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Is there a common response to ultraviolet-B radiation by marine phytoplankton?

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ABSTRACT: Global increases in ultraviolet-B radiation (UVBR) have the potential to alter marine primary production and to affect carbon cycles and marine trophic dynamics. Estimates of UVBR induced photoinhibition have varied greatly, indicating that a common dose-response by marine phytoplankton may not occur from place to place. An action spectrum describing the wavelength specific effects of UVBR on carbon fixation was determined by comparing responses of phytoplankton exposed to natural and artificial UVBR sources. Application of this new action spectrum to data presented here, as well as data reported previously, indicates that a common photoinhibitory response to UVBR may occur for exposures of several hours.

KEY WORDS: Action spectra · Dose response · Phytoplankton · Ultraviolet-B radiation

INTRODUCTION

The quantitative effects of ultraviolet-B radiation (UVBR: 290 to 320 nm) on marine primary production have been the focus of intensive research almost since stratospheric ozone depletion was discovered (Lorenzen 1979, Smith et al. 1980, 1992, Worrest et al. 1981, Behrenfeld et al. 1992, 1993a, Helbling et al. 1992). Developing a reliable estimate of the dose response of marine phytoplankton to UVBR has been a common goal of this research, yet has remained elusive largely due to an incomplete knowledge of the wavelength-dependent biological effects of UVBR.

Stratospheric ozone depletion will result in disproportionate increases in the 290 to 300 nm range of the UVBR spectrum relative to longer wavelengths (Green et al. 1980). Since shorter UVBR wavelengths cause more biological damage per unit of energy than longer UVBR wavelengths (Jones & Kok 1966, Caldwell 1971, Setlow 1974, Rundel 1983, Caldwell et al. 1986, Cullen et al. 1992, Lubin et al. 1992, Quaite et al. 1992), projected global increases in biologically harmful UVBR will be proportionately greater than total increases in UVBR energy. Biological effects of UVBR at each wavelength are extremely difficult to determine independently in nature. Action spectra (mathematical weighting functions) are therefore applied to irradiation doses from entire radiation spectra when estimates of biological effect are of interest.

Applications of action spectra are optimal when limited to the organisms (or cellular components) from which they are derived. However, experimental determinations of action spectra for every organism or cell component of interest is impractical. Therefore, action spectra are commonly applied to experimental irradiance conditions vastly different from the conditions in which they were originally determined.

The most commonly applied action spectra in studies of UVBR effects on marine phytoplankton were not derived for marine phytoplankton. These action spectra are: (1) the photoinhibition action spectrum (PI) of Jones & Kok (1966), derived from Hill reaction inhibition of spinach chloroplasts by radiation between 260 and >560 nm; (2) the Caldwell (1971) plant action spectrum (CPA), derived from the damage spectra of several terrestrial plants for wavelengths <313 nm; and (3) the DNA action spectrum of Setlow (1974) derived from photoproducts in DNA and the mutation rates and mortality of bacteria and phages at wavelengths between 250 and 370 nm. However, these action spec-

tra may not be appropriate for studies on UVBR inhibition of carbon fixation in marine phytoplankton. Three additional action spectra have recently been reported that are more appropriate for marine phytoplankton (Cullen et al. 1992, Helbling et al. 1992, Lubin et al. 1992), although a wavelength-specific action spectrum for natural phytoplankton assemblages has not yet been described.

The largest data sets available on photoinhibition (decrease in carbon fixation) by UVBR in natural marine phytoplankton are those of Smith et al. (1980) and Behrenfeld et al. (1993a). Their results are dramatically different despite similar methods used to determine photoinhibition. Photoinhibition was described by Smith et al. (1980) as a linear function of UVBR dose weighted by the PI action spectrum. Behrenfeld et al. (1993a) found the DNA action spectrum best described the wavelength specific photoinhibition by UVBR, even though the effect was probably not due to DNA damage. The DNA action spectrum gives shorter wavelengths a relatively greater effective weight than the PI action spectrum. This difference is critical since a 17.4% increase in mid-latitude noon UVBR from ozone depletion (Green et al. 1980, Stolarski et al. 1992) would correspond to an increase in biologically effective UVBR of only 20% when weighted by the PI action spectrum compared to an 85% increase when weighted by the DNA action spectrum.

In the current study, a biological action spectrum, consisting of a simple exponent, was derived from comparisons of phytoplankton responses to different spectral conditions and UVBR doses. Application of the exponential action spectrum to data from previous studies (Smith et al. 1980, Behrenfeld et al. 1993a) indicates a common phytoplankton response to UVBR.

MATERIALS AND METHODS

UVBR exposure experiments were conducted during April 1991 between 48° N, 125° W and 48° N, 128° W on board the NOAA RV 'Discoverer'. Phytoplankton carbon fixation rates were determined using the carbon-14 light and dark bottle technique (Parsons et al. 1984). Phytoplankton samples were collected at or before dawn from the upper 2 m of the ocean surface. Collected samples were dispensed into 250 ml UVBR transparent FEP Teflon® bottles, inoculated with 10 µCi $NaH^{14}CO_3$, and immediately placed in an on-deck incubator (Behrenfeld et al. 1993a). Flow-through seawater maintained the samples at sea-surface temperatures during the incubations. Samples were incubated for 4 to 8 h. Five replicates were used in each of 4 treatments: (1) UVBR excluded; (2) ambient UVBR; (3) UVBR enhanced above ambient; and (4) dark (Fig. 1). UVBR enhancements were not initiated until solar intensities were fairly bright (i.e. 0.5 to 4 h after samples were placed in the incubator).

