Vol. 40: 283–292, 2005 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published October 18

Photoreactivation in two freshwater ciliates: differential responses to variations in UV-B flux and temperature

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ABSTRACT: The effects of UV-B radiation on 2 ciliate species (*Glaucoma* sp. and *Cyclidium* sp.) from a clear oligotrophic lake were examined under laboratory conditions with and without photoreactivating radiation (PRR: UV-A and visible light). *Glaucoma* sp. was exposed to 3 UV-B intensities at 4 temperatures to simulate a range of environmentally relevant conditions. Population growth of *Glaucoma* sp. declined with increasing levels of UV-B exposure in treatments receiving PRR; blocking PRR generally resulted in 100% mortality. Occurrence of cyclobutane pyrimidine dimers (CPDs $[mb\ DNA]^{-1}$) was significantly reduced in *Glaucoma* sp. receiving PRR relative to those without PRR. These data indicate that photoenzymatic repair is a major component of UV-B tolerance in *Glaucoma*. At UV-B levels that *Glaucoma* sp. tolerated, *Cyclidium* sp. suffered 100% mortality and accumulated a similar level of CPDs whether or not PRR was blocked. Incubation of the 2 ciliates under UV-transparent and UV-blocking acrylics in the oligotrophic lake confirmed their relative sensitivities to UV radiation (UVR). Photoenzymatic repair in *Glaucoma* sp. was more efficient at 20°C than at 10, 15 and 25°C. The temperature-dependent nature of photoenzymatic repair underscores the need to consider the interactive effects of temperature and UVR on biota, particularly in the face of global climate change and rising incident UVR due to ozone depletion.

KEY WORDS: Ultraviolet radiation · UV-B · Photoreactivation · Photoenzymatic repair · Ciliates · Cyclobutane pyrimidine dimers · Temperature · *Glaucoma* · *Cyclidium*

INTRODUCTION

Erosion of the stratospheric ozone layer has altered the influx of solar ultraviolet radiation in recent years and caused a disproportional increase in the amount of damaging ultraviolet-B (UV-B, 280 to 320 nm) reaching aquatic systems in polar and temperate latitudes (Rex et al. 1997, Madronich et al. 1998, Houghton et al. 2001). UV-B penetration into the water column is highly variable, with 1% attenuation depths ranging from a few centimeters to >10 m. Differences in UV-B attenuation result from multiple factors that include living and detrital particulates, pH, salinity and chromophoric dissolved organic matter (CDOM) concentrations (Morris et al. 1995, Schindler et al. 1996, Arts et al. 2000). Despite the potential for strong vertical gradients of damaging ultraviolet radiation and consequential depth refugia from UV-B exposure in aquatic systems, negative effects of UV-B including inhibition of photosynthesis and damage to nuclear DNA are well documented in marine and freshwater organisms (Neale et al. 1998b, Sommaruga & Buma 2000, Zagarese et al. 2003, MacFadyen et al. 2004).

Absorption of UV-B by DNA disrupts its structure — primarily by formation of pyrimidine dimers — which interferes with replication and transcription (Mitchell & Karentz 1993). Once damage to DNA is incurred, it can be offset by several molecular repair mechanisms. Photoreactivation, or photoenzymatic repair (PER), is a taxonomically widespread, but not ubiquitous, DNA repair process that removes cyclobutane pyrimidine dimers from damaged DNA. PER requires the enzyme CPD photolyase plus photoreactivating radiation (PRR:

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ultraviolet-A [UV-A, 320 to 400 nm] and/or visible light [400 to 700 nm]). The second most prevalent type of DNA damage from UV-B, the (6-4) pyrimidine-pyrimidone dimer, is much less common but potentially more damaging than the CPD to DNA structure (Mitchell 2004). Photoenzymatic repair of (6-4) dimer lesions has been reported for many organisms; however, the efficiency of (6-4) photolyase is very low compared with CPD photolyase (Mitchell 2004). In addition to light-dependent DNA repair, there are multi-step enzymatic repair mechanisms (such as nucleotide excision repair, NER) that do not require light. These dark-repair mechanisms are increasingly important in organisms with reduced PER (Mitchell 1995).

UV-B damage to DNA has generally been found to be independent of temperature (Cadet & Vigny 1990, Pakker et al. 2000), but as an enzyme-dependent process, PER is expected to have an optimum temperature range. Consequently, increases in global temperature (Houghton et al. 2001) and increases in the incident flux of solar ultraviolet radiation may have interactive effects on species and populations by modifying the balance of DNA damage and repair (Williamson et al. 2002). Additionally, changes in global temperature and atmospheric ozone may interact in unexpected ways to alter ecosystem-level processes including biogeochemical cycling and trophic structure (Bothwell et al. 1994, Rae & Vincent 1998, Shindell et al. 1998, Pienitz & Vincent 2000, Zepp et al. 2003). Irrespective of the effects of climate change on regional temperature, temperature and UV intensity vary seasonally and can undergo rapid change over relatively small vertical distances in the water column of lakes and oceans. Thus, the potential interactions of temperature with currently increasing levels of UV-B should be considered when predicting the effects of ozone depletion on aquatic systems.

