Plankton Biol. Ecol. 51 (2): 82–94, 2004

plankton
biology & ecology

Photoinduction of mycosporine-like amino acids and cell volume increases by ultraviolet radiation in the marine dinoflagellate *Scrippsiella sweeneyae*

HITOMI TAIRA[1], JOAQUIM IGNACIO GOES[2], HELGA DO ROSARIO GOMES[2], KAZUO YABE[3†] & SATORU TAGUCHI[4*]

[1] *Department of Bioengineering, Faculty of Engineering, Soka University, Tokyo, Japan*
[2] *Bigelow Laboratories for Ocean Sciences, Boothbay Harbor, USA*
[3] *Department of Marine Science and Technology, Hokkaido Tokai University, Sapporo, Japan*
[4] *Department of Environmental Engineering for Symbiosis, Faculty of Engineering, Soka University, Tokyo, Japan*

Received 2 October 2003; Accepted 19 January 2004

Abstract: The photoinduction of ultraviolet radiation (UVR) absorbing mycosporine-like amino acids (MAAs) was examined under photosynthetically active radiation (PAR) with UVR in exponentially growing cultures of the marine algal bloom-forming dinoflagellate *Scrippsiella sweeneyae*. Exposure to PAR with UVR resulted in marked increases in cellular levels of MAAs in comparison with the PAR controls. Shinorine and mycosporine-glycine were the dominant MAAs followed by palythine, porphyra-334, and palythene. The concentrations of shinorine and mycosporine-glycine were largely responsible for the prominent absorption peak centered at ~323 nm and the shoulder located at 360 nm in the chlorophyll *a* normalized absorption spectra. The increases in cellular MAA contents under UVR were accompanied closely by increases in cell volume. Growth rates, however, between the UVR-exposed and PAR control did not differ appreciably leading us to conclude, that the overall strategy of *S. sweeneyae* when exposed to UVR is to maintain similar growth rates with optimizing cellular MAA contents and cell volume. The cell appears to achieve this acclimation which in concert, helps lengthen the path of light impinging on individual cells thus mitigating possible UVR-mediated DNA damage.

Key words: cell volume, dinoflagellate, mycosporine-like amino acids, photoinduction, ultraviolet radiation

Introduction

The detection of an ozone hole over the Antarctic continent (Farman et al. 1985), and the accelerated pace at which stratospheric ozone destruction has been taking place at high latitudes (Jones & Shanklin 1995), have raised concerns about its consequences for life on this planet. As the ozone hole increases, solar ultraviolet radiation (UVR), mainly ultraviolet-B radiation (UVB: 280–315 nm) incident on the surface of the Earth is enhanced. Solar UVB can penetrate to biologically significant depths within the euphotic zone in marine ecosystems (Smith & Baker 1981; Smith et al. 1992; Helbling et al. 1994; Kuwahara et al. 2000a) and a growing body of evidence suggests that this could lead to impairment of light-dependent responses of marine phytoplankton (Häder et al. 1998). UVB has a high energy level per photon and is readily absorbed by nucleic acids, proteins and pigments. However, it has deleterious effects on phytoplankton, such as damage to the photosynthetic machinery (e.g., PS II) and nuclear DNA (Karentz et al. 1991; Buma et al. 1995) with consequent inhibition of photosynthesis and cell growth rates (Jordan et al. 1991), loss or decrease in activity of the key photosynthetic enzyme, ribulose bis-phosphate carboxylase (Rubisco) (Lesser et al. 1996), decrease of nitrogen uptake (Döhler et al. 1997), alternation of metabolism and protein synthesis (Goes et al. 1995), and loss of photo-orientation and motil-

Abbreviations: MAAs, mycosporine-like amino acids; PAR, photosynthetically available radiation (400–700 nm); UVA, ultraviolet-A radiation (315–400 nm); UVB, ultraviolet-B radiation (280–315 nm); UVR, ultraviolet radiation (280–400 nm).

† Present address: Australian Institute of Marine Science, PMB 3, Townsville MC, Townsville 4810, Queensland, Australia.
* Corresponding author: Satoru Taguchi; e-mail, staguchi@t.soka.ac.jp

ity in diatoms (Sundbäck et al. 1997) and dinoflagellates (Nielsen & Ekelund 1993).

Several aquatic plants, including microalgae, are believed to possess the capability to offset the negative effect of UVR exposure via photoprotective and photorepair mechanisms. These mechanisms aid in decreasing the biological effectiveness of UVR exposure and thus allow the cell to grow in a UVR-enhanced environment. Understanding such mechanisms is critical to assessing the effect of changes in incident solar UVR on the marine ecosystem.

One potential strategy by which marine organisms protect themselves from UVR is via the synthesis of mycosporine-like amino acids (MAAs) (Dunlap & Shick 1998; Neale et al. 1998; Shick et al. 1999; Cockell & Knowland 1999). Mycosporine is the generic name given to water-soluble UVR-absorbing fungal metabolites with a cyclohexenone or a cyclohexenimine chromophore conjugated with the nitrogen substituent of amino acids or amino alcohol (Hirata et al. 1979). These compounds are synthesized by marine algae (Karentz 1994) and have absorption maxima ranging from 310 nm to 360 nm (Nakamura et al. 1982). The protective function of MAAs has been experimentally demonstrated in dinoflagellates *Gymnodinium sanguineum* (Neale et al. 1998) as well as in the eggs of the sea urchin *Strongylocentrotus droebachiensis* fed during reproductive development on red-algae having different concentrations of MAAs (Adams & Shick 1996). The effectiveness and mode of protection by MAAs however is not well understood.

An extensive survey on marine microalgae has revealed that cellular levels of MAAs are particularly high in dinoflagellates (Jeffrey et al. 1999). This taxonomic group represents organisms that are particularly well adapted to marine environments experiencing high PAR and UVR exposure. Therefore dinoflagellates have been considered appropriate experimental organisms to study protective mechanisms under the effect of UVR in marine ecosystems. The production rates of MAAs have been studied in a variety of marine dinoflagellates including *Alexandrium excavatum* (Carreto et al. 1990a, b), *Symbiodinium microadriaticum* (Banaszak & Trench 1995), *Prorocentrum micans* (Vernet et al. 1989; Lesser 1996), *Gonyaulax polyedra* (Vernet et al. 1989; Vernet & Whitehead 1996), *Heterocapsa triquetra* (Wängberg et al. 1997), *Gyrodinium dorsum* (Klisch & Häder 2000, 2002), and *Gymnodinium sanguineum* (Neale et al. 1998; Litchman et al. 2002). The general consensus is that high fluence PAR (Neale et al. 1998; Carreto et al. 1990a; Hannach & Sigleo 1998), blue light (400–500 nm), ultraviolet radiation-A (UVA: 315–400 nm) (Carreto et al. 1990b) and UVB wavelengths (Lesser 1996; Wängberg et al. 1997) can all lead to enhanced production of MAAs. In experiments with cultures of the dinoflagellate *Gyrodinium dorsum* however, when the cells were exposed to polychromatic radiation ranging between PAR and UVR, the highest induction of MAAs was due to UVR irradiation around 310–320 nm (Klisch & Häder 2000, 2002). These results lead us to believe that MAA induction in dinoflagellates varies depending on the quantity and spectral distribution of PAR and UVR. However, the link between the induction of MAAs and the protective function of the induced MAAs by UVR dose is not clear.

Our objective was to investigate the induction of MAAs in response to changing intensities and spectral quality of UVR in exponentially growing cells of the marine dinoflagellate *Scrippsiella sweeneyae* Balech. This species forms extensive red tides in marine coastal and estuarine waters, one such area being the Seto Inland Sea in Japan. It has been considered that the induction of UV absorbing compounds in the living cell by UVR provides strong evidence of a specific UV-screening function (Cockell & Knowland 1999). Therefore the cellular MAA contents were measured to quantify the MAA induction response to UVR exposure. The activity of MAA induction, defined as MAA photoinducibility, was also estimated as the cellular content of MAAs normalized to chlorophyll *a* (Chl *a*), because the induction of MAAs can be dependent on photosynthetic activity (Carreto et al. 1990b; Moisan & Mitchell 2001). Another protective strategy of phytoplankton is known to be the enhancement of cell volume (Behrenfeld et al. 1992; Garcia-Pichel 1994). The protective role of MAAs was analyzed with reference to increases of MAA induction and MAA photoinducibility and also quantified as the ability to enhance cell volume to accommodate more MAAs in the cell without a loss in cellular growth rates and physiological activity.

