

# Effects of UV-A (320 to 399 Nanometers) on Grazing Pressure of a Marine Heterotrophic Nanoflagellate on Strains of the Unicellular Cyanobacteria *Synechococcus* spp.

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**In the open ocean, where turbidity is very low, UV radiation may be an important factor regulating interactions among planktonic microorganisms. The effect of exposure to UV radiation on grazing by a commonly isolated marine heterotrophic nanoflagellate, *Paraphysomonas bandaiensis*, on two strains of the cyanobacteria *Synechococcus* spp. was investigated. Laboratory cultures were exposed to a range of irradiances of artificially produced UV-B (290 to 319 nm) and UV-A (320 to 399 nm) for up to 10 h. At a UV-B irradiance of  $0.19 \text{ W m}^{-2}$ , but not  $0.12 \text{ W m}^{-2}$ , grazing mortality of *Synechococcus* spp. and nanoflagellate-specific grazing rates were reduced compared to mortality and grazing rates with UV-A treatment. Within 6 h of exposure, UV-A alone suppressed grazing mortality at irradiances as low as  $3.02 \text{ W m}^{-2}$ . The extent to which grazing mortality and nanoflagellate-specific grazing rates were suppressed by UV-A increased with both irradiance and duration of exposure. Over a 6-h exposure period, differences in grazing mortality were largely attributable to differential survival of nanoflagellates. Over a longer period of exposure, there was impairment by UV-A alone of nanoflagellate-specific grazing rates. Rates of primary productivity of *Synechococcus* spp. were also reduced by UV-A. The extent to which *Synechococcus* productivity was reduced, compared to the reduction in *Synechococcus* grazing mortality, depended on the duration of UV-A exposure. These results support the hypothesis that UV-A alone influences the composition and biomass of marine microbial communities by affecting predator-prey interactions and primary production.**

Away from coastal regions, production and consumption of organic matter in the ocean are dominated by prokaryotic and eukaryotic organisms with diameters of  $<2$  to  $3 \mu\text{m}$ , or picoplankton (28, 30, 37, 40, 41). Heterotrophic picoplankton are primarily bacteria. Phototrophic picoplankton encompass a wide variety of cyanobacteria and eukaryotic algae. The most well studied of the phototrophic picoplankton are single-celled cyanobacteria in the genus *Synechococcus*. *Synechococcus* spp., and other phototrophic picoplankton, constitute more than 50% of the autotrophic biomass and total primary production in the euphotic zone of the open ocean (16, 40, 44). Much of this production is consumed by heterotrophic nanoflagellates, flagellated protozoa less than  $20 \mu\text{m}$  in diameter, which may occur in open-ocean plankton in densities exceeding  $1,000 \text{ ml}^{-1}$  (3, 5, 40).

Photosynthesis of some strains of marine *Synechococcus* spp. is saturated at low light levels (33), but maximum numbers of *Synechococcus* spp. are often found close to the surface, where the degree of solar irradiance is relatively high (24, 44). Photosynthetically active solar radiation (PAR) includes wavelengths of 400 nm and longer. Wavelengths between 290 and 400 nm are UV radiation and include UV-A (320 to 399 nm) and UV-B (290 to 319 nm). Atmospheric ozone strongly absorbs UV-B but not UV-A (17). Seasonal damage to the stratospheric ozone layer in the last 2 decades, probably resulting from air pollution, has led to enhanced penetration of UV-B at high and mid-latitudes (6, 38).

In response to the thinning of the ozone layer, more attention has been paid recently to the effects on biological processes of UV-B than to those of UV-A. However, there is

abundant evidence that solar UV-A alone can impair biological processes, including nutrient assimilation (9), photosynthesis (7, 21, 26, 31), and motility (10), of marine and freshwater phytoplankton. Recently, Sommaruga et al. (39) reported that the rate of grazing of *Bodo saltans*, a heterotrophic nanoflagellate, on freshwater bacterioplankton is inhibited by both UV-B and UV-A alone. UV-A, relative to UV-B, may be particularly important in aquatic ecosystems, where, as a function of the concentration of seston and dissolved organic matter, UV-B is more rapidly attenuated with depth than is UV-A (1, 32).

In this study, we had two primary objectives. The first was to determine if UV-A alone, compared to treatment with both UV-A and UV-B, would suppress grazing of *Paraphysomonas bandaiensis*, a marine heterotrophic nanoflagellate, on *Synechococcus* spp. Our second objective was to compare the effect of UV-A on the impact of nanoflagellate grazing on a *Synechococcus* population with the effect of UV-A on the rate of *Synechococcus* primary production. As pointed out by Bothwell et al. (2), to predict the net effects of UV on the growth rate or population size of an organism, it is necessary to consider not just the direct effects of UV on the organism but also the responses of predators or symbionts of the organism to UV exposure. Here, we describe the effects of a range of UV-A irradiances on nanoflagellate grazing and on rates of primary production of two strains of *Synechococcus* spp.

