Marine Biology 111, 183–190 (1991)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Inhibition of photosynthesis by ultraviolet radiation as a function of dose and dosage rate: results for a marine diatom *

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Data of final manuscript acceptance: July 23, 1991. Communicated by R. O'Dor, Halifax

Abstract. The effects of ultraviolet radiation on phytoplankton are usually described as a function of dose (J m^{-2}, weighted appropriately). Experiments conducted in 1988 and 1989 on a marine diatom, *Thalassiosira pseudonana* (Clone 3H), demonstrate that during light-limited photosynthesis in visible radiation, the inhibition of photosynthesis by supplemental ultraviolet radiation (principally UV-B: 280 to 320 nm) is a function of irradiance (W m^{-2}) as well as of dose: for equal doses of UV-B, a relatively short exposure to high UV-B irradiance is more damaging to photosynthesis than a longer exposure to lower irradiance. In fact, photoinhibition by UV-B is well described as a monotonic, nonlinear function of irradiance for time scales of 0.5 to 4 h. A nitrate-limited culture was about nine times more sensitive to UV-B than was a nutrient-replete culture, but the kinetics of photoinhibition were similar. These results have some bearing on efforts to describe the effects of ultraviolet radiation on marine primary productivity. Action spectra of photoinhibition by UV can be constructed, but they should only be used to describe photoinhibition for specified time scales. Vertical profiles of relative photoinhibition must be interpreted cautiously because photoinhibition by UV-B is likely to be a function of incubation time and results must therefore be interpreted in the context of vertical mixing.

Introduction

Middle ultraviolet radiation (UV-B; 280 or 290 nm to 320 nm) can be harmful to phytoplankton: when wavelength-selective screens or supplemental sources of irradiance are used to isolate the effects of ultraviolet radiation on natural and cultured phytoplankton, a detrimental effect of UV-B is frequently found (review: Smith and Baker 1989). Studies have shown that UV-B affects photosynthesis (Smith et al. 1980), nitrogen metabolism (Döhler and Biermann 1987), locomotion (Häder and Häder 1988), and growth rate (Jokiel and York 1984) of phytoplankton, but results are difficult to extrapolate, so the extent to which UV-B affects these processes in aquatic systems is largely unknown. Thus, it is difficult to say how much and in what manner UV-B radiation influences primary productivity in the sea.

We consider here photosynthesis and its inhibition by UV-B radiation. Of all the effects of UV-B on phytoplankton, this is the best studied, yet key uncertainties persist (Smith and Baker 1982, Smith 1989). We do not know with confidence the wavelength-dependence (action spectrum) of photoinhibition, a weighting function which is crucial to accurate modeling (Smith et al. 1980, Caldwell et al. 1986, Coohill 1989). Also, there is no quantitative method to assess the mitigating influence of vertical mixing in the water column on the inhibition of photosynthesis by UV-B (cf. Kullenberg 1982). It is therefore difficult to use the results of experimental incubations to describe photosynthesis and photoinhibition in natural marine systems (Smith et al. 1980, Smith and Baker 1982). Another uncertainty, important, but not considered here, is the effect of UV-B on growth and survival of phytoplankton (Calkins and Thordardottir 1980, Jokiel and York 1984, Smith and Baker 1989).

Although photosynthesis and its inhibition by excess visible light are usually reported as functions of irradiance (W m^{-2} or μmol m^{-2} s^{-1}; cf. Neale 1987), commonly the effects of ultraviolet radiation are related to cumulative dose (J m^{-2}, weighted appropriately: Smith et al. 1980), even though time scales are specified. To compare results between experiments or to produce general models, it is then necessary to assume reciprocity (i.e., that the effect is a function of dose, regardless of dosage rate). This assumption has been made cautiously (Smith and Baker 1982). If reciprocity is not satisfied for the inhibition of phytoplankton photosynthesis by UV-B radiation, models of the effects of UV-B on primary productivity must be reconsidered.

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Here we present relationships between exposure of a marine diatom to UV-B radiation and inhibition of photosynthesis. Consistent with studies on the effects of visible light (Kok 1956, Marra 1978a, Samuelsson et al. 1985) and UV-B radiation (254 nm; Redford and Myers 1951), we show that photoinhibition is a function of both dosage rate and dose. The dependence of photoinhibition on dosage rate and the resultant failure of reciprocity complicates, but does not preclude, the determination of wavelength dependence. The partial dependence on dose imposes a temporal component on the determination of photoinhibition such that the time scale of incubation must be reconciled with the time scale of vertical mixing (Harris 1980, Legendre and Demers 1984). Consequently, the kinetics of photoinhibition and the vertical motions of phytoplankton in nature must be understood in order to describe the effects of UV-B on photosynthesis by natural marine phytoplankton.

