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PRIMARY RESEARCH PAPER

Effects of nutrients and dissolved organic matter on the response of phytoplankton to ultraviolet radiation: experimental comparison in spring versus summer

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Abstract The effects of nutrients and dissolved organic matter (DOM) on the response of phytoplankton community structure to ultraviolet radiation (UVR) was studied using natural phytoplankton assemblages from Lake Giles (Northeastern Pennsylvania), a temperate, oligotrophic, highly UVR-transparent lake. Microcosm experiments were conducted in 1-l bags in the spring and summer. A factorial design was used, with two UVR treatments (ambient and reduced), two nutrient treatments (control with no nutrients added, and nitrogen and phosphorus addition together), and two DOM treatments (control of 1 mg l^{-1} and doubled). In April, UVR affected the overall phytoplankton community structure, causing a shift in the dominant species. Significant interactive effects of UVR $\times$ nutrients and UVR $\times$ DOM were found on total phytoplankton biovolumes. In July, all taxa responded positively to the N + P addition, and were affected differentially by the UVR treatments. The initial communities varied in April and July, but *Synura* sp. and *Chroomonas* sp. were present in both seasons. *Synura* sp. responded positively to the addition of DOM in April and the reduction of UVR in July. *Chroomonas* sp. responded positively to the reduction of UVR in April and the addition of nutrients in July. The differential sensitivity of these two species suggests that changing environmental factors between spring and summer promoted differences in the relative importance of UVR in changing phytoplankton community structure.

Keywords Phytoplankton · Ultraviolet radiation · Seasonal effects · Nutrients

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Introduction

While phytoplankton require solar radiation for photosynthesis, ultraviolet wavelengths can be damaging. The shortest wavelength of ultraviolet radiation (UVR) to reach the earth's surface is UVB (280–320 nm), which can damage deoxyribonucleic acids

(DNA) and potentially inhibit photosynthesis by interfering with the electron transport chain, photosystem II, and pigment stability (Jones & Kok, 1966; Noorudeen & Kulandaivelu, 1982). UVA (320–400 nm) can also be damaging but can stimulate repair of DNA damage caused by UVB (Williamson et al. 2001a). The damage caused by UVR can lead to lower biomass production and slower growth rates in phytoplankton (Nilawati et al., 1997; Hiriart et al., 2002; Xenopoulos et al., 2002).

However, phytoplankton have developed ways to deal with UVR. Flagellated phytoplankton can avoid UVR exposure by migrating to deeper water. Phytoplankton that are exposed to UVR can prevent damage by using photoprotective compounds such as carotenoids and mycosporine-like amino acids (MAAs) (Laurion et al., 2002). Phytoplankton can also deal with UVR by repairing the damage. Photoenzymatic repair is stimulated by UVA and photosynthetically active radiation (PAR), and is important for cell survival (Karentz et al., 1991). Algae can also use nucleotide excision repair, a light-independent process that is not as specific as photoenzymatic repair (Sinha & Häder, 2002; MacFadyen et al., 2004).

As DNA repair is an enzymatic reaction, it is temperature dependent (Pakker et al., 2000). The rate of damage and the extent of photoprotection, however, are not known to be temperature dependent. Therefore, a larger proportion of the UVR-induced damage can be repaired as temperature increases, up to 25°C (MacFadyen et al., 2004), and the overall inhibition by UVR can be reduced (Roos & Vincent, 1998; Doyle et al., 2005).

The response of phytoplankton to UVR can also be affected by nutrient concentrations. Nitrogen (N) is needed for the synthesis of pigments, nucleic acids, amino acids, and proteins, while phosphorus (P) is required for synthesis of DNA and RNA. Therefore the cell's capacity for protection and repair may depend on available nutrients and the relative concentrations of N and P. As compared to nutrient replete conditions, nutrient limiting conditions have both increased (Hiriart et al., 2002; Litchman et al., 2002) and decreased the effects of UVR on phytoplankton growth (Xenopoulos & Frost, 2003; Doyle et al., 2005).

The damaging effect of UVR can be mediated by the concentration of colored dissolved organic matter (DOM) in a lake. Since colored DOM absorbs UVR, the concentration of DOM affects how deeply UVR can penetrate into a lake, and thus how much UVR algae are exposed to (Morris et al., 1995). Indirect effects of UVR through interactions with DOM may produce reactive oxygen species that may also influence phosphatase and other enzyme activity (Scully et al. 2003). Allochthonous inputs of DOM to aquatic systems are strongly controlled by precipitation (Schindler et al., 1996).

Due to differences in the efficiency of prevention or repair of UVR-induced damage, species exhibit differential sensitivity to UVR. Therefore, exposure to UVR cannot only cause a reduction in overall population growth rates and/or biomass, it can also cause a shift in the structure of a community (Davidson et al., 1996; Fauchot et al., 2000; van Donk et al., 2001) by decreasing the abundances of the more sensitive species. UVR-induced changes in community structure are also dependent on environmental factors, as Xenopoulos & Frost (2003) found that different species became abundant under different nutrient concentrations, and Doyle et al. (2005) found community structure shifts under different temperatures and nutrient concentrations.

