

DIRECT AND INDIRECT EFFECTS OF ULTRAVIOLET RADIATION AND
DISSOLVED ORGANIC MATTER ON FRESHWATER FLAGELLATES

A Dissertation Submitted
to the Temple University Graduate Board

In Partial Fulfillment
Of the Requirements for the Degree of
Doctor of Philosophy

By:
Amy L. Macaluso
August, 2010

Major Advisor:

Dr. Robert W. Sanders, Temple University, Department of Biology

Examining Committee Members:

Dr. Edward R. Gruberg, Temple University, Department of Biology

Dr. Joel B. Sheffield, Temple University, Department of Biology

Dr. Wade H. Jeffrey, University of West Florida, Department of Biology

ABSTRACT

The purpose of this study was to examine the direct and indirect effects of ultraviolet-B (UV-B) radiation on freshwater protists. Laboratory experiments were conducted in order to determine the importance of photoenzymatic repair (PER) of UVB-induced DNA damage in the heterotrophic nanoflagellate *Paraphysomonas* sp. Investigations into the combined effects of UV-B and chromophoric dissolved organic matter (CDOM) were conducted in laboratory experiments in which protist cultures were exposed to UV-B radiation in the presence and absence of water amended to a higher CDOM concentration in order to determine the ability of CDOM to act as a UV-B filter and as a potential nutrient source. Field experiments examined the responses of natural communities of protists and bacteria to ultraviolet radiation (UVR) in the presence of high and low concentrations of CDOM.

Ultraviolet-B radiation (UV-B; 280 – 320 nm) negatively affects many aquatic organisms, including heterotrophic flagellates, by directly damaging DNA. The quantity of UV-B reaching the surface of a lake varies with atmospheric chemistry, including stratospheric ozone, and the presence of large holes (Alldredge 1977) in this ozone shield during the last decade resulted in historically high UV-B levels. In aquatic systems, the nature of the damage to organisms depends on the intensity and duration of solar radiation, plus its attenuation in the water column. The amount of UV-B damage is highly dependent on the concentration of CDOM in the water column because CDOM strongly absorbs UV-B radiation. This protective role of CDOM is likely to be reduced in areas where warmer, drier climate decreases watershed runoff, which ultimately results in acidification and increased CDOM photodegradation. However, CDOM also may also

act as an organic carbon source for bacteria and stimulate growth of the microbial food web, including bacterivores like heterotrophic flagellates. The effect of UV and CDOM interactions on the microbial food web is not well understood, but climate-related increase in CDOM in an oligotrophic lake could increase the heterotrophic microbial food web impact by reducing UV-B damage and increasing available resources. Since aquatic organisms, including protists, are differentially susceptible to UV-B radiation, climate change effects on CDOM and UV-B attenuation are likely to alter the ecology and community structure of aquatic systems.

This thesis describes investigations into the direct and indirect effects of UV-B radiation on freshwater protozoa. The role of PER of direct UV-B induced DNA damage was examined in laboratory experiments that compared the survival and population growth of the heterotrophic nanoflagellate *Paraphysomonas* sp. at two environmentally relevant temperatures. The results from these experiments demonstrated the reliance of *Paraphysomonas* sp. on PER, with 100% mortality in the absence of the photorepair radiation that activates photoenzymatic repair enzymes. The ability of *Paraphysomonas* sp. to recover from exposure to UV-B radiation declined in flagellates adapted to 15°C relative to the same exposures at 20°C. Experiments examining the direct and indirect effects of UV-B radiation and CDOM on freshwater protists conducted in the laboratory and in an oligotrophic lake in the Pocono Mountains showed that potential DNA damage resulting from UV exposure is reduced and microbial growth may be enhanced with an increase in CDOM concentration.

ACKNOWLEDGEMENTS

Dr. Robert W. Sanders my mentor and friend, who peaked my interest in the tiny organisms on which this dissertation is based. Dr. Sanders has displayed a perfect balance of understanding and constructive criticism that has guided me through my long journey as a graduate student. This work was supported by grants DEB-9973758 and IRCEB DEB-0210972 to Dr. Robert W. Sanders.

Dr. Edward Gruberg, Chair of the Graduate Committee and committee member, who has supported me and encouraged my progress every step of the way.

Dr. Joel Sheffield, teacher and committee member, who taught me that sometimes less is more and the importance of clarity when displaying data.

Dr. Wade Jeffrey, collaborator and committee member, who has always spoken his mind and shaped my understanding of ecological experimentation.

Dr. David Mitchell and Dr. Sandi Connelly, collaborators, for their assistance in processing DNA damage samples.

Jason Porter, collaborator and friend, for his help in processing DOC water samples and his insight on the many aspects of CDOM in natural waters.

Linda and Dennis O'Brien, my parents, for their love and understanding.

Linda and Richard Heimberg, my in-laws, for their support.

Connor Macaluso, my son, who has brought a new perspective to my life.

Dr. Christopher Macaluso, my husband, who has supported me in so many ways in our life together and without whom this work would not have been possible.

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ACKNOWLEDGMENTS	iv
LIST OF FIGURES	vii
LIST OF TABLES	viii
Chapter	
1. Introduction.....	1
Heterotrophic protists in freshwater ecosystems	1
Direct effects of UV-B radiation on biota.....	1
Indirect effects of UV-B radiation on biota	4
Implications for freshwater systems	5
2. Materials and Methods	8
Laboratory Experiments	8
UV lamp-phototron - experimental design	8
UV-B induced DNA damage and repair in <i>Paraphysomonas</i> sp.	11
Immunofluorescence assay for CPDs within <i>Paraphysomonas</i> sp. cells	11
CDOM: UV protection versus food web stimulation – experimental design	13
Culture origin, maintenance and acclimation	16
Preparation and testing of heat-killed bacteria.....	18
Lake Experiments	18
CDOM: UV protection versus food web stimulation – experimental design	18
General Methods.....	21
Microscopic enumeration of flagellates and bacteria	21
Determination of <i>Paraphysomonas</i> sp. growth rates.....	21
DNA damage analysis – DNA dosimeters and <i>Paraphysomonas</i> sp.	22

Preparation of natural chromophoric dissolved organic matter (CDOM)	23
Statistical analyses.....	24
3. Results	25
Direct effects of UV-B radiation on the heterotrophic flagellate <i>Paraphysomonas</i> sp.	25
Survival and growth response to UV-B	25
Dosimeter DNA damage	30
DNA damage and repair in <i>Paraphysomonas</i> sp.	34
Immunofluorescence assay for CPDs within <i>Paraphysomonas</i> sp. cells	34
Heat-killed Bacteria as food	36
Interactive effects of UV-B radiation and CDOM on <i>Paraphysomonas</i> sp.	36
DOC-specific absorbance	36
Survival and growth responses to UVR and CDOM	37
Potential DNA damage measured with dosimeters	44
Lake Experiment: CDOM and UV radiation effects on a natural microbial food web	44
Temperature, oxygen and DOC changes	44
Survival and growth responses of microorganisms to UVR and CDOM	48
Dosimeter DNA damage	54
4. Discussion.....	57
5. Conclusion and Future Studies.....	70
REFERENCES CITED	72

LIST OF FIGURES

	Page
2.1 UV-lamp phototron	9
2.2 Laboratory Experimental design: UV-shielding versus stimulation.....	14
2.3 Photomicrograph of <i>Paraphysomonas</i> sp. (strain GflagA)	17
2.4 Containment Racks in Lake Giles	20
3.1 Abundance changes of <i>Paraphysomonas</i> sp. exposed in UV-lamp phototron at 20°C +PRR	26
3.2 Abundance changes of <i>Paraphysomonas</i> sp. exposed in UV-lamp phototron at 20°C -PRR	27
3.3 Abundance changes of <i>Paraphysomonas</i> sp. exposed to 6 kJ m ⁻²	28
3.4 Abundance changes of <i>Paraphysomonas</i> sp. exposed in UV-lamp phototron at 15°C +PRR	31
3.5 Abundance changes of <i>Paraphysomonas</i> sp. exposed in UV-lamp phototron at 15°C -PRR	32
3.6 Cyclobutane pyrimidine dimers in dosimeters exposed in the UV-lamp phototron	33
3.7 DOC-specific absorbance at 305nm in laboratory CDOM-additions	38
3.8 Abundance changes of <i>Paraphysomonas</i> sp. over 4 days in CDOM-shielded and -unshielded treatments	40
3.9 Change in <i>Paraphysomonas</i> sp. abundance 12 hours post UVR exposure in CDOM-shielded and -unshielded treatments	41
3.10 Abundance changes of a mixed bacterial community over 42 hours in CDOM-shielded and unshielded treatments.....	43
3.11 Percentage of DNA damage in dosimeters in CDOM shielded and –unshielded treatments	45
3.12 Temperature and oxygen profiles for Lake Giles taken late June 2004	46
3.13 Initial and final DOC concentrations and DOC-specific absorbance at 305 nm during June 2004 lake experiment.....	49
3.14 Abundance changes of bacteria during June 2004 lake experiment.....	51
3.15 Abundance changes of heterotrophic nanoflagellates (HNAN) during June 2004 lake experiment	53
3.16 Abundance changes of photosynthetic nanoflagellates (PNAN) during June 2004 lake experiment.....	55
3.17 DNA damage for dosimeters incubated under natural solar radiation in Lake Giles, PA with or without added CDOM and shielded or unshielded from UVR	56