Ciliate species are globally distributed in aquatic ecosystems and are important components of planktonic food webs (Sanders & Wickham 1993, Finlay et al. 1999, Jürgens et al. 1999), yet relatively little is known about the effect of UV radiation on these heterotrophic protists (Sommaruga & Buma 2000). This study presents results from laboratory experiments designed to elucidate interactive effects of ultraviolet radiation and temperature on ciliates from an oligotrophic lake. The efficacy of photoenzymatic repair in the ciliate *Glaucoma* sp. was altered by changing temperature and UV-B intensity over environmentally relevant ranges. *Cyclidium* sp., another ciliate isolated from the same water sample as *Glaucoma* sp., was much more sensitive to UV-B exposure and showed no evidence of photoenzymatic repair. These data add to the growing evidence of species-specific responses to UV-B exposure.

MATERIALS AND METHODS

Maintenance of cultures. Two freshwater ciliates, *Glaucoma* sp. and *Cyclidium* sp., were isolated from enrichment cultures of a water sample collected in October 1999 at a depth of 3.5 m from the pelagic zone (z_{max} = 23 m) of Lake Giles (41° 23′ N, 75° 06′ W). Lake Giles is an optically clear oligotrophic lake ($k_{d\ 305}$ = 0.4 m^{-1}; $k_{d\ PAR}$ = 0.13, where PAR = photosynthetically active radiation) in the Pocono Mountains region of eastern Pennsylvania, USA (Morris et al. 1995). The ciliates were identified to genus using light microscopy and by comparison of sequences of the 18S ssrRNA gene using BLAST against the GenBank nucleotide database. *Glaucoma* sp. and *Cyclidium* sp. showed greatest sequence similarity to *G. chattoni* (97%) and to *C. glaucoma* (98%), respectively. Partial sequences obtained in this study have been deposited in GenBank (Accession Nos. DQ090841 and DQ090842 for *Cyclidium* sp. and *Glaucoma* sp., respectively). Stock cultures were kept at 20°C with a 14:10 h light:dark regime (cool white fluorescent bulbs). The culture medium was 0.05% Cerophyll® adjusted to a pH of 6.0, with an unidentified bacterial isolate from the original enrichment culture present as food.

UV-lamp phototron. Laboratory experiments were performed in a controlled-temperature walk-in chamber using a UV-lamp phototron to expose the 2 species of ciliates to damaging UV-B radiation in the presence and absence of longer wavelength photoreactivating radiation (PRR). The lamp phototron consists of a horizontally rotating wheel set flush into a circular opening in the top of an opaque box containing sources of PRR (visible and UV-A radiation supplied by two 40 W cool white fluorescent bulbs and two 40 W Q-Panel 340 fluorescent bulbs, respectively). The box is ventilated with a high-speed fan. UV-B radiation is provided from above the box by a Spectroline XX15B UV-B lamp (Spectronics). The shortest wavelengths of UV-B and UV-C radiation not present in incident solar radiation were filtered out by covering the UV-B lamp with a cellulose acetate sheet that was replaced after every 12 h exposure.

The wheel has circular openings for up to 40 custom-made flat-bottomed quartz dishes (50 mm diameter, approximately 18 mm deep, 30 ml volume) with quartz lids. Dishes can be exposed to damaging UV-B and repair radiation sources separately, by blocking 1 light source with an opaque disk, or simultaneously by removing the disks. A black skirt around the wheel and PVC collars surrounding each dish minimize stray radiation among dishes. The spectral composition of the damaging and repair radiation was checked at 1 nm resolution with a custom-made spectral radiometer by C. E. Williamson (Williamson et al. 2001). Further details of the UV lamp phototron approach, including

spectral information of the lamps, have been described elsewhere (Grad et al. 2001, Williamson et al. 2001).

General experimental design. The overall design was to expose ciliates to different intensities of UV-B radiation in the presence or absence of PRR at several temperatures. Exposures were for a single 12 h period, after which all treatments were maintained in the dark. By following population growth after exposure in the lamp phototron, tolerance of UV-B could be separated into 2 components: dark repair plus photoprotection, and photoenzymatic repair. Survivorship and population growth in treatments that did not receive PRR were attributed to dark repair mechanisms or photoprotection; increased survival or growth in treatments receiving PRR compared to treatments without PRR is attributed here to photoenzymatic repair.