Following incubation, each sample was filtered through a 0.45 µm polycarbonate filter (Millipore®) at 70 kPa. The filters were then fumed over concentrated HCl for 3 min to remove inorganic carbon. Carbon uptake rates (mg C m^{-3} h^{-1}) were determined by liquid scintillation counting. Counts per minute measured by the scintillation counter (Packard Model 2000CA) were corrected for background and quenching using a radioactive standard. Carbon-14 uptake in the dark treatment was subtracted from the light treatments.

Ultraviolet fluorescent lamps (UVB 313, Q-Panel Co.), preburned for 100 h, were used to create the enhanced UVBR treatment. The lamps were located beneath an incubation tank with a UVBR transparent acrylic (Acrylite OP-4, CYRO Industries) bottom. A 0.13 mm sheet of cellulose acetate between the lamps and the enhanced UVBR treatment bottles eliminated lamp radiation <290 nm. A 0.13 mm sheet of Mylar® between the lamps and all other bottles eliminated lamp radiation <315 nm. Bottles in the UVBR-excluded treatment were wrapped with a 0.13 mm sheet of Mylar® to eliminate all solar wavelengths <315 nm. All other bottles were wrapped with a 0.13 mm sheet of cellulose acetate to reduce spectral differences between treatments at wavelengths >347 nm. Cellulose acetate and Mylar® films were replaced periodically to avoid photodegradative changes in transmittance properties. Imperfect cutoff characteristics of Mylar® resulted in spectral divergence between treatments for wavelengths <347 nm (Fig. 1). Therefore, UVBR doses reported herein include treatment differences for wavelengths <347 nm. Differences between transmittance properties of cellulose acetate and mylar at longer wavelengths caused total radiation doses between 348 and 750 nm to vary by <1% between treatments. One or two layers of neutral density screen (gray plastic mesh) were used between the lamps and the incubation tank to adjust the intensity of the enhanced UVBR dose.

Ambient solar radiation (285 to 800 nm) was continuously monitored using an Optronic Model 752 spectroradiometer clear of all shading mounted on the uppermost deck of the ship. The spectroradiometer was calibrated for wavelength offset by scanning a mercury arc lamp and for intensity using a halogen lamp traceable to the National Institute of Standards and Technology. The calibrated Optronic 752 has a reported wavelength accuracy of ±0.3 nm, wavelength precision of ±0.1 nm, and an intensity accuracy of ±2 to 4% for the entire range of 285 to 800 nm. Ambient UVBR in the sample bottles during each incubation

was calculated from the solar spectral scans by correcting for transmittance characteristics of the teflon bottles and integrating between scans. Enhancement of UVBR from lamps beneath the incubation chamber was measured with the Optronic 752 spectroradiometer at various positions within the chamber through a section of teflon bottle using both new and aged Mylar® and cellulose acetate filters.

RESULTS

Phytoplankton were sampled both inside and outside of the coastal upwelling region off the Washington state coast. Carbon fixation rates were high (12.1 to 39.2 mg C m^{-3} h^{-1}) inside and low (0.3 to 0.9 mg C m^{-3} h^{-1}) outside the upwelling region (Table 1). Compared to uptake in the ambient UVBR treatment, exclusion of solar UVBR resulted in enhanced carbon fixation rates, while enhancement of UVBR depressed carbon fixation (Table 1).

Comparisons of the percent decrease in carbon fixation per total unweighted UVBR dose between the ambient and excluded treatments and the ambient and enhanced treatments reveal separate, linear dose-responses (Fig. 2a). Lamp radiation produced greater decreases in carbon fixation per unit UVBR energy than did solar radiation (Fig. 2a). The ultraviolet fluorescent lamps produce a spectrum enriched in the short UVBR wavelengths compared to the solar spectrum (Fig. 3) and, therefore, a greater biologically effective dose per unit of energy. The lesser effect of solar UVBR compared to artificially enhanced UVBR (Fig. 2a) indicates, once again, that application of an action spectrum to the spectral data is necessary to compare dose-responses to the different UVBR spectra.

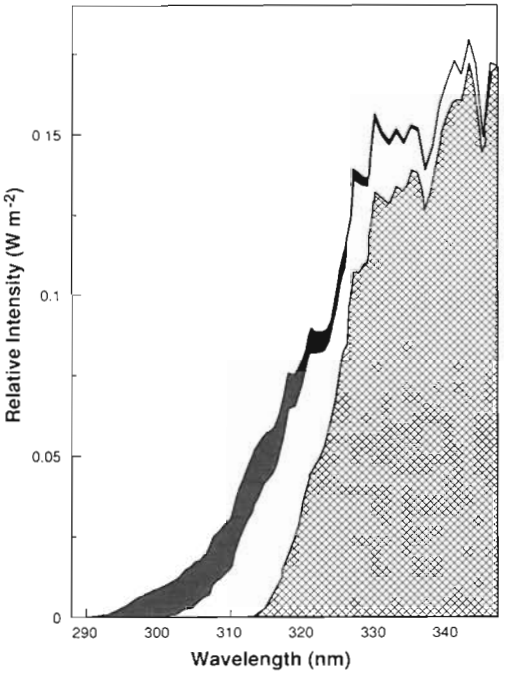

Fig. 1 Spectra in the ultraviolet-B radiation (UVBR) treatments during clear sky conditions at approximately local apparent noon. Shaded: UVBR enhanced; unshaded: ambient UVBR; crosshatched: UVBR excluded

The correct, or 'best fit', action spectrum will result in convergence of the slopes and intercepts of dose responses calculated independently from divergent radiation spectra and the highest regression coefficient for the combined data (Smith et al. 1980). The PI, CPA, and the DNA action spectra all give more biologically effective weight to short wavelengths than long wavelengths. Of the 3 action spectra, the PI spectrum has the least slope and the DNA spectrum has the greatest

Table 1. Incubation times, UVBR doses, and carbon fixation rates (± SD) during each experiment. UVBR doses are total doses compared to the UVBR excluded treatment and are weighted by the exponential action spectrum (Eq. 2)