LIST OF TABLES

	Page
1.1 Abbreviations of commonly used terms.....	2
3.1 Maximum specific growth rates of <i>Paraphysomonas</i> sp. exposed to different UV levels	29
3.2 DNA damage and population change for <i>Paraphysomonas</i> sp. after acute UV-B exposure	35
3.3 DOC concentration, dissolved absorbance and DOC specific absorbance in CDOM-shielded and -unshielded treatments	39
3.4 Maximum growth rates of <i>Paraphysomonas</i> sp. in a laboratory UV-shielding experiment ...	42
3.5 Repeated measured ANOVA table for temperature and oxygen concentration between sample bags incubated at the surface of Lake Giles June 2004	47
3.6 ANOVA table for final DOC concentration between treatments incubated at the surface of Lake Giles June 2004	50
3.7 Maximum specific growth rates of bacteria and flagellate assemblages incubated at the surface of Lake Giles June 2004	52

CHAPTER 1

INTRODUCTION

Heterotrophic protists in freshwater ecosystems

The importance of heterotrophic protists in aquatic ecosystem processes has been well established (Sherr & Sherr 1984, Sherr & Sherr 1989, Carrick et al. 1991, Sanders et al. 1992). Protists, and especially heterotrophic nanoflagellates, are major predators of bacteria, prey of zooplankton and contribute to recycling of essential nutrients. Consequently, factors that negatively affect biomass and alter the structure of protistan communities will influence both higher and lower trophic levels. Some of the most common heterotrophic nanoflagellates are in the genus *Paraphysomonas*, which are distributed worldwide in freshwater and marine systems; *P. vestita* is the most commonly isolated species due to the range of acceptable conditions under which it grows (Finlay & Clarke 1999). Therefore, responses of *Paraphysomonas* species to aspects of global climate change, including UV-B and dissolved organic carbon (DOC) flux, reflect important potential effects on aquatic microbial food webs.

Direct effects of UV-B radiation of biota

Ultraviolet-B radiation (UV-B; 280 – 320 nm) negatively affects many aquatic organisms primarily through the formation of DNA lesions. Cellular DNA strongly absorbs UV-B radiation, which induces the formation of two major types of lesions between adjacent pyrimidine bases. Cyclobutane pyrimidine dimers (CPDs) account for a majority of UV-B induced photoproducts, but pyrimidine [6-4] pyrimidinone dimer ([6-4] photoproduct) are also produced in measurable amounts (Mitchell & Nairn 1989). Both types of these bulky lesions inhibit DNA replication and transcription, which in turn can

CDOM	Chromophoric or Colored Dissolved Organic Material	Breakdown products of non-living organic material, both from within the lake and from terrestrial sources, with high molecular weights that can cause natural waters to be dark in color; responsible for a majority of the absorption of damaging ultraviolet B wavelengths in aquatic ecosystems
CPD	Cyclobutane Pyrimidine Dimers	Lesions formed between adjacent pyrimidine bases within DNA resulting from ultraviolet B radiation exposure
DOC	Dissolved Organic Carbon	Dissolved carbon compounds found in natural waters that are released by living or dead biota
HNAN	Heterotrophic Nanoflagellate	Protistan plankton, in a size range of 2 to 20 μm that obtain nutrition by digesting organic compounds; one of the most important consumers of bacteria
MSGR	Maximum Specific Growth Rate (μd^{-1})	Determined from changes in microorganism concentrations (cells per ml) over a time period of highest growth and normalized to a 24 h period. Quantified using the exponential growth equation: $\mu = (\ln N_2 - \ln N_1) / (t_2 - t_1)$, where N_2 and N_1 are protist concentrations at time t_2 and t_1 and $(t_2 - t_1)$ is the time interval between sampling (usually 1 d)
NER	Nucleotide Excision Repair	A light-independent, multi-step DNA repair mechanism that utilizes the enzymes endonuclease and DNA polymerase to eliminate cyclobutane pyrimidine dimers
PAR	Photosynthetically Active Radiation	Solar radiation from 400 to 700 nanometers that photosynthetic organisms are able to use in the process of photosynthesis
PER	Photoenzymatic Repair	DNA repair mechanism that directly reverses cyclobutane pyrimidine dimers using the enzyme photolyase and PRR
PNAN	Phototrophic Nanoflagellate	Protistan plankton, in a size range of 2 to 20 μm , that acquire energy from photosynthesis
PRR	Photoenzymatic Repair Radiation	Wavelengths of radiation in the visible (400-700 nm) and ultraviolet A (320-400 nm) parts of the solar spectrum used as an energy source by DNA repair enzymes, such as photolyase
UVR	Ultraviolet Radiation	Solar radiation from 100 to 400 nanometers broken down into: UV-A: Ultraviolet A (320 – 400 nanometers) UV-B: Ultraviolet B (280 – 320 nanometers) UV-C: Ultraviolet C (100 – 280 nanometers)

Table 1.1 Abbreviations of commonly used terms

lead to cell death if essential genes are affected. Higher UV-B exposure increases DNA damage and can affect species survival if organisms are unable to repair or avoid this additional damaging radiation.

The extent of the damage depends on the intensity and duration of exposure to solar radiation plus the attenuation of ultraviolet radiation (UVR) in the water column. Depending on water clarity, organisms living in the surface waters of aquatic systems can encounter injurious levels of UVR and consequently must acquire mechanisms to minimize its negative effects. Behavioral and biochemical responses to UVR exposure, which are not mutually exclusive, are known for a broad range of species, and include avoidance by daily vertical migration within the water column (Leibold & West 1993, Leech & Williamson 2001), the production of photoprotective compounds (Banaszak & Trench 2001, Tartarotti et al. 2001, Hoyer et al. 2002), and use of DNA repair mechanisms (Smith-Sonneborn 1979, Malloy et al. 1997). DNA repair mechanisms include light-independent nucleotide excision repair (NER) and light-dependent photoenzymatic repair (PER). NER is a multi-step process that utilizes the enzyme endonuclease, which cleaves the damaged DNA strand before and after the lesion, and results in the removal of an oligonucleotide containing the dimer. DNA polymerase then fills in the gap using the opposite DNA strand as a template. All organisms have the ability to repair DNA damage via NER, while only some groups have been shown to possess the enzymes needed to utilize PER.

PER has been shown to be an important mechanism of DNA repair in some amphibian species (Blaustein et al. 1994), several zooplankton species (Malloy et al. 1997, Grad et al. 2001), bacteria (Peccia & Hernandez 2002) and protozoa (Smith-

Sonneborn 1979, Sanders et al. 2005). PER directly cleaves the bond formed between pyrimidine bases in certain UVR-generated lesions via the enzymes CPD photolyase and [6-4] photolyase. These enzymes require absorption of photoreactivating radiation (PRR) in the visible (400-700 nm) and UV-A (320-400 nm) parts of the spectrum to provide the energy needed to drive the repair reaction.

DNA damage is independent of temperature, but enzymatic DNA repair has been shown to be temperature dependent. In the crustacean *Daphnia pulcaria*, PER efficiency varied over a gradient between 5°C and 25°C with increased CPD removal at the higher temperature (MacFadyen et al. 2004). Across a similar temperature range, the ciliate *Glaucoma* sp. had a PER temperature optima at 20°C (Sanders et al. 2005). Although DNA repair mechanisms can reduce mortality of exposed organisms, higher UV-B intensities cause more damage, which makes determining the resultant effects of increased UV-B exposure particularly important for the small single-celled organisms comprising the freshwater microbial loop. Solar radiation, including UV-B flux, increases rapidly in early spring when water temperature remains below the optima of enzymatic repair processes for some species. Thus, in temperate regions, there is potential for community composition to be shaped by species tolerance to seasonal changes that include shifts in the ratio of UV-B exposure to temperature.

Indirect effects of UV-B radiation on biota

UV-B intensity, temperature and the concentration of dissolved organic carbon (DOC) within aquatic ecosystems vary between seasons creating complex circumstances that may influence UV-B damage to organisms. The quantity of UV-B reaching the surface of a lake varies with atmospheric chemistry, including stratospheric

ozone, but the depth to which UVR penetrates in the water column is strongly affected by the amount of colored/chromophoric dissolved organic matter (CDOM). CDOM is responsible for a majority of the absorption of damaging UV-B wavelengths (Nielsen & Ekelund 1993) and effectively attenuates UV-B radiation to a greater extent than UV-A or photosynthetically active radiation (Morris et al. 1995).