Stainless steel mesh covers placed over the quartz lids of individual dishes modified the levels of damaging radiation from the UV-B lamp. At a given temperature (see next paragraph), the levels of ultraviolet radiation from the UV-B lamp were 100, 78, and 40% of maximum; the total exposures over a 12 h period were 58, 45 and 23 kJ m^{-2} (primarily in the 295 to 320 nm range). All references to the UV exposures refer to the total UV from the Spectroline XX15B lamp, ignoring contribution from the lamps used to supply PRR. For comparison, the irradiance spectrum of the UV-B lamp was weighted by the Setlow DNA action spectrum integrated across 285 to 339 nm and normalized to 300 nm (Setlow 1974). Equivalent Setlow-weighted exposures with the different meshes were 9.7, 7.5 and 3.9 kJ m^{-2}, respectively. An additional treatment with a UV-B intensity of 11 kJ m^{-2} (1.8 kJ m^{-2} Setlow-weighted) was added for *Cyclidium* sp., and a final experiment that further reduced the UV-B lamp intensity to 6 kJ m^{-2} (0.97 kJ m^{-2} Setlow-weighted) by adding mesh directly over the UV-B lamp was performed for both species at 20°C. Each treatment and control was run in triplicate in all experiments. The maximum UV exposure (100%) over 12 h was approximately equal to ambient UV exposure on a sunny day around summer solstice at 40° N latitude when weighted for the spectral sensitivity of the cladoceran *Daphnia pulicaria* (Williamson et al. 2001).

For *Glaucoma* sp., experiments were run at 10, 15, 20 and 25°C to span the range of environmentally relevant temperatures that the ciliates would encounter in Lake Giles. *Cyclidum* sp. was tested for its response to UV-B exposure only at 20 and 25°C because preliminary tests indicated negligible growth at 15°C. Ciliates were pre-adapted to experimental temperatures for 3 to 5 d. Ciliates in logarithmic growth phase were transferred from the pre-adapted stock culture to 0.22 µm-filtered Lake Giles water and adjusted to a targeted initial abundance of 500 to 1000 ciliates ml^{-1}. Since UV radiation could have a direct negative effect on live bacteria, and consequently have an indirect effect on the ciliates via food limitation, heat-killed bacteria (HKB) were added as a food source at a final concentration of approximately 1×10^7 HKB ml^{-1}. Preliminary experiments demonstrated that both ciliate species had similar growth dynamics in the Cerophyll® medium with live bacteria and in sterile-filtered lakewater from Lake Giles supplemented with HKB.

Ciliate abundance was determined from 3.5 ml samples taken at 0 h (prior to UV exposure), 12 h (end of UV exposure), 24, 48, 72, and 96 h, and preserved with Lugol's iodine. Prior to fixation, each sample was examined for ciliate movement and morphology since changes in these parameters have been previously identified as potential sublethal effects of UV radiation in protozoans (Sommaruga et al. 1996). Ciliates were enumerated at 400× using Utermöhl chambers on a Zeiss Axiovert microscope.

Heat-killed bacteria. A culture of the bacterium *Pasteurella* sp. was grown to late exponential growth in 0.1% yeast extract and killed by heating the culture in a 70°C water bath for 1 h (Sanders et al. 1990). All subsequent steps for HKB preparation used aseptic techniques. The killed cells were centrifuged in sterile 250 ml bottles at 8000 rpm ($8230 \times g$) for 20 min. Pellets were resuspended in autoclaved distilled water and the wash step was repeated. HKB were then filtered through a sterile 5 µm Nuclepore filter to remove aggregated cells and were further dispersed by a probe sonicator immediately prior to addition to experiments. Sterility of HKB was checked at the beginning of each experiment by inoculation into 0.1% yeast extract.

DNA damage. To confirm that DNA damage in ciliates exposed to UV-B was consistent with presumed repair mechanisms and growth responses, additional experiments were performed following the standard UV-lamp phototron procedure. Replicate quartz dishes of *Cyclidium* sp. and *Glaucoma* sp. were exposed to UV-B at the maximum level (58 kJ m^{-2}) with or without PER at 20°C over a 12 h time course. Controls for background levels of photoproduct consisted of replicate dishes kept in the dark during the exposure period. Immediately after exposure, ciliates were collected on Nuclepore polycarbonate filters and stored frozen until DNA was extracted from the cells. Concentrations of cyclobutane pyrimidine dimer (CPD) photoproducts of DNA damage were then quantified by radioimmunoassay (Mitchell 1996).

Exposure to natural solar radiation. *Glaucoma* sp. and *Cyclidium* sp. were exposed to natural solar radiation in Lake Giles on successive sunny days in late June 2001 (5 and 6 d after summer solstice). Stock cultures were grown in incubators under fluorescent light at

23°C in 0.2 µm-filtered Lake Giles water supplemented with HKB. Ciliates were diluted to several hundred per milliliter with filtered lake water plus HKB in UV-transparent Bitran polyethylene bags (5 replicate bags per treatment) in the laboratory and transported to Lake Giles in coolers. Bags were suspended at a depth of 0.5 m under flat sheets of UV-blocking acrylic (OP-2, CYRO) or UV-transparent acrylic (OP-4, CYRO). Water temperature during incubations was 23°C. *Glaucoma* sp. was exposed for 8 h beginning at 08:00 h Eastern Standard Time (EST), while the more UV-sensitive *Cyclidium* sp. was exposed for 4 h beginning at 09:30 h EST the following day. After exposure, the bags were returned to the laboratory and maintained at 23°C in the dark for an additional 48 h. Samples for microscopic enumeration were fixed with Lugol's iodine at the beginning and end of the exposure periods, and at 24 and 48 h post-exposure.