Date (1991)	Incubation time (h)	UVBR dose ($J\ m^{-2}\ EXP_{300}$)		Carbon fixation rate ($mg\ C\ m^{-3}\ h^{-1}$)		
		Ambient	Enhanced	Excluded	Ambient	Enhanced
18 Apr	6.0	834	3062	0.65 (± 0.04)	0.55 (± 0.03)	0.37 (± 0.04)
19 Apr	7.5	734	3457	0.74 (± 0.05)	0.68 (± 0.03)	0.48 (± 0.02)
20 Apr	6.5	759	2864	0.74 (± 0.02)	0.65 (± 0.05)	0.51 (± 0.04)
21 Apr	6.5	943	3171	0.75 (± 0.04)	0.60 (± 0.08)	0.39 (± 0.03)
22 Apr	7.0	979	2826	16.7 (± 0.93)	15.5 (± 0.57)	13.4 (± 1.26)
23 Apr	7.0	456	3757	12.1 (± 0.76)	12.4 (± 0.69)	7.8 (± 1.00)
24 Apr	7.8	867	2004	20.4 (± 1.22)	19.3 (± 2.30)	16.4 (± 0.22)
25 Apr	7.2	780	1917	0.92 (± 0.30)	0.87 (± 0.11)	0.75 (± 0.26)
26 Apr	7.5	1254	3978	0.73 (± 0.16)	0.60 (± 0.07)	0.37 (± 0.05)
27 Apr	8.0	1445	4292	39.2 (± 3.31)	30.7 (± 0.76)	18.7 (± 1.94)
28 Apr	6.8	1655	2934	35.3 (± 4.25)	28.0 (± 2.04)	22.1 (± 4.36)

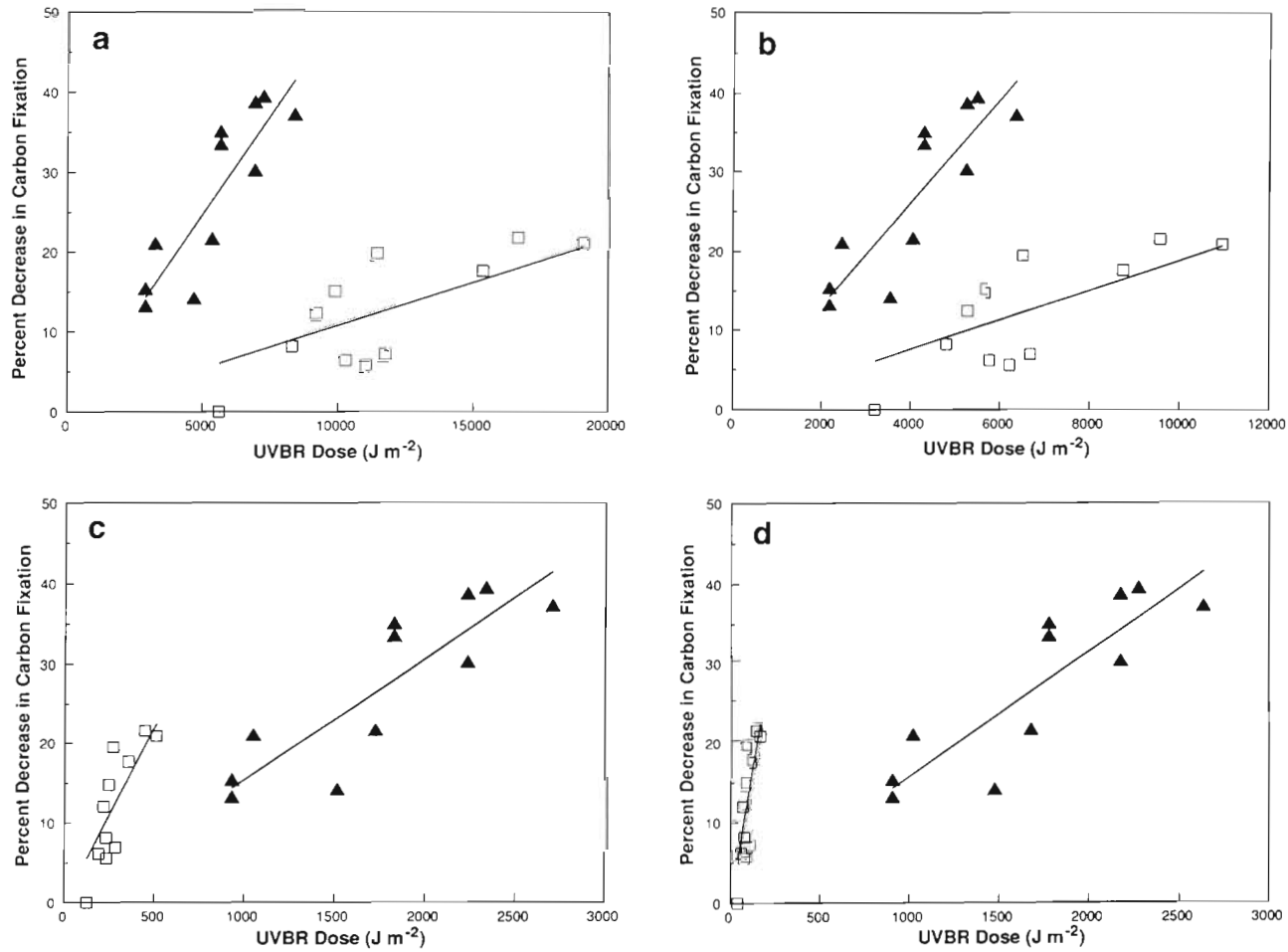

Fig. 2. Percent decrease in carbon fixation as a function of ultraviolet-B radiation (UVBR) dose (the incremental dose relative to the ambient treatment). (a) Unweighted UVBR doses; (b) UVBR weighted by the photoinhibition action spectrum of Jones & Kok (1966); (c) UVBR doses weighted by the Caldwell (1971) plant action spectrum; and (d) doses weighted by the DNA action spectrum of Setlow (1974). Action spectra used for weighting the UVBR doses were all normalized to 1 at 300 nm. (□) Decreases between the UVBR-excluded and ambient UVBR treatments; (▲) decreases between the ambient and enhanced UVBR treatments

slope (Fig. 4a). None of these predetermined action spectra satisfy the criterion for the 'best fit' action spectrum (Fig. 2b, c, d), although the 'best fit' action spectrum clearly lies between the PI and the DNA spectra.