CDOM is a product of the breakdown of non-living organic material, both from within the lake and from external sources, such as terrestrial plant litter. At DOC concentrations above 2 mg C L⁻¹, UV-B penetration is very low but as concentrations decrease to approximately 1-2 mg C L⁻¹, UV-B penetration increases exponentially (Schindler et al. 1996, Williamson 1996). This suggests that even a small decrease in CDOM concentration could significantly increase the UV-B exposure to aquatic organisms in low DOC systems.

In addition to its direct effect as a UV-B shield, CDOM may have indirect effects on protozoan communities, and these may alter protozoan community structure in opposing ways. Absorption of UV-B and photosynthetically active radiation (PAR) results in photodegradation of CDOM into low molecular weight molecules, that are more biologically available and are taken up by bacteria (Lindell et al. 1995). By enhancing population growth of bacterial food resources, the photochemical breakdown of CDOM indirectly stimulates protozoan population growth. However, because photochemical breakdown reduces the UV-B absorptive properties of CDOM it also increases UV-B exposure, which may result directly in protozoan population decline.

Implications for freshwater systems

Some aquatic environments, such as alpine lakes, have small watersheds that receive relatively little runoff and thus are already areas of low CDOM and potentially

high UVR impact (Strecker et al. 2004). Effects of global climate change are likely to influence CDOM concentration and composition in freshwater systems. In areas where watershed runoff is reduced, typical of warmer, drier climates, acidification and increased CDOM photodegradation is predicted to reduce the photoprotective role of CDOM (Schindler et al. 1996, Yan et al. 1996, Zepp et al. 2007). There is a growing potential for reduced ozone levels (Kerr & McElroy 1993, Madronich et al. 1998), which will enhance biologically-damaging UV-B penetrating into aquatic ecosystems. Although the ozone decrease has been largest in the Southern Hemisphere, the 305 nm clear-sky irradiance increases since 1979 are similar at the latitudes of Buenos Aires, Argentina (34.5° N) and Washington DC (38.5° N) – 9% and 7%, respectively (Herman 2010). As my data shows, dealing with UV-B exposure in these current environmental conditions, not just potentially higher UV-B levels in the future, is a challenge for aquatic organisms. Investigation of the ecological consequence that UVR has on freshwater organisms, including microorganisms, has been spurred by the potential of increasing UVR impact.

Aquatic organisms including protists are differentially susceptible to UV-B radiation. The changes in the *in situ* levels of UV-B, whether due to climate change or other factors, are likely to alter the ecology and community structure of aquatic systems (Sommaruga 2003). Long-term effects of increasing UV-B radiation on aquatic ecosystems are not known, but potential changes in species diversity due to differential sensitivity to UVR could modify patterns of predation and competition, ultimately altering carbon and nutrient cycling. Understanding the UVR tolerance of individual species can aid in predicting the ecological impacts of changes in UVR.

This thesis will describe research that examined the direct and indirect effects of ultraviolet radiation and dissolved organic matter on freshwater flagellates. The response of the freshwater heterotrophic nanoflagellate *Paraphysomonas* sp. to UV-B was dependent on UV-intensity and temperature. The strain of *Paraphysomonas* sp. studied relied heavily on photoenzymatic repair to counter the lethal effects of UV-B, but was highly adapted to UVR overall. Additionally, naturally-derived CDOM was an effective UV-B shield for both *Paraphysomonas* sp. in laboratory experiments and for lake populations of heterotrophic flagellates. My hypothesis that natural CDOM exposed to UVR would act as a strong stimulant to the microbial food web was not supported in these experiments.

CHAPTER 2

MATERIALS AND METHODS

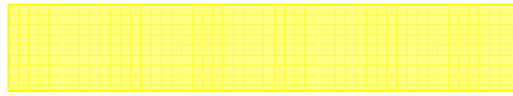
Laboratory Experiments

UV lamp-phototron - experimental design

To assess the effects of UV-B radiation on survival and growth of *Paraphysomonas* sp. (strain GflagA, see section on culture origin), cultures were exposed to UV-B radiation over 12 hours with or without simultaneous photorepair radiation (PRR) using a UV-lamp phototron (Williamson et al. 2001). This apparatus consists of a horizontal rotating wheel (5 rpm) set into an opaque box that houses photorepair lamps consisting of two 40W cool white fluorescent bulbs (PAR) and two 40W Q-Panel 340 fluorescent bulbs (UV-A) (see Williamson et al. 2001 for energy spectra of the PRR). Damaging UV-B radiation was supplied from above the box by a Spectroline XX15B UV-B lamp (Spectronics) covered with cellulose acetate to remove short UV-C wavelengths (Figure 2.1).

Because the energy output of individual UV-B lamps is known to vary, we followed an approximately annual calibration schedule using a custom-made spectral radiometer. The spectral radiometer consisted of a scanning monochromator (model SP300i, Acton Research Corporation, Acton, Massachusetts, USA) with a UV-sensitive PMT (1P28 photomultiplier tube) connected to a 3-m fiber-optic cable and a cosine-corrected flat diffuser collector. The response was calibrated for wavelength using a mercury lamp and for irradiance using a 1000-W NIST-traceable standard lamp (National Institute of Standards and Technology [U.S. Department of Commerce], Gaithersburg, Maryland, USA) (Williamson et al. 2001).

DAMAGING
RADIATION



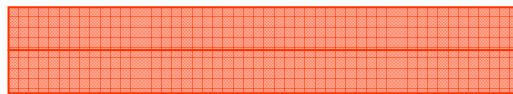
UV-B
SOURCE



SAMPLES



REPAIR
RADIATION



UV-A and PAR
LIGHT SOURCES



Figure 2.1 UV-lamp phototron: Conceptual diagram and photograph.

Holes arranged in two rows around the outer edge of the wheel accommodated custom-made quartz treatment dishes (55mm diameter, 30ml volume) with quartz lids. Depending on the treatment, one or both of the light sources could be blocked by the placement of an opaque disk either below (interrupting PRR) or above the dish (interrupting UV-B). The opaque disks were not used for treatments with the quasi-natural combination of both UV-B and PRR. A black PVC collar surrounded each dish to reduce stray radiation. Stainless steel mesh screens placed within the collars on the lid of individual dishes were used to modify the UV-B exposure for cumulative UV-B doses of 23, 45 or 58 kJ m⁻² over 12 hours. The 12 hour maximum UVR exposure, determined from the lamps spectral output, was approximately equal to ambient UVR exposure at 40°N to 44°N latitude on a sunny day near the summer solstice (Williamson et al. 2001, Marangoni et al. 2004). An additional exposure level of 6 kJ m⁻² UV-B was tested separately at 20°C, and required an additional mesh screen surrounding the UV-B lamp. Each treatment and control was run in triplicate in all experiments.

Experiments were performed at 15°C and 20°C, temperatures within the range that *Paraphysomonas* sp. would naturally experience in Lake Giles. Prior to UV-B exposure, cell density of *Paraphysomonas* sp. was reduced to a target abundance of 5,000 flagellates mL⁻¹ by dilution with <0.22mm filtered Lake Giles water. Heat-killed bacteria (HKB), a food source unaffected by UV-B (preparation description follows), were added to a final concentration of 1 x 10⁷ cells mL⁻¹, and the flagellates with HKB were then transferred to the individual quartz dishes. This assured that the initial food and flagellate concentrations were identical in all treatments. The dishes were exposed to the UV-B lamps with or without PRR lamps for 12 hours and sampled prior to and

immediately following exposure, 12 hours following exposure, and every 24 hours thereafter for 4 days.

UV-B induced DNA damage and repair in Paraphysomonas sp.

In order to investigate UV-B induced DNA damage and the DNA repair ability of *Paraphysomonas sp.*, cells were briefly exposed to UV-B lamps and then incubated in darkness or light. For these acute UVR exposures, the UV-lamp phototron apparatus was modified by mounting two additional UV-B lamps above the rotating wheel without using PRR lamps. The *Paraphysomonas sp.* culture was diluted with <0.22mm filtered Lake Giles water to approximately 9,000 cells mL⁻¹, and cells were exposed for 5 minutes for a total UV-B exposure of 13 kJm⁻² without PRR. Immediately after exposure, samples were taken for DNA damage analyses and microscopic enumeration, and replicate dishes were transferred to PRR or placed in the dark for 48 hours. Control dishes were never exposed to UV-B and were kept in the dark for the duration of the experiment. This experiment was conducted within a temperature controlled environmental chamber at 20°C. Additional samples to determine DNA damage and cell abundance were taken after 1.5, 6, 24 and 48 hours. Cells for DNA damage analysis (see general methods) were collected on sterile 3 mm polycarbonate filters under low-pressure filtration (approximately 100 mm Hg in vacuum). Filters were placed in sterile 1.5 ml microcentrifuge tubes and stored at -20°C until DNA was extracted (see general methods). Cell abundance samples were fixed and enumerated as described in general methods.