## MATERIALS AND METHODS

***Synechococcus* strains, nanoflagellates, and media.** We used two strains of *Synechococcus* spp. in these experiments. *Synechococcus* sp. strain WH8012 is coccoid with a diameter of between 1 and  $1.5 \mu\text{m}$ . *Synechococcus* sp. strain WH7803 is ovoid with a lengthwise diameter of about  $2 \mu\text{m}$ . Both strains were maintained in SN medium (44) prepared with Instant Ocean artificial seawater (composition per liter: 18.7 kg of  $\text{Cl}^-$ , 10.4 kg of  $\text{Na}^+$ , 2.6 kg of  $\text{SO}_4^{2-}$ , 1.3 kg of  $\text{Mg}^{2+}$ , 0.4 kg of  $\text{Ca}^{2+}$ , 0.4 kg of  $\text{K}^+$ , 0.2 kg of  $\text{HCO}_3^-$ , 0.006 kg of B, and 0.008 kg of  $\text{Sr}^{2+}$ ). The nanoflagellate used in all experiments was *P. bandaiensis*, a colorless chryomonad commonly isolated from open ocean plankton samples

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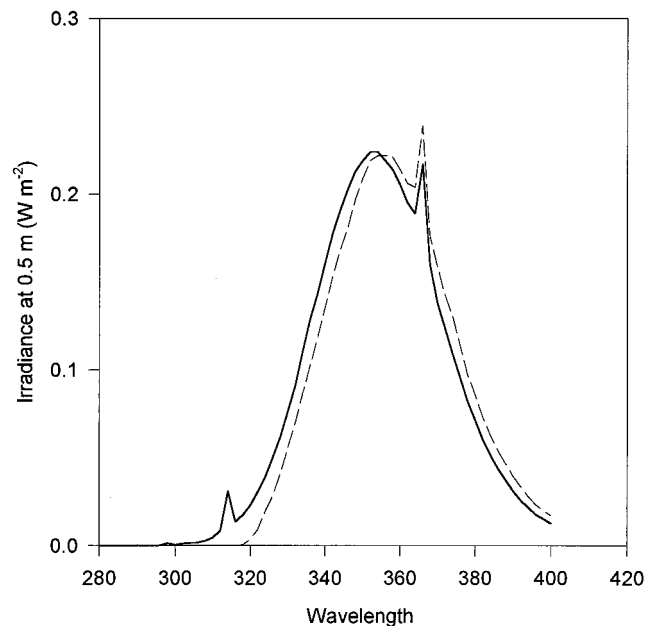


FIG. 1. Irradiance of UV source at 0.5 m. The solid line indicates the maximum irradiance used in experiments in which organisms were exposed to UV-A+UV-B. The broken line indicates the maximum irradiance used in experiments in which organisms were exposed to UV-A alone. Irradiance is in watts per square meter per nanometer. Wavelength is in nanometers.

(4) which readily consumes *Synechococcus* spp. in culture (34). *P. bandaiensis* was maintained on a mixed bacterial assemblage growing in 0.01% yeast extract made with artificial seawater and amended with rice grains. All organisms were cultured at 20°C under a light-dark cycle (12 h of light:12 h of darkness) at a PAR irradiance of 30  $\mu\text{mol s}^{-1} \text{m}^{-2}$ .

**UV and visible irradiance.** The UV source was a Psoralite series 2400 light system fitted with F24T12BL/HO UV-A fluorescent lamps situated behind an acrylic shield. The spectrum of UV irradiance to which organisms were exposed during experiments (Fig. 1) was determined by a model 742 Optronics spectroradiometer calibrated with an Optronics OL-200H calibration standard. UV irradiance was regulated by the use of various numbers of neutral-density screens situated between the UV source and the samples. A Plexiglas sheet was used to remove both UV-A and UV-B. To remove UV-B selectively, we used two sheets of Mylar 500D, which reduced the irradiance of wavelengths below 320 nm by more than 99.5%. The ranges of irradiances of UV-A and UV-B used were 0.00 to 9.48 and 0.00 to 0.20  $\text{W m}^{-2}$ , respectively. In all experiments, continuous PAR of approximately 40  $\mu\text{mol s}^{-1} \text{m}^{-2}$  was provided by a bank of fluorescent lights (tube no. RB15T8) positioned on the side of the samples opposite that of the UV source. The PAR source was shielded by a polycarbonate lens to remove any UV that might have been produced.

Gleason and Wellington (15) measured mean broadband UV irradiances at 10 m in the Caribbean of 15.70 (UV-A) and 0.32 (UV-B)  $\text{W m}^{-2}$ . Thus, the broadband UV-A and UV-B irradiances used in these experiments approximate solar broadband UV. The maximum wavelength-specific irradiance to which organisms were exposed in experiments was between 0.22 and 0.24  $\text{W m}^{-2}$  at 354 to 366 nm (Fig. 1); at these wavelengths, this irradiance occurs down to approximately 8 to 10 m in clear ocean water (1). Compared to sunlight, however, irradiance at longer wavelengths was depleted relative to irradiance at 354 nm (27).

**Basic experimental protocol.** Twenty-four hours prior to an experiment, a portion of the nanoflagellate culture was filtered in series through a Whatman GF/C filter and a 1.0- $\mu\text{m}$ -pore-size Nuclepore filter. This procedure removed all nanoflagellates but allowed heterotrophic bacteria to pass through the filters and repopulate the medium. The filtered medium was used for controls in which grazing on *Synechococcus* spp. was eliminated.