Statistical treatments. The significance of UV-B intensity levels and temperature on maximum population sizes (as percentages of dark controls) and on the efficiency of photoenzymatic repair (growth rates in treatments receiving PRR) were tested using 2-way ANOVA. Prior to analyses, data were (square root +1)-transformed for growth rates and arcsine-transformed for relative (percent) population size.

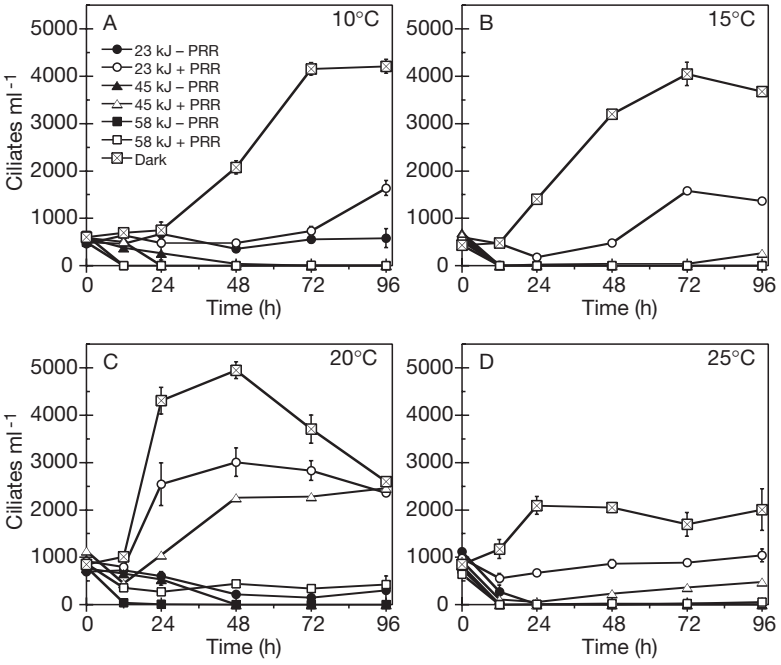

Fig. 1. *Glaucoma* sp. Changes in the abundance (ciliates ml^{-1} ± SE) after exposure to UV-B with (+) or without (–) photorepair radiation (PRR) at different temperatures (kJ = kJ m^{-2})

RESULTS

Glaucoma sp. and *Cyclidium* sp. varied dramatically in their responses to UV-B exposure in the presence and absence of photoreactivating radiation. When exposed to UV-B, population growth of both species was generally inhibited relative to dark controls, but *Glaucoma* sp. survived and grew at much higher exposure levels than *Cyclidium* sp.

Table 1. *Glaucoma* sp. Maximum specific growth rate (d^{-1}) (mean ± SE, n = 3). UV-B (kJ m^{-2}) lamp exposures were with (+) or without (–) photoreactivating radiation (PRR). S: some survival, but no growth; M: complete mortality

UV-B	Temperature (°C)			
	10	15	20	25
Dark	0.88 ± 0.13	1.2 ± 0.15	2.9 ± 0.08	1.3 ± 0.35
23 + PRR	0.82 ± 0.16	0.70 ± 0.13	2.3 ± 0.39	0.91 ± 0.39
23 – PRR	S	M	S	S
45 + PRR	S	S	1.1 ± 0.03	0.47 ± 0.03
45 – PRR	M	M	M	M
58 + PRR	M	M	0.13 ± 0.19	S
58 – PRR	M	M	M	M

Glaucoma sp. had the highest growth rate and reached the maximum population size in dark controls (Table 1, Fig. 1). When exposed to UV-B without PRR, it usually suffered complete mortality although survival without population growth was sometimes observed (Table 1). After UV-B exposure, growth rate and survival were both temperature dependent with maxima at 20°C. *Glaucoma* sp. in the dark controls at 20°C had the highest maximum growth rate (μ_{max} = 2.9 d^{-1}) and abundance (approximately 5000 cells ml^{-1}) relative to any combination of UV-B exposure and temperature (Table 1, Fig. 1). The effects of UV-B intensity and temperature on growth rates were both significant ($p < 0.001$), and ANOVA indicated a significant interaction effect.

The most efficient photoenzymatic repair also was observed at 20°C, where *Glaucoma* sp. grew or survived at all UV-B exposure levels in treatments that received PRR (Fig. 2). There was a trend of increasing maximum growth rate and maximum population size with decreasing UV-B exposure level if PRR was present (Table 1, Fig. 1C). Equivalent exposure levels without PRR resulted in 100% mortality except for the lowest exposure treatments. In the lowest UV-B plus PRR treatment (23 kJ m^{-2}) at 20°C, *Glaucoma* sp. had