Immunofluorescence assay for CPDs within Paraphysomonas sp. cells

Attempts were made to examine the direct effects of UV-B radiation on aquatic protists, specifically the formation and repair of CPDs, using anti-thymine-dimer

antibodies and fluorescently labeled secondary antibody to quantify CPDs within the nucleus of whole cells. The protocol used to detect dimers in individual bacterial cells was modified from Peccia and Hernandez (2002) with the aim of demonstrating PER within *Paraphysomonas* sp. following UV-B exposure. The use of this model protozoan organism to detect CPDs within cells was an attempt to link UV-B induced DNA damage to cell death and the absence of population recovery.

The protocol described in Peccia and Hernandez (2002) was modified as described below. *Paraphysomonas* sp. cultures were exposed to the highest level of UV-B radiation used in the standard lamp phototron exposures (55 KJ m^{-2} over 12 hours) with and without PRR. Controls for UV-B exposure included cultures kept in the dark, and cultures exposed to PRR alone. Following exposure cells were fixed with formaldehyde (final concentration 3.7%) and drawn down onto $0.4 \mu\text{m}$ polycarbonate filters. The filters were soaked with phosphate buffered saline with Tween-20 (PBST) buffer containing 1% Bovine serum albumin (BSA) for 30 minutes, followed by incubation with mouse monoclonal antibody directed against CPDs (Kamiya Biomedical Co., Seattle, WA) for 18 to 21 hours. Filters were washed 2 times with PBST and incubated with fluorescently labeled rabbit anti-mouse secondary antibody (Molecular Probes, Eugene, OR) for 3 hours. Cells were washed 2 times with PBS and then stained with DAPI (4', 6-diamidino-2 phenylindole) for 10 minutes. The filters were then mounted on slides and examined by epifluorescent microscopy.

For this method, changes in the quantity of CPDs before and immediately following exposure were expected to be measurable using epifluorescent microscopy. Decreases were anticipated in CPDs over time in those treatments receiving PRR, and therefore a decrease in fluorescence. Imaging software was to be used to score

individual cells for mean fluorescence units correlating to the presence of CPDs allowing the ratio of damaged to non-damaged cells within each treatment to be calculated.

The inability to visualize CPDs within whole protist cells required troubleshooting in which many adjustments in protocol were made including increasing the concentration of primary and secondary antibodies (1:5,000 to 1:40) and the addition of a tertiary antibody to amplify fluorescence. Since pH is known to affect the fluorescence of some fluorophores, various immersion oils and mounting liquids of differing pH levels were tested. Two detergents (Tween-20 and Triton-X), in a range of concentrations (0.1% to 1%), were used in attempts to permeabilize the cell membrane of protist cells to allow for entry of the antibodies. Adjustments made to the protocol did not lead to the expected results of visualizing DNA damage within the flagellate cells tested.

CDOM: UV protection versus food web stimulation - experimental design

Chromophoric dissolved organic matter (CDOM) acts as a filter that absorbs UV-B radiation to a greater extent than other wavelengths of the solar spectrum. CDOM also has the potential to stimulate bacterial growth and thus indirectly bacterial predators including heterotrophic flagellates. To separate the relative importance of CDOM as a UV-B filter (direct effect) and as a food web stimulant (indirect effect), laboratory experiments using a modification of the UV-lamp phototron design were performed (Figure 2.2).

The experiments described here were conducted separately for bacteria and protists. Equal initial abundances of *Paraphysomonas* sp. or a mixed bacterial community (originating from co-culture with *Paraphysomonas* sp.) were inoculated into 55 mm, 27.5 ml quartz dishes (described in UV-lamp phototron methods) containing

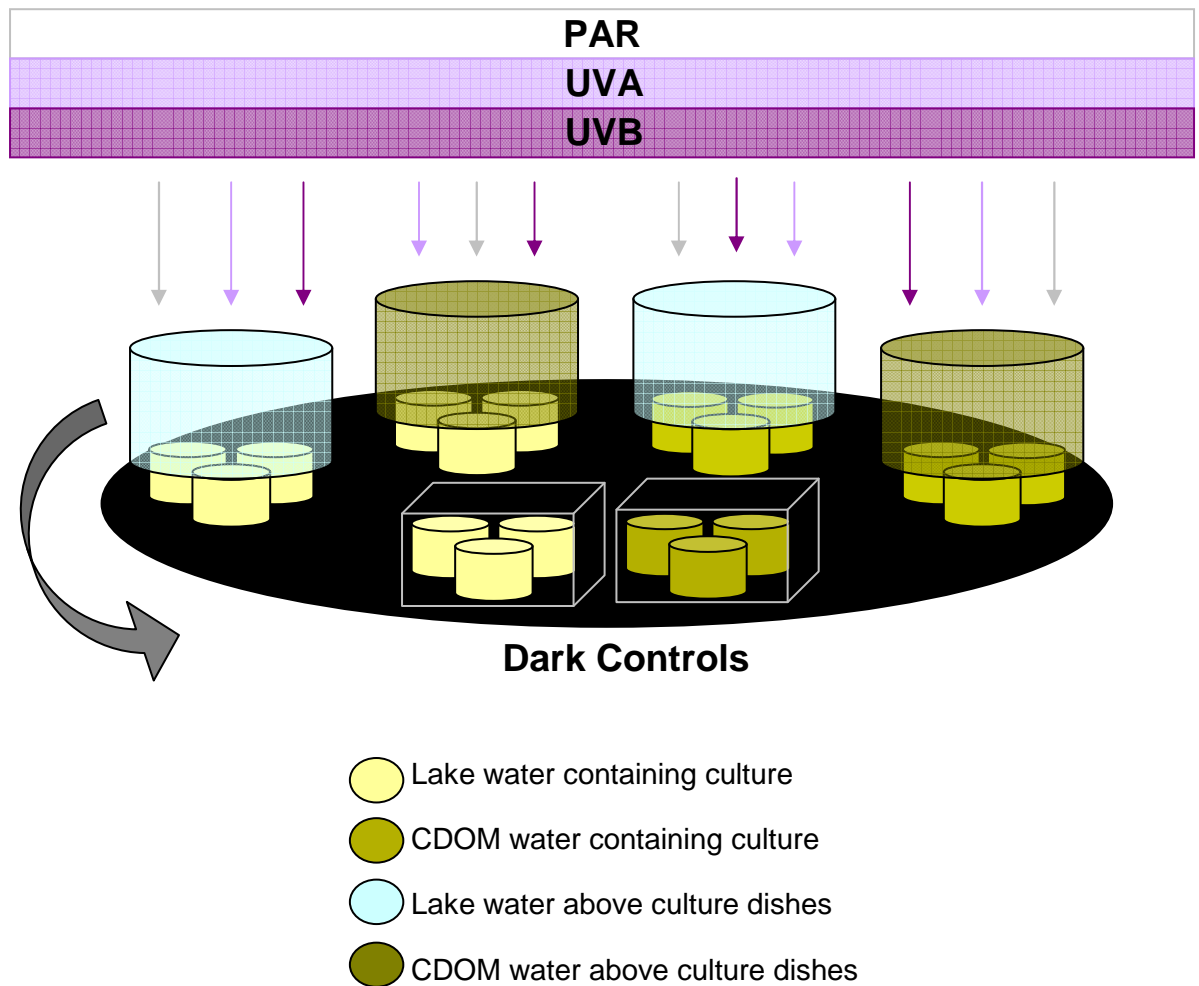


Figure 2.2 Experimental setup separating roles of chromophoric dissolved organic matter (CDOM) as a UV-protective filter and as a stimulatory carbon source for microorganisms.

either low CDOM lake water (approximately 1 mg C L⁻¹ DOC) or lake water with added CDOM (final concentration 2.4 mg C L⁻¹ DOC). Live bacteria were added to protist dishes as a source of food.

Triplicate dishes containing *Paraphysomonas* sp. or bacterial cells in low or high CDOM lake water were placed on a rotating wheel beneath quartz “UVB-filtering” dishes (1,500 ml, 5.5 cm deep) containing either the low or the high CDOM lake water. UV-B and photorepair radiation (PRR) lamps were suspended above the filtering dishes (Figure 2.2). This design led to four replicated treatments: 1) cells in unamended lake water shielded by a dish of high CDOM water; 2) cells in high CDOM water shielded by a dish of high CDOM water; 3) cells in unamended lake water under a dish of unamended lake water; and 4) cells in high CDOM water under a dish of unamended lake water. Replicated controls of flagellate and bacterial cells in unamended lake water and in high CDOM water were maintained in the dark.

The dishes were exposed to the UV-B and PRR lamps for 12 hours (total UV-B of 58 kJ m²). Flagellate treatments were sampled prior to and immediately following exposure, 6 hours following exposure, and every 24 hours thereafter for 5 days. Bacterial community treatments were sampled prior to and immediately following exposure, as well as 6, 24 and 30 hours following exposure. CDOM properties (DOC concentration and DOC-specific absorbance 305 -320 nm) were determined to assess the extent of CDOM photodegradation. In addition, DNA dosimeters (see general methods) were exposed under the same treatment conditions as *Paraphysomonas* sp. and bacterial cells to determine the maximum potential DNA damage for each treatment. Experimental temperature, maintained in a controlled-temperature walk-in incubator, was 20°C.