Materials and Methods

Culture and experimental conditions

Scrippsiella sweeneyae Balech (NIES-684) was obtained from the algal collection at the National Institute of Environmental Science, Japan. The algae were grown at 25°C in GPM medium (Loeblich & Smith 1968), prepared with filtered sea-water from Sagami Bay, Japan. The growth medium was supplemented with 10^{-8} M sodium selenite (MERCK, Germany) which was required to stimulate the growth and cell yields of bloom-forming phytoplankton species (Doblin et al. 1999).

Algal cultures were incubated in quartz bottles wrapped with a PAR transparent-UV Guard film (FUJIFILM, Japan) (Fig. 1) and exposed to PAR only at 20.7 W m^{-2} from cool white fluorescence tubes (FLR40S, Toshiba, Japan) over a 12 h light and 12 h dark cycle. To ensure that the cultures were exposed to equal amounts of light and to prevent the cells from sedimenting during incubation, the bottles were gently rotated in a plankton wheel (TAITEC, Japan) in the incubation chamber. Semi-continuous cultures kept in exponential phase under the conditions mentioned above for a period of one month were used in these experiments.

UVR in the experimental incubation chamber was provided by Toshiba fluorescence tubes Model FL202E (Fig.

2). Growing cells were subjected to four different combinations of PAR and UVR obtained by using combinations of three filter materials. UV Guard film, mylar film (PANAC, Japan), and acetate film (Polymer Plastics Corporation, USA), which screened out 99% radiation shorter than 410, 313, and 290 nm, respectively (Fig. 1). These filters were carefully wrapped around the quartz bottles for each treatment defined as below. PAR-only cultures using UV Guard film and UVA transparent cultures using mylar film were defined as the control (>410 nm treatment) and the >313 nm treatment, respectively. UVB transparent cultures using acetate film were defined as the >290 nm treatment and the cultures without any films were defined as the >280 nm treatment. To study the response on MAA induction in *S. sweeneyae* by UVR intensity, neutral density filters (DAIM, Japan) were then employed to obtain five different UVR intensities, referred to as Experiments 1 to 5 (Table 1). These various combinations of UVR screening films and neutral density screens resulted in a total of 20 different light treatments including various intensities of PAR in the control due to visible light released from the UV lamp (Table 1). Both PAR and UVR, expressed as

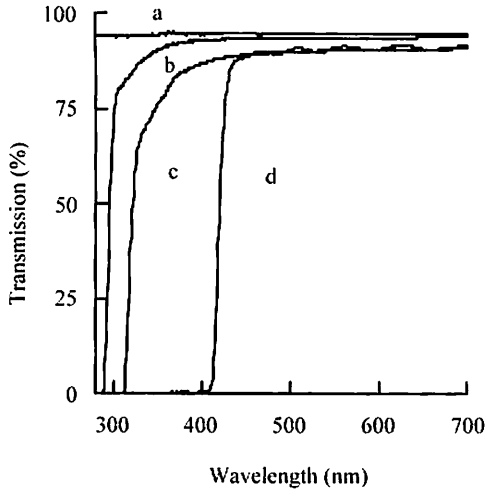

Fig. 1. Spectra of the filters used to achieve the various irradiance conditions for testing MAA induction in *Scrippsiella sweeneyae*. Quartz (a), acetate (b), mylar (c), and UV Guard filters (d) allow longer wavelengths than 280 nm, 290 nm, 313 nm and 410 nm to pass through, respectively.

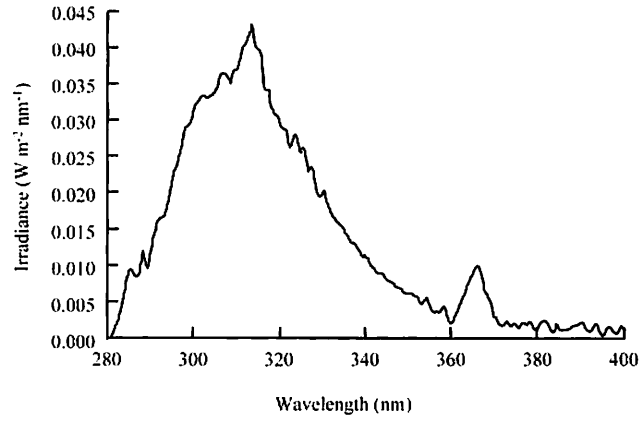

Fig. 2. Spectral distribution of the UVR lamp (TOSHIBA fluorescence tube model FL202E).

Table 1. Irradiances for PAR treatment (>410 nm) and UVR treatments (>313 nm, >290 nm, and >280 nm) during UVR exposure experiment of *Scrippsiella sweeneyae*.

Experiment	Treatment	PAR (W m^{-2}) (400–700 nm)	UVA (W m^{-2}) (315–400 nm)	UVB (W m^{-2}) (280–315 nm)
1	>410 nm	17.42	0.00	0.00
	>313 nm	18.70	0.23	0.00
	>290 nm	19.46	0.28	0.040
	>280 nm	19.64	0.30	0.052
2	>410 nm	21.99	0.00	0.00
	>313 nm	23.61	0.49	0.00
	>290 nm	24.58	0.60	0.36
	>280 nm	24.80	0.63	0.50
3	>410 nm	23.65	0.00	0.00
	>313 nm	25.39	0.71	0.00
	>290 nm	26.43	0.87	0.64
	>280 nm	26.67	0.92	0.89
4	>410 nm	31.11	0.00	0.00
	>313 nm	33.39	1.09	0.0066
	>290 nm	34.76	1.34	1.13
	>280 nm	35.08	1.41	1.56
5	>410 nm	31.37	0.00	0.00
	>313 nm	33.67	1.46	0.010
	>290 nm	35.05	1.80	1.62
	>280 nm	35.37	1.89	2.24

$\mu W\,cm^{-2}\,s^{-1}$, were measured by Biospherical Instruments Radiometer Models GMR 610 and PUV 510 (USA). The former instrument provided PAR measurements at 412, 443, 490, 555, 665, 683, and 710 nm, while the latter provided UVR measurements at 305, 320, 340, and 380 nm. The photon fluence density at 280 nm was assumed to be $0\,\mu W\,cm^{-2}\,s^{-1}\,nm^{-1}$, because the FL202E UV lamp does not emit light <280 nm (Fig. 2). In order to obtain absolute values of UVB, light intensities were integrated from 280 nm to 315 nm for the >280 nm treatment, or 290 nm to 315 nm for the >290 nm treatment assuming no significant fluctuation in UVR between the two wavelengths measured. UVA and PAR intensity were also integrated from 315 nm to 400 nm and from 400 nm to 700 nm, respectively (Table 1).

Exponentially growing PAR-only cultures provided the inoculation for all treatments in the present study. The inocula were introduced to obtain an initial concentration of 90 ± 24 cells ml^{-1} in 8 L medium in a polycarbonate bottle wrapped with UV Guard film during all experiments. When the cultures approached the beginning of logarithmic growth phase, subsamples were harvested and used for time zero. The culture was divided into four quartz bottles (370 ml) for each treatment (>410 nm, >313 nm, >290 nm, and >280 nm treatment). Then the UVR exposure was started. The UVR exposure was run over a period of logarithmic growth phase that lasted 2 days. On the first and second days of exponential growth phase, triplicate subsamples were collected at the midway point of the light cycle. All subsamples were used for microscopic determinations of cell numbers and cell volume, HPLC analyses of pigment and MAAs, and spectrophotometric measurements of the absorption characteristics of the cells.

Cell number and cell volume measurements

Cell density of the cultures was monitored microscopically. The length, width, and height of 100 cells from each treatment were measured on random cells under the microscope and cell volumes were estimated assuming an ellipsoid shape for the cells (Hillebrand et al. 1999).

Volume specific growth rate

Since cell volume increase was observed in the present study, cell volume was also considered in calculation of the growth rate. Thus, the volume specific growth rate μ_v (d^{-1}) was determined, as follows,

$$\mu_v = \frac{\mathrm{Ln}[N(t_2)\cdot V(t_2)] - \mathrm{Ln}[N(t_1)\cdot V(t_1)]}{(t_2 - t_1)} \quad (1)$$

where $N(t)$ is the cell density and $V(t)$ is the cell volume at time t.

