellular green alga *Dunaliella parva* 19/9. *Photosynth. Res.* 25:129–36.

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