Culture origin, maintenance and acclimation

Cultures of the heterotrophic nanoflagellate *Paraphysomonas* sp. (strain GflagA, Figure 2.3) used in these experiments were established using single-cell isolations from water samples collected in June 2001 from a depth of 0.5 m within the pelagic zone of Lake Giles (41°23'N, 75°06'W). Located in the Pocono Plateau of northeastern Pennsylvania, Lake Giles is an oligotrophic lake with a low DOC concentration [~1.7 mg C liter⁻¹ as measured in June 2004] (see Lake Experiment for detailed description of Lake Giles). The flagellates were identified to the genus *Paraphysomonas* by general morphology and comparison of a partial 18S rRNA gene sequence to the GenBank nucleotide database with a sequence similarity of >99% to *P. vestita* when a variable A/T-rich insertion was removed from analysis (Caron et al. 1999). The sequence obtained for the GflagA strain was submitted to GenBank (Accession No. FJ528656).

Cultures were acclimated to temperature in light- and temperature-regulated growth chambers with a 14:10 hour light:dark cycle (cool white fluorescent bulbs) for several weeks at 15 or 20°C depending on the planned experimental temperature. Culture media used for general maintenance was 0.1% Cerophyll® containing an assemblage of co-isolated unidentified bacteria as food. This mixed bacterial community was also maintained separately in culture, grown in yeast extract media. Prior to experiments, *Paraphysomonas* sp. and bacterial cells were transferred to filtered, autoclaved Lake Giles water and grown at the experimental temperatures for at least one week. Experiments were started using acclimated populations in exponential growth phase. HKB were used as the sole food source for *Paraphysomonas* sp. and were added to starting the experiment.

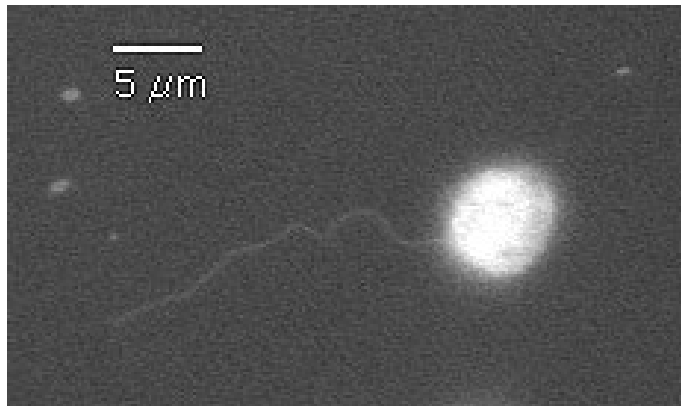


Figure 2.3 Photomicrograph of *Paraphysomonas* sp. (strain GflagA) taken using darkfield microscopy.

Preparation and testing of heat-killed bacteria

A batch culture of the bacterium *Pasteurella* sp. was grown to stationary phase in 0.1% yeast extract. Bacteria were then heat-killed by submerging the culture-containing flask in a 70°C water bath for 1 hour (Sanders et al. 1990). Using aseptic technique, the cells were washed two times via centrifugation in sterile 250mL bottles at 8,000 rpm (8,230 x g) for 20 min and resuspended in sterile distilled water. Resuspended cells were filtered through sterile Nuclepore filters (47 mm, 5µm pore-size) to remove clumps. Sterility of the heat-killed bacteria (HKB) solution was verified by inoculation into 0.1% yeast extract and observation for signs of bacterial growth (none detected).

The effect of experimental levels of UV-B on the food quality of HKB was compared to that of HKB without UV-B exposure using the UV-lamp phototron (see previous description). HKB were exposed in replicate quartz dishes to UV-B (with PRR) at 58 kJm⁻² at 16°C following our standardized method (above). Controls were kept in the dark on the lamp-phototron wheel. After exposure, *Paraphysomonas* sp. was added to each UV-exposed dish and dark controls. Subsamples were preserved with Lugol's immediately after the addition of flagellates and every 24 hours until population growth ceased. Initial HKB concentration in both treatments was 5.1 x 10⁶ mL⁻¹. Flagellates were enumerated as described in general methods.

Lake Experiment

CDOM: UV protection versus food web stimulation - experimental design

The purpose of this experiment was to examine the effect of chromophoric dissolved organic matter (CDOM) on the microbial food web in the presence and absence of environmental UV radiation. Lake Giles, the experimental site, is located in

Pike County within the Pocono Mountains of Pennsylvania (latitude: 41 22' 34" N; longitude: 75 05' 33" W) at an elevation of 428m. The surface area of the lake is 0.5km² with an average depth of 10.1m and maximum depth of 23.0m. Seasonal surface water temperatures range from ~2°C in January to ~24°C in July. Over the course of the experiment described here executed in June of 2004 the temperatures ranged between 20.7°C and 22.4°C at the surface and between 12.3°C and 13.3°C at 9m of depth.

An integrated water sample (surface to 1.5 m) was collected from Lake Giles and passed through a 48 µm mesh to remove large zooplankton and then distributed into two holding tanks. Prefiltered CDOM was added to one of the holding tanks (4.5 mg C L⁻¹ DOC, final concentration) and an equal volume of filtered lakewater was added to the other holding tank. Water with microplankton (and with or without added CDOM) was then distributed to replicate 4-liter UV-transparent Bitran Series "S" watertight plastic bags, which were suspended at a depth of 0.5 m in containment racks with plastic covers that either transmitted (OP-4) or excluded (OP-2) UV radiation (Figure 2.4). This established 4 treatments: +CDOM/-UV; +CDOM/+UV; -CDOM/-UV; and -CDOM/+UV (environmental exposure). For each treatment, three replicate bags were incubated in Lake Giles from June 21 – 30, 2004. Bags were examined in early morning each day and subsamples were fixed for protist and bacterial abundances (see description in general methods). Additionally, for each bag, temperature and oxygen levels were determined using a YSI model 58 digital oxygen/temperature meter.

Potential for DNA damage from exposure to solar radiation was examined by deployment of two replicate DNA dosimeters (see description in general methods) in



Figure 2.4 Containment rack for field experiments examining the interaction of chromophoric dissolved organic matter (CDOM) with UV exposure. Bitran bags, visible within the PVC pipe, were incubated in situ under natural solar radiation covered by either UV-transparent or UV blocking acrylic covers.

each Bitran bag. Temperature and oxygen depth profiles of Lake Giles were determined using the YSI oxygen/temperature meter with a 25 m cable.

General Methods

Microscopic Enumeration of flagellates and bacteria

Samples from laboratory experiments using *Paraphysomonas* sp. were fixed and stained with acid Lugol's solution, and enumerated at 400x magnification in Phycotech settling chambers using a Zeiss Axiovert 10 microscope. Bacterial cells in laboratory experiments were preserved with glutaraldehyde (1% final concentration), collected on black Nuclepore cellulose filters (0.2 μm pore size) and stained with DAPI, 4',6-diamidino-2-phenylindole (Porter & Feig 1980), and counted at 1000X magnification with epifluorescence microscopy (Sanders & Porter 1986).

In field experiments, flagellates were preserved with glutaraldehyde (1% final concentration), stained for 12-24 h with Primulin (final concentration 250 $\mu\text{g ml}^{-1}$) collected on black Nuclepore cellulose filters (0.8 μm pore size), and counted at 1000X magnification with epifluorescence microscopy (Sanders & Porter 1986). Prepared slides were kept frozen until enumerated to preserve autofluorescence of chlorophyll and allow differentiation between heterotrophic and phototrophic flagellates. Bacteria were preserved with glutaraldehyde (1% final concentration), collected on black Nuclepore cellulose filters (0.2 μm pore size) and stained with DAPI, 4',6-diamidino-2-phenylindole (Porter & Feig 1980).

*Determination of *Paraphysomonas* sp. growth rates*

Maximum growth rates (μ , d^{-1}) of *Paraphysomonas* sp. were determined from changes in abundance over the 24 h period during which flagellate numbers increased

the most in a given treatment. Rates were quantified using the exponential growth equation:

$$\mu = (\ln N_2 - \ln N_1) / (t_2 - t_1)$$

where N_2 and N_1 are flagellate concentrations (number of cells per mL) at time t_2 and t_1 and $(t_2 - t_1)$ is the time interval between sampling (1 d). This overall design allowed examination of the potential for photo-dependent recovery with a reasonable experiment endpoint based on the well-documented growth response of protozoa in batch cultures where food becomes limiting as populations grow exponentially.

DNA damage analysis – DNA dosimeters and Paraphysomonas sp.

DNA dosimeters, composed of a sterile solution of salmon sperm DNA within quartz tubing, were exposed to UV-B with and without PRR in the UV-lamp phototron for 12 h in the same apparatus used to test *Paraphysomonas* sp. responses to UV (in quartz dishes immersed in water from Lake Giles). Because salmon sperm lacks the enzymes to repair UV-induced DNA damage, the dosimeters give a metric of the maximum potential DNA damage from the UV-B exposures used in the lamp phototron experiments.