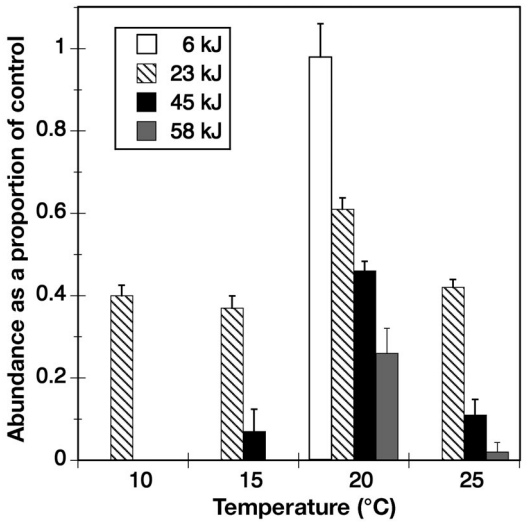

Fig. 2. *Glaucoma* sp. Maximum abundance as a proportion of dark controls during exposure to different temperatures and levels of UV-B in the presence of photorepair radiation

the greatest growth rate (μ_{max} = 2.3 d^{-1}) of any UV-B treatment (Table 1). Maximum specific growth rates in the 45 and 58 kJ m^{-2} UV-B exposures with PRR were significantly reduced (μ_{max} = 1.1 d^{-1} and 0.13 d^{-1}, respectively). In the experiment in which UV-B exposure was reduced to 6 kJ m^{-1}, *Glaucoma* sp. reached the same abundance in dark controls and treatments with PRR (Fig. 2). Without PRR, exposure to 45 and 58 kJ m^{-2} UV-B resulted in 100% mortality within 24 to 48 h (Table 1). At 23 kJ m^{-2} without PRR, abundance declined to about 150 ml^{-1} and no population growth occurred during the experiment (Fig. 1). Survival was much greater at 6 kJ m^{-1}, but even at this lowest exposure level growth in treatments without PRR was negligible.

For any given treatment, population growth rates and/or survival declined when incubation temperature was increased or decreased from 20°C (Table 1). At 25°C, *Glaucoma* sp. growth rate in the control was 45% of the 20°C control; similar inhibition was observed for the 45 and 58 kJ m^{-2} with PRR treatments relative to those treatments at 20°C (Table 1). The maximum abundance at 25°C was significantly lower compared to the 20°C experiment, and survival in treatments without PRR was observed only at the lowest level of UV-B exposure at 25°C (Fig. 1D). At 15°C, the maximum specific growth rates in the dark control and in the 23 kJ m^{-2} with PRR treatment were not significantly different from those treatments at 25°C (Table 1). There was no population growth at 15°C in the 58 kJ m^{-2} with PRR treatment. However, after the initial decline in abundance in the 45 kJ m^{-2} with PRR, a small but stable population of survivors was present and there was a small increase in ciliate abundance during the last 24 h of the experiment (Fig. 1B). At 10°C, positive growth was observed only in the dark control and at the lowest UV-B level tested (23 kJ m^{-2}), while there was 100% mortality within 12 to 48 h at higher exposures of UV-B (Fig. 1A). *Glaucoma* sp. in the dark controls at 10°C showed a moderate growth rate during the 96 h sampling period (μ_{max} = 0.88 d^{-1}), and reached a maximum abundance of approximately 4000 ciliates ml^{-1} (Fig. 1A). In the 10°C treatment with 23 kJ m^{-2} UV-B plus PRR, abundance was indistinguishable from that in the minus-PRR treatment until the 72 to 96 h time period, when growth over the final 24 h of the experiment was 0.82 d^{-1} (Table 1, Fig. 1A).

The major trends in the lamp-phototron experiments with *Glaucoma* sp. can be summarized by comparing the maximum cell abundance in each treatment receiving PRR to the maximum abundance in the dark control at that temperature. Maximum abundance relative to the control occurred at the lowest exposure level at every temperature tested. At 20°C and an exposure of 6 kJ m^{-2} from the UV-B lamp, abundance was not significantly different between the control and plus PRR treatment (Fig. 2). At 15, 20 and 25°C, abundance relative to control decreased with increasing UV-B intensity. At 10°C, the ciliates did not survive the higher levels of UVR exposure (Table 1), but their abundance relative to the dark control in the 23 kJ m^{-2} plus PRR was similar to that at 15°C (Fig. 2). Both temperature and UV-B intensity had significant effects on the maximum population size in the presence of PRR ($p < 0.001$), and ANOVA also indicated a significant interaction component ($p < 0.001$).

Cyclidium sp. was much more sensitive to UV-B exposure than *Glaucoma* sp., and suffered complete mortality in the 58 and 23 kJ m^{-2} treatments with and without added PRR at both 20 and 25°C (Table 2). Experiments were not performed at lower temperatures for this species because preliminary experiments indicated very low population growth at 15°C. When *Cyclidium* sp. was exposed to 11 kJ m^{-2} of UV-B, survival was observed in treatments with and without

Table 2. *Cyclidium* sp. Maximum abundance (percentage of the dark control) during exposure to UV-B (kJ m^{-2}) lamps with (+) or without (–) photoreactivating radiation (PRR) at 20 and 25°C; mean ± SE, n = 3. M: complete mortality; nd = no data

