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Acclimation of phytoplankton to UV-B radiation: oxidative stress and photoinhibition of photosynthesis are not prevented by UV-absorbing compounds in the dinoflagellate *Prorocentrum micans*

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ABSTRACT: Experiments on the temperate marine dinoflagellate *Prorocentrum micans* showed that cultures acclimated to moderate intensities (120 µmol quanta m^{-2} s^{-1}) of visible radiation and supplemental ultraviolet (UV) radiation exhibited significant inhibition of photosynthesis. This inhibition of photosynthesis caused a significant 30% decrease in specific growth rates for those cells exposed to UV radiation by the end of the 21 d culture. The mechanism for this decrease in chlorophyll specific photosynthetic rate does not appear to have been damage to photosystem II, as suggested for many acute exposure experiments. Rather, significant decreases in chlorophyll per cell and the specific activities of the carboxylating enzyme, Rubisco, explain the observed decrease in photosynthesis. The decrease in cellular chlorophyll and Rubisco activities occurs despite the presence and accumulation of mycosporine-like amino acids, whose UV absorbing properties have been suggested as an important protective mechanism against the deleterious effects of UV radiation. Our results also implicate oxidative stress, most likely a result of photodynamic interactions, as the cause for the decrease in Rubisco activities. Action spectra generated from these experiments show a significant decrease in the wavelength-dependent effects of UV radiation in cultures exposed to UV radiation, suggesting that UV-absorbing compounds do provide some, if not complete, protection. Previous predictions about specific changes in the shape of action spectra were centered around the absorption maximum of individual UV-absorbing compounds. The observed changes in the overall shape of the UV action spectra for photosynthesis in *P. micans* can be attributed to the broad overlapping absorption spectra of the suite of UV-absorbing compounds.

KEY WORDS: UV-B radiation · Oxidative stress · Photoinhibition · Phytoplankton · Photosynthesis

INTRODUCTION

The decrease of stratospheric ozone from anthropogenic inputs of chlorinated fluorocarbons has resulted in an increase in the amount of harmful ultraviolet (UV) radiation, specifically UV-B, reaching the sea surface (Baker et al. 1980, Solomon 1988). Special attention is focused on the Antarctic, where the autocatalytic destruction of stratospheric ozone ('the ozone hole') leads to enhanced fluxes of UV-B (Frederick & Snell 1988, Smith & Baker 1989) and a decrease in primary productivity (Smith et al. 1992, Holm-Hansen et al. 1993, Neale et al. 1994). In tropical waters, UV radiation, both UV-A (320 to 400 nm) and UV-B (290 to 320 nm) can penetrate to depths greater than 30 m (Jerlov 1950, Smith & Baker 1979, Fleischmann 1989). In southern oceanic waters UV-A can be measured at depths up to 100 m (Helbling et al. 1994) and UV-B to depths greater than 30 m (Smith et al. 1992, Helbling et al. 1994). Wavelengths within the UV portion of the

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spectrum are known to have a detrimental effect on photosynthesis and growth in phytoplankton (Lorenzen 1979, Smith & Baker 1982, Jokiel & York 1984, Cullen & Lesser 1991, Cullen et al. 1992, Helbling et al. 1992, Smith et al. 1992, Behrenfield et al. 1994, Lesser et al. 1994, Neale et al. 1994) The molecular targets of UV radiation-induced damage include DNA and proteins, oxidation of membrane lipids, and photooxidation of chlorophyll or damage to photosystem II (Freeman & Crapo 1982, Asada & Takahashi 1987, Renger et al. 1989, Neale et al. 1993, Vincent & Roy 1993).

Ozone depletion is proceeding at a faster rate than anticipated (Rodriguez et al. 1991), and recent studies have indicated a significant decline in stratospheric ozone throughout the northern hemisphere (Blumthaler & Ambach 1990, Stolarski et al. 1992). These new results suggest that temperate ecosystems may be exposed to higher fluxes of UV radiation, particularly the UV-B portion of the spectrum, than they have been historically. Of particular concern is that ozone depletion results in an increase in damaging UV-B wavelengths without a proportional increase in longer UV-A and blue wavelengths involved in photoreactivation and photorepair (Smith 1989, Vincent & Roy 1993). Despite the greater absorption of UV radiation by chlorophyll and dissolved organic matter, generally found in higher concentrations in temperate ecosystems (Smith & Baker 1979, 1989), the increase in UV-B radiation is likely to have biological effects. Studies on the biological effects of UV-B radiation for these highly productive temperate latitudes are required in order to understand the effects of present day fluxes of UV-B radiation and to make predictions about increases due to ozone depletion. Specifically, and as in the Antarctic, changes in the flux of UV-B radiation are expected to have biological effects on phytoplankton.