Materials and methods

Cultures of the marine diatom *Thalassiosira pseudonana* (Clone 3H, obtained from the Provasoli-Guillard Culture Collection for Marine Phytoplankton) were grown under a 12 h light:12 h dark cycle at 20 °C. The containers were polycarbonate and the cultures were bubbled with acid-scrubbed air and stirred. Nutrient-replete 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^{-1} using f/2 growth medium with 50 μM nitrate. Illumination was from Vita-Lite full-spectrum fluorescent lamps providing a quantum scalar irradiance of 75 $\mu mol\ m^{-2}\ s^{-1}$ (photosynthetically active radiation, PAR) as measured by a Biospherical Instruments QSL 100 4π sensor immersed in a water-filled culture vessel.

To observe the short-term effects of UV-B on photosynthesis, samples from the cultures were placed in open glass dishes and exposed in an experimental incubator to uniform visible light from below and ultraviolet radiation from above. Air temperature was controlled at 20 °C. Visible light was provided by Vita-Lite fluorescent lamps shone through UV-B opaque acrylic (cutoff = 385 nm) and neutral-density mylar screen. The irradiance (75 $\mu mol\ m^{-2}\ s^{-1}$) was well within the light-dependent portion of the photosynthesis-irradiance relationship for the cultured algae (data not shown). Ultraviolet radiation, predominantly UV-B, came from two fluorescent lamps (FS40 T12-UVB, Light Sources Inc., illuminated 200 h prior to use) suspended 60 cm above the samples. The lamps were turned on for several hours prior to the experiment to stabilize output. Six different intensities of ultraviolet radiation (2, 8, 14, 37, 72 and 100% of incident) were obtained by placing perforated nickel screens over the dishes. It is assumed that during any one experiment the spectral quality of UV radiation was the same between treatments. An acrylic plate, opaque to UV-B (cutoff = 385 nm), was placed over another dish, which served as a control. A rotating table was used to stir the samples continuously during the experiment, except during sampling. For one set of experiments, the UV-B lamps were unfiltered, except for the screens placed over the dishes. For the other set, cellulose acetate film (0.13 mm thickness, aged 100 h at 3 cm from two FS40 lamps) was used to attenuate shorter wavelengths that are not encountered in nature (Caldwell et al. 1986).

Photosynthesis was measured as the uptake of ^{14}C-bicarbonate. At the outset, a portion of the culture was harvested and inoculated with a solution of ^{14}C-bicarbonate to a final specific activity of 2.6×10^4 Bq ml^{-1} (0.7 $\mu Ci\ ml^{-1}$). 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. This was a check for uniformity of visible light between treatments. Subsequently, both the UV and visible lamps were illuminated and subsamples were taken periodically over the next 4 h. 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 ^{14}C. Later, scintillation fluid was added (Ecolume, ICN) and the activity of organic ^{14}C was determined with a Beckman LS7500 scintillation counter, using the H# method of quench correction. Subsamples (20 µl) were placed in 4 ml fluor + 0.2 ml phenethylamine to determine the amount of label added. It was determined in a pilot experiment that the amount of label did not change significantly over several hours in the open dishes. Results were corrected for time-zero controls and expressed as cumulative uptake (P, $mg\ m^{-3}$) relative to control (P_{cont}, UV-B excluded) for the period of exposure to UV-B.

A relatively small amount of PAR came from the FS40 lamps (about 4 $\mu mol\ m^{-2}\ s^{-1}$ at the 100% UV-B dish and at the control dish, proportionally less in the other treatments). Thus, photoinhibition would be slightly overestimated in the low-UV treatments because they received less PAR. No correction has been made for this second-order effect. 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 experiments. We conclude that during the experiments, differences between samples are attributable to ultraviolet radiation (predominantly UV-B) rather than to differences in visible light. Accordingly, we will hereafter attribute observed photoinhibition of photosynthesis to UV-B radiation.

Spectral irradiance was determined with a diode-array spectroradiometer system (EG & G). The system included a model 1420 intensified 1024-channel diode-array detector with a blue-enhanced photocathode and high-quantum-efficiency option. The detector was connected to a model 1235 spectrograph (monochromator) with a 600 groove/mm 300 blaze grating, optimal resolution between 200 and 450 nm. A 2.9 m quartz fiber-optic probe (C Technologies, Inc.) fitted with a diffuser suitable for UV and visible radiation (Oriel Corp.) was used for spectral input. The detector was controlled by Macintosh computer and software (MacOma) using a model 1461 detector inferface.