Experiments were initiated by introduction into sterile artificial seawater of either *Synechococcus* sp. strain WH8012 or WH7803 to a final concentration of  $10 \times 10^6 \text{ ml}^{-1}$  or  $5 \times 10^6 \text{ ml}^{-1}$ , respectively. For experimental treatments, *P. bandaiensis* was introduced to a final concentration of approximately 25,000 cells  $\text{ml}^{-1}$ . Controls containing *Synechococcus* organisms but no nanoflagellates were prepared for each UV irradiance. To controls, an aliquot of nanoflagellate-free filtrate was added, equal in volume to the additions containing nanoflagellates. Incubation was in UV-transparent polyethylene bags at 25°C. All bags were left

open at the top and periodically gently mixed. For each treatment there were four replicates of 25 ml.

The bags were suspended 0.5 m from the UV source, and their contents were exposed to UV as explained above. At various times, samples were withdrawn for enumeration of *Synechococcus* cells, heterotrophic bacteria, and nanoflagellates. Samples preserved with unbuffered formalin (final concentration, 5%) were filtered onto 0.4- $\mu\text{m}$ -pore-size polycarbonate filters for enumeration by epifluorescence microscopy. *Synechococcus* cells were visualized by autofluorescence (29). Nanoflagellates and heterotrophic bacteria were counted after being stained with acridine orange (23).

We also measured in vivo fluorescence of the *Synechococcus* photosynthetic pigments. Open-ocean isolates of *Synechococcus* spp., including the two strains used in this study, contain phycoerythrin as an accessory light-harvesting pigment (44). Phycoerythrins absorb green light and fluoresce yellow-orange (29). The fluorescence of unpreserved aliquots was measured in a 1-cm-wide cuvette by a Sequoia-Turner fluorometer using a 540-nm shortwave pass filter for excitation and a 585-nm longwave pass filter for emission. Both changes in the number of *Synechococcus* cells and the rate of change of population fluorescence were used for estimating grazing (see below).

**Effect of UV-A and UV-B on nanoflagellate grazing.** Two experiments were performed to compare the effect of UV-A alone versus that of UV-A plus UV-B (UV-A+UV-B) on grazing of *P. bandaiensis* on *Synechococcus* spp. (Table 1, experiments 1 and 2). In both experiments, we used *Synechococcus* sp. strain WH8012. In the first experiment, UV-A+UV-B-treated organisms were exposed to 5.65 W of UV-A and 0.12 W of UV-B  $\text{m}^{-2}$ . Organisms exposed to UV-A alone received an irradiance of 5.47  $\text{W m}^{-2}$ . In the second experiment, UV-A+UV-B-treated organisms were exposed to 9.36 W of UV-A and 0.19 W of UV-B  $\text{m}^{-2}$ , and those exposed to UV-A alone received an irradiance of 9.48  $\text{W m}^{-2}$ . After 6 and either 9 or 10 h of irradiation, samples were collected and processed as described above.

**Effect of a range of UV-A irradiances on nanoflagellate grazing and *Synechococcus* population production.** In two other experiments, we compared the effect of UV-A alone, at a range of irradiances, on grazing of *P. bandaiensis* on *Synechococcus* spp. and on primary production of the *Synechococcus* population (Table 1, experiments 3 and 4). In these experiments, the contents of all bags were shielded from UV-B by two sheets of Mylar 500D. Neutral-density screening and Plexiglas were used to produce UV-A irradiances of 0.00, 3.02, 5.47, and 9.48  $\text{W m}^{-2}$ . Samples were collected and processed as described above.

*Synechococcus* population production was determined by measuring the rate of synthesis of  $^{14}\text{C}$ -labeled biomass in controls.  $\text{NaH}^{14}\text{CO}_3$  was added to an initial activity of 0.04  $\mu\text{Ci ml}^{-1}$ . At 3-h intervals for 9 h, 1-ml aliquots were withdrawn, acidified with 100  $\mu\text{l}$  of 1 N HCl, and shaken overnight to remove unincorporated radiolabel. Radioactivity in the samples was measured in a Beckman LS6500 scintillation counter. Rates are expressed as disintegrations per minute incorporated per milliliter per hour.

**Calculation of *Synechococcus* grazing mortality and nanoflagellate-specific grazing rates.** Gross growth rates ( $b$ ) of the *Synechococcus* population in each control were estimated by the formula  $b = (1/t_1 - t_0) \ln(I_{1C}/I_{0C})$ , where  $I_{1C}$  and  $I_{0C}$  are the relative fluorescence intensities for samples without nanoflagellates at times  $t_1$  and  $t_0$ , respectively. Net rates of growth ( $a$ ) for each of the experimental treatments were estimated similarly by the formula  $a = (1/t_1 - t_0) \ln(I_{1E}/I_{0E})$ , where  $I_{1E}$  and  $I_{0E}$  are the relative fluorescence intensities in samples containing nanoflagellates at times  $t_1$  and  $t_0$ , respectively. Net and gross growth rates over more than one sampling interval (for example, from 0 to 9 h) were determined as the weighted average of growth rates calculated over individual sampling intervals (for example, from 0 to 6 and 6 to 9 h).