One mechanism to ameliorate the effects of solar UV radiation is to block the potentially harmful wavelengths with UV-absorbing compounds. A group of these compounds has an absorption maximum centered around 320 nm. These 'S-320 compounds' were first detected in several reef-building corals and a cyanobacterium by Shibata (1969). Since then a group of compounds, now known as mycosporine-like amino acids (MAAs), have been identified in cyanobacteria (Garcia-Pichel & Castenholz 1993a) and found in a wide variety of marine organisms, including dinoflagellates (Carreto et al. 1990a, b), macroalgae (Sivalingam et al. 1974, Wood 1989), echinoderms (Nakamura et al. 1981, Shick et al. 1992), numerous corals, zoanthids, sea anemones and other anthozoans (Takano et al. 1978, Hirata et al. 1979, Dunlap & Chalker 1986, Shick et al. 1991), and a phyletic assortment of Antarctic marine organisms (Karentz et al. 1991b).

A protective role for these compounds has been inferred from their UV-absorbing properties and their logarithmic decrease in concentration with increasing depth in corals (Scelfo 1984, Dunlap et al. 1986). The concentrations of these compounds may also change after shielding from UV exposure or transplantation of organisms to shallower depths (Jokiel & York 1982, Scelfo 1984, Wood 1989, Shick et al. 1991).

The studies by Carreto et al. (1989, 1990a, b) on dinoflagellates appear to be the only published accounts which demonstrate the ability of temperate-zone species to change their concentrations of MAAs in response to changing irradiance. Elevated concentrations of MAAs in the red-tide dinoflagellate *Alexandrium excavatum* were observed when the irradiance was increased from 20 to 200 µmol quanta m^{-2} s^{-1}, without exposure to UV radiation, suggesting a role for visible radiation in the biosynthesis of MAAs (Carreto et al. 1990a, b). The synthesis of MAAs at high irradiances could be prevented by simultaneous exposure to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of photosynthesis (Carreto et al. 1990a).

Whether these compounds provide full protection from the damaging effects of UV radiation is largely unknown or correlative. Recent evidence for the cyanobacterium *Gleocapsa* sp., which does produce MAAs after exposure to UV radiation, suggests that MAAs do not provide full protection for the photosynthetic apparatus but augment other cellular acclimatory responses to UV radiation (Garcia-Pichel & Castenholz 1993a, b). Studies on Antarctic phytoplankton communities have shown that MAAs provide protection. Vernet et al. (1994) found that Antarctic communities dominated by prymnesiophytes with strong absorption in the UV range (320 to 330 nm), presumably MAAs, had significantly less UV-induced photoinhibition, while Lesser et al. (unpubl.) observed significantly higher MAA concentrations in McMurdo Sound phytoplankton communities dominated by diatoms exposed to UV radiation. The accumulation of MAAs is one explanation for the lack of any significant differences in the action spectra for UV-induced photoinhibition between cultures of McMurdo Sound phytoplankton communities acclimated with and without UV radiation (Neale et al. 1994).

An alternative approach is to predict the effect of UV-B, and the role of MAAs, based on a quantitative description of the relationship between photosynthetic rate and exposure of phytoplankton to UV radiation. The core of such an approach is the biological weighting function (BWF), or action spectrum (Smith et al. 1980, Rundel 1983, Caldwell et al. 1986, Coohill 1989, Smith 1989). The BWF defines the biologically effective fluence rate (or dose rate) for the biological effect under study, in this case photosynthesis. If the

presence of UV-absorbing compounds plays a role in the protection of a cell from UV exposure, then the action spectrum for photosynthesis should change its shape in those regions represented by the absorption of MAAs.

The functional aspects of accumulating MAAs for free-living microalgae and cyanobacteria may also depend directly on the optical pathlengths, on the order of microns, of these cells. These small pathlengths can limit the protection of intracellular targets, even with highly absorbing compounds such as MAAs (Garcia-Pichel & Castenholz 1993a, Garcia-Pichel 1994).

Presented here are experimental results from cultures of the temperate free-living dinoflagellate *Prorocentrum micans*. These experiments were initiated to assess the long-term effects of chronic exposure to UV-B radiation, as many studies utilize acute exposures to UV radiation, and the role of UV-absorbing compounds in the photoacclimation process to UV radiation for this phytoplankter.