Fortuitously, the PI, CPA, and DNA action spectra are closely approximated between 290 and 347 nm by an exponential model:

$$\varepsilon(\lambda)_{290-347} = \alpha e^{c\lambda} \quad (1)$$

where $\varepsilon(\lambda)$ = wavelength-dependent biological efficiency for photoinhibition; c = a constant describing the slope of the curve; λ = wavelength between 290 and 347 nm; and α = a normalization factor (Fig. 4a). An exponential action spectrum (where $c = -0.022$) describes the PI spectrum almost exactly between 290 and 347 nm. The DNA spectrum deviates from an exponential model at the shortest UVBR wavelengths and between 320 and 347 nm (Fig. 4b). The CPA spectrum only weights wavelengths ≤313 nm.

The action spectrum for UVBR induced photoinhibition of phytoplankton carbon fixation can be determined from Eq. (1) by choosing a value for c which satisfies the 'best fit' criterion (Rundel 1983). Advantages of such an action spectrum are its direct relevance to the cellular target(s) of interest and the fewer assumptions required for its application compared to more complex action spectra used previously. The best fit exponential action spectrum, normalized to 1 at 300 nm, was:

$$\varepsilon(\lambda)_{290-347} = 3.318 \times 10^{17}\, e^{-0.0448\lambda} \quad \text{(Fig. 4a)} \quad (2)$$

Weighting UVBR doses by the exponential action spectrum described by Eq. (2) results in a single linear dose-response for all treatment data (Fig. 5a). The dose

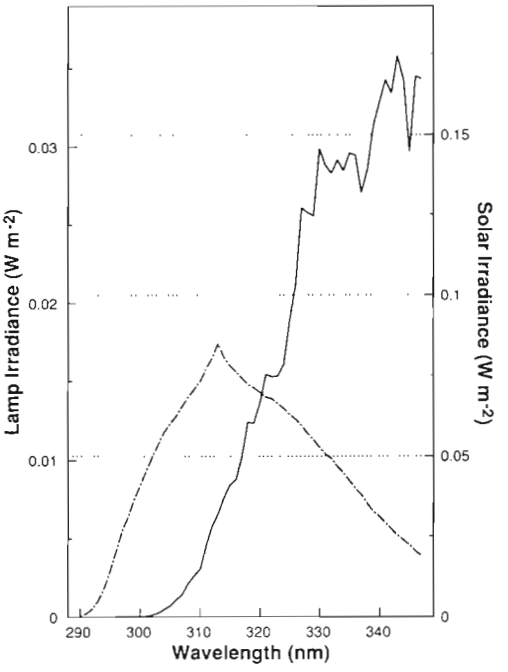

Fig. 3. Spectral distribution and relative intensity of solar (—) and lamp + cellulose acetate (– –) radiation measured with an Optronic model 752 spectroradiometer

range can be expanded by comparing carbon fixation rates in the ambient and enhanced UVBR treatments to the UVBR excluded treatment (Fig. 5b), resulting in the dose-response relationship:

$$P_C = 0.0116 Q_{EXP} \quad (3)$$
$$(r^2 = 0.86,\ p < 0.001,\ n = 22)$$

where P_C = percent photoinhibition calculated as $[(CF_{EXCL} - CF_{UVB})/CF_{EXCL}] \times 100$; where CF_{EXCL} = carbon fixation in the UVBR excluded treatment, and CF_{UVB} = carbon fixation in either the ambient or enhanced treatment; and Q_{EXP} = cumulative UVBR dose (J m^{-2}) between 290 and 347 nm weighted by the 'best fit' exponential action spectrum (Eq. 2), which is normalized to 1 at 300 nm (i.e. EXP_{300}). The regression slope (Eq. 3) is half as steep as the regression slope reported by Behrenfeld et al. (1993a) ($P_C = 0.022\, Q_{DNA}$), where cumulative UVBR dose (Q_{DNA}) was weighted by the DNA action spectrum normalized to 1 at 300 nm (i.e. DNA_{300}). However, lower slope of the dose-response could have been anticipated since the 'best fit' action spectrum (Eq. 2) is approximately half as steep as the DNA action spectrum (Fig. 4a, b).

Experimental results reported herein must be interpreted with caution. Experiments were of short duration (4 to 8 h) and may not be representative of the longer term effects of UVBR on growth and biomass