Assuming that the gross growth rates were equal in the control and experimental treatments, the rate of grazing mortality of *Synechococcus* spp.,  $1/\text{time}$ , is the difference between  $b$  and  $a$  (12); i.e., grazing mortality =  $b - a$ . Grazing mortality is a community-level measurement, reflecting both the abundance of nanoflagellate predators and the grazing rate of individual nanoflagellates. For calculation of nanoflagellate-specific grazing rates (nanoliters of prey cleared per nanoflagellate per hour), grazing mortality was divided by the average number of nanoflagellates per volume in the time interval (20).

In a recent article, Ochs (34) discusses the use of monitoring population fluorescence versus *Synechococcus* abundance for quantifying rates of nano-

TABLE 1. Description of experiments examining effects of UV on nanoflagellate grazing

Expt no.	<i>Synechococcus</i> strain <sup>a</sup>	Nanoflagellate <sup>a</sup>	UV spectrum or spectra tested
1	WH8012 (10,000)	<i>P. bandaiensis</i> (25)	UV-A+UV-B; UV-A
2	WH8012 (10,000)	<i>P. bandaiensis</i> (25)	UV-A+UV-B; UV-A
3	WH7803 (5,000)	<i>P. bandaiensis</i> (25)	UV-A
4	WH8012 (10,000)	<i>P. bandaiensis</i> (25)	UV-A

<sup>a</sup> Numbers in parentheses indicate the densities of organisms (in cells  $\times$  1,000 per milliliter) at the start of the experiment.

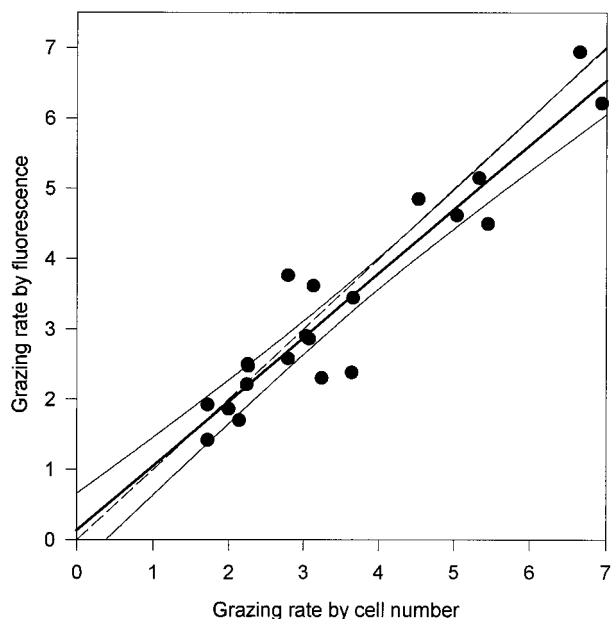


FIG. 2. Relationship of specific grazing rates of *P. bandaiensis* as determined by changes in fluorescence and changes in *Synechococcus* sp. cell numbers:  $Y = 0.91X + 0.14$ ;  $r^2 = 0.91$ . All results for which both measurements were obtained were included; 95% confidence intervals are indicated. The broken line shows a 1:1 relationship. Units are nanoliters cleared per nanoflagellate per hour.

flagellate grazing. Briefly, as long as changes in population fluorescence at a particular UV irradiance and incubation period represent proportionately equivalent changes in cell number across the range of *Synechococcus* abundance in treatments with and without grazers, these methods will provide identical results. We found this condition to be satisfied for *Synechococcus* sp. strain WH8012 and nearly satisfied for *Synechococcus* sp. strain WH7803 (34). In the present study, we directly compared grazing rates measured by both methods.

**Statistical analysis.** Differences in grazing mortality, nanoflagellate-specific grazing rates, nanoflagellate abundance, and primary production for the various treatments were examined by analysis of variance (ANOVA). Data that did not meet the criteria of a parametric test were analyzed by the Kruskal-Wallis one-way ANOVA on ranks. For isolation of treatment differences, the Student-Newman-Keuls test was used. All tests were two tailed with statistical significance set at  $P < 0.05$ .

**RESULTS**

There was a close correspondence in the two methods of calculating grazing rates of *P. bandaiensis* (Fig. 2). As indicated by a comparison of the mean coefficient of variation for all

estimates of nanoflagellate-specific grazing rates by fluorescence (0.13; standard deviation [SD] = 0.08,  $n = 22$ ) with the mean coefficient of variation for estimates made by changes in cell number (0.30; SD = 0.18,  $n = 22$ ), grazing measurements made by determining changes in fluorescence were more precise. For our discussion of results in individual experiments, therefore, we report only values of grazing mortality and nanoflagellate-specific grazing rates determined by fluorescence changes.

In experiment 1, there were no significant differences in grazing mortality between UV-A+UV-B-treated organisms and those exposed to UV-A alone, calculated over both 6 and 10 h of incubation (Table 2). Nanoflagellate-specific grazing rates with UV-A+UV-B and UV-A treatments also did not significantly differ. Thus, UV-B, at the relatively low irradiance used in experiment 1, appears not to have been a factor affecting nanoflagellate grazing. *Synechococcus* grazing mortality and nanoflagellate-specific grazing rate were highest for organisms shielded from UV-A+UV-B.