MATERIALS AND METHODS

Culures and experimental conditions. Cultures of the dinoflagellate *Prorocentrum micans* (Clone PRORO III), obtained from the Provasoli-Guillard Culture Collection for Marine Phytoplankton, were grown on a 12 h light: 12 h dark cycle at 20°C. Two containers (3 l) made of polycarbonate were fitted with a UV transparent (UVT, 50% cutoff ~285 nm) and UV opaque (UVO, 50% cutoff ~385 nm) Plexiglas covering. The cultures were bubbled with acid-scrubbed air, and nutrient-replete semi-continuous cultures were kept in exponential phase using f/2 growth medium (Guillard 1975). Visible radiation was from Vita-Lite full-spectrum fluorescent lamps providing a quantum scalar irradiance of 120 µmol quanta m^{-2} s^{-1} (photosynthetically active radiation, 400 to 700 nm) as measured by a Biospherical Instruments QSL 100 4π sensor immersed in a water-filled culture vessel. These irradiances are known to be saturating for photosynthesis and growth in dinoflagellates with acclimation occurring well within the 21 d of this experiment (Richardson et al. 1983). Ultraviolet radiation, predominantly UV-B, came from 2 aged (100 h) fluorescent lamps (FS40 T12-UVB, Light Sources Inc.) covered with cellulose acetate film (0.13 mm thickness, aged 48 h at 3 cm from 4 FS40 lamps). The cellulose acetate was used to attenuate shorter wavelengths that are not encountered in nature (Caldwell et al. 1986) and was changed every 3 d. Both the UV and visible radiation sources were suspended 50 cm above the cultures. Although these laboratory conditions result in a spectrally unbalanced light regime, if the cells' light-dependent processes are at irradiance saturation, then a spectral imbalance should have little effect and provide important mechanistic information. The cultures were acclimated under these conditions for 21 d.

Spectral irradiance from the UV lamps was measured using a calibrated diode-array spectroradiometer system (Cullen & Lesser 1991). Biologically effective irradiances for different treatments were determined as described by Smith & Baker (1979) and Smith et al. (1980) using the DNA weighting function of Setlow (1974), the generalized plant function of Caldwell (1971), the chloroplast photoinhibition function of Jones & Kok (1966), and the dinoflagellate photoinhibition weighting function of Cullen et al. (1992).

Biomass, fluorescence, and growth analyses. Chlorophyll, *in vivo* fluorescence, and particulate C and N were analyzed prior to, and after, each experiment. 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 dimethyl sulfoxide (DMSO) (6:4 vol/vol) at −4°C in the dark for at least 24 h.

The same fluorometer was used for discrete measurements of the fluorescence of chlorophyll *in vivo*. Fluorescence was measured after at least 30 min adaptation in the dark and then again after exposure to 3×10^{-6} M DCMU in ethanol (Vincent et al. 1980). The initial fluorescence reading (F_0) was made after 15 s in the fluorometer, then DCMU was added. Fluorescence in the presence of DCMU (F_m) was recorded after 45 s in the fluorometer. Distilled water served as the blank. $F_m - F_0$ is equivalent to variable fluorescence (F_v). The cellular fluorescence capacity (CFC) index was then calculated (F_v/F_m) as a qualitative measure of the relative number of functional PS II units, or the quantum efficiency of PS II (Vincent et al. 1980).

Triplicate specimens of 50 ml were taken at the beginning of each experiment 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 analysis. 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; pre-filtered culture medium was passed through baked filters and used as blanks. Cell counts were obtained using a Coulter counter model ZM fitted with a 100 µm orifice.

Flow cytometery. Chlorophyll per cell, measured as autofluorescence, was measured using a Becton-Dickinson FACS analyzer Single cell measurements were

made as cells passed through a focused mercury lamp beam at an excitation wavelength of 488 nm. The emitted fluorescent signals were recieved by photomultiplier tubes fitted with 630 nm long-pass filters. Chlorophyll and cell size (forward angle light scatter, FALS) measurements were made on 5000 cells to yield frequency distribution histograms. The flow cytometer was calibrated with fluorescent beads of known size and checked against cultures of free-living phytoplankton whose cell volume and fluorescent characteristics are known.

Fluorescein diacetate (FDA) was used as a cellular probe to assess the metabolic activity of phytoplankton after the acclimation period. FDA is a lipophilic fluorescent probe that enters the cell and is subsequently cleaved by cellular esterases, resulting in fluorescent fluorescein which can be quantified by flow cytometry. Since these enzymes are involved in phospholipid turnover, the presence of active enzyme is correlated with metabolic activity. The FDA method described by Dorsey et al. (1989) was used to assess the metabolic activity of *Prorocentrum micans*. FDA is excited using an excitation wavelength of 488 nm, and the emitted yellow-green fluorescent signals were recieved by photomultiplier tubes fitted with a 530 nm short-pass filter to separate that signal from the red autofluorescence of chlorophyll.