Spectral absorption analysis

Cell suspensions were harvested using a centrifuge (Kubota centrifuge Model 2010, Japan) at 2,500 rpm for 10 min at room temperature. Samples were extracted in 1 ml of 80% [v/v] aqueous methanol (HPLC grade, Wako, Japan). Cells were then sonicated for 1 min in an ice bath. The solvent mixture extracted both UVR-absorbing MAAs and photosynthetic pigments from cells, leaving a colorless pellet. The solvent mixture was centrifuged (Kubota 1910, Japan) again at 10,000 rpm for 5 min. The absorption spectrum of the supernatant was measured from 190 nm to 850 nm in a spectrophotometer (Shimadzu spectrophotometer Model UV-2450, Japan) using 80% methanol as the blank control. The absorbance spectra were normalized to absorbance at 750 nm.

Determination of chlorophyll *a*

Cell suspensions were filtered onto 25 mm Whatman GF/A filters, and extracted in 1.5 ml of 100% methanol (HPLC grade). Subsamples were then sonicated for 5 min in an ice bath and extracted for 24 h at −4°C in the dark. Extracts were filtered through 0.2 μm membrane filters. The methanol extracts were analyzed in a Beckman HPLC Model System Gold (USA) with C18 reversed-phase Ultrasphere 3 μm column using a solvent gradient system similar to that described by Head & Horne (1993). Integrated peak areas were quantified with external standards obtained from the International Agency for ^{14}C Determination, Denmark.

Identification and quantification of mycosporine-like amino acids

Cell suspensions were filtered onto 25 mm Whatman GF/A glass fiber filters, and extracted in 1.5 ml of 20% methanol (HPLC grade) to remove non-polar photosynthetic pigments (Klisch & Häder 2000) for at least 24 h at 37°C in the dark. The solvent mixture was then centrifuged at 10,000 rpm for 5 min. The supernatant (1 ml) was evaporated under reduced pressure, and re-dissolved in 1 ml of 100% methanol, a process known to remove proteins and salts which frequently pose a problem during the HPLC runs (Klisch & Häder 2000). The extracts were vortexed and centrifuged again at 10,000 rpm for 5 min and the supernatant carefully separated and evaporated to complete dryness at 45°C (Klisch & Häder 2000, 2002). The residue was redissolved in 100 μl of MilliQ water, and filtered through a 0.2 μm membrane filter for HPLC analysis.

MAAs were separated by reverse phase HPLC using a Shiseido® CAPCELL PAK C18 UG120, 5 μm (250 mm ×4.6 mm I.D., Japan) and Shiseido® SG 120 guard column, 5 μm (35 mm×4.6 mm I.D.). During each run, the column was equilibrated with a 100% solvent A [80% MilliQ water and 20% (v/v) 0.5 M ammonium acetate (Wako, Japan)] run at a flow rate of 1 ml min^{-1}, followed by a 100% eluent

B [75% MilliQ water, 25% (v/v) methanol, and 0.2% acetic acid (Wako, Japan)] run for 20 min. Elution of MAAs from the column was detected by measuring absorbance at both 310 nm and 334 nm (Adams & Shick 1996). MAA concentrations were calculated from peak areas detected at 334 nm using peak areas for individual MAA standards during a calibration run. Common MAAs were identified by their retention time, absorption spectra from 290 nm to 400 nm, and co-chromatography with authentic standards shinorine, palythine, porphyra-334, and palythene, which were obtained from the red algae *Tichocarpus crinitus*, *Chondrus yendoi*, *Porphyra yezoensis*, and *Palmaria palmate*, respectively. Mycosporine-glycine was obtained from cultures of the dinoflagellate *Alexandrium tamarense* (Carreto et al. 2001). Quantification was done using the following molar extinction coefficients: shinorine: ε_{334}=44,700 (Tsujino et al. 1980), palythine: ε_{320}=36,200 (Takano et al. 1978a), porphyra-334: ε_{334}=42,300 (Takano et al. 1979), mycosporine-glycine: ε_{310}=28,100 (Ito & Hirata 1977), and palythene: ε_{360}=50,000 (Takano et al. 1978b), respectively.

Statistical analysis

Two-way ANOVA without replication was used to assess the changes in the variability in the cell volume, cellular Chl *a* contents, cellular MAA contents and Chl *a* specific total MAA contents under the >410 nm treatment during the incubation duration for all the UVR exposure experiments. It was also used to assess the variation in cell volume, cellular Chl *a* contents, cellular MAA contents, and Chl *a* specific total MAA contents when exposed to different intensities and wavelength compositions of UVR. Standard error (SE) was calculated as a measure of variation and sampling error.

Results

Cell volume and growth

Average cell densities at time zero were 392±55 cells ml^{-1} in all experiments. Cell densities increased to 1,390±251 cells ml^{-1} in the control (>410 nm treatment) on Day 2 of the UVR exposure experiment. The average cell volume at time zero was 9.85×10^3±153 μm^3 cell^{-1}. In the control samples, the cell volume did not show any significant variability during the incubation duration between any of the UVR exposure experiments (two-way ANOVA, p>0.05), and the overall average cell volume was 9.81×10^3±436 μm^3 (Fig. 3a). Volume specific growth rate ranged from 0.63±0.04 to 0.74±0.13 d^{-1} in the controls (Table 2). There was no significant difference in volume specific growth rate between any of the controls.

Cell densities increased to 1,563±147 cells ml^{-1} on Day 2 of the UVR exposure experiment in all UVR treatments.

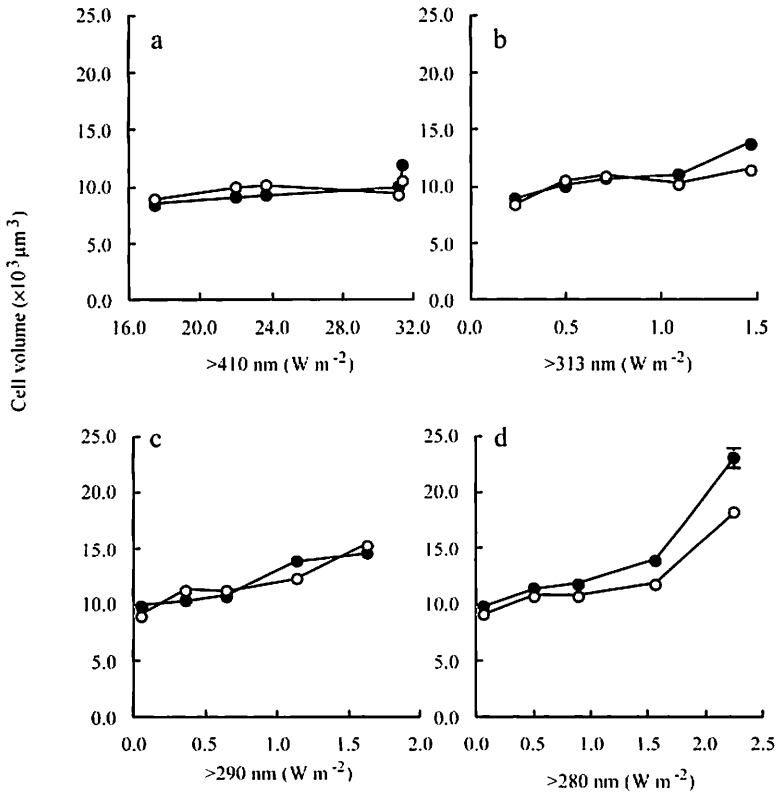

Fig. 3. Cell volume of *Scrippsiella sweeneyae* cultured under different light intensities on Day 1 (○) and Day 2 (●) of UVR exposure experiments in the >410 nm (a), >313 nm (b), >290 nm (c), and >280 nm treatments (d). Vertical bars indicate one standard error (SE). Ratios of SE to the mean are smaller than 0.036.

Table 2. Mean volume specific growth rate (μ_v in d^{-1}) with one standard error (SE) observed in the >410 nm treatment as a control and the different UVR treatments.