UV-B	20°C		25°C	
	+ PRR	– PRR	+ PRR	– PRR
6	96 ± 8	42 ± 2	nd	nd
11	21 ± 1	51 ± 7	18 ± 5	39 ± 5
23	M	M	M	M
58	M	M	M	M

PRR. The population size remained relatively stable in treatments without PRR, but declined in the presence of PRR at both 20 and 25°C. Finally, at an exposure level of 6 kJ m^{-2}, *Cyclidium* sp. survived and grew in all treatments. However, population growth was much greater in the treatment with PRR and, as with *Glaucoma* sp., the maximum abundance observed at 6 kJ m^{-2} with PRR was not significantly different from the dark control (Table 2). In treatments without PRR, *Cyclidium* sp. reached a maximum abundance of only 42% of the dark control, indicating that population growth was inhibited in the absence of photoenzymatic repair at the lowest UV exposure.

Cyclobutane pyrimidine dimers per megabase DNA (CPDs Mb^{-1}) were elevated in both species of ciliates exposed to UV-B at 58 kJ m^{-1} relative to background levels in the dark controls (Table 3). For *Glaucoma* sp., treatments receiving photoreactivating radiation had significantly fewer CPDs Mb^{-1} than those without PRR ($p < 0.001$). In contrast, CPDs Mb^{-1} in *Cyclidium* sp. were not significantly different in treatments with and without PRR (Table 3).

Results from exposure to natural solar radiation at Lake Giles confirmed the relative sensitivity to UV radiation observed in the laboratory experiments with the 2 ciliate species. Populations of *Cyclidium* sp. exposed to full sunlight decreased precipitously during and after exposure, while those shielded from UV with OP-2 acrylic grew rapidly (Table 4). Growth rates of *Glaucoma* sp. under UV-shielded and UV-transparent acrylic were positive and indistinguishable from each other (Table 4).

Table 3. *Glaucoma* sp. and *Cyclidium* sp. Cyclobutane pyrimidine dimers per megabase (CPD Mb^{-1}) during maximum experimental UV-B (kJ m^{-2}) lamp exposure with (+) and without (–) photoreactivating radiation (PRR) at 20°C; mean ± SE, n = 3

UV-B	*Glaucoma* sp.	*Cyclidium* sp.
Dark	2.0 ± 0.3	1.9 ± 0.2
58 + PRR	6.5 ± 1.7	23 ± 12
58 – PRR	29 ± 5	18 ± 5

Table 4. *Glaucoma* sp. and *Cyclidium* sp. Growth rates (d^{-1}) during exposure to natural solar radiation at a depth of 0.5 m in Lake Giles in June. UV-exposed treatments were incubated under UV-transparent acrylic (OP-4, CYRO), and UV-shielded treatments under UV-blocking acrylic (OP-2, CYRO)

Treatment	*Glaucoma* sp.	*Cyclidium* sp.
UV-exposed	1.6 ± 0.03	–1.4 ± 0.48
UV-shielded	1.6 ± 0.14	1.6 ± 0.18

DISCUSSION

Effects of UV-B and repair radiation

Deleterious effects of UV-B, including damage to DNA, appear to be species-specific among protists (Karentz et al. 1991, Wickham & Carstens 1998, Sommaruga & Buma 2000). The ciliate *Stentor coeruleus* was sensitive to UVR exposure, but *S. araucanus* showed no difference in the proportion of survivors when treatments were shielded from UVR (Häder & Häder 1991, Modenutti et al. 1998). Likewise, changes in population sizes during incubations of pond communities from Greenland indicated strong negative effects of UV-B on the ciliates *Askenasia* sp. and *Bursaridium* sp., moderate negative effects on the ciliates *Halteria* sp. and *Strombidium* sp., and no apparent effect on *Urotricha* sp. (Wickham & Carstens 1998). Direct negative effects of UV radiation, in the form of accumulated DNA damage, also shows differences across protistan taxa. Sommaruga & Buma (2000) found that the heterotrophic flagellates *Bodo saltans* and *B. caudatus* accumulated greater DNA damage after UV-B exposure than chrysomonad or cryptomonad flagellates, and a *Cyclidium* sp. did not accumulate any CPDs after a Setlow-weighted UV-B exposure of 1.69 kJ m^{-2}. By comparison, growth of the *Cyclidium* sp. isolated from Lake Giles was inhibited by exposure at 11 kJ m^{-2}, but not at 6 kJ m^{-2} (Setlow-weighted, 1.8 and 0.97 kJ m^{-2}, respectively) from our UV-B lamp with photorepair radiation (Table 2). The *Glaucoma* sp. and *Cyclidium* sp. examined here also had very different tolerances to UV-B and accumulated different levels of DNA damage (CPDs) despite being isolated from the same water sample.