While certain environmental factors, such as temperature, nutrient status, and DOM concentration, can alter the effects of UVR on phytoplankton community structure, it is unknown how seasonal changes in these environmental factors alter the importance of UVR in shaping seasonal phytoplankton succession. Seasonal changes in environmental factors may explain why some studies reveal an effect of UVR on phytoplankton community structure (van Donk et al., 2001; Xenopoulos & Frost, 2003) while others find little to no effect (Halac et al., 1997; Fouilland et al., 2003; Roy et al., 2006).

The objective of this study was to determine if seasonal conditions alter the effects of nutrient status or DOM concentration on phytoplankton response to UVR exposure. We manipulated UVR exposure, and nutrient and DOM concentrations in an in situ experiment conducted in April and July, 2005, in an oligotrophic lake. We hypothesized that UVR would have a stronger effect on community structure in the spring because temperatures were lower and cells had not been acclimated to UVR exposure. As a consequence, we further hypothesized that the effects of the DOM addition would be greater in the spring than in the summer. The effect of nutrient concentrations

on phytoplankton response to UVR was predicted to be higher in the summer because nutrient concentrations were lower due to stratification and reduced runoff.

Materials and methods

Site description

Lake Giles is a temperate, dimictic lake located on the Pocono Plateau in northeastern Pennsylvania (41°23′ N, 75°06′ W). It has an average depth of 10 m with a maximum depth of 24 m. Lake Giles has an area of 48 ha and a forested watershed of 183 ha. It is an oligotrophic lake with a DOC concentration of about 1–1.5 mg l^{-1} C, which makes the lake one of the more transparent lakes in the northeastern United States (Morris et al., 1995).

In situ experiments

The growth responses of phytoplankton species were assessed with a set of experiments carried out in Lake Giles in April and July, 2005. Weak thermal stratification became established prior to the April experiment. A full factorial design was used, manipulating UVR (ambient and reduced), DOM concentration (ambient and doubled), and nutrients (control, and nitrogen and phosphorus addition). Using a van Dorn sampler, we collected water containing the natural phytoplankton assemblages from 3 m (the midpoint of the epilimnion). Water was collected between 0900 and 1100 h. The water was then passed through a 100 μm mesh to remove large zooplankton grazers. Initial samples for soluble reactive phosphorus (SRP), DOM, and total dissolved nitrogen (TDN) were taken directly from Lake Giles and immediately filtered with a 0.7 μm GF/F filter (Whatman). SRP was analyzed by the ascorbic acid method (APHA, 1998) and DOM and TDN were analyzed by a high temperature combustion method using a TOC autoanalyzer (Shimadzu model TOC-V_{CSH}) with an attached TDN measuring unit (model TNM-1). Initial silicon analyses were also included prior to the July experiment. Water samples for silicon analysis were syringe filtered (0.45 μm polypropylene, Whatman) in the field, acidified to pH 2 with ultrapure nitric acid, and stored in polyethylene bottles until analysis. Silicon was determined using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Thermo Elemental X-Series, Winsford, UK) with pneumatic nebulization. Concentrations were quantified using a six-point standard calibration curve with an absolute accuracy of ~5% and a precision better than 3%.

Each treatment was replicated four times. For each treatment, all of the water was collected at the same time and combined in a 4-l carboy to reduce variability among replicates. Nutrients and/or DOM additions, if used, were added to the carboys. In nutrient treatments, N was added in the form of $NaNO_3$ to achieve an enrichment factor of 8 μmol l^{-1} and P was added in the form of NaH_2PO_4 to achieve an enrichment factor of 0.5 μmol l^{-1}. DOM was concentrated by reverse osmosis from a nearby bog lake (see description below). The initial DOM concentration of unaltered lake water was approximately doubled in the +DOM treatments by an addition of 1 mg l^{-1}. Water from a DOM-addition carboy was filtered through 0.7 μm GF/F filters (Whatman) for DOM and TDN analysis, and through 0.45 μm polypropylene filters (Whatman) for silicon analysis.

The water was then added to 1-l liquid-tight specimen bags (Bitran S series) made of UVR-transmitting polyethylene (transmits 94% PAR 400–700 nm and 86% solar UVR 295–399 nm, 50% transmittance at 234 nm), and then placed on racks. Each rack consisted of one-inch PVC pipe frames. Bungee cords separated the rack into 12 sections, and bird netting was secured to the bottom to hold the bags. Half of the racks were covered with Aclar, a long-wave-pass plastic that in water transmits both PAR (100% 400–700 nm) and most UVR (98% of UVB 295–319 nm, 99% UVA 320–399 nm, with a sharp wavelength cutoff and a 50% transmittance point at 212 nm). The other half of the racks were covered with Courtgard, a long-wave-pass plastic that transmits PAR (95% 400–700 nm in water) but blocks most UVR (transmits no UVB 295–319 nm, and only 9% of UVA 320–400 nm with a sharp wavelength cutoff and a 50% transmittance point at 400 nm). The racks floated on the surface of Lake Giles, attached to an anchor line. To compensate for the high light levels at the surface, each bag was enveloped in one layer of window screen mesh, which served as a neutral density filter to further reduce light levels to 62% of ambient.