DNA was extracted from *Paraphysomonas* sp. cells that had been concentrated on polycarbonate filters. Just enough solution to cover the filter (35 ml of QuickExtract™ DNA Extraction Solution 1.0, Epicenter Biotechnologies) was added to microcentrifuge tubes, which were placed in a 65°C heat block for 30 minutes followed by 98°C for 15 minutes with mixing after 7 minutes. Filters were removed and the DNA samples were stored at -20°C until analyzed for DNA damage. For both filters and dosimeters, DNA damage was measured as cyclobutane pyrimidine dimers (CPDs) per megabase of DNA

using radioimmunoassay (Mitchell et al. 1985). Processing of DNA damage samples was completed by Dr. David Mitchell's laboratory at The University of Texas.

Preparation of natural chromophoric dissolved organic matter (CDOM)

To examine the role of chromophoric dissolved organic matter (CDOM) in responses of the microbial food web to UV radiation, CDOM was concentrated from Beaver Pond, a dystrophic pond in an adjoining watershed to Lake Giles. Beaver Pond water was filtered through a 5 μm prefilter (Ace Hardware) and concentrated using a reverse osmosis unit built in the Morris 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 to 100. 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 microorganisms. Water from Beaver Pond was concentrated 17-fold to make the CDOM concentrate ($[\text{DOC}] \sim 100 \text{ mg L}^{-1}$, $a_{320} [\text{DOC}]^{-1} = 4.42 \text{ m}^{-1}$). Preparation and analysis (below) of CDOM was completed by Jason Porter in the laboratory of Dr. Don Morris at Lehigh University.

Concentrated CDOM was stored in the dark until it was added experimentally to water from Lake Giles, with target concentrations of 2.5 mg C L^{-1} for laboratory experiments and 4 mg C L^{-1} for the lake experiment, to mimic concentrations of less UV transparent lakes. Final concentration of CDOM in experiments was determined using a total carbon analyzer (TOC-5000, Shimadzu) after filtration through a Whatman GF/F glass fiber filter and acidification with 2N HCl (Sharp et al. 1993). DOC-specific absorbances were determined by a scanning spectrophotometer (Shimadzu, 200-800

nm) as described in (Morris & Hargreaves 1997). Water samples were collected from the Bitran Series “S” watertight plastic bags on Days 0 (start) and 9 (end).

Statistical analyses

The significance of UV-B intensity levels and temperature effects on growth rates in treatments receiving PRR in laboratory experiments, as well as the significance of CDOM and UV radiation on maximum specific growth rates in field experiments were tested using 2-way ANOVA. Prior to analyses, growth rate data were (square root +1)-transformed. The significance of CDOM and UV radiation on DNA dosimeter damage, final total DOC concentration and changes in DOC-specific absorbance were tested using 2-way ANOVA. The effect of UVR exposure on food quality of HKB (as determined by flagellate growth rate) was tested with 1-way ANOVA. Repeating measures ANOVA was used to test the significance of CDOM and UV radiation on temperature and oxygen levels within the sample bags over the nine days of the field experiment.

CHAPTER 3

RESULTS

Direct effects of UV-B radiation on the heterotrophic flagellate *Paraphysomonas* sp.

Survival and growth responses to UV-B

At 20°C, all populations of *Paraphysomonas* sp. exposed simultaneously to UV-B and PRR survived and grew (Figure 3.1). All treatments at 20°C without PRR experienced complete mortality within 24 hours when exposed to greater than 6 kJ m⁻² UV-B (Figure 3.2). Maximum abundances of *Paraphysomonas* sp. in the dark control and PRR-only control groups were reached by 24 hours (Figure 3.1). Populations of flagellates exposed to 23 kJ m⁻² UV-B in the presence of PRR had an initial lag in growth relative to controls, but reached a comparable maximum abundance by 36 hours following exposure. At greater UV-B exposure levels increasingly longer lag periods were observed, with flagellates exposed to the highest UV-B level having the longest interval of reduced growth post-exposure. In the separate experiment in which cells were exposed to 6 kJ m⁻² UV-B, maximum abundances comparable to dark control were reached by 12 hours post-exposure in the presence of PRR; cells exposed to 6 kJ m⁻² UV-B in the absence of PRR survived and maintained a constant population with minimal growth (Figure 3.3).

The highest maximum specific growth rates for *Paraphysomonas* sp. at 20°C were observed for cells grown in the dark or exposed to only PRR lamps ($m_{\max} = 2.6 \text{ d}^{-1}$ and 2.5 d^{-1} , respectively) (Table 3.1). The effect of UV-B intensity on maximum specific growth rates was significant ($p < 0.001$) and growth rates were reduced in a

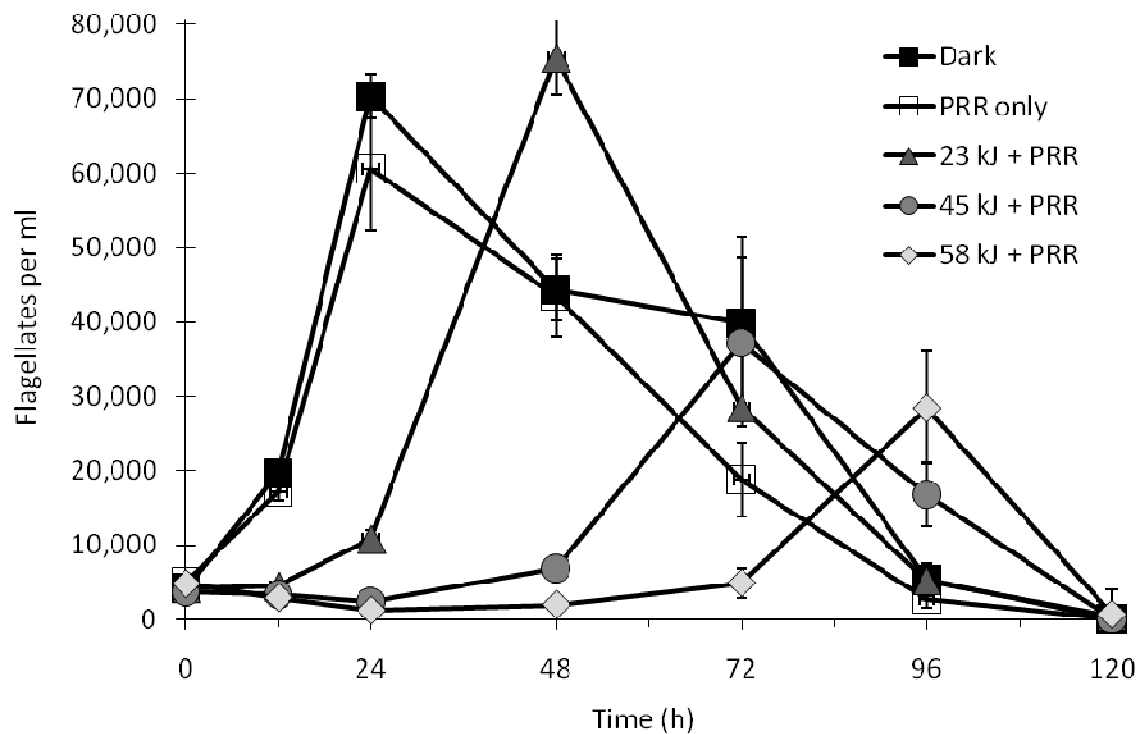


Figure 3.1 Abundance changes (mean \pm SE, $n = 3$) of *Paraphysomonas* sp. after a 12 hour exposure to three environmentally relevant levels of UV-B (23, 45, and 58 kJ m⁻²) with (+) simultaneous photoreactivating radiation (PRR) at 20°C. Dark controls were not exposed to any UVR; PRR controls were exposed only to the photoreactivating radiation lamps. Abundance declines seen post maximum are the result of cell death (bursting).