The spectroradiometer was calibrated using a constant current source and a 1 000 W quartz-halogen tungsten lamp calibrated from 250 to 750 nm to National Bureau of Standards (USA) specifications (power supply and lamp from Optronics Laboratories, Inc.). Special care was taken to achieve robust calibration. The MacOma software assigned wavelengths to diode elements by a linear spline-fit to emission lines from a germicidal lamp. Spectral irradiance from the standard lamp was calculated for each of those wavelengths, using piecewise polynomial fits to the calibration data provided by the supplier. Because longer wavelengths dominated the output from the standard lamp, the influence of stray light on the measurement of ultraviolet radiation was a concern. Accordingly, we used dark measurements for each element as blanks for wavelengths >310 nm, but for the shorter wavelengths substituted readings obtained when a Schott long-pass WG 345 filter was inserted in the light path. We reasoned that if stray light was a problem, spurious readings at short wavelengths would be obtained, and these could be detected when the long-pass filter was used. The problem was not serious, however. Nonetheless, Schott-filter blanks did yield better results for the calibration, so they were used subsequently for all measurements at wavelengths ≤310 nm.

Subsequent to the experiments, spectral output of the FS40 lamps in the experimental system was measured with the spectroradiometer. The lamps had been illuminated for an additional period of about 300 h for a growth experiment, but new cellulose acetate film, aged as described above, was used. The measured spectrum of the unfiltered lamps (Fig. 1) compares very well to one presented by Caldwell et al. (1986; their Fig. 8). Our cellulose acetate film transmitted substantially less UV-B, however, presumably because of more potent aging procedures.

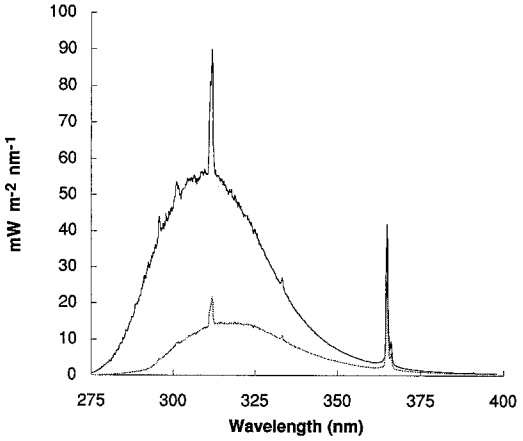


Fig 1. Spectral irradiance of the FS40 lamps, measured at 60 cm, at the surface where the experimental exposures were performed. The upper curve represents unfiltered lamps. The lower curve is for the same lamps filtered by aged cellulose acetate film

Table 1. Biologically effective dose ($mW\,m^{-2}$, 275 to 400 nm) for the FS40 lamps, as employed in the experiments (see Fig. 1). The weightings tabulated by Smith et al. (1980) were used. Weightings for intermediate wavelengths were calculated by linear interpolation. The UV-B weighting is integrated spectral irradiance, 280 to 320 nm. Due to the unnatural irradiance regime, these doses should not be used to describe the effects of UV-B exposures in nature (see text)

Weighting function	Source of irradiance	
	Unfiltered lamps	Filtered lamps
DNA	100	7.7
Plant	285	31
Photoinhibition	1014	223
UV-B	1582	303

Biologically effective dose for the the filtered and unfiltered lamps (Table 1) was determined as described by Smith et al. (1980) using the DNA weighting function of Setlow (1974), the generalized plant function of Caldwell (1971), and the chloroplast photoinhibition function of Jones and Kok (1966).

Results

Photosynthesis of *Thalassiosira pseudonana* was inhibited by UV-B radiation. When a nutrient-replete culture was exposed to a range of UV-B irradiance from unfiltered lamps, photosynthetic rates (P/P_{cont}) for each relative irradiance were depressed to asymptotic values more rapidly than our sampling could resolve (Fig. 2A). The asymptotic rates were inversely correlated with UV-B irradiance. The pattern for the nitrate-limited culture, exposed to filtered lamps, was nearly the same (Fig. 2B). The kinetics of photoinhibition in these experiments were very similar to those observed during exposure to intense visible light (e.g. Samuelsson et al. 1985).

Relative photoinhibition $[(P_{cont}-P)/P_{cont}]$ is plotted vs cumulative dose of UV-B in Fig. 3A, C. At any one time during the experiment, photoinhibition was a monotonic,