We used DNA dosimeters to quantify the total damage to DNA. DNA dosimeters are made of UVR-transparent quartz tubes, approximately 5 cm in length and 1 cm in diameter. They were filled with a sterile buffer solution containing DNA from salmon testes (Sigma-Aldrich Co., St. Louis, MO, USA) and hermetically sealed with rubber stoppers on each end. Because the dosimeters contained only naked DNA, there was no DNA repair or photoprotection occurring within the dosimeters.

DNA dosimeters were placed in one replicate of each treatment as the bags were filled with water, and removed at the end of the experiment and covered immediately with aluminum foil. The dosimeters were later analyzed for DNA damage by quantifying the amount of photoproducts (cyclobutane pyrimidine dimers) formed per mega base of DNA using a radioimmunoassay (RIA) specific for this lesion. Briefly, the RIA is a competitive binding assay between very small amounts of radiolabeled DNA and sample DNA for anti-sera raised against UV-irradiated DNA. Details of the methodology and the sensitivity and specificity of the RIA, as well as the standards used for quantification, are described by Mitchell (1996, 2006).

Vertical profiles of underwater UVR (305, 320, 340, and 380 nm) and PAR (400–700 nm) were collected at the start of each experiment using submersible profiling ultraviolet radiometers (Biospherical PUV-501 and PUV-501b). The 1% attenuation depths for 320 nm UVR and PAR were then estimated using these profiles. Vertical temperature profiles were also measured using these instruments.

The bags were incubated for six days during spring (April 16–22, 2005) and summer (July 7–13, 2005). At the end of the experiments, three 50-ml subsamples were taken from each bag and preserved with Lugol's iodine solution for phytoplankton counts.

DOM source and concentrating techniques

The source water for the concentrated DOM addition was from Beaver Lake, a small, shallow (maximum depth = 10 m) lake in a sphagnum bog habitat surrounded by mixed deciduous-coniferous forests located approximately 2 km northeast of Lake Giles, in an adjoining watershed. The pH of Beaver Lake is 5.7, compared to a pH of 5.9 in Lake Giles. During late spring and summer Beaver Lake is highly colored, with DOC concentrations ranging from 5 to 14 mg l^{-1} and a DOC-specific absorbance (320 nm) of 4.15 m^{-1}. Because of its chromophoric properties and proximity to Lake Giles, Beaver Lake water was used as the DOM source for the experiment.

Beaver Lake water was filtered through a 5 µm prefilter (Ace Hardware) and concentrated using a reverse osmosis unit built in Don Morris's lab at Lehigh University. Lake water was pressurized to 20 psi and delivered to two pressurized sleeves fitted with thin-film membranes (DESAL, GE Osmonics) with an exclusion efficiency of 95% for molecules between a molecular weight of 70–100 Daltons (i.e., molecules smaller than this size were lost to waste water). The concentrate was subsequently filtered through a 1 µm prefilter (Polydepth Filter Cartridge, Pentrek Filtration) and 0.2 µm process filter (Memtrex Filter, GE Osmonics, Inc.) to remove particulates and most microorganisms. Reverse osmosis does not substantially increase most nutrient concentrations. The addition of 1 mg l^{-1} of DOC to the experimental bags added 0.017 µM of total phosphorus, of which 0.007 µM was in the form of soluble reactive phosphorus. Silica additions, however, were substantial in this case, providing 6.65 µM of silica in the DOM addition bags.

UV exposure estimates

The UV exposures during the two experimental periods were estimated from data collected at 12 min intervals with a Smithsonian Environmental Research Center SR18 spectroradiometer located on a tower near Lake Giles. The data from the 320 nm sensor (2 nm bandwidth) were used to estimate UV exposure in exposure days. One 320 nm exposure day is equivalent to 10.9 kJ m^{-2} nm^{-1}, the amount of 320 nm UV reaching the surface of Lake Giles during summer solstice (Cooke and Williamson 2006). This exposure day metric is based on values averaged from June 15 to 25 with a radiative transfer model (RT Basic, Biospherical Instruments, Inc., San Diego, CA, U.S.A.) and ozone levels typical for this region (332 Dobson Units, from http://toms.gsfc.nasa.gov/). Exposure days provide a convenient and biologically relevant metric of solar UV exposures (Williamson et al., 1999, 2001a, b).

Cell counts

Cell counts were performed by settling 25–50 ml (depending on cell densities) of each sample in an Utermöhl-style chamber and counting individuals of each phytoplankton species under an inverted microscope at 400× magnification (Nikon TS100). Genera were identified according to Wehr & Sheath (2003). For each sample, at least 500 total cells were counted. Counts were then converted to biovolume by first estimating the volume (in μm^3) of at least 20 individual cells of each taxon which were approximated to the nearest geometric shape. The average cell volumes were then multiplied by the cell numbers (in ml^{-1}) to estimate biovolume.

Statistical analysis

Using SPSS version 11.5 (SPSS, 1999), we performed a two-way ANOVA to assess the effects of UVR exposure and DOM concentration on the amount of DNA damage in the dosimeters. Changes in phytoplankton communities were examined for April and July experiments with a principal components analysis (PCA) on the treatment averages of biovolumes for each species. Treatment averages were $\log_{10}$ transformed for each season, and the analyses were run in R (version 2.1.1) with symmetric scaling. We also performed three-way ANOVAs to assess the effects of UVR, DOM concentration, and nutrient status on the total final biovolume of phytoplankton as well as of each phytoplankton species, using a $P < 0.05$ significance level.