Experiment	Treatment	Volume specific growth rate (d^{-1})
		$\mu_v \pm SE$
1	>410 nm	0.67±0.04
	>313 nm	0.75±0.10
	>290 nm	0.79±0.08
	>280 nm	0.71±0.15
2	>410 nm	0.63±0.09
	>313 nm	0.70±0.04
	>290 nm	0.71±0.04
	>280 nm	0.73±0.11
3	>410 nm	0.70±0.07
	>313 nm	0.83±0.14
	>290 nm	0.76±0.13
	>280 nm	0.82±0.23
4	>410 nm	0.72±0.16
	>313 nm	0.75±0.12
	>290 nm	0.76±0.19
	>280 nm	0.76±0.13
5	>410 nm	0.74±0.13
	>313 nm	0.83±0.09
	>290 nm	0.84±0.30
	>280 nm	0.76±0.25

The differences in cell volume between the control and the UVR treatments were significant, especially on Day 2 during all of the UVR exposure experiments (two-way ANOVA, $p<0.001$) (Fig. 3b, c, and d). These differences were also significant between the UVR treatments (two-way ANOVA, $p<0.001$), particularly where the >280 nm treatment induced larger cell volumes than for the >313 nm and >290 nm treatments. The cell volume increased with UVR intensity in all UVR treatments (two-way ANOVA, $p<0.001$). In the >280 nm treatment the cells attained volumes of about $23.0\times10^3\ \mu m^3$ at the highest intensity of UVR (Experiment 5). Volume specific growth rate comparisons revealed no significant differences between the UVR intensities or between the UVR treatments (Table 2). Additionally there were no differences in volume specific growth rate between the controls and UVR treatments.

Cellular chlorophyll *a* content

Cellular Chl *a* contents ranged from 21.7 to 38.1 pg Chl *a* cell $^{-1}$ at time zero with an average of 27.5±2.9 pg Chl *a* cell $^{-1}$. In the control samples, cellular Chl *a* contents did not show any significant variability during the incubation duration between any of the UVR exposure experiments (two-way ANOVA, $p>0.05$). Although intracellular Chl *a* increased during the UVR exposure experiments, no patterns of variability were discernible between the control and the UVR treatments. A statistical test of the differences between the UVR treatments also indicated no significant differences (two-way ANOVA, $p>0.05$).

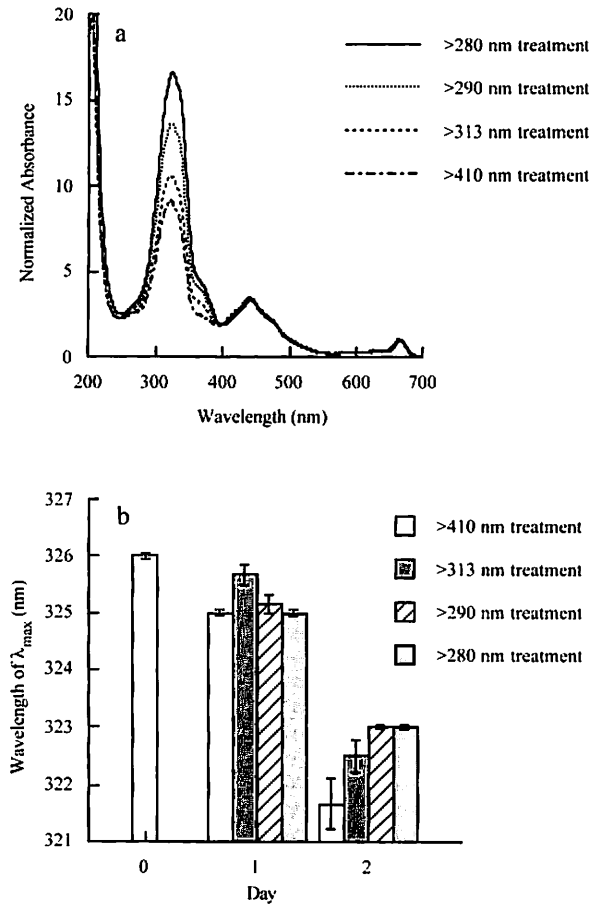

Fig. 4. The absorption spectra of methanolic extracts from *Scrippsiella sweeneyae*. The absorption was normalized to the chlorophyll *a* peak at 665 nm for the cultures exposed to different spectral compositions in Experiment 5 on Day 2 (a). Changes in the wavelength of maximum absorption of extracts exposed to different spectral compositions, plotted as a function of the number of days exposure in Experiment 5 (b). The vertical bar indicates one standard error.

Spectral absorption

Chl *a*-normalized absorption spectra of methanolic extracts of *S. sweeneyae* in the control samples were characterized by one peak centered at around 323 nm (a_{323}) and a prominent shoulder at 360 nm (a_{360}) in the UV region, (Fig. 4a). Exposure to UVR clearly resulted in an enhancement in the UV absorption peaks as compared to the controls. The UV absorption maxima (λ_{max}), centered at around 323 nm, fluctuated between ca. 329 and ca. 321 nm. In Experiment 5 the absorption maxima shifted from 326 nm to 321–323 nm in all treatments (Fig 4b).

Mycosporine-like amino acids

HPLC analysis of MAAs indicated the presence of a mixture of MAAs in *S. sweeneyae* (Fig. 5a and b). Of these, five MAAs, shinorine (wavelength of maximum absorbance

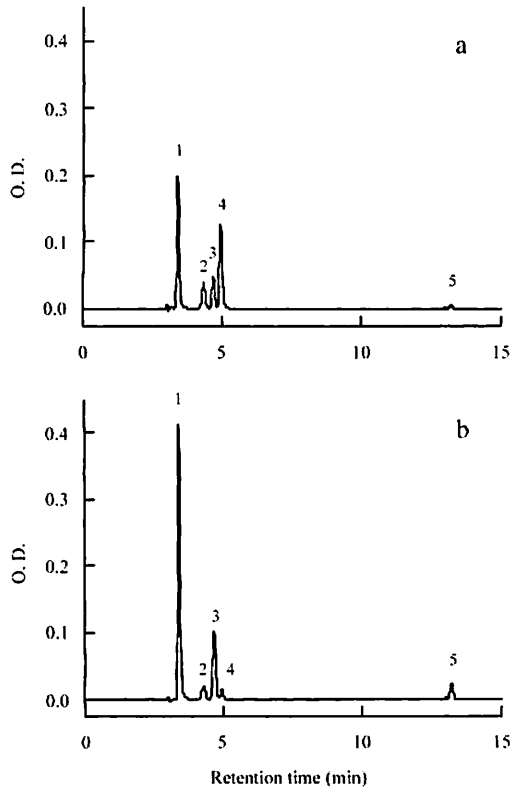

Fig. 5. HPLC chromatogram of MAAs from *Scrippsiella sweeneyae* detected at 310 nm (a) and 334 nm (b). Numbers 1 to 5 indicate shinorine (retention time: RT=3.4 min), palythine (RT=4.2 min), porphyra-334 (RT=4.8 min), mycosporine-glycine (RT=4.9 min), and palythene (RT=13.0 min), respectively.

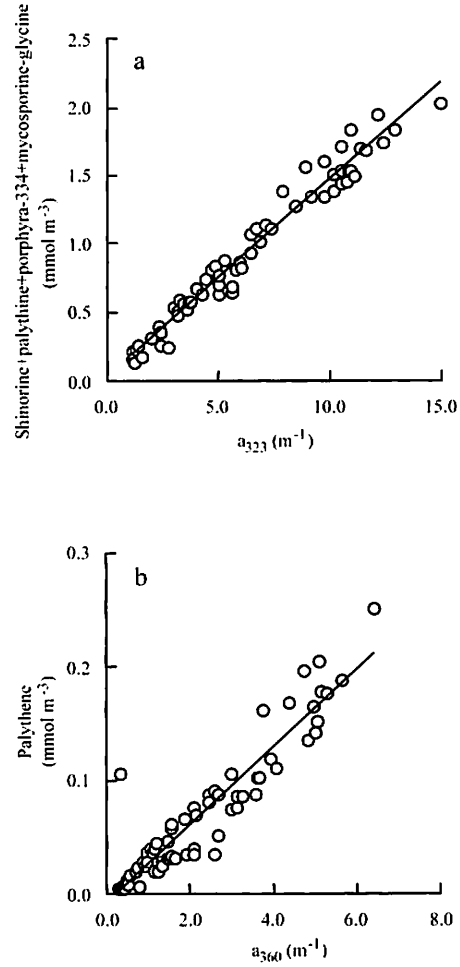

Fig. 6. Relationship between the absorption peaks at 323 nm (a) and 360 nm (b) and the concentration of MAAs. The solid line is a least-square regression fit of the data.