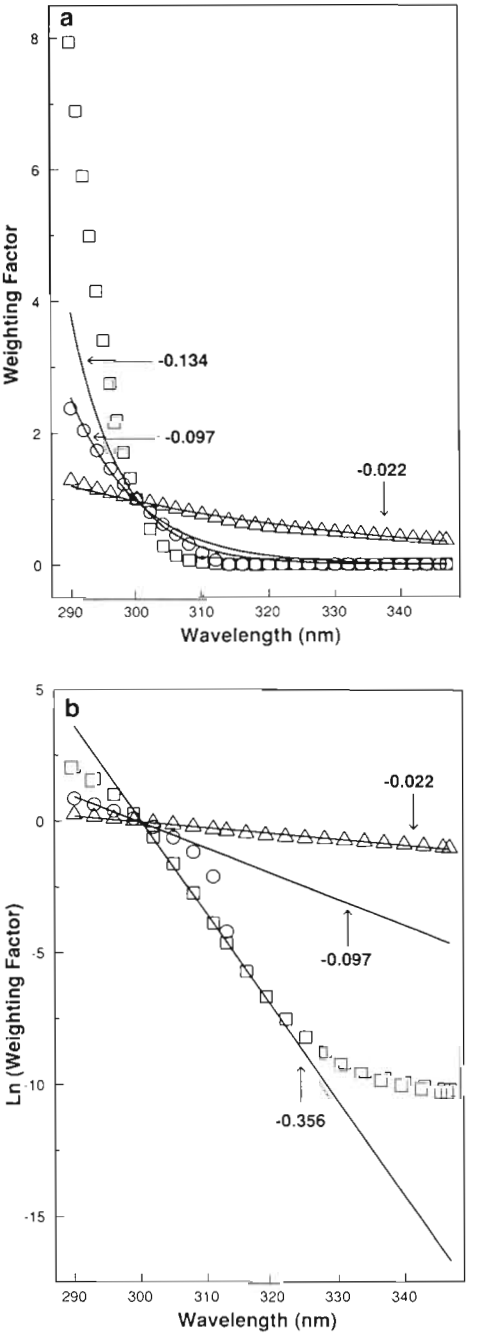

Fig. 4. Comparison of (Δ) photoinhibition, (O) CPA and (□) DNA action spectra between 290 and 347 nm (a) with exponential curves and (b) expanded to a ln:normal scale to show the relationships more clearly. Exponent values are indicated for each curve. (a) DNA action spectrum compared to 'best fit' exponential (–0.13448; Eq. 2). (b) Exponent derived from the linear portion of the ln-transformed DNA action spectrum (–0.356). All action spectra normalized to 1 at 300 nm

(Behrenfeld et al. 1992, 1993b). UVBR effects measured during incubations of several hours may also not be representative of natural conditions when the aver-

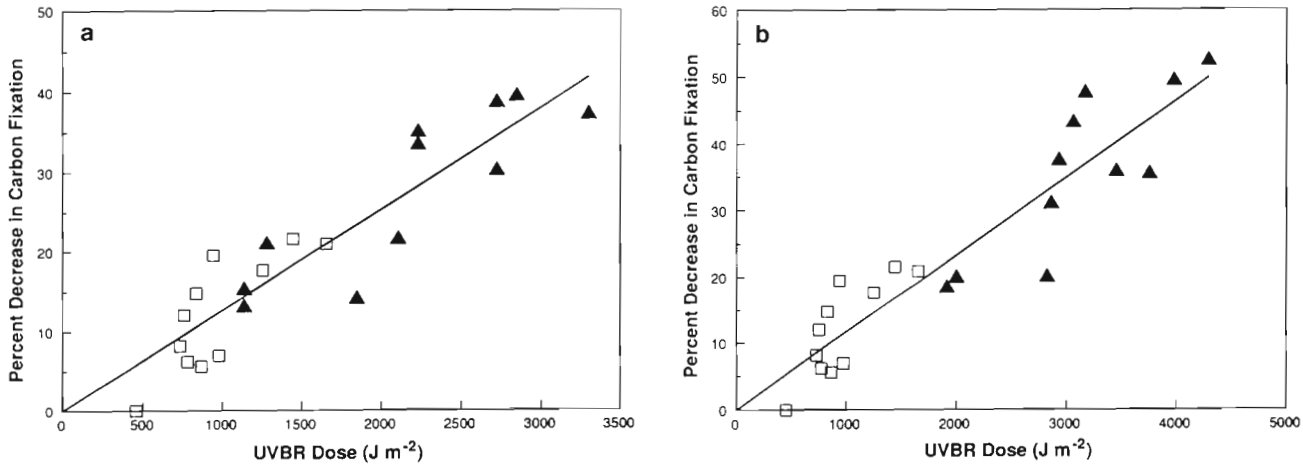

Fig. 5. Dose response using the 'best fit' exponential action spectrum (Eq. 2). (a) Dose response for percent decrease in carbon fixation between ambient and excluded ultraviolet-B radiation (UVBR) treatments and ambient and enhanced UVBR treatments (R^2 = 0.83). (b) Same dose response as in (a) except with the range of doses expanded by making all comparisons to the UVBR excluded treatment (R^2 = 0. 86). (□) Effect of solar UVBR; (▲) effect of enhanced UVBR

age exposure of phytoplankton to surface UVBR is limited to much shorter periods by rapid vertical mixing. Furthermore, these experiments were performed using closed bottles which very likely create unnatural effects.

DISCUSSION

We found cumulative inhibition of carbon uptake by UVBR during incubations of several hours to be a linear function of UVBR dose weighted by the 'best fit' exponential action spectrum (Eq. 2). Dose responses previously reported vary from linear to sigmoidal for biologically weighted and unweighted UVBR doses. Inhibition of carbon uptake by UVBR has also been reported as a function of dose rate, rather than cumulative UVBR dose (Cullen & Lesser 1991). A complete review of UVBR literature is beyond the scope of this report. However, we have made an intercomparison between our dose response (Eq. 3) and dose responses from the 2 largest published data sets for UVBR inhibition of phytoplankton carbon fixation (Smith et al. 1980, Behrenfeld et al. 1993a). Conversion of all UVBR doses to doses weighted by our exponential action spectrum results in a common dose-response for all 3 studies. Our linear dose-response is also discussed with regard to previously reported sigmoidal dose-responses (e.g. Helbling et al. 1992). Finally, we compared measured photoinhibition-rates to UVBR dose rates to determine whether any effect of dose-rate could be identified within the range of exposures used during our study.