In experiment 2, the intensities of both UV-A and UV-B were higher than those used in experiment 1. Over 6 h, there were significant differences in grazing mortality and the nanoflagellate-specific grazing rate between organisms treated only with UV-A and those treated with UV-A+UV-B. Over a 9-h incubation, there was a significant difference in grazing mortality between the UV-A-treated organisms and those treated with UV-A+UV-B, but the difference between their nanoflagellate-specific grazing rates was not significant. As in experiment 1, the highest values for grazing mortality and nanoflagellate-specific grazing rate were measured in organisms shielded from all UV (Table 2).

In each of the two experiments in which only UV-A was used, there was an inverse relationship between UV-A irradiance and both grazing mortality and nanoflagellate-specific grazing rate (Table 3). In experiment 3, significant differences in the rate of *Synechococcus* sp. strain WH7803 grazing mortality with the various treatments were evident after 6 h. At the highest UV-A irradiance, grazing mortality over 6 h was suppressed by approximately one-third compared to mortality with treatment involving complete shielding from UV-A. Over the longer exposure period, grazing mortality was suppressed by up to 75% by UV-A. Nanoflagellate-specific grazing rates were not significantly different from each other after the 6-h incubation, although the pattern of mean rates is suggestive of a weak UV-A effect. Over a 9-h incubation period, nanoflagellate-specific grazing rates were significantly higher with lower UV-A irradiances. At the highest irradiance, there was a 56%

TABLE 2. Effects of UV-A alone or UV-A+UV-B on grazing mortality (GM) of *Synechococcus* spp. and nanoflagellate-specific grazing rates (GR) of *P. bandaiensis*

Expt no.	Irradiance ( $W\ m^{-2}$ )		Grazing measurement <sup>a</sup>			
	UV-A	UV-B	0-6 h <sup>b</sup>		0-9 (10) h <sup>b</sup>	
			GM	GR	GM	GR
1	0.00	0.00	0.08 (0.02) a	6.7 (1.6) a	0.13 (0.05) a	10.8 (2.7) a
	5.47	0.00	0.05 (0.01) b	5.5 (1.0) a	0.06 (0.00) b	6.8 (0.7) b
	5.65	0.12	0.05 (0.01) b	5.8 (0.8) a	0.06 (0.01) b	6.7 (0.4) b
2	0.00	0.00	0.06 (0.00) a	2.9 (0.4) a	0.08 (0.00) a	5.1 (0.5) a
	9.48	0.00	0.04 (0.01) b	2.5 (0.3) a	0.05 (0.01) b	3.3 (0.5) b
	9.36	0.19	0.03 (0.01) c	1.7 (0.1) b	0.03 (0.00) c	2.6 (0.1) b

<sup>a</sup> Units: GM, 1/hours; GR, nanoliters cleared per nanoflagellate per hour. Numbers in parentheses are SD. All grazing measurements were calculated by determining changes in in vivo population fluorescence. In each experiment, values followed by the same letter are not significantly different from each other ( $P > 0.05$ , ANOVA).  
<sup>b</sup> The duration of UV-A exposure over which the grazing rates were calculated. In experiment 1, the longer period was 10 h.

TABLE 3. Effects of UV-A exposure on grazing mortality (GM) and nanoflagellate grazing rates (GR) of *P. bandaiensis* on *Synechococcus* spp.

Expt no.	UV-A irradiance (W m <sup>-2</sup> )	Grazing measurement <sup>a</sup>			
		0-6 h <sup>b</sup>		0-9 h <sup>b</sup>	
		GM	GR	GM	GR
3	0.00	0.03 (0.00) a	2.4 (0.3) a	0.04 (0.00) a	3.2 (0.2) a
	3.02	0.03 (0.01) a,b	2.2 (0.6) a	0.03 (0.00) b	2.3 (0.2) b
	5.47	0.02 (0.00) b,c	2.3 (0.2) a	0.02 (0.00) c	2.0 (0.3) b
	9.48	0.02 (0.00) c	1.9 (0.1) a	0.01 (0.00) d	1.4 (0.2) c
4	0.00	0.10 (0.00) a	4.9 (0.2) a	0.14 (0.01) a	6.3 (0.4) a
	3.02	0.09 (0.00) b	5.1 (0.3) a	0.12 (0.01) b	5.5 (0.4) b
	5.47	0.07 (0.01) c	4.5 (0.8) a	0.10 (0.01) c	4.7 (0.4) c
	9.48	0.06 (0.02) c	3.6 (1.2) a	0.06 (0.01) d	3.8 (0.8) c

<sup>a</sup> Units: GM, 1/hours; GR, nanoliters cleared per nanoflagellate per hour. Numbers in parentheses are SD. All grazing measurements were calculated by determining changes in *in vivo* population fluorescence. In each experiment, values followed by the same letter are not significantly different from each other ( $P > 0.05$ , ANOVA).