λ_{max}=334 nm), palythine (λ_{max}=320 nm), porphyra-334 (λ_{max}=334 nm), mycosporine-glycine (λ_{max}=310 nm), and palythene (λ_{max}=360 nm) were identified in the present study. With the exception of palythene, the sum of these four MAA concentrations, possessing maximum absorption peaks from 310 nm to 334 nm, correlated strongly with the absorption peak at 323 nm ($p<0.001$) (Fig. 6a). The palythene concentration correlated significantly with the absorption shoulder observed at 360 nm ($p<0.001$) (Fig. 6b).

Cellular MAA contents at time zero of UVR exposure ranged from 0.20±0.01 of shinorine to 0.020± 0.002 pmol cell^{-1} of palythene (Table 3). The relative amounts of individual MAA per cell at time zero estimated from the absolute concentrations of individual MAAs revealed that shinorine, followed by mycosporine-glycine, was the most dominant MAA in *S. sweeneyae*. Both compounds accounted for between 30 and 40% of the total MAAs. Palythine or porphyra-334 accounted for less than 14% of the total MAAs. Palythene was the least abundant MAA in *S. sweeneyae* accounting for less than 5.0% of the total MAAs.

Table 3. Mean cellular MAA contents with one standard error and relative abundance of MAAs at time zero.

	Cellular concentration (pmol/cell)	Relative abundance (%)
Shinorine	0.20±0.01	39.7±2.6
Palythine	0.067±0.01	12.7±0.9
Porphyra-334	0.055±0.01	10.4±1.3
Mycosporine-glycine	0.17±0.02	33.9±1.7
Palythene	0.020±0.002	4.0±0.5
Total	0.51±0.05	

Induction of mycosporine-like amino acids

Variations in the induction of MAAs, measured as the cellular MAA content, were not significant in the control (two-way ANOVA, $p>0.05$) (Fig. 7a and e), even though PAR intensity varied from 17 to 31 W m^{-2} (Table 1). MAA induction at Day 1 of the UVR exposure experiment in the control let to higher values than on Day 2 (two-way ANOVA, $p<0.05$). The increase in MAA induction on Day 1 was also observed in the UVR treatments. MAAs were induced more on Day 2 than Day 1 at the two highest UVR intensities (Fig. 7b, c, d, f, g, and h).

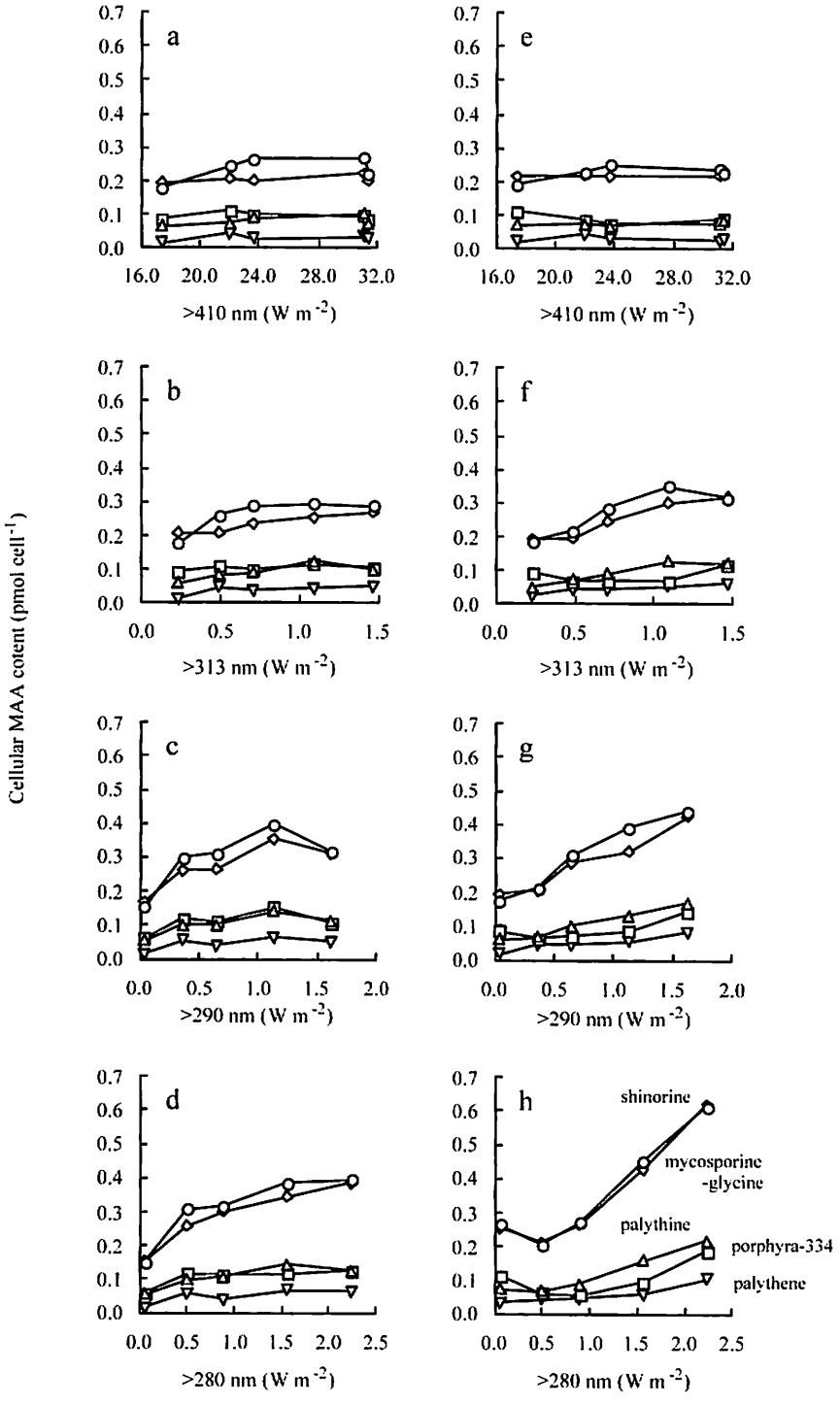

Fig. 7. Induction of MAAs in *Scrippsiella sweeneyae* by different light intensities at >410 nm on Day 1 (a) and Day 2 (e), >313 nm on Day 1 (b) and Day 2 (f), >290 nm on Day 1 (c) and Day 2 (g), >280 nm treatment on Day 1 (d) and Day 2 (h). Shinorine, mycosporine-glycine, palythine, porphyra-334, and palythene are indicated with circles, diamonds, triangles, squares, and inversed triangles, respectively.

Each MAA induction increased significantly with increasing UVR intensities (two-way ANOVA, $p<0.001$). The induction of MAAs also increased appreciably with UVR incorporating shorter wavelengths (two-way ANOVA, $p<0.001$). The most prominent increases were observed for the major MAAs, shinorine and mycosporine-glycine, in the >280 nm treatment, where they were about 3-fold greater compared to the concentrations at time zero (Fig. 7h). The minor MAA, palythene, showed the greatest increase over the experimental period, i.e., the concentration

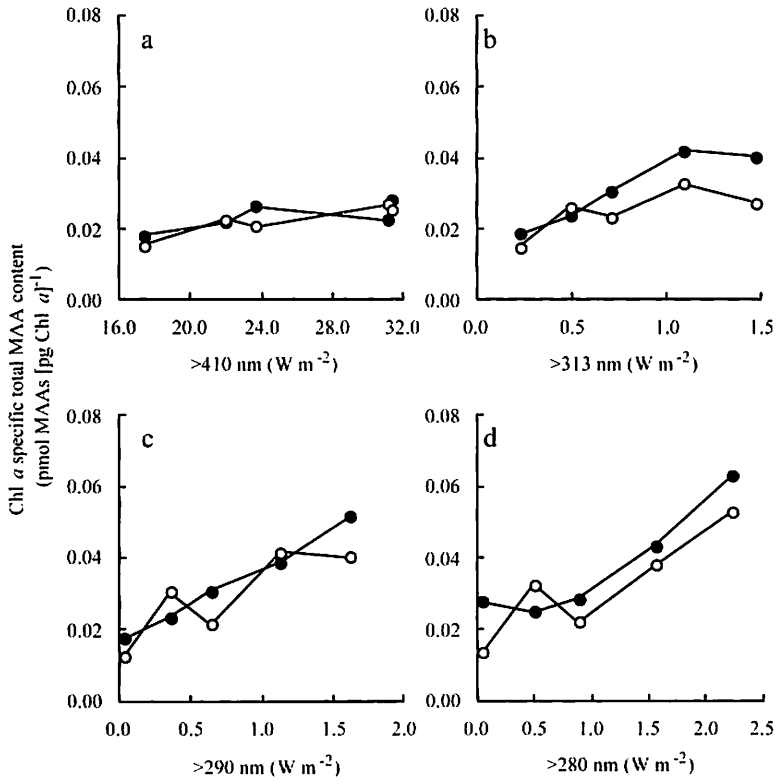

Fig. 8. The chlorophyll *a* specific total MAA contents in *Scrippsiella sweeneyae* under different light intensities on Day 1 (○) and Day 2 (●) in the >410 nm (a), >313 nm (b), >290 nm (c), and >280 nm treatments (d).

was about 5 times greater than that recorded at time zero in the >280 nm treatment (Fig. 7h).