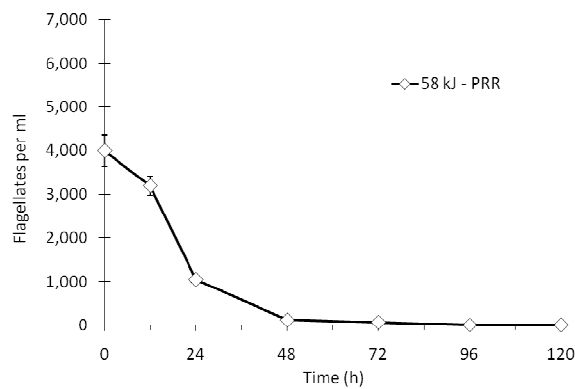
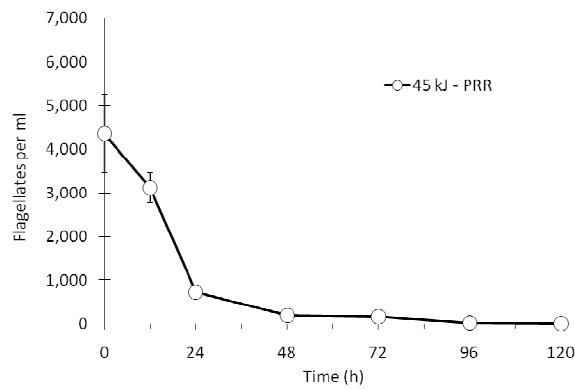
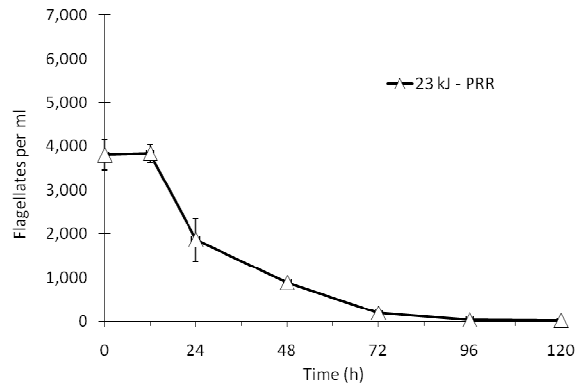


Figure 3.2 Abundance changes (mean \pm SE, n = 3) of *Paraphysomonas* sp. after a 12 hour exposure to 23 kJ m⁻², 45 kJ m⁻² and 58 kJ m⁻² with without (-) simultaneous photoreactivating radiation (PRR) at 20°C.

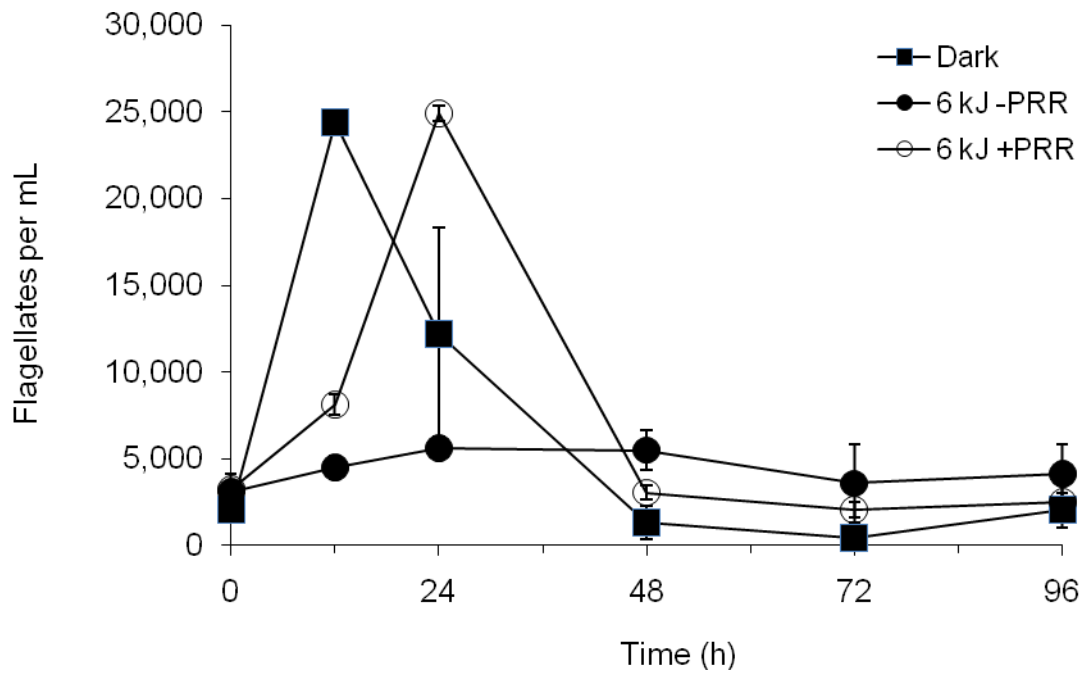


Figure 3.3 Abundance changes (mean \pm SE, n = 3) of *Paraphysomonas* sp. after a 12 hour low intensity (6 kJ m^{-2}) UV-B lamp exposure with (+) or without (-) simultaneous photoreactivating radiation (PRR) at 20°C .

<u>UV Treatment</u>	<u>Growth Rate (d⁻¹)</u>	
	20 °C	15 °C
Dark	2.6 ± 0.41	2.0 ± 0.14
PRR only	2.5 ± 0.25	1.8 ± 0.11
6 – PRR	0.73 ± 0.3	nd
6 + PRR	2.2 ± 0.10	nd
23 – PRR	M	M
23 + PRR	2.0 ± 0.09	1.4 ± 0.10
45 – PRR	M	M
45 + PRR	1.7 ± 0.35	M
58 – PRR	M	M
58 + PRR	1.1 ± 0.14	M

Table 3.1 Maximum specific growth rates of *Paraphysomonas* sp. (mean ± SE, n = 3) at 20 and 15°C exposed to various levels of UV-B (kJ m⁻²) with (+) or without (-) simultaneous photoreactivating radiation (PRR). M = complete mortality; nd = no data.

dose-dependent manner in cells exposed to UV-B with simultaneous PRR. Exposure to the highest UV-B level with PRR reduced maximum specific growth rate to less than half that of controls ($m_{\max} = 1.1 \text{ d}^{-1}$). In the absence of PRR, a growth rate of 0.73 d^{-1} was observed in the lowest UV-B exposure level (6 kJ m^{-2}), but all other exposure levels without PRR led to complete mortality (Table 3.1).

The ability of *Paraphysomonas* sp. to recover from exposure to UV-B radiation declined in flagellates adapted to 15°C relative to the same exposures at 20°C . Populations exposed to 23 kJ m^{-2} UV-B and PRR recovered most quickly and began to grow after approximately 48 hours. Unlike the populations in corresponding UV-B intensities at 20°C , flagellates in all other UV-B plus PRR treatments failed to rebound after exposure (Figure 3.4). There was a time lag before population growth started in the 15°C PRR-only control treatment for the duration of the PRR exposure that did not occur in the dark control. These two control groups had the greatest maximum specific growth rates at 15°C (Dark $m_{\max} = 1.8 \text{ d}^{-1}$ and PRR $m_{\max} = 2.0 \text{ d}^{-1}$) (Table 3.1), but these rates were only 75 % of those at 20°C . Flagellates that were not exposed to simultaneous PRR at 15°C experienced 100% mortality by 24 hours (Figure 3.5). The effects of UV-B intensity and temperature were significant ($p < 0.001$), and ANOVA indicated a significant interaction effect.

Dosimeter DNA damage

Maximum potential DNA damage (as CPDs) from exposure levels used in the lamp phototron experiments were quantified using DNA dosimeters of salmon sperm DNA in quartz tubes exposed to the UV-B lamp with and without PRR lamps. Data demonstrate dose-dependent DNA damage confirming that greater UV-B exposure results in a greater number of CPDs in the absence of DNA repair (Figure 3.6). The

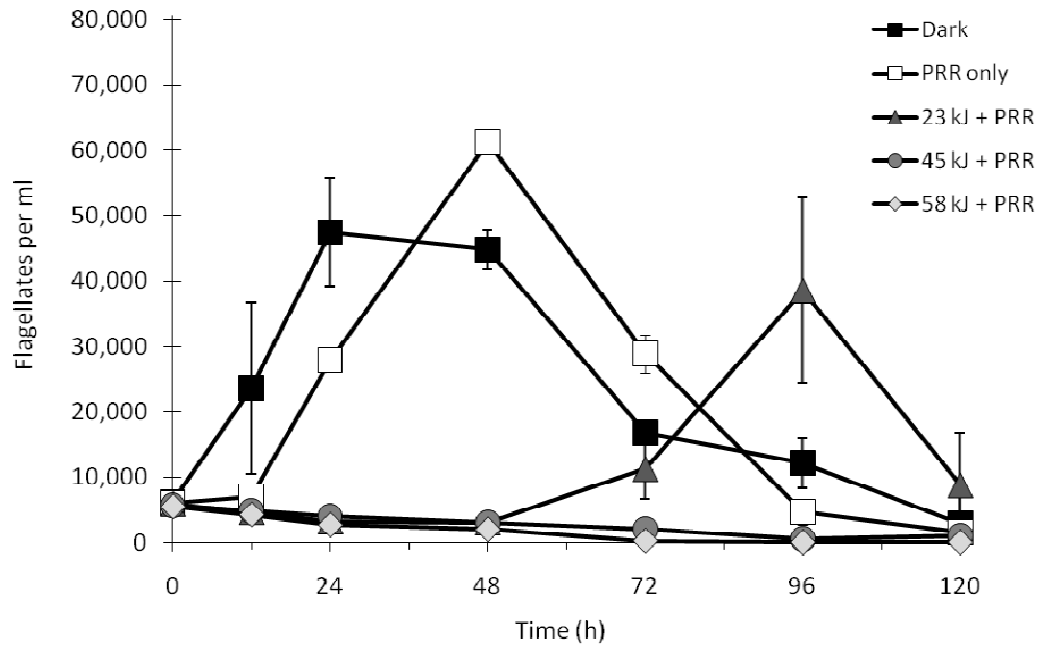


Figure 3.4 Abundance changes (mean \pm SE, n = 3) of *Paraphysomonas* sp. after a 12 hour exposure to UV-B (kJ m^{-2}) lamps with (+) simultaneous photoreactivating radiation (PRR) at 15°C.