<sup>b</sup> The duration of UV-A exposure over which the grazing rates were calculated.

suppression of nanoflagellate-specific grazing rates after 9 h compared to the rates with treatment involving complete shielding from UV-A.

The results of experiment 4, in which the prey were *Synechococcus* sp. strain WH8012, were similar to results of experiment 3 (Table 3). At the highest UV-A irradiance, there was a 40% suppression of grazing mortality after 6 h and a 57% reduction after 9 h compared to the mortality of organisms shielded from UV-A. As in experiment 3, nanoflagellate-specific grazing rates did not differ significantly from each other until 9 h of UV-A exposure, at which point there were significant differences between treatments that corresponded to the gradient in UV-A irradiance. At an irradiance of 9.48 W m<sup>-2</sup>, there was a 40% reduction of grazing after 9 h compared to that with UV-shielded treatment.

In the two experiments using UV-A alone, *Synechococcus* abundance in controls either remained stable or increased over the course of the incubations, and there was a decline in *Synechococcus* abundance when nanoflagellates were present (Fig. 3). Although the decline in abundance was slight in experiment 3, it was notable considering the increase of *Synechococcus* organisms in all four controls, especially those exposed to the lowest irradiances of UV-A. With the highest and lowest UV-A irradiances, the ratios of numbers of *Synechococcus* organisms to total numbers of heterotrophic bacteria at the beginning of the experiment and after 9 h of exposure were not significantly different (data not shown). Thus, for the various treatments, differences in the relative amounts of heterotrophic bacteria, an alternative prey, do not explain the differences in nanoflagellate grazing.

Nanoflagellate abundance declined with all treatments in both experiments using only UV-A (Fig. 4). In general, the decline was greatest with higher levels of UV-A. Between 6 and 9 h there was generally either no further decline or an increase in nanoflagellate numbers with all treatments.

Primary productivity of *Synechococcus* spp. was increasingly suppressed as UV-A irradiance increased (Fig. 5). In both experiments, *Synechococcus* productivity was suppressed to a greater degree than was grazing mortality over 6 h. Unlike reductions in grazing mortality, which tended to become more severe with a longer incubation time, reductions of primary productivity were similar over both exposure periods. Consequently, as exposure time became longer, the degree to which grazing mortality was suppressed by UV-A tended to become more similar to the degree of suppression of primary productivity.

## DISCUSSION

These experiments demonstrate that both grazing mortality of *Synechococcus* spp. and specific rates of grazing of *P. bandaiensis* on *Synechococcus* spp. are suppressed by exposure to UV for between 6 and 9 or 10 h at irradiances approximating surface water conditions of the oligotrophic ocean. Considering the well-known detrimental impact of UV-B absorption on biological molecules, especially nucleic acids, it is not surprising that UV-A+UV-B treatment had a somewhat greater im-

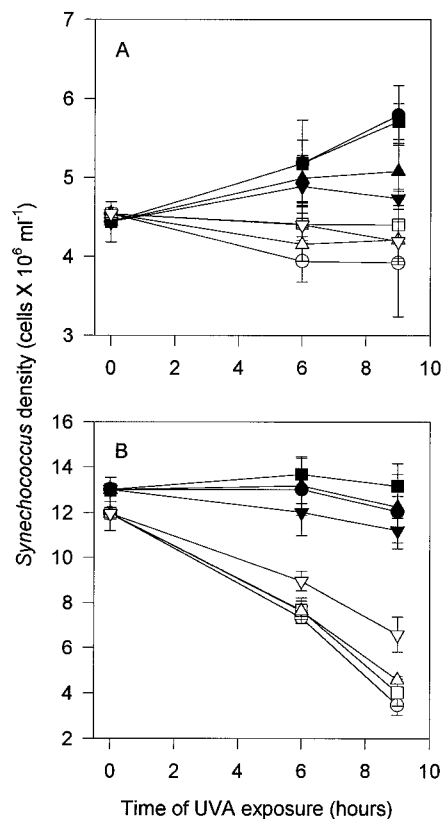


FIG. 3. *Synechococcus* abundance in experiments 3 (A) and 4 (B) (see Table 1). Symbols for UV-A intensities: ▼, 9.48 W m<sup>-2</sup>; ▲, 5.47 W m<sup>-2</sup>; ■, 3.02 W m<sup>-2</sup>; ●, 0.0 W m<sup>-2</sup>. Open symbols indicate corresponding treatments in the presence of nanoflagellates.



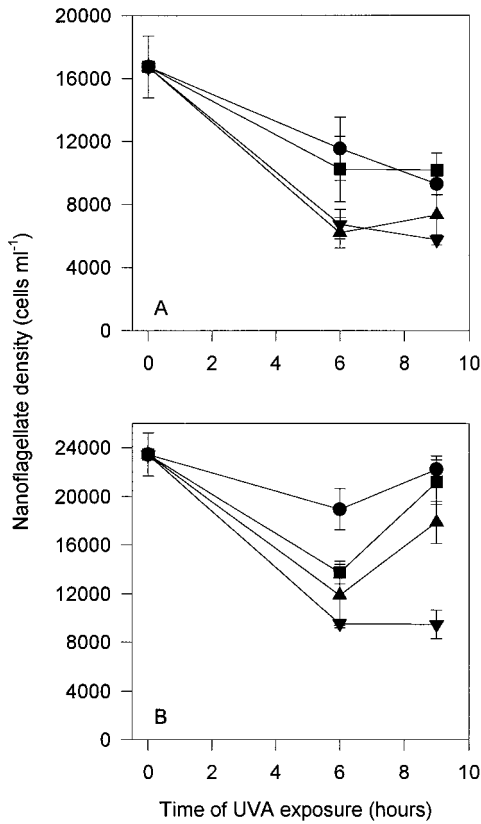


FIG. 4. Nanoflagellate abundance in experiments 3 (A) and 4 (B) (see Table 1). See the legend to Fig. 3 for definitions of symbols.