The MAA photoinducibility, defined as the Chl *a* specific content of total MAAs at time zero, was 0.019±0.00 in all experiments. The MAA photoinducibility did not change significantly in the control during the incubation duration in any of the UVR exposure experiments (two-way ANOVA, $p>0.05$). The MAA photoinducibility on Day 1 and Day 2 of UVR exposure was significantly higher than in the control, especially on Day 2 in the >280 nm treatment (two-way ANOVA, $p<0.001$) (Fig 8a, b, c, and d). The MAA photoinducibility was also clearly greater in the cells exposed to shorter wavelengths of UVR (two-way ANOVA, $p<0.01$). Significant increases in the MAA photoinducibility were also observed particularly in the >290 nm and >280 nm treatments with increasing UVR intensities (two-way ANOVA, $p<0.001$) (Fig 8c and d).

Discussion

Scrippsiella sweeneyae is a marine unicellular dinoflagellate adapted to environments with high-irradiance. This species exhibits positive phototaxis at high irradiance as observed for other species of dinoflagellates (Blasco 1978) but is unlikely to be photobleached significantly by even high-intensities of PAR. Natural light intensities ranged from 39 to 339 for PAR, 3.7 to 28.3 for UVA, and 0.14 to 1.2 W m^{-2} for UVB in 1998 at the Manazuru Peninsula in Sagami Bay (35°09′49″N, 139°10′33″E) (Kuwahara et al. 2000b) where the latitude is similar to the Seto Inland sea where this species forms red tides. However, the integrated light intensities in this study ranged from 17 to 35 for PAR, 0.23 to 1.9 for UVA, and 0.04 to 2.2 W m^{-2} for UVB (Table 1). Although a direct comparison cannot be made due to the different spectral composition between solar radiation and the UV lamp used in this study, a relatively high UVB intensity was employed particularly to assess features of the accumulation of cellular MAAs in response to harmful UVB radiation without significant effects on growth rate.

The presence of MAAs in cells grown solely under visible light, however, indicates that the biosynthesis of MAAs is not solely a UVR-dependent process but an inherent physiological feature of *S. sweeneyae*. Although the role of MAAs is believed to be primarily photoprotective against UVR (Cockell & Knowland 1999; Dunlap & Shick 1998; Neale et al. 1998; Shick et al. 1999), 1) the ability of *S. sweeneyae* to accumulate these compounds when grown for prolonged periods under visible light as was observed in the pre-experimental cultures, and 2) the lack of appreciable changes in intracellular MAA contents under various intensities of visible light during the experiments, suggest that *S. sweeneyae* is capable of consistently diverting a fraction of photosynthetically-fixed energy towards the production and maintenance of optimal MAA concentrations within the cell. The ability of *S. sweeneyae* to produce MAAs under visible light, however, does not seem to be unusual for di-

noflagellates. A survey of 206 strains of marine phytoplankton for the presence of MAAs (Jeffrey et al. 1999) also showed that several surface bloom-forming phytoplankton, in particular dinoflagellates, are capable of producing large amounts of MAAs even when grown for several generations under visible light. The capacity to biosynthesize MAAs appears to be an inherent physiological trait in surface bloom-forming organisms (Jeffrey et al. 1999) which experience high intensities of incident irradiance during photosynthesis over the course of the day. *S. sweeneyae* is a surface bloom-forming phytoplankton species and has been implicated in the formation of extensive red-tides in estuarine and coastal marine environments. The ability of this organism to synthesize large amounts of MAAs therefore does not appear to be unusual.

The nearly constant intracellular levels of MAAs maintained by *S. sweeneyae* under different intensities of visible light observed in our study differs from earlier reports of induction of MAAs by high fluence rates of visible light (Carreto et al. 1990a; Riegger & Robinson 1997). The reason for the discrepancy between our findings and those reported earlier is unknown and certainly deserves further study. One possibility is that *S. sweeneyae* requires a much greater intensity of visible light regime to stimulate MAA synthesis above the optimal levels maintained by the cells.

The MAA pool in *S. sweeneyae* was made up of a mixture consisting of five MAAs i.e., shinorine, mycosporine-glycine, palythine, porphyra-334, and palythene. The composition of MAAs of *S. sweeneyae* was similar to that of *Gymnodinium sanguineum* (Litchman et al. 2002) another bloom-forming dinoflagellate. However, the relative abundance of individual MAAs between the two species was different, even when grown under similar PAR regimes. In *S. sweeneyae* the two predominant MAAs were shinorine (ca. 40%) and mycosporine-glycine (ca. 34%), whereas in *G. sanguineum* it was palythene (ca. 37%). This comparison suggests that the biosynthesis of MAAs is a species-dependent process.

The absorption peak of spectra in *S. sweeneyae* shifted from 326 nm towards shorter wavelengths in all the treatments at the highest intensities of UVR (Experiment 5). Accompanying the shifts in position of the UV absorbance peak were changes in the relative abundance of the two-major MAAs, i.e., a decrease in the relative concentrations of shinorine (13% decrease compared to time zero) and an increase in mycosporine-glycine (4% increase compared to time zero). Since the absorption maxima of mycosporine-glycine is 310 nm and that of shinorine is about 334 nm, the most plausible reason for the shift in the UVR absorption maxima towards short wavelengths appears to be the decrease in relative concentrations of shinorine. UVR-mediated shifts in absorption peaks of MAAs towards short wavelengths have also been reported in *Gyrodinium dorsum* (Klisch & Häder 2000). In this organism the peak shift was associated with a decrease in the relative concentrations of porphyra-334 and an increase in mycosporine-glycine. A notable difference between *S. sweeneyae* and *G. dorsum*, however, was that the change in the relative abundances of MAAs in the former could be induced either or both by PAR and UVR, whereas in *G. dorsum*, the changes were observed solely following exposure of the cells to UVR.

The increase in MAAs in *S. sweeneyae* cells exposed to shorter wavelengths of UVR was highly conspicuous. Photoinduction of MAAs by UVR has been reported in several phytoplankton strains (Carreto et al. 1990a; Lesser 1996; Klisch & Häder 2000, 2002; Marchant et al. 1991; Riegger & Robinson 1997). The induction of MAAs, however, appears to be a process that is wavelength and phytoplankton-species dependent. In *S. sweeneyae*, the greatest increase in cellular MAA contents was observed in cells exposed to wavelengths >280 nm. In *Anabaena* sp., a cyanobacterium (Sinha et al. 2002), the maximum induction of MAAs was found when the cells were exposed to >290 nm UVR. In the green algae *Prasiola stipitata*, the increase was greatest with >300 nm UVR (Gröniger & Häder 2002), whereas in the dinoflagellate *Gyrodinium dorsum* (Klisch & Häder 2000, 2002) the greatest increase was observed at >310 nm. These wavelength-associated differences in MAA accumulation could be related to the differences in light signal perception by the photoreceptors within the organisms (Halldal 1962). It is also possible that the induction of MAAs takes place only after a certain threshold of UVR intensities is crossed, as was observed in the present study. Since the induction of MAAs was clearly dependent on the dose rate of UVR and differed at different wavelengths, MAA function was suggested to be for specific UV-screening (Cockell & Knowland 1999). These observations are in agreement with those reported for *Phaeocystis pouchetii* in which the induction of UV-absorbing compounds was found to be clearly related to the dose rate of UVB (Marchant et al. 1991).