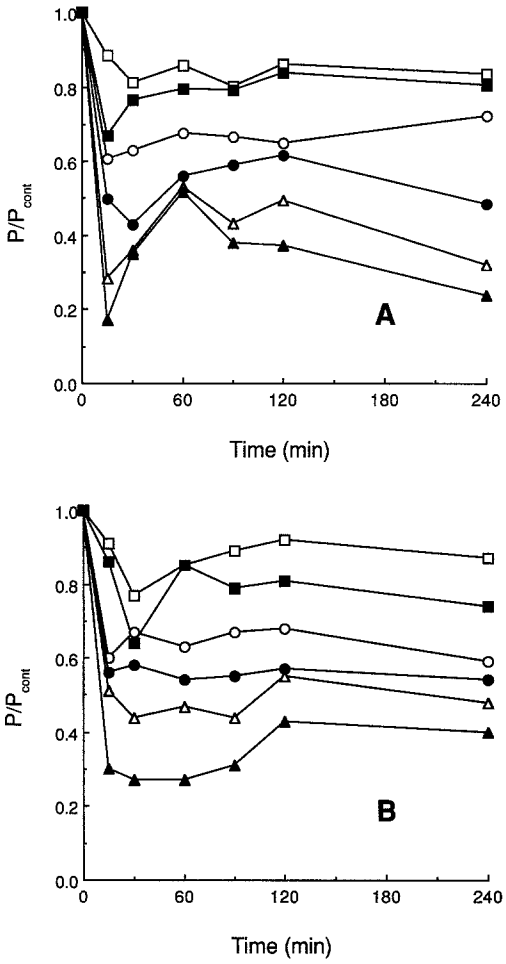
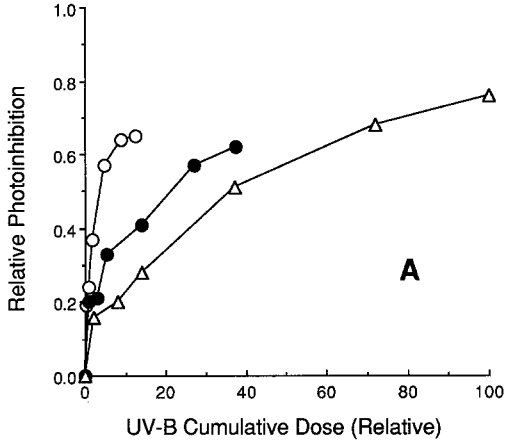


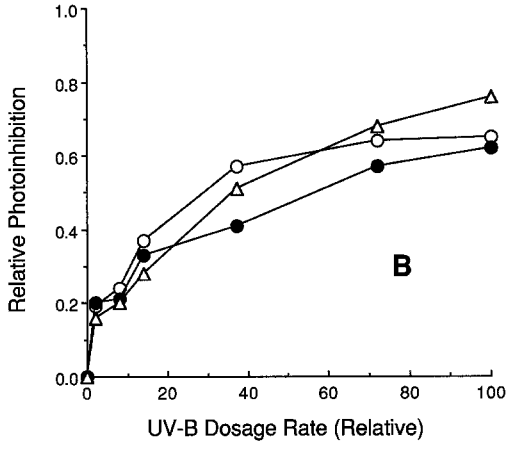
Fig. 2. *Thalassiosira pseudonana.* Relative photosynthesis (P/P_{cont}, cumulative uptake of ^{14}C-bicarbonate, where P_{cont} = uptake by control culture) as a function of supplemental UV-B radiation. Relative dosage rates: (□) 2%, (■) 8%, (○) 14%, (●) 37%, (△) 72%, (▲) 100%. Depression of photosynthesis is a monotonic function of dosage rate for each experiment. (A) Nutrient-replete culture, unfiltered FS40 lamps. (B) Nitrate-limited culture, FS40 lamps filtered with cellulose acetate

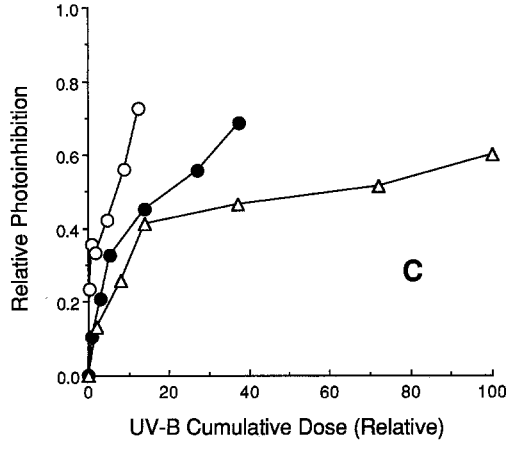
nonlinear function of UV exposure. The relationship is clearly dependent on time scale, however: for equal doses, a relatively short exposure to high UV-B irradiance is more damaging to photosynthesis than a longer exposure to lower irradiance. This result is called a failure of reciprocity (cf. Smith et al. 1980).