impact on grazing by *P. bandaiensis* than UV-A alone. However, only at the higher irradiance of UV-B was there a significant suppression of grazing mortality or the nanoflagellate-specific rate of grazing compared to the effect of UV-A alone (Table 2).

UV-A alone suppressed the grazing mortality of *Synechococcus* spp. in all experiments. Differences in grazing mortality over the 6-h exposure periods are largely attributable to differential survival of nanoflagellates, as indicated by the nanoflagellate-specific grazing rates. Is it possible that in non-turbid seawater a diel effect of UV-A is to kill heterotrophic nanoflagellates? If this is so, the rate of nanoflagellate reproduction at night, or at times of the day (or on days) when UV-A irradiance is minimal, must be sufficient to replace individuals lost on sunny days. Available data on diel patterns in heterotrophic nanoflagellate abundance in the ocean do not permit resolution of this question. In measurements made in Woods Hole Harbor and in the oligotrophic Mediterranean Sea, Hagström et al. (19) observed decreases in nanoflagellate numbers of approximately 50% from midnight to midday. In other studies, there were equally large diel changes in nanoflagellate abundance, but in the opposite direction (13, 45). The maximum length of time in which samples were collected in these studies was 36 h, which makes it impossible to evaluate the consistency of the patterns observed. It is evident, however, assuming diel UV-induced mortality of nanoflagellates in the open ocean of up to 50%, that nanoflagellate growth rates are sufficient for recovery during the nightly UV-free period.

Differences in grazing mortalities with the various treat-

ments were more pronounced after 9 h than after 6 h in both experiments 3 and 4, but there was little change or even an increase in numbers of nanoflagellates after between 6 and 9 h of UV-A exposure. Consequently, nanoflagellate-specific grazing rates, evaluated over 9 h, exhibited significant treatment effects, with grazing rates decreasing as UV-A exposure increased. These results suggest that after prolonged exposure to UV-A, the major factor contributing to differences in nanoflagellate-specific grazing rates was impairment of grazing by individual nanoflagellates. The mechanism of UV-A-induced impairment of nanoflagellate grazing is not clear. UV-A alone, as well as UV-B, interferes with the motility of phototropic flagellates of various kinds, at least in some cases by causing the loss or retraction of flagella (8, 18, 22, 42). We have observed that *P. bandaiensis*, following exposure to UV-A at the irradiances used in these experiments, tends to swim more slowly, and somewhat more erratically, than unexposed organisms, which is suggestive of flagellar damage (34). A freshwater nanoflagellate exhibited similar impairment of motility and suppression of grazing after exposure to artificial and natural UV-A (39). Impairment of flagellar movement may result in less-frequent encounters with potential prey, both by interfering with nanoflagellate motility and by disrupting flagellum-

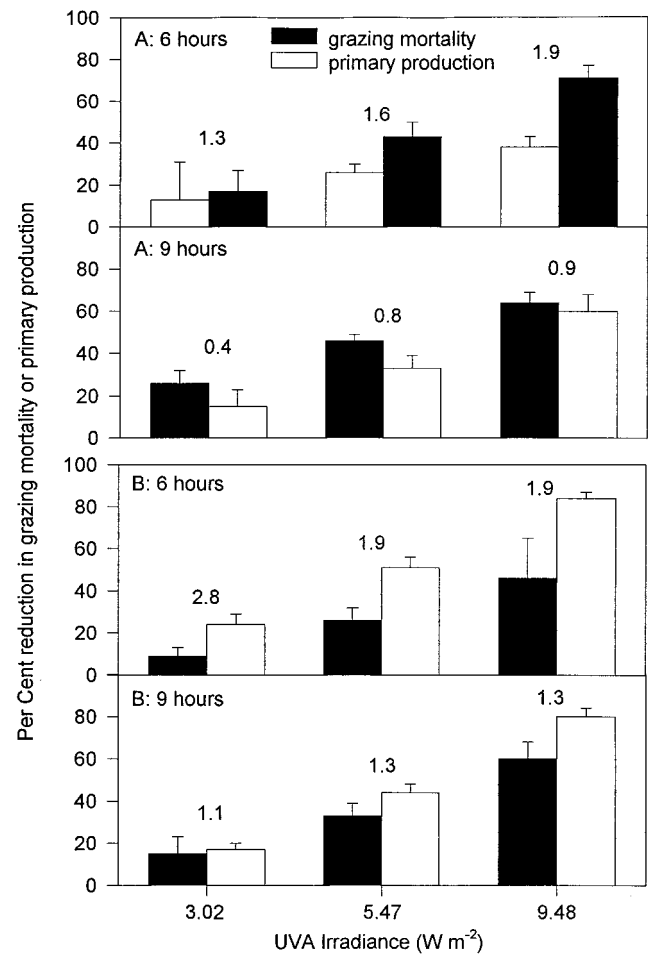


FIG. 5. Percent reduction of grazing mortality or primary production, compared to treatments involving complete shielding from UV-A, in experiments 3 (A) and 4 (B). Results are shown over two time periods, 0 to 6 h and 0 to 9 h. The number over each pair of bars is the mean ratio of the reduction in productivity to the reduction in grazing mortality.