A prominent change that *S. sweeneyae* cells underwent following exposure to UVR was an increase in cell volume. This increase ranged from 10 to 130% over the controls and was greatest in the >280 nm treated cells. Variations in cell size are considered to be a photoprotective strategy against UVR and visible light when they become detrimental to cell physiology and growth (Garcia-Pichel 1994). An increase in cell volume results in an increase in the pathlength of light traveling to the cells. Such an increase in the pathlength can afford the cells protection against UVR by protecting sensitive sites, such as cellular DNA from the harmful effects of UVR (Garcia-Pichel 1994). In *S. sweeneyae* the increase in cell volume that resulted in the UVR treated samples, without compromising cell growth, might suggest that DNA was being protected from the harmful effects of UVR via a two-pronged strategy of an increase in cell size and the accumulation of MAAs within the cells.

Photoinduction of MAAs and cell volume increases were minimal in *S. sweeneyae* cells exposed to visible light. This result is a clear indication that the intensities of visible light

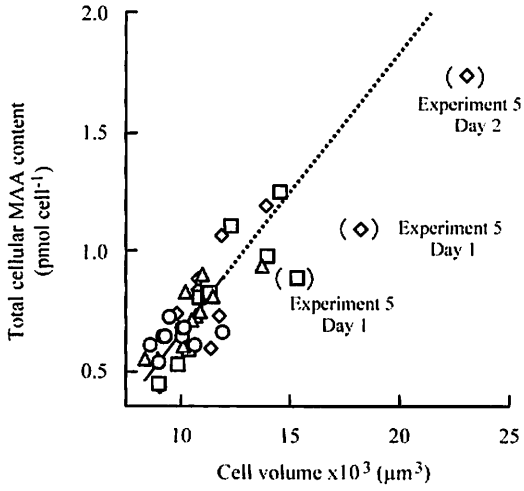

Fig. 9. The relationship between the cell volumes and total cellular MAA contents in the >410 nm (○), >313 nm (△), >290 nm (□), and >280 nm treatments (◇). Symbols in the parentheses indicate data for the >290 nm treatment on Day 1 and >280 nm treatment on Day 1 and Day 2 of UVR exposure. The solid line was fitted to data under UVR treatment within the same range of variation in cell volume (8.5×10^3 to 12×10^3 μm^3) in the >410 nm treatment ($p<0.001$). The dashed line indicates the extrapolation of the solid line.

that the cells were subject to in our experiments were not stressful. Furthermore, these observations lend credence to the view that cell volume enhancement and photoinduction of MAAs (over and above minimal cellular MAA contents) are processes that are triggered only when the cells are exposed to the harmful intensities of UVR. The highly significant relationship ($p<0.001$) between cell volume and cellular MAA contents under UVR treatments within the same range of variation in cell volume in the control (Fig. 9) suggests that cell volume increases and photoinduction of MAAs are physiological processes that are not mutually exclusive when grown under UVR. This relationship seems to be quite robust up to cell volumes of about 15.0×10^3 μm^3. The three outliers seen in Fig. 9 are the >290 nm treatment on Day 1 and the >280 nm treatment on Day 1 and Day 2 of UVR exposure in Experiment 5. Although cellular MAA contents for the UVR treatment in Experiment 5 were higher compared to the other treatments, the points were clearly offset from the line of best fit. The overall photoprotective strategy that the cells adopt as they adapt when MAA production cannot keep pace with increasing UVR is through enhancement of the pathlength of UVR by enhancing cell volume.

Literature Cited

Adams, N. L. & J. M. Shick 1996. Mycosporine-like amino acids provide protection against ultraviolet radiation in eggs of the green sea urchin, *Strongylocentrotus droebachiensis*. *Photochem. Photobiol.* **64**: 149–158.

Banaszak, A. T. & R. K. Trench 1995. Effects of ultraviolet (UV) radiation on marine microalgal-invertebrate symbioses. II. The synthesis of mycosporine-like amino acids in response to exposure to UV in *Anthopleura elegantissima* and *Cassiopeia xamachana*. *J. Exp. Mar. Biol. Ecol.* **194**: 233–250.

Behrenfeld, M. J., J. T. Hardy & Lee II 1992. Chronic effects of ultraviolet-B radiation on growth and cell volume of *Phaeodactylum tricornutum* (Bacillariophyceae). *J. Phycol.* **28**: 757–760.

Blasco, D. 1978. Observations on the migration of marine dinoflagellates off the Baja California Coast. *Mar. Biol.* **46**: 41–47.

Buma, A. G. J., E. J. van Hannen, L. Roza, M. J. W. Veldhuis & W. W. C. Gieskes 1995. Monitoring ultraviolet-B-induced DNA damage in individual diatom cells by immnofluorescent thymine dimer detection. *J. Phycol.* **31**: 314–321.

Carreto, J. I., M. O. Carignan, G. Daleo & S. G. De Marco 1990a. Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds? *J. Plankton Res.* **12**: 909–921.

Carreto, J. I., V. A. Lutz, S. G. De Marco & M. O. Carignan 1990b. Fluence and wavelength dependence of mycosporine-like amino acid synthesis in the dinoflagellate *Alexandrium excavatum*, p. 275–279. In *Toxic Marine Phytoplankton*. (ed. Graneli, E., L. Edler, B. Sundström & D. M. Anderson). Amsterdam. (Elsevier)

Carreto, J. I., M. O. Carignan & N. G. Montoya 2001. Comparative studies on mycosporine-like amino acids, paralytic shellfish toxins and pigment profiles of the toxic dinoflagellates *Alexandrium tamarense*, *A. catenella* and *A. minutum*. *Mar. Ecol. Prog. Ser.* **223**: 49–60.

Cockell, C. S. & J. Knowland 1999. Ultraviolet radiation screening compounds. *Biol. Rev.* **74**: 311–345.

Doblin, M. A., S. I. Blackburn & G. M. Hallegraeff 1999. Comparative study of selenium requirements of three phytoplankton species: *Gymnodinium catenatum*, *Alexandrium minutum* (Dinophyta) and *Chaetoceros* cf. *tenuissimus* (Bacillariophyta). *J. Plankton Res.* **21**: 1153–1169.

Döhler, G., G. Drebes & M. Lohmann 1997. Effect of UV-A and UV-B radiation on pigments, free amino acids and adenylate content of *Dunaliella tertiolecta* Butcher (Chlorophyta). *J. Photochem. Photobiol. B: Biol.* **40**: 126–131.

Dunlap, W. C. & J. M. Shick 1998. Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: a biochemical and environmental perspective. *J. Phycol.* **34**: 418–430.

Farman, J. C., B. G. Gardiner & J. D. Shanklin 1985. Large losses of total ozone in Antarctica reveal seasonal ClO_x/No_x interaction. *Nature* **315**: 207–210.

Garcia-Pichel, F. 1994. A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. *Limnol. Oceanogr.* **39**: 1704–1717.

Goes, J. I., N. Handa, S. Taguchi & T. Hama 1995. Changes in the patterns of biosynthesis and composition of amino acids in a marine phytoplankton exposed to ultraviolet-B radiation: nitrogen limitation implicated. *Photochem. Photobiol.* **62**: 703–710.

Gröniger, A. & D.-P. Häder 2002. Induction of the synthesis of an UV-absorbing substance in the green alga *Prasiola stipitata*. *J. Photochem. Photobiol. B: Biol.* **66**: 54–59.

Häder, D.-P., H. D. Kumar, R. C. Smith & R. C. Worrest 1998. Effects on aquatic ecosystems. *J. Photochem. Photobiol. B: Biol.*

46: 53–68.

Halldal, P. 1962. Taxes. p.583–593. In *Physiology and biochemistry of algae*. (ed. Lewin, R. A.). New York. (Academic Press)

Hannach, G. & A. C. Sigleo 1998. Photoinduction of UV-absorbing compounds in six species of marine phytoplankton. *Mar. Ecol. Prog. Ser.* **174**: 207–222.

Head, E. J. H. & E. P. W. Horne 1993. Pigment transformation and vertical flux in an area of convergence in the North Atlantic. *Deep-Sea Res.* **40**: 329–346.