Results

The epilimnetic temperature of Lake Giles was nearly 15°C higher, and initial SRP and TDN concentrations lower, in July than in April (Tables 1, 2). Initial dissolved organic carbon (DOC) concentrations were similar in the two seasons, but the lake was more transparent in July (deeper 1% attenuation depths for PAR and UVR). The DOM addition approximately doubled the DOC concentrations in each season, and included the addition of a small amount of nitrogen but a substantial amount of silica (Table 2).

The UVR exposure levels were very similar during the two exposure periods due to the sunnier conditions in April than in July. Accounting for the 38% reduction in ambient UV by the mesh screens, the 1.5% reduction due to the Aclar, and the 14% reduction due to the Bitran bags, the exposure levels in the experimental bags were: 2.16 exposure days or 23.53 kJm^{-2} nm^{-1} of 320 nm UV during the April incubation and 2.10 exposure days or 22.92 kJm^{-2} nm^{-1} of 320 nm UV for the July incubation. Thus, during the 6 day incubations the experimental bags during both months were exposed to the equivalent of approximately 2.5 days of solar UV at the very surface of Lake Giles during summer solstice under cloudless conditions if there were no mesh screens or other materials to reduce

Table 1 Seasonal differences in the mixing depth, 1% depth for PAR and 320 nm UVR, epilimnetic temperature, and initial soluble reactive phosphorus (SRP) concentrations in Lake Giles

Season	Mixing depth (m)	$Z_{1\%}$PAR (m)	$Z_{1\%}$320 nm (m)	Temperature (°C)	SRP (μmol l^{-1})
April	6	13	1.9	9.8	0.12 (0.07)
July	6	16	3.7	24.3	0.01 (0.00)

Standard errors (SE) are indicated in parentheses, $n = 2$

Table 2 Average DOC, TDN and silica (Si) concentrations in Lake Giles initial and DOM addition samples

Sample	April		July		
	DOC (μmol l^{-1})	TDN (μmol l^{-1})	DOC (μmol l^{-1})	TDN (μmol l^{-1})	Si (μmol l^{-1})
Giles	100.0 (3.3)	9.03 (0.29)	106.7 (9.2)	7.68 (0.38)	3.6*
DOM addition	197.5 (12.5)	11.95*	165.0 (11.7)	10.22 (0.02)	17.9*

Standard errors are indicated in parentheses, $n = 2$

*$n = 1$

UV. The 1% attenuation depths for 320 nm UV were used to estimate 320 nm UV exposure levels in the surface mixed layer of the lake assuming random thorough mixing in this surface layer, exponential attenuation of UV with depth, and a 6 m mixing depth. In April these exposure levels were estimated to be 7% of surface irradiance, while the greater UV transparency in July gave an exposure estimate of 13% of surface irradiance.

The dosimeters showed that the UVR manipulation was effective in both seasons; there was much more DNA damage in the +UVR treatments than in the −UVR treatments ($P < 0.001$ for both April and July, Fig. 1). The DOM addition also reduced the amount of DNA damage in the +UVR treatment in April, showing that some UVR was attenuated by the addition of DOM ($P = 0.042$). The dosimeters did not show an effect of the nutrient manipulation on the amount of DNA damage (data not shown).

The initial phytoplankton communities were determined in April and July (Fig. 2). In April, there were five common species from three phyla. The two dominant species were *Synura* sp. and *Chroomonas* sp., with *Tabellaria* sp., and two *Mallomonas* species (sp. a and b) also present. The *Synura* sp. was most similar to *Synura sphagnicola*, and *Mallomonas* sp. b was most similar to *Mallomonas caudata*; both these species have been found previously in Lake Giles sediments (Kodama et al., 1997) and water samples (Robert Moeller, personal communication). However, as we did not have access to the appropriate electron microscopy facilities to examine the scales of any of these taxa, we are not assigning species names to these groups, and leave all classification at the genus level. In July, the *Synura* sp. and *Chroomonas* sp. observed in April were still common, although not dominant, along with five other species from four phyla: *Gymnodinium* sp., *Merismopedium* sp., *Chrysodidymus* sp., another *Mallomonas* species (sp. c; appearing similar to *Mallomonas heterospina*, which has also been identified in Lake Giles sediments (Kodama et al., 1997)) and an unidentified small flagellate. The community in July was more uniform; however, *Gymnodinium* sp. and *Merismopedium* sp. were the most abundant. Data for taxa comprising less than 5% of the total biovolume of the assemblage are not presented here.