Flow cytometry was also used to assess the level of oxidative stress in the treatment and control cultures (Kobzik et al. 1990). Three specific metabolic probes were utilized: 2',7'-dichlorofluorescein-diacetate [DCF, 25 µl of a 1 mM solution (in dimethylformamide) to 250 µl of culture and incubate for 5 min at room temperature] detects intracellular hydrogen peroxide (H_2O_2); dihydrorhodamine 123 [DHR, 25 µl of a 40 µM solution (in dimethylformamide) to 250 µl of culture and incubate for 15 min at room temperature] is a more sensitive indicator of H_2O_2 concentrations; and hydroethinide [HE, 25 µl of a 250 µM solution (in dimethylformamide) to 250 µl of culture and incubate for 15 min at room temperature] detects intracellular superoxide radical (O_2^-) formation. The excitation wavelength for all 3 probes was 488 nm, and the detection wavelength was 515 to 555 nm for DCF and DHR, and 600 to 630 nm for HE. The fluorescent signals of UVO and UVT cultures were collected on the initial day of the cultures, Days 3, 7, 9, 17 and 21 The statistical analysis for all flow cytometry results was done on 1000 cells for each culture on each sampling day using a repeated measures ANOVA.

Antioxidant enzymes. Activities of superoxide dismutase (SOD) and ascorbate peroxidase (ASPX) were assayed spectrophotometrically as described by Lesser & Shick (1989). Assays were performed on the supernatants of 100 ml of culture material that were filtered onto GF/F filters (N = 5), resuspended in 100 mM phosphate buffer (pH 7.0) and sonicated for 1 min on ice to disrupt the cells. The sonicated suspension was centrifuged for 30 min at 10000 rpm in a microcentrifuge, and the supernatant retained for the assay of antioxidant enzymes. The protein content of the algal supernatants was measured by the Bradford (1976) method. All enzyme assays were performed in a Shimadzu UV/visible spectrophotometer at 20°C. Results are expressed as units (U) of enzyme activity per mg protein, where 1 U = 1.0 µmol substrate converted min^{-1}.

Carbon uptake. The method of Lewis & Smith (1983) was used to make measurements of photosynthesis as a function of irradiance (*P-I*). Samples were inoculated with ^{14}C-bicarbonate (final activity, about 10 µCi ml^{-1}), and aliquots of 1 ml were dispensed into scintillation vials (7 ml capacity) in a temperature-controlled aluminum block. A range of irradiance was provided from below with 2 ENH projection lamps directed through a heat filter of circulating water and attenuated with neutral density screens. Quantum scalar irradiance in each position was measured with a Biospherical Instruments QSL-100 4π sensor with a modified collector. Incubations began within 30 min of sampling and were terminated after 30 min. The *P-I* equation of Platt et al. (1980) was used to model the results where P_i^B [µg C (µg chl *a*)$^{-1}$ h^{-1}] is the instantaneous rate of photosynthesis normalized to chl *a* at irradiance *I* and α, the light-limited rate of carbon uptake, is estimated during the non-linear fitting of the data. The maximum photosynthetic capacity, P_{max} [µg C (µg chl *a*)$^{-1}$ h^{-1}], was calculated according to Platt et al. (1980).

Activities of 1,5 ribulose bisphosphate carboxylase/oxygenase (Rubisco) were measured using the method of Glover & Morris (1979), which follows the uptake of ^{14}C-bicarbonate (0.25 µCi µmol^{-1}) at 20°C using the optimal substrate conditions for the enzyme, and were expressed on a chlorophyll basis to allow comparison of these activities directly to the *P-I* curves.

Mycosporine-like amino acids (UV- absorbing compounds). At the end of the experimental period 100 ml aliquots (N = 3) were filtered as described above and placed in 3 ml of 100% methanol. Extraction, separation, and quantification of MAAs were performed using reverse-phase high performance liquid chromatography (HPLC), as originally described by Dunlap & Chalker (1986) and modified by Karentz et al. (1991b) and Shick et al. (1992). Individual MAAs were separated by reverse-phase isocratic HPLC on a Brownlee RP-8 column protected with an RP-8 guard, in an aqueous mobile phase including 0.1% acetic acid and 25 or 55% methanol. Detection of peaks was by UV absorbance at 313 and 340 nm. Identities of peaks were confirmed by co-chromatography using known standards in an aqueous mobile phase of 0.1% acetic

Table 1 Biologically weighted UV irradiance (mW m^{-2}, 290–400 nm) and unweighted UV-B irradiance (mW m^{-2}, 290–320 nm) for the cellulose acetate filtered FS40 lamps used for the experiments on *Prorocentrum micans*. The weightings tabulated by Smith et al. (1980) were used unless otherwise noted

Weighting function	Treatment	UV irradiance (mW m^{-2})
DNA	UVO	0.00
	UVT	0.61
Plant	UVO	0.00
	UVT	5.38
Photoinhibition	UVO	1.52
	UVT	72.37
Photoinhibition[a]	UVO	0.28
	UVT	13.41
Unweighted UV-B (290–320 nm)	UVO	0.00
	UVT	88.44
Unweighted UV (290–400 nm)	UVO	17.98
	UVT	255.24

[a]Dimensionless; using biological weighting function on *P. micans* from Cullen et al. (1992)

acid and 55% methanol. MAA concentrations were calculated using standards for mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, and palythinol, and were normalized to soluble protein.