Helbling, E. W., V. Villafaneand & O. Holm-Hansen 1994. Effects of ultraviolet radiation on Antarctic marine phytoplankton photosynthesis with particular attention to the influence of mixing, p. 207–227. In *Ultraviolet Radiation in Antarctica: Measurements and Biological Effects*. Antarct. Res. Ser. Vol. 62 (ed. Weiler, C. S. & P. A. Penhale). Washington, DC. (American Geophysical Union)

Hillebrand, H., C.-D. Dürselen, D. Kirschtel, U. Pollingher & T. Zohary 1999. Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.* **35**: 403–424.

Hirata, Y., D. Uemura, K. Ueda & S. Takano 1979. Several compounds from *Palythoa tuberculosa* (Coelenterata). *Pure Appl. Chem.* **51**: 1875–1883.

Ito, S. & Y. Hirata 1977. Isolation and structure of a mycosporine from the zoanthid *Palythoa tuberculosa*. *Tetrahedron Lett.* **28**: 2429–2430.

Jeffrey, S. W., H. S. MacTavish, W. C. Dunlap, M. Vesk & K. Groenewoud 1999. Occurrence of UVA- and UVB-absorbing compounds in 152 species (206 strains) of marine microalgae. *Mar. Ecol. Prog. Ser.* **189**: 35–51.

Jones, A. E. & J. D. Shanklin 1995. Continued decline of total ozone over Halley, Antarctica, since 1985. *Nature* **376**: 409–411.

Jordan B. R., W. S. Chow, Å. Strid & J. M. Anderson 1991. Reduction in *cab* and *psb* A RNA transcripts in response to supplementary ultraviolet-B radiation. *FEBS Lett.* **284**: 5–8.

Karentz, D., J. E. Cleaver & D. L. Mitchell 1991. Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. *J. Phycol.* **27**: 326–341.

Karentz, D. 1994. Ultraviolet tolerance mechanisms in Antarctic marine organisms, p. 93–110. In *Ultraviolet Radiation in Antarctica: Measurements and Biological Effects*. Antarct. Res. Ser. Vol. 62 (ed. Weiler, C. S. & P. A. Penhale). Washington, DC. (American Geophysical Union)

Klisch, M. & D.-P. Häder 2000. Mycosporine-like amino acids in the marine dinoflagellate *Gyrodinium dorsum*: induction by ultraviolet irradiation. *J. Photochem. Photobiol. B: Biol.* **55**: 178–182.

Klisch, M. & D.-P. Häder 2002. Wavelength dependence of mycosporine-like amino acid synthesis in *Gyrodinium dorsum*. *J. Photochem. Photobiol. B: Biol.* **66**: 60–66.

Kuwahara, V. S., T. Toda, K. Hamasaki, T. Kikuchi & S. Taguchi 2000a. Variability in the relative penetration of ultraviolet radiation to photosynthetically available radiation in temperate coastal waters, Japan. *J. Oceanogr.* **56**: 399–408.

Kuwahara, V. S., H. Ogawa, T. Toda, T. Kikuchi & S. Taguchi 2000b. Variability of bio-optical factors influencing the seasonal attenuation of ultraviolet radiation in temperate coastal waters of Japan. *Photochem. Photobiol.* **72**: 193–199.

Lesser, M. P., P. J. Neale & J. J. Cullen 1996. Acclimation of Antarctic phytoplankton to ultraviolet radiation: ultraviolet-absorbing compounds and carbon fixation. *Mol. Mar. Biol. Biotechnol.* **5**: 314–325.

Lesser, M. P. 1996. 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*. *Mar. Ecol. Prog. Ser.* **132**: 287–297.

Litchman, E., P. J. Neale & A. T. Banaszak 2002. Increased sensitivity to ultraviolet radiation in nitrogen-limited dinoflagellates: Photoprotection and repair. *Limnol.Oceanogr.* **47**: 86–94.

Loeblich A. R. & V. E. Smith 1968. Chloroplast pigments of the marine dinoflagellate *Gyrodinium resplendens*. *Lipids* **3**: 5–13.

Marchant, H. J., A. T. Davidson & G. J. Kelly 1991. UV-B protecting compounds in the marine alga *Phaeocystis pouchetii* from Antarctica. *Mar. Biol.* **109**: 391–395.

Moisan, T. A. & B. G. Mitchell 2001. UV absorption by mycosporine-like amino aids in *Phaeocystis antarctica* Karsten induced by photosynthetically available radiation. *Mar. Biol.* **138**: 217–227.

Nakamura, H., J. Kobayashi & Y. Hirata 1982. Separation of mycosporine-like amino acids in marine organisms using reversed-phase high-performance liquid chromatography. *J. Chromatogr.* **250**: 113–118.

Neale, P. J., A. T. Banaszak & C. R. Jarriel 1998. Ultraviolet sunscreens in *Gymnodinium sanguineum* (Dinophyceae): mycosporine-like amino acids protect against inhibition of photosynthesis. *J. Phycol.* **34**: 928–938.

Nielsen, T & N. G. A. Ekelund 1993. Effects of UV-B radiation and humic substances on growth and motility of *Gyrodinium aureolum*. *Limnol. Oceanogr.* **38**: 1570–1575.

Riegger, L. & D. Robinson 1997. Photoinduction of UV-absorbing compounds in Antarctic diatoms and *Phaeocystis antarctica*. *Mar. Ecol. Prog. Ser.* **160**: 13–25.

Shick, J. M., S. Romaine-Lioud, C. Ferrier-Pagès & J.-P. Gattuso 1999. Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral *Stylophora pistillata* despite decreases in its population of symbiotic dinoflagellates. *Limnol. Oceanogr.* **44**: 1667–1682.

Sinha, R. P., J. P. Sinha, A. Gröniger & D.-P. Häder 2002. Polychromatic action spectrum for the induction of a mycosporine-like amino acid in a rice-field cyanobacterium, *Anabaena* sp. *J. Photochem. Photobiol. B: Biol.* **66**: 47–53.

Smith, R. C. & K. S. Baker 1981. Optical properties of the clearest natural waters (200–800 nm). *Appl. Opt.* **20**: 177–184.

Smith, R. C., B. B. Prézelin, K. S. Baker, R. R. Bidigare, N. P. Boucher, T. Coley, D. Karentz, S. MacIntyre, H. A. Matlick, D. Menzies, M. Ondrusek, Z. Wan & K. J. Waters 1992. Ozone depletion: Ultraviolet radiation and phytoplankton biology in Antarctic waters. *Science* **255**: 952–958.

Sundbäck, K., S. Odmark, A. Wulff, C. Nilsson & S.-Å. Wängberg 1997. Effects of enhanced UVB radiation on a marine benthic diatom mat. *Mar. Biol.* **128**: 171–179.

Takano, S., D. Uemura & Y. Hirata 1978a. Isolation and structure of a new amino acid, palythine, from the zoanthid *Palythoa tuberculosa*. *Tetrahedron Lett.* **26**: 2299–2300.

Takano, S., D. Uemura & Y. Hirata 1978b. Isolation and structure of two amino acids, palythinol and palythene, from the zoanthid

Palythoa tuberculosa. *Tetrahedron Lett.* **49**: 4909–4912.

Takano, S., A. Nakanishi, D. Uemura & Y. Hirata 1979. Isolation and structure of a 334 nm UV-absorbing substance, porphyra-334 from the red algae *Porphyra tenera* Kjellman. *Chem. Lett.* 419–420.

Tsujino, I., K. Yabe & I. Sekikawa 1980. Isolation and structure of a new amino acid, shinorine, from the red alga *Chondrus yendoi* Yamada et Mikami. *Bot. Mar.* **23**: 65–68.

Vernet, M., A. Neori & F. T. Haxo 1989. Spectral properties and photosynthetic action in red-tide populations of *Prorocentrum micans* and *Gonyaulax polyedra*. *Mar. Biol.* **103**: 365–371.

Vernet, M. & K. Whitehead 1996. Release of ultraviolet-absorbing compounds by the red-tide dinoflagellate *Lingulodinium polyedra*. *Mar. Biol.* **127**: 35–44.

Wängberg, S.-Å, A. Persson & B. Karlson 1997. Effects of UV-B radiation on synthesis of mycosporine-like amino acid and growth in *Heterocapsa triquetra* (Dinophyceae). *J. Photochem. Photobiol. B: Biol.* **37**: 141–146.