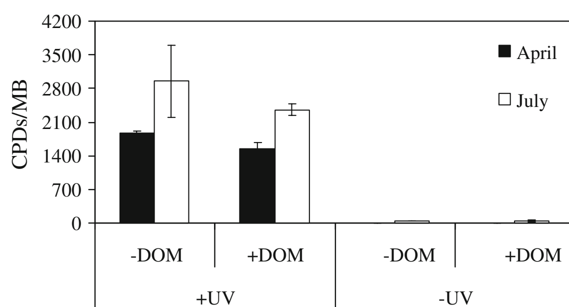

Fig. 1 Amount of DNA damage (number of cyclobutane pyrimidine dimers per megabase (CPDs/MB)) in each treatment in April and July

In April, an examination of the results by single factors (Fig. 3) revealed that the dominant species shifted from *Synura* sp. with UVR exposure to *Chroomonas* sp. without UVR exposure. This shift was driven by an increase in the biovolume of *Chroomonas* sp. in the −UVR treatments and not a decrease in the biovolume of *Synura* sp. in these treatments (Fig. 4A–B). UV × nutrient ($P = 0.037$) and UV × DOC ($P = 0.015$) effects were observed for the total biovolumes across treatments (Table 3). The PCA for April (Fig. 5A) further illustrated these effects, revealing that *Chroomonas* sp. and the two Mallomonas taxa dominated the −UV N + P +DOC treatment, while *Tabellaria* was abundant in the −UV N + P −DOC treatment. In contrast, *Synura* sp. dominated the +UV N + P +DOC treatment.

In July, the relative proportion of *Mallomonas* sp. c and *Merismopedium* sp. decreased, while that of *Chroomonas* sp., *Synura* sp., and the small flagellate all increased in the nutrient addition (N + P) treatment (Fig. 3F). Overall, based on the PCA for July (Fig. 5B), all taxa responded to the N + P addition, and were further separated by the UV treatments. The significant interaction ($P = 0.001$) between UV and nutrients for the total biovolume ANOVA in July further illustrates this (Table 3). *Chroomonas*, *Chrysodidymus*, *Mallomonas*, and *Gymnodinium* were more abundant in the +UV treatments, while *Synura*, *Merismopedium*, and a small flagellate clustered in the −UV treatments (Fig. 5B).

The response to these environmental parameters varied by season and by species, as illustrated by the responses of the two species that were present in both seasons, *Synura* sp. and *Chroomonas* sp. (Fig. 4). The three-way ANOVAs for these taxa (Table 3) revealed that, in April, the biovolume of *Synura* sp. increased with the addition of DOM ($P = 0.001$). It also

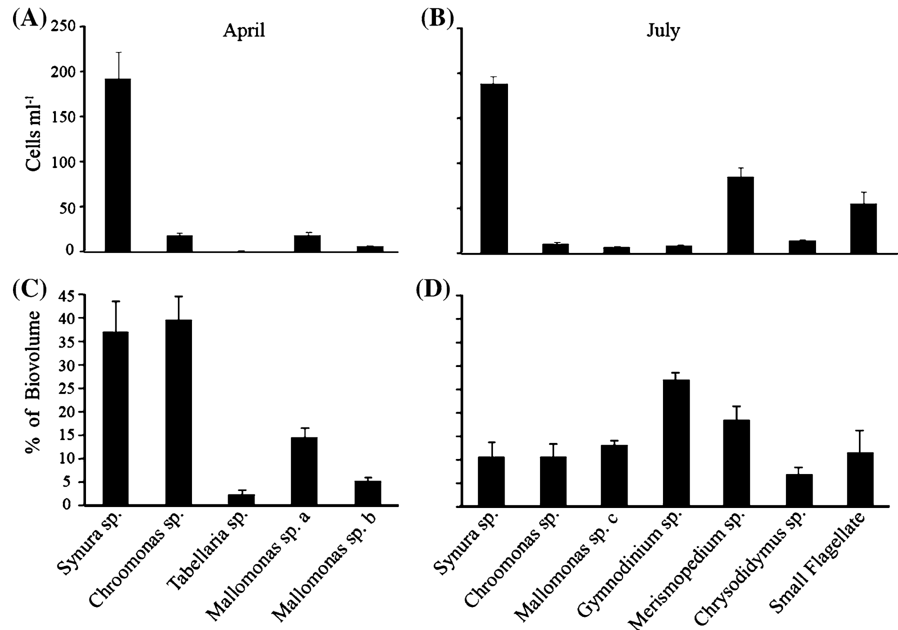

Fig. 2 Initial phytoplankton communities in April (**A**, **C**) and July (**B**, **D**) in Lake Giles. The top two plots (**A**, **B**) are by cells ml^{-1}, the lower two plots (**C**, **D**) by % of total biovolume. Error bars represent standard error ($n = 4$)

increased in treatments with the addition of nutrients ($P = 0.033$) and with the reduction of UVR ($P = 0.049$). In July, the biovolume of *Synura* sp. increased with reduced UVR ($P < 0.001$) and with the addition of nutrients ($P < 0.001$). For *Chroomonas* sp., however, there was a significant three-way interaction between UVR, DOM, and nutrients in both April and July ($P = 0.047$ in April and $P < 0.001$ in July, Table 3). For *Chroomonas* sp. in April, the removal of UVR caused an overall increase in biovolume. When UVR was removed, *Chroomonas* sp. responded positively to the addition of N and P. In July, UVR alone did not affect growth; however, the addition of N and P increased biovolume regardless of UVR exposure.