Intercomparison of dose-responses

UVBR dose-response data from previous field studies (Behrenfeld et al. 1993a), when recalculated using the new action spectrum (Eq. 2), correspond closely with data herein and also indicate no distinguishable threshold (Fig. 6). The dose response for the combined data is:

$$P_C = 0.007 Q_{EXP} \quad (4)$$
$$(r^2 = 0.53,\ p < 0.001,\ n = 90)$$

Slope of the dose response for the combined data is slightly lower than the dose response described in Eq. (3). These combined data represent the largest published set of observations on UVBR effects on carbon uptake by natural phytoplankton and include observations from coastal areas, open ocean gyres, equatorial and mid-latitude upwelling areas, and the Antarctic convergence and include both surface and deep (20 to 40 m) samples.

The next largest available data set on marine phytoplankton photoinhibition by UVBR was reported by Smith et al. (1980). They compared dose responses based on PI, CPA, and DNA weighted doses, and found the greatest convergence between treatments when UVR doses were weighted by the PI action spectrum. They compared action spectra using data from one experiment (4 data points per treatment comparison) (Fig. 3 in Smith et al. 1980). Interestingly, regression slope for their DNA weighted ambient UVR dose-response data ($P_C \approx 0.018\, Q_{DNA}$) used in the action spectrum comparison was similar to the slope of the dose response re-

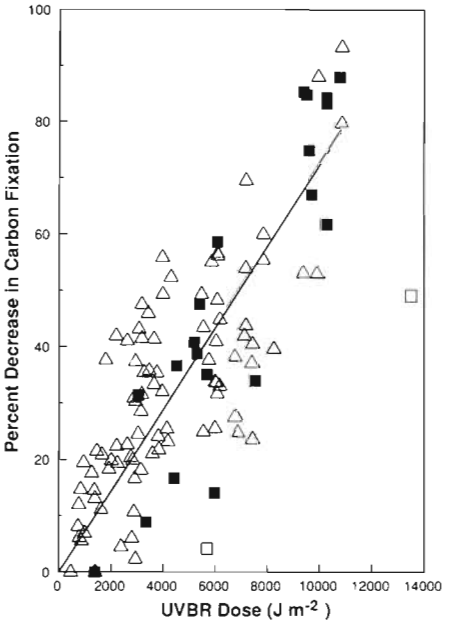

Fig. 6. Dose response for combined data describing photoinhibition as a function of UVBR doses weighted by the 'best fit' action spectrum (n = 112, R^2 = 0. 65). (Δ) 1991 data from Washington coast and data from Behrenfeld et al. (1993a); (■) ambient treatment data from Smith et al. (1980) converted from photoinhibition doses to EXP_{300} doses; (□) ambient data of Smith et al. (1980) not used in calculation of combined regression coefficient

ported by Behrenfeld et al. (1993a; $P_C = 0.022\, Q_{DNA}$). We therefore converted all the ambient UVR doses reported by Smith et al. (1980) into UVR doses weighted by the exponential action spectrum (Eq. 2) using simple conversion factors, and found their ambient UVBR dose responses converge with our dose response (Fig. 6).

Conversions of UVBR doses weighted by one action spectrum to another are possible using simple conversion factors when radiation spectra are constant. Conversion of our ambient and enhanced UVBR doses weighted by the DNA_{300} action spectrum to doses weighted by our new exponential action spectrum (Eq. 2) results in standard errors of only ±7% and ±0.1% respectively. Error in the ambient UVBR conversion could result entirely from variability in the solar spectrum. Lamp UVBR doses can be converted almost exactly because lamp spectra are constant.

We converted the ambient UVR doses weighted by the PI_{270} action spectrum reported by Smith et al. (1980; their Fig. 4) to doses weighted by the exponential action spectrum (i.e. EXP_{300}) by: (1) multiplying the DNA_{265} doses reported in their action spectrum comparison by 30.6525 to convert to DNA_{300} doses; (2) converting PI_{270} doses to DNA_{300} doses using the factor 0.0106 (calculated from their action spectrum comparison data); and (3) converting DNA_{300} doses to EXP_{300} doses using the factor 4.30 (calculated from our ambient UVR data). The converted ambient UVBR dose response data of Smith et al. (1980) thus closely correspond to our dose response data (Fig. 6) and increase the regression coefficient (R^2 = 0.65) for the combined data. Conversion of ambient UVR doses of Smith et al. (1980) from DNA_{300} to EXP_{300} using a conversion factor calculated from our ambient UVBR data should not result in large errors because both studies utilized surface solar radiation spectra for their ambient UVBR treatments. However, our conversion factors should not be used for dose conversions between treatments of different spectral quality (e.g. UVBR doses at different depths).

Dose-response slope for the 4 enhanced UVBR observations used by Smith et al. (1980, their Fig. 3a) for their action spectrum comparison does not correspond to the slope of our dose response. This discrepancy may have resulted from using enhanced UVBR response data collected from exposures to unfiltered UV lamps, which emit wavelengths <290 nm (i.e. ultraviolet-C radiation: UVCR). Unfiltered UV lamp radiation weighted by the DNA_{300} action spectrum represents a much greater biologically effective dose than filtered lamp radiation weighted by the DNA_{300} action spectrum. Smith et al. (1980) used FS40 Westinghouse UV lamps to enhance the UVBR dose above ambient. Unfiltered FS40 Westinghouse lamps measured in our laboratory produce a biologically effective dose (DNA_{300}) approximately 8× greater than the same lamp configuration filtered by cellulose triacetate. The unshaded enhanced UVBR dose used in the action spectrum comparison by Smith et al. (1980) was approximately 14× greater than the unshaded ambient solar UVBR dose, when weighted by the DNA action spectrum. Such an enhancement could only have been created by using a very large number of filtered UV lamps or a few unfiltered UV lamps. Thus, discrepancy between the enhanced UVBR dose-response data of Smith et al. (1980; their Fig. 3a) and our dose-response data (Eq. 3), the dose response of Behrenfeld et al. (1993a), and the ambient response data of Smith et al. (1980) may result if the exponential action spectrum and the DNA action spectrum do not adequately describe the effectiveness of wavelengths <290 nm in causing photoinhibition of carbon fixation in marine phytoplankton.