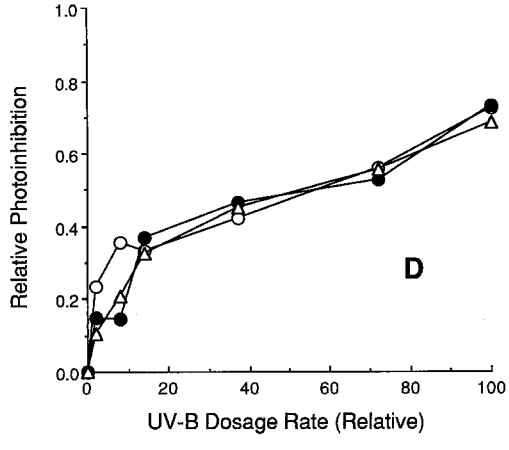
Because photosynthetic rates reached asymptotic values rapidly with respect to sampling times (Fig. 2), photoinhibition could be described as a function of irradiance (Neale and Richerson 1987). For both the nutrient-replete and nitrate-limited cultures, photoinhibition was a monotonic, nonlinear function of irradiance with little discernible dependence on time scale between 30 and 240 min (Fig. 3B, D).

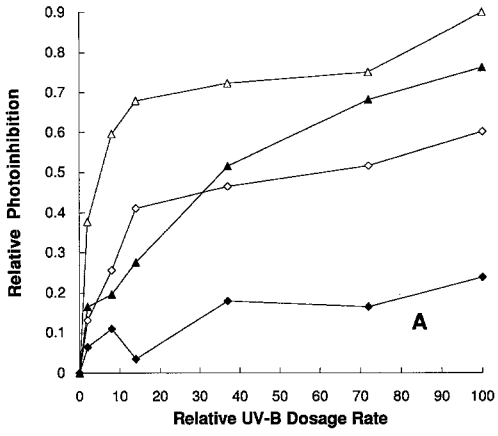
Four experiments were performed for this study: nutrient-replete culture with unfiltered lamps; nutrient-replete with filtered lamps; nitrate-limited with unfiltered lamps; nitrate-limited with filtered lamps (Fig. 4A). As expected, it was found that the unfiltered UV-B lamps were more damaging to photosynthesis than were the











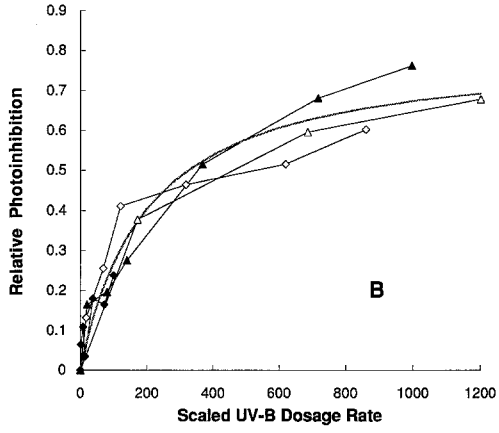


Fig. 4. *Thalassiosira pseudonana.* Influence of spectral irradiance and nutrition on relative photoinhibition $[(P_{cont}-P)/P_{cont}$, where P_{cont} = uptake of ^{14}C-bicarbonate by control culture] over 240 min. (A) Relative photoinhibition as a function of % maximum exposure: (◇) nitrate-limited with filtered lamps; (△) nitrate-limited with unfiltered lamps; (◆) nutrient-replete with filtered lamps; (▲) nutrient-replete with unfiltered lamps. (B) Relative photoinhibition as a function of scaled exposure; symbols as in (A). Irradiance for the nutrient-replete culture under filtered lamps is scaled from 0 to 100, as in Fig. 2. Relative irradiance for nitrate-limited cultures is multiplied by 8.61 and irradiance from unfiltered lamps by 9.97. These factors were obtained from a nonlinear fit to Eq. (1). The smooth curve is the result ($n=28$, $r^2=0.985$)

lamps filtered with cellulose acetate. Also, nitrate-limited cultures were much more sensitive to UV-B than were nutrient-replete cultures. The rapid depression to an irradiance-dependent asymptote (as in Fig. 2) and the nonlinear dependence on relative irradiance were similar for all treatments.

We compared results between experiments by determining and applying sensitivity factors associated with spectral irradiance (filtered vs unfiltered lamps) and nu-