The PCA plots also support the observation that these two taxa responded differently during the two experimental periods. *Chroomonas* plotted near the −UV N + P +DOC treatment in April, while in July it plotted near the +UV N + P −DOC treatment (Fig. 5). *Synura* plotted near the +UV N + P +DOC in April, while in July it plotted near the −UV N + P −DOC treatment.

Discussion

In Lake Giles, the phytoplankton community consisted primarily of synurophytes and other flagellates throughout the spring and summer. Flagellates are typically considered to be more sensitive to UVR exposure than other groups of phytoplankton (Villafañe et al., 1995; Barbieri et al., 2002), yet in this study we found a range in sensitivities. We also found evidence that seasonal changes in environmental conditions played a role in modulating the UVR sensitivity of the two species that were common in both spring and summer. Therefore, with the continued dominance of flagellates and the differences within these two species, changes in the responses of these two species to UVR in April versus July were likely due, in part, to changing environmental factors and not solely due to the phytoplankton community composition, as has been suggested by other studies (Gala & Giesy, 1991; Xenopoulos et al., 2002).

UVR had a strong negative effect on the growth of *Chroomonas* sp. in April, but this species dominated in a +UV treatment in July. The higher temperature in July may have been responsible for the decreased sensitivity to UVR. Other studies have also found decreased sensitivity to UVR at higher temperatures (Roos & Vincent, 1998; Doyle et al., 2005). The higher temperatures in the summer may have increased the rate of photoenzymatic repair, thus decreasing the sensitivity of *Chroomonas* sp. in July. MacFadyen et al. (2004) have demonstrated that higher temperatures increased the rate of repair in zooplankton.

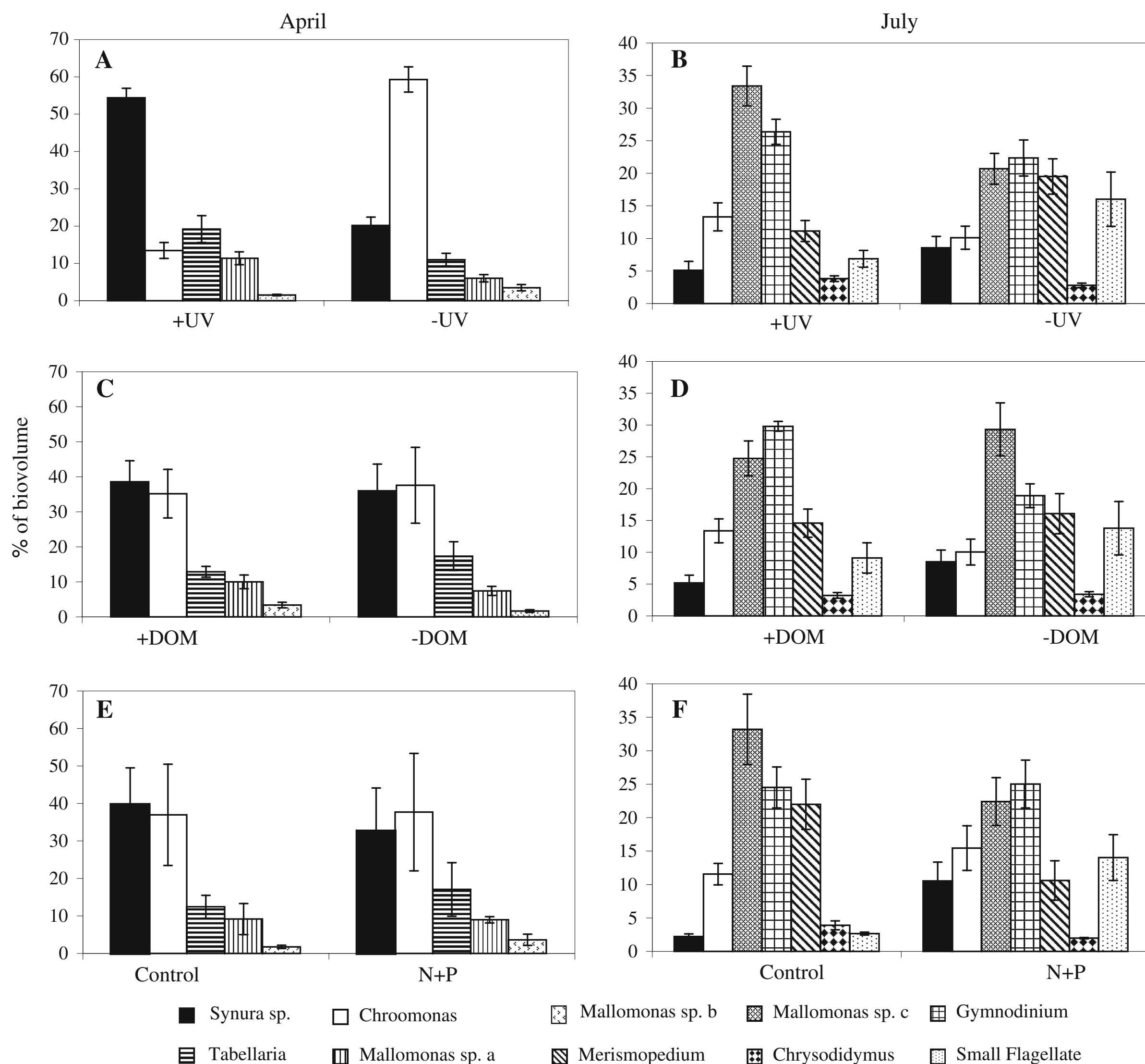

Fig. 3 Average percent biovolume of each common species in April (**A**, **C**, **E**) and July (**B**, **D**, **F**) grouped by UVR (**A–B**), DOM (**C–D**), and nutrient (**E–F**) treatments. Error bars represent standard error (n = 4). Note the change in scale