Our results and the dose responses reported by Behrenfeld et al. (1993a) are similar when all UVBR doses are weighted by the new exponential action spectrum (Eq. 2) (Fig. 6). Conversion of ambient UVBR doses reported by Smith et al. (1980) from PI_{270}- to EXP_{300}- weighted doses results in convergence of their dose responses with our results (Fig. 6). This compari-

son, however, is only an approximation. We compared carbon fixation rates in the ambient and enhanced UVBR treatments to the UVBR excluded treatment [which included near ambient intensities of solar ultraviolet-A radiation (UVAR: 320 to 400 nm)] to calculate percent inhibition by UVBR for our dose response. Smith et al. (1980) calculated percent inhibition of carbon uptake by comparison to an estimated maximum photosynthetic rate in the absence of all UVAR and UVBR. Thus, dose responses may not be directly comparable between studies. Final resolution of the discrepancy between our results and those of Smith et al. (1980) would require a reanalysis of their data in which: (1) dose responses are shown for all DNA_{300} weighted doses (rather than results for a single experiment); (2) responses in each different UVR treatment are distinguishable; and (3) percent inhibition is calculated by comparison with measured carbon uptake in one of the UVR treatments (rather than with an estimated maximum uptake).

Threshold for UVBR effects

We assume that linear responses to UVBR occur within any given wavelength and, therefore, to any constant spectrum. Lamp spectra remain constant during the course of any experiment and between experiments. In contrast, the solar spectrum changes constantly with time, place, and atmospheric conditions. Small deviations from linearity in dose-responses that occurred under lamp spectra (Fig. 2a) compared to the deviations from linearity that occurred under solar spectra (Fig. 2a) appear to support our assumption. Thus, without *a priori* reasons for assuming more complex dose-responses to UVBR, a linear response to UVBR by marine phytoplankton appears to be the most parsimonious assumption.

Rejection of a linear dose-response to UVBR (e.g. Helbling et al. 1992) is unwarranted without testing spectral data for a wavelength specific response (i.e. use of an action spectrum). A sigmoid dose-response, for example, could be fit to the unweighted solar data presented here (Fig. 2a), suggesting a threshold effect. Evidence for such a sigmoidal response is highly dependent upon the response at the lowest UVBR intensity, which occurred on the most heavily overcast day. On overcast days, short UVBR wavelengths are attenuated more than longer wavelengths. The biologically effective dose is then lower per unit UVBR energy than during a clear day. This discrepancy is accounted for by using the exponential action spectrum.

Dose versus dose rate

We describe the cumulative inhibition of carbon uptake by UVBR as a function of total dose (Eqs. 3 & 4), and thus assume that the effect of a given UVBR dose is independent of dose rate (i.e. the 'law of reciprocity'; Smith et al. 1980). Cullen & Lesser (1991) reported inhibition of carbon fixation by UVBR in monocultures of the marine diatom *Thalassiosira pseudonana* as

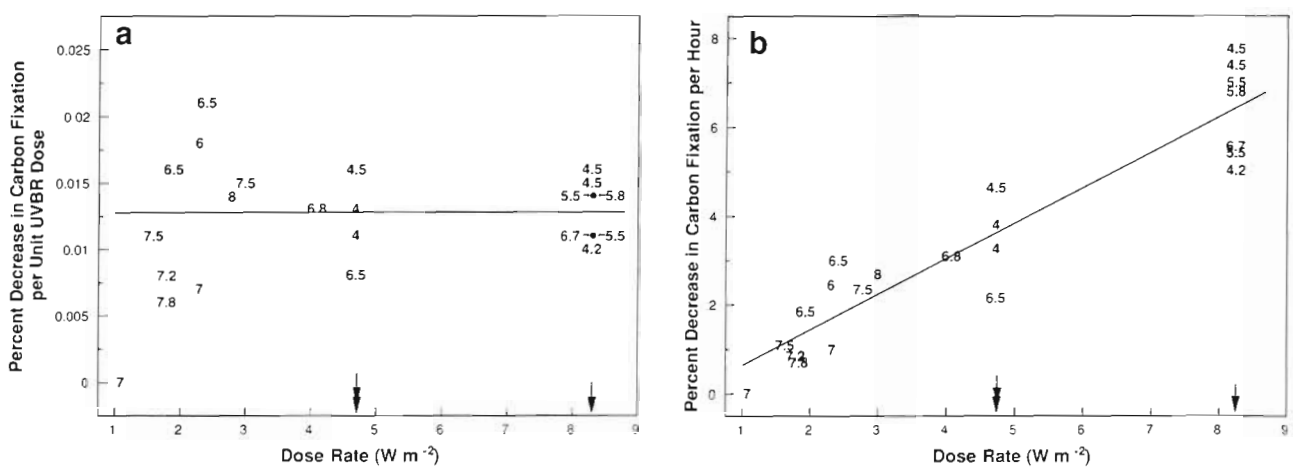

Fig. 7 Photoinhibition of carbon uptake as a function of ultraviolet-B radiation (UVBR) dose rate. (a) Test for dose-rate dependence. (b) Test for a change in photoinhibition rate with increasing dose rate. Percent decrease in carbon uptake, compared to uptake in the ambient UVBR treatment, is plotted using hours of incubation (ranging from 4 to 8 h). Incubation times for the ambient UVBR effects are longest because lamp enhancements were delayed for 0.5 to 4 h after incubations were initiated. Dose rates are cumulative dose divided by time of incubation (W m^{-2} EXP_{300}). Solid line is regression for all data. Data corresponding to the UVBR enhanced treatment occur above the arrows. Single arrow = 1 screen over lamps; double arrow = 2 screens over lamps

dose-rate dependent, indicating that the 'law of reciprocity' does not hold for UVBR inhibition of carbon uptake. Determining whether UVBR inhibition of carbon uptake is predominantly dose or dose-rate dependent has important implications for modeling the effects of solar UVBR on natural surface marine phytoplankton populations.