Action spectra. A newly constructed analytical model of photosynthetic response to irradiance was used to describe the interaction of UV and photosynthetically active radiation (PAR, ca 400 to 700 nm) on photosynthesis. In this biological weighting function/*P-I* model, photoinhibition is dependent on both absolute UV irradiance and UV relative to PAR (Cullen et al. 1992). The long-pass filter cut-off wavelengths were chosen to define irradiance treatments containing progressively greater amounts of first UV-A and then UV-B, added to a constant background of PAR. This 'polychromatic' approach was chosen to obtain environmentally relevant action spectra (Caldwell et al. 1986). The weightings obtained using these procedures are absolute rather than relative and can be compared directly without normalizing the action spectrum to some nominal wavelength at which the greatest wavelength-dependent effects are observed. A detailed description of the model and analytical approach is given in Cullen et al. (1992) and Neale et al. (1994)

RESULTS

Biologically effective irradiances of UV radiation

Semi-continuous cultures of *Prorocentrum micans* grown at saturating PAR irradiances were exposed to an unweighted UV-B irradiance (Table 1, 88.44 mW m^{-2}) that was considerably less than the irradiance observed at the surface (1 m, 1.26×10^3 mW m^{-2}) during June in the Gulf of Maine, USA (Lesser unpubl.). In spite of the spectral imbalance of the laboratory photic regime this represents only 7% of the ambient UV-B dose for typical mid-latitude waters at the surface. Biologically effective irradiances of UV-B radiation were calculated using several different weighting functions (Table 1). The photoinhibition weighting functions show an almost 50-fold difference in biologically effective UV-B dose between experimental and control cultures (Table 1).

Growth rates

Significant effects of UV radiation on specific growth rates, C:N ratios, chlorophyll per cell, and cell concentrations were observed. UVT cultures had significantly lower rates of chlorophyll-specific growth, decreased chlorophyll per cell, and a decrease in cell concentrations after the 21 d acclimation period (Table 2). Cell-specific growth rates were not significantly different from one another (Table 2). The C:N ratios for UVT cultures were significantly higher, while no differences were observed for the CFC ratio (Table 2)

Table 2. Effects of UV radiation on growth, biomass, and biochemistry for UVO and UVT cultures of *Prorocentrum micans* (mean ± SD). Probability (p) values are from an ANCOVA analysis of slopes for the growth rates and from an unpaired Student's *t*-test at the 5% significance level for the remaining analyses

Parameter	Treatment	Result	p
Growth (chlorophyll specific, d^{-1})	UVO	0.111 ± 0.0093	0.044
	UVT	0.076 ± 0.0109	
Growth (cell specific, d^{-1})	UVO	0.265 ± 0.064	>0.05
	UVT	0.215 ± 0.061	
Cell numbers ($\times 10^3$ ml^{-1})	UVO	8.21 ± 0.49	0.002
	UVT	5.67 ± 0.52	
Chlorophyll *a* $cell^{-1}$	UVO	13.93 ± 1.03	0.004
	UVT	8.26 ± 1.26	
C:N ratio	UVO	5.33 ± 0.11	0.027
	UVT	5.69 ± 0.33	
CFC ratio	UVO	0.61 ± 0.022	0.41
	UVT	0.59 ± 0.018	

Table 3. Effects of UV radiation on metabolic activity and oxidative stress for UVO and UVT cultures of *Prorocentrum micans* (mean ± SD). Probability (p) values are from a repeated measures ANOVA (flow cytometry data) or an unpaired Student's *t*-test (antioxidant enzyme activities) at the 5% significance level

Flow cytometry	Treatment	Result	p
FDA fluorescence cell^{-1}	UVO	38 ± 15	> 0.05
	UVT	36 ± 15	
HE (H_2O_2) fluorescence cell^{-1}	UVO	267 ± 106	0.0001
	UVT	295 ± 114	
DHR (H_2O_2) fluorescence cell^{-1}	UVO	290 ± 89	0.005
	UVT	299 ± 83	
DCF (O_2^-) fluorescence cell^{-1}	UVO	333 ± 155	0.02
	UVT	347 ± 175	
SOD activity (U mg^{-1} protein)	UVO	2.73 ± 2.60	0.009
	UVT	6.42 ± 1.09	
ASPX activity (U mg^{-1} protein)	UVO	2.63 ± 0.65	0.0002
	UVT	11.45 ± 3.40	

Flow cytometry

All measurements of the cellular concentration of hydrogen peroxide and superoxide radicals showed a significant treatment effect, with UVT cultures always having higher concentrations of these reduced oxygen products (Table 3). Additionally, both chlorophyll (measured as autofluorescence) and cell size (measured as FALS) showed a significant effect of exposure to UV radiation. Chlorophyll content per cell decreased, supporting the bulk chlorophyll analysis, and cell size increased significantly in cells exposed to UV radiation. For all cases, the repeated measures ANOVA detected a significant independent and interactive effect of time ($p < 0.05$) which showed an increase in the effects of UV radiation on chlorophyll, cell size, and cellular concentration of active species of oxygen with time or exposure. There was no significant ($p < 0.05$) difference in FDA-staining characteristics, indicating that there was no difference in metabolic rates measured by this assay.