Previous acclimation to higher UVR exposure in July, compared to April, may also have been responsible for the decreased sensitivity to UVR. Not only were cells exposed to UVR for a longer time prior to the July experiment than the April experiment, daily exposure was also higher in July than April because UVR penetrated nearly twice as deep in July and cells were mixed to the same depth. As cells are exposed to UVR, they produce more photoprotective compounds, such as MAAs (Karentz et al., 1991, Helbling et al., 1996). Tartarotti and Sommaruga (2006) found MAA concentrations were much higher in the late summer compared to just after ice-out. It is known that marine cryptomonads have a high proportion of UVR absorbing compounds (Jeffrey et al., 1999), so *Chroomonas* sp. may also have been able to produce UVR-absorbing compounds and increase its photoprotection capacity as the summer progressed.

In contrast, UVR had the opposite effect on the growth of *Synura* sp.—a strong negative effect in July and a much weaker effect in April, with no

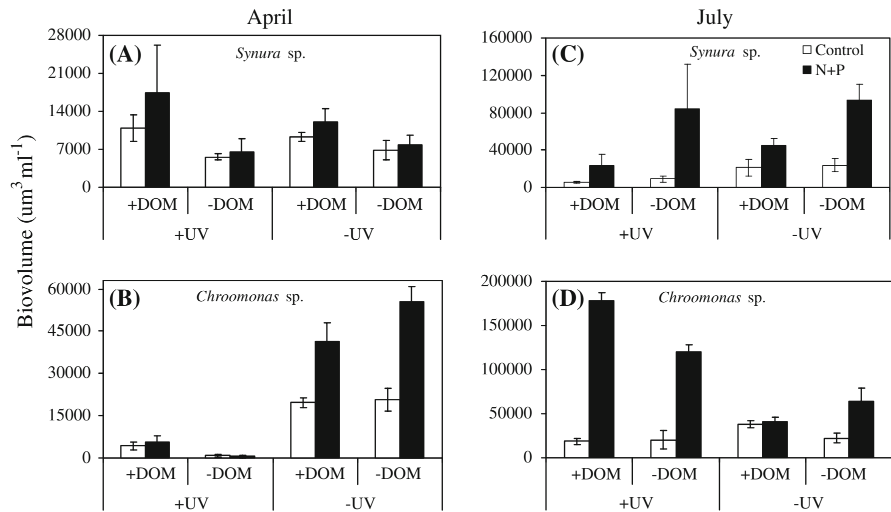

Fig. 4 Average final biovolumes in the UVR, DOM, and nutrient addition treatments across the seasons, April (**A**–**B**) and July (**C**–**D**). Panels **A** and **C** are of *Synura* and panels **B** and **D** are of *Chroomonas*. Error bars represent standard error (n = 4). Note the change in scale

Table 3 Results of the three-way ANOVA to assess the effects of UVR, nutrients, and DOM on the total biovolumes as well as those of *Synura* and *Chroomonas*

Treatment	April			July		
	Total biovolume	*Synura*	*Chroomonas*	Total biovolume	*Synura*	*Chroomonas*
UVR	**<0.001**	**0.049**	**<0.001**	0.179	**<0.001**	0.104
Nutrients	**0.004**	**0.033**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
DOM	**0.018**	**0.001**	0.315	0.551	0.055	**<0.001**
UVR × nutrients	**0.037**	0.613	**<0.001**	**0.001**	0.487	**<0.001**
UVR × DOM	**0.015**	0.429	0.332	0.139	0.441	0.108
Nutrients × DOM	0.389	0.932	**0.037**	0.561	**0.009**	**0.001**
UVR × nut × DOM	0.872	0.538	**0.047**	0.449	0.501	**<0.001**

Significant results ($P < 0.05$) are shown in bold

differences in colony structure observed. This opposite effect of UVR may be due to differences in acclimation or possibly photoprotective capacity. This species may also rely more heavily on avoidance of UV within the water column during this time of year, which was not possible during incubation in the bags.

It is unclear why there was a positive effect of DOM on the biovolume of *Synura* sp. in April. A positive effect of DOM is usually thought to occur due to UVR attenuation; however, this does not appear to be the case here. While the dosimeter data showed a decrease in the exposure to UVR with the addition of DOM, the complete removal of UVR in April had a weaker effect on *Synura* sp. than the DOM addition. Also, the DOM addition failed to increase the growth rate of any other species, despite their responses to UVR removal, suggesting DOM was not an effective screen for UVR. It is unlikely that this positive effect on *Synura* sp. was due to an increase in heterotrophy, as synurophytes are not known to be heterotrophic (Jansson et al., 1996). The positive effect on *Synura* sp. may very likely have been due to the substantial silica addition that accompanied the DOM addition, as synurophytes require silica for their scales. Silica measurements from Lake Giles in April are not available; as indicated above, the July data reveal that the DOM addition substantially increased silica concentrations in the +DOM treatments.