We tested for dose-rate dependence in our response data by calculating the regression slope for the comparison between UVBR dose-rate (W m^{-2} EXP_{300}) and percent decrease in carbon fixation per unit weighted UVBR dose (P_C/Q_{UVB}) (Fig. 7a). Average ambient UVBR dose-rates were used for this comparison because solar UVBR dose-rates varied from 0 to 7.7 W m^{-2} EXP_{300}, according to cloud conditions and time of day. Exact dose-rates could be calculated for the enhanced UVBR responses. These enhanced dose-rates were 8.25 and 4.73 W m^{-2} EXP_{300} when 1 or 2 screens were placed over the lamps, respectively. Regression slope for the comparison between UVBR dose-rate and percent decrease in carbon fixation per unit weighted UVBR dose was not significantly different from zero ($p \ll 0.001$), which is consistent with a dose-dependent, rather than a dose-rate-dependent, relationship (Fig. 7a). Variability in this dose-rate-dependence test was not ordered according to exposure time, as would be expected if a dose-rate effect was hidden within the variance, and thus adds support to a dose-dependent relationship.

The efficiency of UVBR at inhibiting carbon uptake in *Thalassiosira pseudonana* was also reported by Cullen & Lesser (1991) to decrease with increasing dose-rate (i.e. a curvilinear response). We tested our response data for a similar curvilinear relationship by comparing photoinhibitory efficiency (i.e. percent decrease in carbon fixation per unit time) to dose-rate (Fig. 7b). The linear relationship ($r^2 = 0.88$) resulting from this comparison indicates a constant photoinhibitory efficiency within the range of dose-rates and incubation times used during our study (Fig. 7b). Again, variance in this test was not ordered according to exposure time and thus supports a dose-dependent relationship.

Our experimental design utilized 3 different UVBR spectra and a single sampling and thus preclude a definitive test whether UVBR inhibition of carbon uptake in natural marine phytoplankton is predominantly dose dependent or dose-rate dependent. Results of our comparisons between photoinhibition rate and dose rate (Fig 7a, b) are consistent with a dose-dependent relationship for these several hour UVBR exposures. Such a consistency may not occur for shorter or longer exposures. Variability within these comparisons statistically prevents rejection of dose-rate dependence, although there is no distinguishable pattern between the hours of exposure and the magnitude of divergence from the dose-dependent models described by the regressions. Further research is needed which specifically addresses the importance of dose rate on UVBR inhibition of carbon uptake in natural marine phytoplankton.

CONCLUSIONS

Photoinhibition of carbon uptake during several hour exposures to UVBR was best described as a linear function of total dose (Eq. 4) and the wavelength specific biological effectiveness of UVBR in reducing carbon fixation is adequately defined by a simple exponential action spectrum (Eq. 2). The exponential action spectrum applies only to wavelengths between 290 and 347 nm (i.e. the waveband of spectral divergence between treatments). The exponential action spectrum (Eq. 2) does not apply to wavelengths >347 or <290 nm and may not apply to biological processes other than carbon fixation. Three additional action spectra for photoinhibition of phytoplankton photosynthesis by UVBR (Cullen et al. 1992, Helbling et al. 1992, Lubin et al. 1992) are similar to the exponential action spectra for the UVBR waveband. Rundel's (1983) action spectrum for inhibition of carbon uptake by the terrestrial plant *Rumex patientia* is also comparable to our exponential action spectrum and was derived in a similar manner. Lack of a threshold in our dose-response (Fig. 6) does not imply that adaptive mechanisms are not important. Indeed, much of the scatter around the regression (Fig. 6) could be due to differences in the UVBR tolerances of the phytoplankton sampled during any one experiment.

Our dose-response model and action spectrum are appropriate for estimating the change in surface photoinhibition resulting from a change in UVBR intensity relative to photosynthetic rates under current solar radiation intensities (i.e. the additional effects of UVBR superimposed upon photoinhibition by longer UVAR and PAR wavelengths). Spectral changes in UVBR resulting from the wavelength specific absorption by seawater can be accounted for by calculation of biologically effective dose using the exponential action spectrum. Our dose response and action spectrum do not, however, allow estimation of total surface photoinhibition resulting from all wavelengths of the solar spectrum. Solar UVAR and PAR wavelengths can inhibit surface photosynthesis to a greater extent than UVBR (Smith et al. 1980, Hobson & Hartley 1983, Maske 1984, Bühlmann et al. 1987, Cullen et al. 1992, Helbling et al. 1992). However, UVAR and PAR wavelengths are not significantly affected by stratospheric ozone depletion.

Depletion of the stratospheric ozone layer results in a proportionately larger increase in short UVBR wavelengths than in longer UVBR wavelengths (Green et al. 1980). Slope of our new action spectrum indicates that this increase in UVBR would represents a greater increase in the biologically damaging dose at the oceans' surface than the lower sloped PI action spectrum used by Smith et al. (1980) to describe photoinhibition of phytoplankton carbon fixation. However, short wavelengths of UVBR are attenuated more rapidly in the oceans' surface than longer wavelengths (Zaneveld 1975) and, therefore, attenuation of biologically effective dose would be more rapid for doses weighted by our exponential action spectrum than a PI type action spectrum.

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