The variation in UV sensitivity between protistan species is likely to reflect differences in both physiology and behavior. Various strategies for avoiding damage from UV radiation known in other eukaryotes are also known in protists. Attenuation in the water column would reduce the negative effects of UV-B for species that either remain in deep water or migrate deeper into the water column during peak daylight hours. Several species of ciliates appear to detect and avoid UV-B radiation (Barcelo & Calkins 1979, Lenci et al. 1997), although this does not appear to be the case for the species of *Glaucoma* and *Cyclidium* examined here. Experiments in 20 cm columns indicated that vertical distributions for both *Glaucoma* sp. and *Cyclidium* sp. exposed to unidirectional illumination from UV lamps or natural sunlight were no different from UV-shielded or dark controls after 1 to 2.5 h (A. L. Macaluso pers. obs.). Some organisms also use photoprotective agents to prevent or reduce UV-induced damage. Production of mycosporine-like amino acids

(MAA) and carotenoids that absorb strongly in the UV range of the spectrum are known for a variety of photosynthetic protists (Neale et al. 1998a, Jeffrey et al. 1999), and these compounds along with their photoprotective utility can be passed to consumers (Rocco et al. 2002, Moeller et al. 2005). To our knowledge there is no indication that any heterotrophic ciliates produce photoprotective compounds, and extractions of the experimental food source (HKB) in 25% aqueous methanol showed no absorption peaks in the UV portion of the spectrum (data not shown). Even when avoidance behavior or photoprotection are apparent in organisms, neither is 100% effective, and the ability to repair UV-induced damage to DNA should be as widespread in protists as it is in other organisms (Mitchell & Karentz 1993, Blaustein et al. 1994, Malloy et al. 1997, Grad et al. 2001).

Photoenzymatic repair (PER) — a major mechanism for mending DNA damage caused by UV-B radiation — has been demonstrated for the ciliate *Paramecium tetraurelia*, some photosynthetic dinoflagellates (Smith-Sonneborn 1979, Litchman et al. 2002), and in Antarctic diatoms (Karentz et al. 1991). Karentz et al. (1991) found that DNA repair was enhanced in the presence of PRR in several diatoms; however, the importance of PER relative to 'dark repair' mechanisms such as NER is relatively unknown for protists. Use of the lamp phototron allowed separation of photorepair wavelengths from UV-B, thus allowing evaluation of PER relative to other mechanisms of UV tolerance. The increased survival, growth rates and final abundances of *Glaucoma* sp. in the treatments receiving PRR relative to those without PRR (Table 1, Fig. 1) strongly suggest that PER is an important component of the overall tolerance of UV-B for this species. Without PRR, *Glaucoma* sp. suffered 100% mortality when exposed to UV-B at intensities >23 kJ m^{-2}; at 23 kJ m^{-2} without PRR, populations either declined to a low stable abundance with no apparent growth or died out (Table 1). Even at the lowest level of UV-B tested (6 kJ m^{-2} at 20°C) growth without PRR was very low (data not shown), while populations with PRR were indistinguishable from the dark controls (Fig. 2). Further evidence of the importance of PER to *Glaucoma* sp. is the significantly reduced accumulation of CPDs in the presence of PRR (Table 3).

In contrast to *Glaucoma* sp., *Cyclidium* sp. showed little tolerance of UV-B and photoenzymatic repair played a reduced role. UV-B exposures at which *Glaucoma* sp. had positive population growth resulted in 100% mortality for *Cyclidium* sp., irrespective of PRR (Table 2). The similar accumulations of CPDs in populations of *Cyclidium* sp. exposed to UV-B with or without PRR indicate that PER was very inefficient in this ciliate (Table 3). Only at the lowest UV-B exposure tested (6 kJ m^{-2}) did *Cyclidium* sp. grow better in the presence of PRR. At 11 kJ m^{-2}, *Cyclidium* sp. survived but did not grow, and its abundance was reduced more in the presence of PRR than in its absence (Table 2). Since exposure of *Cyclidium* sp. to PRR alone did not significantly inhibit growth relative to dark controls (data not shown) and harmful consequences of PER exposure was not apparent at 6 kJ m^{-2}, the negative effect of PRR in the presence of 11 kJ m^{-2} of UV-B suggests that UV-A and UV-B may act as multiple stressors in this species. Sensitivity to UV-A is known for many species, including several heterotrophic and photosynthetic protists. The freshwater kinetoplatid flagellate *Bodo saltans*, and 2 marine flagellates, *Paraphysomonas bandaiensis* and *P. imperforata*, had reduced motility and feeding when exposed to UV-A radiation alone or in combination with UV-B (Sommaruga et al. 1996, Ochs 1997, Ochs & Eddy 1998). Reduced motility was also reported for the ciliate *Stentor coeruleus* in treatments exposed to the UV-A portion (320 to 400 nm) of natural solar radiation relative to treatments exposed only to wavelengths >400 nm (Häder & Häder 1991). Alternatively, the small amount of UV-B emitted by the UVA-340 lamps supplying PRR (MacFadyen et al. 2004) may have had an additive effect that exceeded a threshold in *Cyclidium* sp. for UV-B tolerance.