By design, only cells <100 μm were included in this study in an effort to focus on the relatively direct effects of UVR on the phytoplankton community, as opposed to effects of UVR through higher trophic

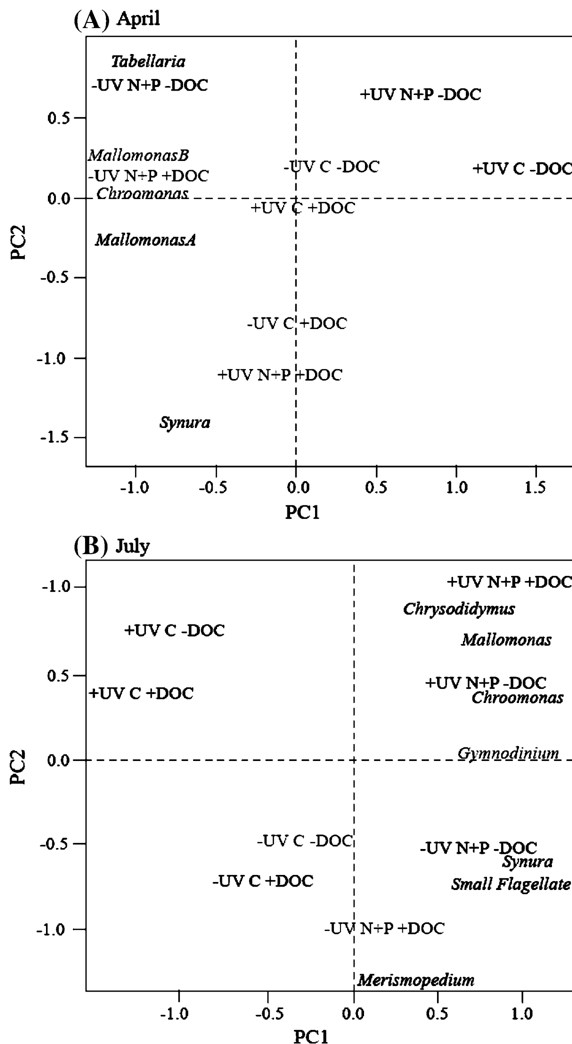

Fig. 5 Principal components analysis (PCA) of phytoplankton communities (based on average biovolume measurements) in the eight different treatments for (**A**) April and (**B**) July. Taxon names are indicated in italics

levels. However, we cannot completely eliminate the possibility that grazing occurred within our experimental bags. Some microzooplankton may have been small enough to pass through the 100 μm mesh. However, these small grazers are unlikely to have been very important in driving the community shifts we observed because they were rare (although exact densities in the bags are unknown, they were rarely encountered during microscopic examination for phytoplankton counts). Grazing could also have occurred by the phytoplankton cells themselves, as some species are known to ingest other smaller cells. For example, large *Gymnodinium* species can ingest small *Chroomonas* species (Fields & Rhodes, 1991).

The incubation procedure kept the phytoplankton at the surface within the range of UVR, not allowing them to be mixed out of UVR for periods of time as would occur under natural conditions. In addition, the study was conducted in the absence of invertebrate grazers, hence extrapolating the results of this study to make conclusions about natural community responses to UVR should be done with caution.

The results from the present study may provide insight into why previous studies have found mixed effects of UVR on phytoplankton (Halac et al., 1997; van Donk et al., 2001; Fouilland et al., 2003). As different phytoplankton taxa appear to use different mechanisms to deal with UVR, the relative importance of factors such as temperature and nutrient status in affecting the response of phytoplankton to UVR can be expected to vary interspecifically. It is possible that phytoplankton taxa that rely on photoenzymatic repair to deal with UVR would benefit from higher temperatures later in the summer, whereas phytoplankton taxa which rely on photoprotection would benefit from higher nutrient concentrations earlier in the spring. Therefore, depending on the taxa present, results from experiments conducted in different seasons would be expected to differ.

As environmental conditions appear to strongly influence the response of phytoplankton to UVR, we would expect that future environmental changes in temperate, oligotrophic lakes may alter the importance of UVR in shaping phytoplankton community structure. For example, higher spring temperatures could increase the rate of repair of UVR-induced damage, making spring phytoplankton less sensitive to UVR. They could also lead to the earlier establishment of thermal stratification, which would cause nutrients to become limiting earlier in the summer, thereby possibly negatively influencing any nutrient-dependent mechanisms for dealing with UVR exposure and increasing the sensitivity of certain summer phytoplankton.

In this temperate, oligotrophic lake, UVR and nutrients had strong, interactive effects on phytoplankton community structure in the spring and summer. For individual taxa, however, there were seasonal differences in the effects of UVR, such that some taxa showed a greater change in growth with the removal of UVR in the spring, while others were

more affected by UVR in the summer. In this study, we have demonstrated differences in the effects of UVR on individual phytoplankton taxa as well as on phytoplankton community structure in spring versus summer, and that changes in environmental factors may have caused this variation in the importance of UVR.

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