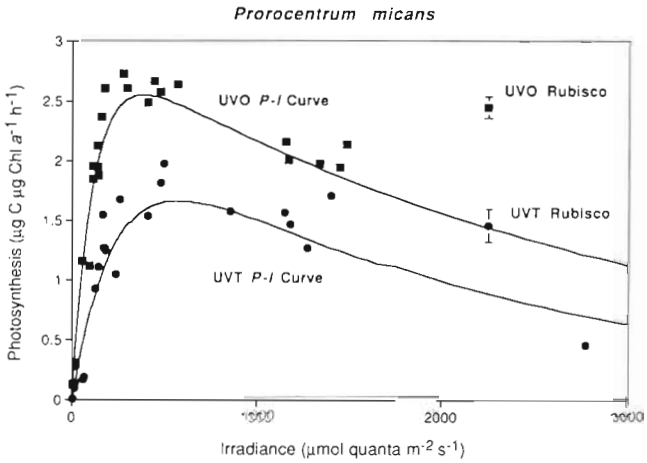

Fig. 1. Photosynthesis-irradiance (*P-I*) curves from UVO (opaque to UV radiation) and UVT (transparent to UV radiation) from Day 21 of the long-term cultures. Values for P_{max} [UVO = 2.66 ± 0.09 (SE), UVT = 1.63 ± 0.076 (SE)], and α [UVO = 0.025 (SE) ± 0.003, UVT = 0.010 ± 0.004 (SE)] were significantly different from one another (*t*-test, $p < 0.05$). Chlorophyll-specific values for Rubisco [N = 3; UVO = 2.45 ± 0.09 (SD), UVT = 1.46 ± 0.14 (SD)] were also significantly different from each other [ANOVA, $p < 0.05$; Student-Newman-Keuls (SNK) multiple comparison test, $p < 0.05$] while the UVO treatment was not significantly different from pre-UV radiation exposure activities [2.15 ± 0.07 (SD)] of the culture (ANOVA, $p < 0.05$; SNK, $p < 0.05$)

Antioxidant enzymes

Supporting the direct measurement of active species of oxygen by flow cytometry are the specific activities of the antioxidant enzymes, superoxide dismutase and ascorbate peroxidase. Significant differences in the specific activites of these enzymes were observed with UVT cultures expressing higher activities (Table 3).

Photosynthesis-irradiance curves

After 21 d of acclimation the UVT culture showed a significant depression of maximum photosynthetic capacity (P_{max}) and light-limited rate of carbon uptake (α) (Fig. 1). Additionally, a significant decrease in the chlorophyll-specific activities of Rubisco was detected (Fig. 1), which corresponds with the observed changes in the maximum photosynthetic capacity of these cultures (Fig. 1).

Mycosporine-like amino acids

Quantitative chromatography showed the presence of 4 different MAA compounds. Significantly ($p < 0.05$) higher concentrations of mycosporine-glycine, shinorine, and porphyra-334 were noted in UVT cultures (Fig. 2), while asterina-330 was significantly higher in UVO cultures. Total MAA concentration was significantly higher in UVT (38.9 ± 1.3 nmol mg^{-1} protein) than in UVO (17.5 ± 2.4 nmol mg^{-1} protein) cultures.

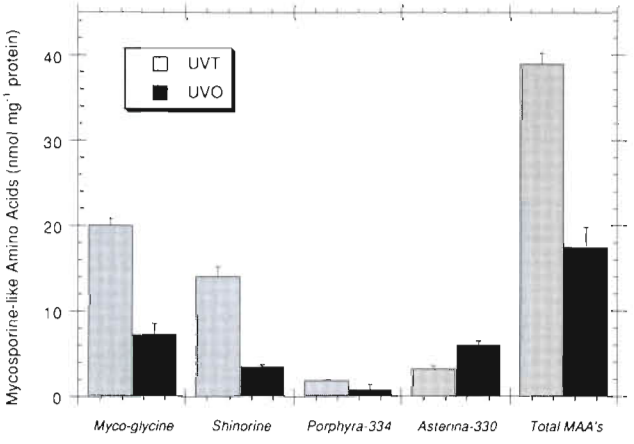

Fig. 2. Concentrations of mycosporine-like amino acids (MAAs; mean ± SD) from UVT and UVO cultures of *Prorocentrum micans* normalized to soluble protein. Differences in the concentrations of MAAs were assessed using an unpaired Student's *t*-test at the 0.05 level of probability. Significant ($N = 3$; $p < 0.05$) differences of all individual MAAs and total MAA concentrations were observed

Action spectra

The biological weighting functions (action spectra) for UVT and UVO cultures exhibit similar shapes, and there is some suggestion of a specific shape change in the UVT treatment between 330 and 350 nm, an area of high absorbance for MAAs (Fig. 3). However, the absolute magnitudes of these biological weighting functions are significantly (no overlap in their 95% confidence intervals of the estimates) different in their wavelength-dependent UV radiation effects on photosynthesis between 285 and 345 nm, allowing only subjective comments on differences in the shape, and the cause of any changes in shape, in the area between 285 and 345 nm.