Fig. 3. *Thalassiosira pseudonana.* Relative photoinhibition $[(P_{cont}-P)/P_{cont}$, where P_{cont} = uptake of ^{14}C-bicarbonate by control culture] as a function of supplemental exposure to UV-B radiation. Same experiments as in Fig. 2. (A, B) Nutrient-replete culture, unfiltered FS40 lamps; (C, D) nitrate-limited culture, filtered lamps. (A, C) photoinhibition as a function of cumulative dose (relative units); (B, D) photoinhibition as a function of relative dosage rate (irradiance; relative units). (○) 0 to 30 min, (●) 0 to 90 min, (△) 0 to 240 min

trition (nutrient-replete vs nitrate-limited). A saturation function was used to describe photoinhibition:

$$PI = PI_{max} \times \frac{I f_1 f_2}{K_i + I f_1 f_2} \quad (1)$$

where PI is relative photoinhibition, PI_{max} is the theoretical maximum, I is relative irradiance, and K_i is a saturation parameter. The relative sensitivity factors f_1 and f_2 are for spectral irradiance and nutrition, respectively. For example, $f_1 = 10$ indicates that exposure to the unfiltered lamps at 10% transmission is as damaging as exposure to the filtered lamps at 100% transmission. Likewise, a value of 10 for f_2 indicates that a nitrate-limited culture exposed to UV-B will be inhibited to the same extent as a nutrient-replete culture exposed to 10 times that irradiance. A nonlinear fitting routine (SYSTAT for Macintosh, procedure NONLIN) was used to find the best-fit estimates for f_1 and f_2. The results (Fig. 4B) showed that the unfiltered lamps were 10.0 times ($\pm$2.04 SE) as damaging as the filtered lamps and that nitrate-limited cultures were 8.6 times ($\pm$1.83 SE) more sensitive to UV-B than were nutrient-replete cultures.

We conclude that nitrate-limited cultures of *Thalassiosira pseudonana* are much more sensitive to UV-B than are nutrient-replete cultures and that neither the unnatural wavelengths (<290 nm) emitted by unfiltered lamps nor nutritional differences significantly altered the kinetics of photoinhibition induced by UV-B radiation.

Discussion

Photoinhibition vs dose and dosage rate

When effects of ultraviolet radiation on marine phytoplankton are expressed as a function of dose, results can be generalized only if the effect is independent of the time scale of exposure, i.e., if reciprocity is satisfied (cf. Smith and Baker 1982). We have shown that during exposure to UV-B of 0.5 to 4 h, photoinhibition in a marine diatom is poorly described as a function solely of dose: reciprocity fails. In fact, photoinhibition induced by UV-B was described better as a function of UV-dosage rate (Fig. 3). The failure of reciprocity has been observed in *Chlorella pyrenoidosa* for the inhibition of photosynthesis by UV-C radiation (254 nm; Redford and Myers 1951) and for UV-B induced photoinhibition of photosynthesis in the seagrass *Halodule wrightii* (Trocine et al. 1981).

Possible mechanisms

The dependence of photoinhibition on dosage rate (irradiance) rather than dose is attributed to the activity of repair mechanisms (Kok 1956, Van Baalen 1968, Hirosawa and Miyachi 1983, Samuelsson et al. 1985, Neale 1987). The kinetics of photoinhibition in *Thalassiosira pseudonana* seem to indicate that a balance between damage and repair is established less than 30 min after UV-B exposure begins. Consistent with this interpretation, experiments showed that *T. pseudonana* was much more susceptible to UV-B in the presence of the translation inhibitor streptomycin (Lesser and Cullen unpublished). Streptomycin, and other inhibitors of 70s ribosomal protein synthesis (e.g., chloramphenicol), inhibit the de novo synthesis of chloroplast-encoded proteins required for the photosynthetic integrity of the thylakoid membrane (Ohad et al. 1984, Samuelsson et al. 1985, Kyle 1987). Ohad et al. (1984) and Richter et al. (1990a) used different approaches to show that the degradation of one of these proteins (D1: associated with the Q_B binding site of photosystem II) is involved in photoinhibition. Richter et al. (1990b) also showed that toxic oxygen species cause the degradation of the D1 protein in spinach, and Lesser and Shick (1989) present experimental evidence supporting the involvement of toxic forms of oxygen in the photoinhibition of photosynthesis in symbiotic dinoflagellates (=zooxanthellae) of sea anemones when exposed to ultraviolet radiation.

Generality of the result

Although there is broad-based evidence for the existence of repair mechanisms which lead to the failure of reciprocity during inhibition of photosynthesis by UV-B radiation, we cannot assume that our results for *Thalassiosira pseudonana* apply to marine phytoplankton in general. For example, Trocine et al. (1981) demonstrated a failure of reciprocity and light-activated repair in the seagrass *Halodule wrightii*, but two other species of seagrass did not show photorepair, and unlike *H. wrightii*, those species demonstrated reciprocity in UV-B induced photoinhibition. Inhibition of net photosynthesis in another higher plant, *Rumex patentia*, was also well described as a function of dose (Caldwell et al. 1986), and reciprocity has been satisfied during other experiments to determine the sensitivity of photosynthesis to UV-B (Sisson 1986).

It is apparent that photoinhibition of photosynthesis by UV-B cannot be described generally as a function either of dose or of dosage rate. For any particular time scale, however, the two are equivelent if irradiance is constant. Thus, relationships between exposure and response can be compared between experiments on similar phytoplankton assemblages if time scales are specified.