induced feeding currents (11). Alternatively, UV may suppress grazing rates by altering the palatability of prey (22); our visual observations, however, suggest that disruption of nanoflagellate flagella is more likely the cause of suppression of grazing.

Few studies have been conducted examining diel patterns in nanoflagellate grazing in the ocean, and these do not agree in their conclusions. Waterbury et al. (44) inferred from data on *Synechococcus* abundance collected over several diel cycles that grazing of nanoflagellates on *Synechococcus* spp., uncorrected for nanoflagellate abundance, occurred principally at night in surface water of the northern Sargasso Sea but continuously at a more turbid coastal water site. In turbid water, the effect of UV on plankton would be expected to be less than in a more clear environment. In two other studies, however, the diel pattern in grazing of marine protozoa was the opposite of that observed by Waterbury et al. (44). Rates of *Synechococcus* grazing mortality in the English Channel were consistently higher during the day than at night (46), and higher daytime grazing rates of nanoflagellates on bacteria were observed at several locations by Wikner et al. (45). None of these studies was designed specifically to test the effect of UV on grazing of nanoflagellates, a behavior which is probably influenced by multiple environmental factors, of which UV exposure may be of importance only at certain places and times.

Effects of UV on trophic-level interactions, as well as on the physiology or growth rates of individual species, may influence community structure and ecosystem-level productivity. Using predator-free enclosures, Bothwell et al. (2) discovered that growth rates of freshwater benthic diatoms were suppressed by exposure to ambient UV-B. In the presence of algivorous chironomid larvae, however, the net growth rate of the diatoms was higher than in controls in which the predators were present but UV-B was eliminated. Bothwell et al. (2) concluded that differential effects of UV-B on the diatoms compared to the chironomids suppressed predation to a greater degree than diatom growth was inhibited, with the net effect of UV-B exposure being to increase diatom abundance. In the present study, after 6 h of exposure, UV-A suppressed community-level primary production to a somewhat greater extent than the grazing mortality of *Synechococcus* was reduced. In terms of ecosystem-level productivity, therefore, the effects of this amount of UV-A exposure on suppression of grazing would partially, but not completely, cancel UV-A-induced reductions in *Synechococcus* productivity. The relative effects of UV-A on productivity and grazing mortality were more similar after 9 h than after 6 h, indicating that evaluations of the influence of UV-A on microbial-community structure must take into consideration both the UV-A irradiance and the duration of exposure.

Considering the simplicity of the system under study, it would be hazardous to noncritically extrapolate these results to a field situation. First, although the broadband UV-A and UV-B irradiances used in these experiments are realistic (15), an artificial source of UV can only approximate the spectral composition of solar UV. Second, the heterotrophic nanoflagellate used in this study appears to be sensitive to UV-A, but other species of nanoflagellates may not be as sensitive. For example, heterotrophic nanoflagellates in the family *Bodonidae* may be more sensitive to UV than are chryomonads (39). Even among closely related chryomonads there may be differences in UV sensitivity, as indicated by observations that grazing of *Paraphysomonas imperforata* was suppressed less by UV exposure than was grazing by the nanoflagellate used in these experiments (34). Finally, and perhaps most importantly, there is the question of whether the organisms used in this study were more sensitive to the effects

of UV-A under the experimental conditions used than they would be in nature. For example, when exposed to UV, many prokaryotic and eukaryotic microorganisms will synthesize compounds that act as UV-absorbing sunscreens and that provide protection against damage to critical biomolecules (14, 25). Cultured organisms not previously exposed to UV may have reduced concentrations of these protective compounds; for these organisms, UV is likely to be particularly stressful or lethal (35, 36, 43). Considering these caveats, the effects of UV-A observed in these experiments perhaps represent an unusually severe or worst-case scenario.

There is a large and growing body of literature describing the effects of UV on microbial physiology, but there have been few studies examining effects on interactions between organisms. Until models of effects of UV-A and UV-B on ecological communities fully incorporate trophic-level interactions, they will be inadequate in helping us understand the role of natural UV irradiance in, or predict the effects of elevated UV on, microbial food webs or microbially mediated biogeochemical processes. Laboratory experiments are valuable as a preliminary means of exploring hypotheses regarding effects of UV on microbial food web interactions, but field studies will provide results that are more clearly relevant to the natural environment. These studies are strongly encouraged, especially in clear oligotrophic marine environments, where UV-A and UV-B may be significant factors influencing the structure and function of microbial food webs.

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