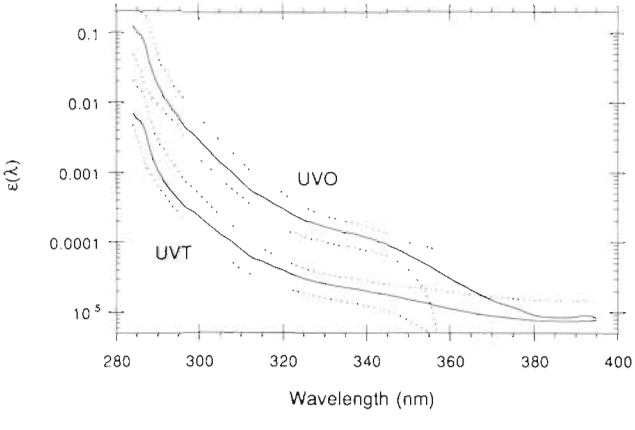

Fig. 3. Polychromatic action spectra (biological weighting function) for UVT and UVO cultures; $\varepsilon(\lambda) = (\text{mW m}^{-2})^{-1}$ Significant differences [no overlap between 95% confidence intervals (dotted lines)] betiween the 2 action spectra are observed between 285 and 345 nm

DISCUSSION

After 21 d of acclimation, cultures exposed to UV radiation show a significant depression of maximum photosynthetic capacity and light-limited photosynthetic rates. The mechanism for this decrease in chlorophyll-specific photosynthetic rate does not appear to be damage to photosystem II (PS II), as suggested by measurements of the quantum yield of fluorescence for PS II (Table 2; CFC), although oxidative stress has been shown to cause damage to PS II at the D1 protein (Richter et al. 1990). There was, however, a transient decrease in the quantum yield of fluorescence in cultures exposed to UV-B radiation initially (data not shown). These values increased over the time course of the experiments and the quantum yield of fluorescence values were not significantly different between the 2 cultures at the end of the experiment, suggesting that the number of functional PS II units was equivalent at that time. Rather than damage to PS II, a significant decrease in the specific activities of the carboxylating enzyme Rubisco, and chlorophyll content per cell, explains the observed decrease in photosynthesis. Decreases in cellular chlorophyll content have been observed in previous experiments on the effects of UV radiation, but these decreases did not explain the lower rates of photosynthesis (Lesser et al. 1994). Rubisco, however, is known to be sensitive to the direct and indirect effects of UV-B radiation in terrestrial plants and phytoplankton (Vu et al. 1984, Strid et al. 1990, Neale et al. 1993), and decreases in the specific activities of Rubisco are known to principally affect maximum photosynthetic capacity (Glover 1989). Alternatively, undetectable damage to PS II, using the techniques in this study, could result in the down regulation of other components of the photosynthetic apparatus, including Rubisco (Strid et al. 1990).

The indirect effects of UV-B radiation on Rubisco are mediated by active species of oxygen. In particular, Rubisco is sensitive to hydrogen peroxide (H_2O_2), which occurs in high concentration in illuminated chloroplasts (Asada & Takahashi 1987). The presence of higher specific activities of superoxide dismutase and ascorbate peroxidase in *Prorocentrum micans* exposed to UV-B radiation suggests that a higher flux of active oxygen species is occurring in these cells. Direct analysis of superoxide radicals and hydrogen peroxide in individual cells by flow cytometry shows a significantly higher concentration of these toxic oxygen species in cells exposed to UV-B radiation, which is likely to have been generated via photodynamic

action with various cellular constituents (Valenzeno & Pooler 1987). Although statistically different and potentially related to UV-B effects on nitrate uptake (Döhler 1986), the differences in C:N ratios are less than 6% and probably had little effect on cellular performance. The decrease in photosynthetic rates and the investment in protective enzymes and UV-absorbing compounds (see below) manifests itself as a significant 34% decrease in chlorophyll-specific growth rate, and, consequently, cell numbers by the end of the experimental period. The cell-specific growth rate, although 19% lower for cells exposed to UV radiation, was not statistically different from control cultures. These results do not preclude the possibility that damage to DNA, induced by the direct and indirect effects of UV-B radiation, partially or fully caused the observed decreases in growth rate (Karentz et al. 1991a)