Nutrient limitation

Nutrient-limited phytoplankton have been shown to be more sensitive than their nutrient-replete counterparts to excess visible light (Kiefer 1973, Prézelin et al. 1986). Here we have shown that nitrate-limitation enhances the susceptibility of *Thalassiosira pseudonana* to UV-B radiation. It should be noted that our nitrate-limited cultures were grown in continuous culture. On a 24 h time scale, they were in balanced growth. Accordingly, photosynthetic performance is visible light, normalized to chlorophyll, was not degraded (Cullen 1990). When phytoplankton in batch culture are subjected to nitrogen star-

vation, however, photosynthetic performance declines (Welschmeyer and Lorenzen 1981, Cleveland and Perry 1987). We do not know how susceptibility of photosynthesis to UV-B is changed during nitrogen starvation.

Relevance to action spectra

Photorepair, second-order effects and the failure of reciprocity can greatly complicate the determination of biological weighting functions (Coohill 1989). Because photosynthesis and associated repair mechanisms interfere with dose-response relationships in studies of photoinhibition, Jones and Kok (1966) excluded them from consideration by measuring partial reactions in chloroplasts to describe the action spectrum of photoinhibition. Their work was important in identifying characteristics of the damage function, yet their action spectrum does not necessarily represent the appropriate biological weighting function for photoinhibition of photosynthesis in the sea.

The spectral sensivity of natural phytoplankton to UV radiation was examined by Smith et al. (1980). Photosynthesis was measured during half-day incubations in UV-transparent quartz bottles in situ or under ambient daylight attenuated with neutral-density screens. Experimental treatments included enhancement of UV-B with filtered FS40 lamps and exclusion of UV-B with mylar or vinyl filters. Different biological weighting functions (action spectra) were used to construct dose-response relationships for photoinhibition of photosynthesis. It was found that when dose was calculated from the weighting function of Jones and Kok (1966), consistent relationships between photoinhibition and calculated exposure were obtained, whereas neither Setlow's (1974) DNA action spectrum nor Caldwell's (1971) generalized action spectrum for effects on plants generated a plot of photoinhibition that was a monotonic function of dose. Accordingly, the action spectrum of Jones and Kok (1966) was tentatively accepted for use in a model of photoinhibition in the sea (Smith et al. 1980, Smith and Baker 1982).

Smith et al. (1980) considered their technique to be valid only if reciprocity holds. Our results show that for at least one marine diatom, reciprocity fails to hold for photoinhibition of photosynthesis. Nonetheless, we feel that the evaluation of action spectra by Smith et al. (1980) is legitimate. The time scale for the measurements was clearly specified, and for any particular time scale photoinhibition is likely to be a monotonic function of cumulative dose.

Results of our experiments (Figs. 2 to 4) could have been reported in terms of biologically weighted dose ($J\,m^{-2}$) or weighted irradiance ($W\,m^{-2}$). Further, relative inhibition by filtered vs unfiltered lamps could have been examined for consistency with different weighting functions (Table 1; cf. Smith et al. 1980). These analyses were performed, but they are not presented here, to avoid any suggestion that the degree of photoinhibition observed in our experiments would be expected for equivalent weighted exposures in nature, where UV-A radiation (320 to 400 nm) and visible light would be much more intense (Smith and Baker 1989). Unnatural radiation regimes were used here to examine the kinetics of photoinhibition, not spectral sensitivity. It was shown that the principal kinetic features for photoinhibition under unnatural irradiance regimes and different culture conditions were similar to those observed in nature (Neale 1987). Different experiments (cf. Maske 1984, Caldwell et al. 1986) are appropriate for examining spectral sensitivity. Such experiments can be interpreted more effectively with knowledge of the kinetics of photoinhibition.

Photoinhibition and vertical mixing

Measurements of photosynthesis in the water column can yield biased results because static incubations at near-surface irradiance often show photoinhibition, whereas vertical mixing in nature can mitigate the effects of excessive light (Marra 1978 b, Gallegos and Platt 1985). The problem has been addressed many times (review: Neale 1987), usually without explicit consideration of ultraviolet radiation. Vertical mixing is nonetheless well recognized as a major complication in studies of the effects of UV-B on phytoplankton (Kullenberg 1982, Smith and Baker 1982).

We have shown here that for a marine diatom, the kinetics of photoinhibition induced by UV-B are similar to the kinetics of photoinhibition in excess visible light (Samuelsson et al. 1985): on time scales greater than ca. 1 h, photoinhibition is a function of irradiance rather than of dose. On shorter time scales, however, as photosynthesis approaches its asymptotic inhibited rate, relative photoinhibition is a function of time (Neale 1987: p. 57).