Temperature effects on recovery from UV exposure

Within species-specific ranges, metabolism of protists and growth rate are generally positively related to temperature (Caron et al. 1990, Jones & Rees 1994). The maximum specific growth rate (μ_{max}) of *Glaucoma* sp. calculated for dark controls follows this trend and increases with increasing temperature from 10°C through 20°C. At 25°C, μ_{max} was reduced to approximately the same value as at 15°C, suggesting that optimal growth in this strain of *Glaucoma* occurs at 20°C. If DNA damage from UV-B were largely independent of temperature, but enzymatically driven repair of UV-B damage were not, changing temperature would be expected to alter the ratio of damage to repair (Williamson et al. 2002). The results from our experiments support this suggestion. At the optimum temperature for growth (20°C), *Glaucoma* sp. populations grew at every UV-B level, but the maximum abundance decreased with increasing UV-B intensity (Fig. 2). When *Glaucoma* sp. was acclimated to experimental temperatures greater or less than 20°C, maximum abundance relative to controls decreased at all UV-B intensities (Fig. 2), indicating that PER (i.e. photolyase activity) was less effective at temperatures that were non-optimal for growth.

Several earlier studies observed temperature-dependent responses to UV radiation by aquatic organisms. Repair of UV-B-induced DNA damage by both PER and NER showed temperature optima in the marine red alga *Palmaria palmata* (Pakker et al. 2000), and the rate of PER increased with increasing temperature in the estuarine killifish *Fundulus heteroclitus* (Malloy et al. 1997). Malloy et al. (1997) also noted efficient PER in krill and 2 Antarctic fish at temperatures ≤1°C. Survival of freshwater zooplankton after exposure to UV-B also showed temperature optima, with greatest survival of the copepod *Leptodiaptomus minutus* and the cladoceran *Daphnia catawba* at 25°C, and the rotifer *Asplanchna girodi* at 20°C (Williamson et al. 2002). Another cladoceran, *D. pulicaria*, was found to have increasingly efficient PER at 25°C compared to 5 and 15°C, although temperature effects were not significant for NER (MacFadyen et al. 2004). Thus, the data for *Glaucoma* sp. fit a general trend, excepting the stenothermal Antarctic species, for an increasing efficiency of PER with increasing temperature within a species-specific range.

Implications for aquatic systems

The effect of temperature on enzymatic repair of UV damage has ramifications on scales beyond individual organisms. For example, environmental seasonal variations in ambient UV radiation and water temperatures can lead to a strong variation in UV:temperature ratios (UV:T). In Lake Giles, where our cultures of *Glaucoma* sp. and *Cyclidium* sp. originated, the highest UV:T conditions occur in spring (March and April) when UV begins increasing and temperatures remain cold; UV:T is lower in summer (June to August) because both temperature and UV influx are high (Williamson et al. 2002). Although our lamp phototron does not exactly simulate solar radiation, it approximates a range of exposures to UV radiation that are relevant to Lake Giles (Williamson et al. 2001). We do not have data for seasonal distributions of these species in Lake Giles, other than incidental observations of *Glaucoma* sp. in depth-integrated samples during the early summer and both species in surface waters in October when they were isolated. Our data for *Glaucoma* sp. suggest that it is likely to be most susceptible to UV exposure at extremes of UV:T ratios because its survival at a given UV exposure is reduced at both low and high temperatures (Figs. 1 & 2). The *Cyclidum* sp. that we isolated would probably be limited to times or to depths where UV-B exposure was minimal (such as October when the ciliates were isolated). Clearly, UV levels in the surface waters of Lake Giles in June could lead to population declines for *Cyclidium* sp. without inhibiting growth in *Glaucoma* sp. (Table 4). Taken together, these data suggest that even at current levels of UV radiation, community structure of protists in clear oligotrophic lakes and oceans is likely to be influenced by ambient UV-B. Predicting the interactive effects of climate change and increased levels of UV-B radiation due to ozone depletion on protistan populations is beyond the scope of these laboratory-based experiments. It is reasonable to expect, however, that global climate change will modify UV-temperature relationships in aquatic systems in several ways — including alteration of seasonal temperatures, ice-out, and vertical mixing (MacFadyen et al. 2004). While information from a few species or a single trophic level must be applied with care in predicting the effects of UV radiation on pelagic food webs (Wickham & Carstens 1998, Mostajir et al. 1999), heterotrophic protists play a central role in pelagic food webs (Müller 1989, Sanders et al. 1989, Weisse et al. 1990); consequently, levels of UV-B that are inhibitory or fatal to protists could alter the transfer of energy between trophic levels and result in indirect impairment of the other populations that depend on the functions of these microorganisms.

Acknowledgements. We thank C. E. Williamson for advice on construction of the UV-lamp phototron and for determining the spectral composition of the damaging and repair radiation for our lamps, P. J. Neale for conversion factors to calculate Setlow weighting of the UV-B lamp, and A.-C. Olsson Allen for laboratory assistance. We also thank the Blooming Grove Hunting and Fishing Club for allowing access to Lake Giles. This research was supported by NSF grants DEB-9973758 and DEB-IRCEB-0210972.

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Editorial responsibility: John Dolan, Villefranche-sur-Mer, France

Submitted: March 31, 2005; Accepted: July 1, 2005
Proofs received from author(s): September 20, 2005