The decrease in photosynthetic performance, chlorophyll content, and Rubisco activities occurs despite the presence and accumulation of MAAs in those cultures exposed to UV-B radiation. High concentrations of UV-absorbing compounds have been shown to cause high *in vivo* absorbances within the UV portion of the spectrum that does not function in photosynthetic light harvesting for *Prorocentrum micans* (Vernet et al. 1989). The total concentration of MAAs in cultures exposed to UV-B radiation is almost twice the concentration in dinoflagellates not exposed to UV-B radiation, with the greatest differences in mycosporine-glycine (λ_{max} = 310 nm) and shinorine (λ_{max} = 334 nm). Using the sunscreen factor (S) for *Gonyaulax polyedra* (S = 0.59; Table 2 therein) from Garcia-Pichel (1994) as the most appropriate based on the size range of *P. micans* (22 to 38 µm), it then follows that the dose of UV radiation required to inhibit photosynthesis in *P. micans* containing MAAs should have been approximately 2.43 times that needed had there been no sunscreens (Garcia-Pichel 1994) The biologically effective dose, using the photoinhibition function of Cullen et al. (1992), of UV radiation for UV-acclimated cultures was almost 50 times that experienced by control cultures. It also follows that, since cells exposed to UV radiation were substantially less inhibited than might be expected from these irradiances, mechanisms are being employed to counteract the damaging effects of UV radiation. Cells of *P. micans* are, therefore, provided protection by the accumulation of MAAs, but not without damage to Rubisco or other cellular targets (e.g. DNA) that might result from oxidative stress which subsequently results in a depression of photosynthetic capacity and growth.

Action spectra generated from these experimental cultures show a substantial decrease in UV effects, both UV-A between 320 and 345 nm and all of the UV-B portion of the spectrum, for cultures exposed to UV-B radiation. Again, differences in the wavelength-dependent effects of UV radiation between the 2 cultures are significant between 285 and 345 nm, supporting a role for UV-absorbing compounds in decreasing the effect of UV radiation on photosynthetic capacity in acclimated cultures. As shown by Lesser et al. (1994), part of the response of phytoplankton to UV radiation is a new equilibrium between damage and repair processes. Therefore, the responses observed in cultures acclimated with UV radiation is a result of this new equilibrium and may not reflect any additional UV radiation stress during the action spectrum measurement, while the control cultures represent an acute exposure. The total doses and dosage rates applied during the action spectrum measurement, however, are higher than the acclimation regime and may result in additional inhibition of photosynthesis even though the action spectrum for UV-acclimated cultures showed a decrease in the wavelength-dependent effects compared to cultures not acclimated with UV radiation. Previous predictions about specific changes in the shape of action spectra were centered around the absorption maximum of individual UV-absorbing compounds. The overall change in the magnitude of the wavelength-dependent effects for the action spectra, rather than a shape change in a specific region, for photosynthesis of *Prorocentrum micans* can be attributed, in part, to the broad overlapping absorption spectra of the suite of MAAs found in *P. micans* and other repair processess or bio-optical properties of these cells.

The data presented here provide strong evidence that MAAs do not provide complete protection for this dinoflagellate, and possibly phytoplankton in general, but are part of an overall response to the direct and indirect effects of UV radiation. The functional aspects of accumulating MAAs for free-living microalgae and cyanobacteria may also depend directly on the optical pathlengths, on the order of microns, for these cells since an increase in cell diameter (pathlength) increases the probability that a photon of UV radiation will interact with a homogeneously distributed UV-absorbing compound (Garcia-Pichel & Castenholz 1993a, b, Garcia-Pichel 1994). Karentz et al. (1991a) showed a negative relationship between the formation of DNA photoproducts, indicating damage to DNA, and cell size. Smaller cells with an increase in the surface area to volume relationship showed more damage per unit DNA. Mean cell size differences in *Prorocentrum micans* are most likely the result of slower growth rates (Gallagher & Alberte 1985) for cultures exposed to UV radiation. The changes in the optical pathlengths in these cells and higher MAA concentration also contribute to preventing further damage. For

larger species of phytoplankton or colony formers, the optical pathlengths will be much longer and presumably result in an increase in the absorbance of UV radiation by these compounds. The optical pathlength of phytoplankton, being dependent upon cell size, will also be affected by photoacclimatory state, nutrient status, and ultrastructural organization (Dubinsky 1992). These varying pathlengths can potentially limit the protection of intracellular targets from UV radiation as shown by Garcia-Pichel (1994), even with the accumulation of UV-absorbing compounds such as MAAs.

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Erratum

Re: MP Lesser

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- The confidence intervals for the action spectra in Fig. 3 are incorrect: the action spectra are *not* statistically different from one another. This error does not affect the interpretation of the experimental work provided in the paper that supports the hypothesis that UV absorbing compounds provide incomplete protection in this species of phytoplankton. The author regrets the error, which is his alone.