It is instructive to examine the consequences of dose-dependence vs irradiance-dependence of photoinhibition on the measurement of photosynthesis in aquatic environments. If relative photoinhibition is a function of dose, photoinhibition is completely time-dependent (Fig. 5A). Thus, when the mixed layer circulates rapidly (time scale less than ca. 1 h), conventional measurements of photoinhibition (full spectrum or UV-B component) are likely to overestimate photoinhibition. In contrast, if photoinhibition is wholly dependent on dosage rate (irradiance), experimental results are independent of time scale and can be considered valid, regardless of the rate of vertical mixing (Fig. 5B).

Our results suggest that photoinhibition is irradiance-dependent only for time scales longer than the induction period for photoinhibition, i.e., >ca. 1 h. Thus, conventional incubations of 4 to 24 h will adequately represent nature only if the water column is relatively stable over the same period. That is, for an experimental result to be valid, the time scale of the measurement must be the same same as the time scale of vertical mixing (e.g. Harris 1980, Legendre and Demers 1984). An alternative approach can be used: independent measurements of short-term photosynthetic performance or fluorescence characteristics can be measured in vertical profile to estimate the influence of irradiance-dependent processes during verti-

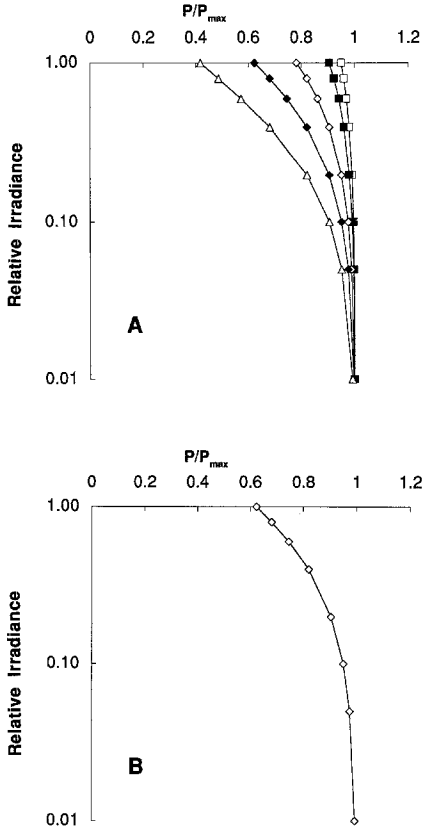


Fig. 5. Relative photoinhibition as a function of time of exposure for hypothetical measurements in a water column. The vertical axis represents relative irradiance, scaled according to its potential to inhibit photosynthesis (logarithmic scale). (A) Photosynthesis (P, relative to maximal photosynthesis P_{max}) modeled as a function of cumulative dose (i.e., time of exposure in dimensionless units relative to the time scale of photoinhibition). Time = (□) 0.1, (■) 0.2, (◇) 0.5, (◆) 1.0, (△) 2.0. The result is strongly a function of incubation time. (B) Photosynthesis modeled as a function solely of irradiance. Result is the same, regardless of incubation time

cal mixing (Lewis et al. 1984, Vincent et al. 1984, Neale and Richerson 1987).

Conclusions

It is not easy to examine directly the effects of the UV-B radiation on marine primary productivity. Natural radiation regimes are extremely difficult to simulate, especially when vertical mixing through a submarine irradiance field is considered. Action spectra are essential to models, but their measurement is problematic. Nonetheless, numerous attempts have been made to estimate the inhibition of phytoplankton photosynthesis by UV-B radiation. These efforts were made when little was known about the effects of UV-B on the kinetics of photoinhibition. Results have previously been expressed as a function of dose, but the importance of time scale has been explicitly recognized (Smith et al. 1980). We have shown that with respect to the kinetics of photoinhibition in a marine diatom, UV-B radiation acts much like excess visible light. We conclude that the voluminous literature on photoinhibition and vertical mixing is likely to apply to UV-B effects as well as to the effects of visible radiation. Depending on the time scale of the measurement and the activity of repair mechanisms, photoinhibition can be a function either of dose or of dosage rate. For a specified time scale, however, relative dose and relative irradiance can be equivalent. With due caution, biological weighting functions can be determined even though reciprocity fails. By carefully considering the fundamental processes involved, oceanographers should be able to assess the effects of ultraviolet radiation on marine photosynthesis even though they cannot simulate nature experimentally.

Acknowledgements. This research was supported by grants from the National Science Foundation (DPP 881768), the Office of Naval Research (N0014-89-J-1066, N0014-89-J-1239) and NASA (NAGW 2072). Dedicated to the memory of Chase Van Baalen. Bigelow Laboratory Contribution No. 91021.

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