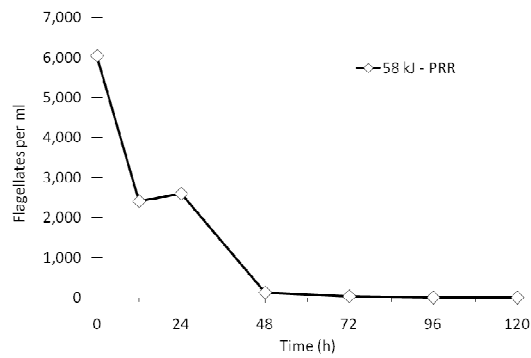
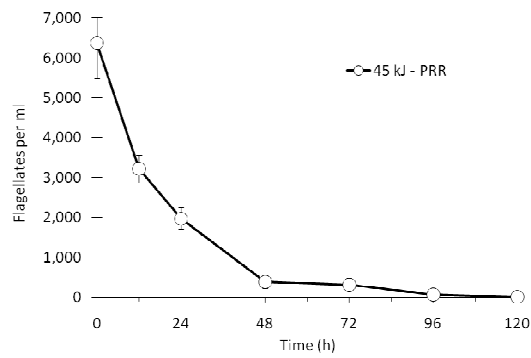
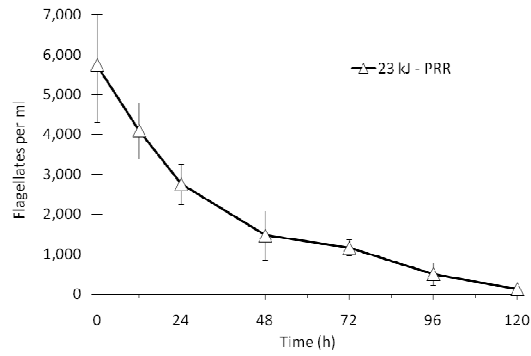


Figure 3.5 Abundance changes (mean \pm SE, n = 3) of *Paraphysomonas* sp. after a 12 hour exposure to UV-B (kJ m^{-2}) lamps without (-) simultaneous photoreactivating radiation (PRR) at 15°C.

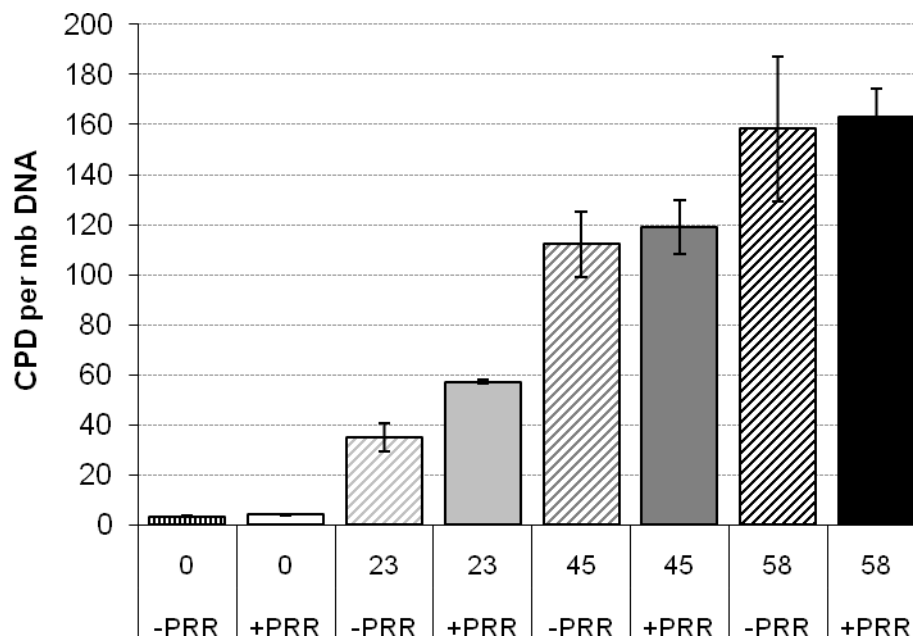


Figure 3.6 Cyclobutane pyrimidine dimers per megabase DNA in dosimeters exposed in the lamp phototron to different levels of UV-B (kJ m^{-2}) with (+) or without (-) simultaneous photoreactivating radiation (PRR) for 12 hours. Dosimeters consisted of isolated salmon sperm DNA with no repair enzymes present and represent the maximum potential accumulation of dimers when no photorepair or photoprotection is present.

highest UV-B level tested (58 kJ m^{-2}) with and without PRR resulted in 162.9 and 158.3 CPD mb^{-1} DNA, respectively. In general, exposure to the PRR lamps did not increase CPD formation. DNA dosimeters exposed to PRR lamps alone had 4.1 CPD mb^{-1} , while those which were kept in the dark had 3.4 CPD mb^{-1} . Dosimeters exposed to 1 UV-B lamp for 12 hours (58 kJ m^{-2}) accumulated 47 times more CPD mb^{-1} DNA compared to dosimeters kept in the dark (Figure 3.6).

DNA damage and repair in Paraphysomonas sp.

During the acute UV-B exposure (13 kJ m^{-2} over a 5 minute time course), DNA damage in *Paraphysomonas* sp. cells accumulated to approximately 55 CPD mb^{-1} . Those cells that were transferred to dark conditions immediately after UV-B exposure showed no significant change in CPD mb^{-1} DNA over the following 48 hours (Table 3.2). However, a reduction in CPD mb^{-1} DNA was seen in cells that were exposed to PRR following UV-B exposure, with only 38% of initial damage remaining by 48 hours (Table 3.2). When compared to the control treatment, cells exposed to PRR following UV-B exposure demonstrated a 53% increase in relative population size at 24 hours, while the population size of flagellates put into the dark decreased by 20% (Table 3.2).

Immunofluorescence assay for CPDs within Paraphysomonas sp. cells.

To determine the direct effects of UV-B on protists at the molecular level attempts were made to quantify CPDs within whole cells using an immunofluorescent microscopy technique directed specifically at thymine-thymine dimers which was modified from Peccia and Hernandez (2002). Repeated attempts were made to visualize CPDs in cultured protozoan cells following UV-B exposure, but these cells yielded no internal fluorescence expected from anti-thymine dimer antibody binding in any of the treatment or control samples. Troubleshooting, such as modification of antibody

Treatment Following UV-B Exposure	CPD mb⁻¹ DNA (0 h)	CPD mb⁻¹ DNA (48 h)	%CPD remaining (48 h)	Change in population size relative to control (24 h)
Moved to Dark	57.5 ± 3.5	58.4 ± 7.2	100	- 0.20 ± 0.10
Moved to PRR	53.6 ± 5.1	20.6 ± 0.4	38	0.53 ± 0.10

Table 3.2 Post-exposure DNA damage (cyclobutane pyrimidine dimers per megabase DNA, mean ± S.E.) and relative change in population size for *Paraphysomonas* sp. in treatments with acute 5-minute UV-B exposure (13 kJ m⁻² total) followed by incubation in the dark or in photorepair radiation (PRR). CPD mb⁻¹ did not change over time in the controls, which remained in darkness for the duration of the experiment.

concentrations (1°, 2° and addition of a 3°), varying detergent type and concentration, and pH sensitivity testing of the fluorescently labeled secondary antibody, was done between each unsuccessful trial, but did not result in the expected labeling of DNA damage.

These unexpected results suggest that the immunofluorescent technique may have failed within the protists because the DNA damage was too low to detect using immunofluorescent microscopy. Another potential problem was that the antibodies may not have penetrated the nuclear membrane. Proteins within protozoan cells have been probed using similar fluorescent antibody techniques, although those proteins are not found within the nuclei (Brugerolle & Adoutte 1988, Peccia & Hernandez 2002, Noel et al. 2003)

Heat-killed Bacteria as Food

The growth rate of *Paraphysomonas* sp. fed with heat-killed bacteria (HKB) exposed to UVR was not significantly different from those fed with HKB stored in the dark. The specific growth rates of flagellates fed with HKB exposed to UVR at the highest intensity and exposure time used in our experiments versus those fed HKB kept in the dark were $1.0 \pm 0.06 \text{ d}^{-1}$ and $1.1 \pm 0.02 \text{ d}^{-1}$, respectively. HKB abundances changed only in the presence of flagellates, irrespective of exposure to UV-B.

Interactive effects of UV-B radiation and CDOM on *Paraphysomonas* sp.

DOC-specific absorbance

There were no significant differences observed in DOC, absorbance at 305 nm, or DOC-specific absorbance ($A_{d305}[\text{DOC}]^{-1}$) within or between any given lake water treatment (lake water, shielded lake water, lake water kept in the dark) following the 12 hour

exposure period (Figure 3.7, Table 3.3). Nor were there treatment (lake water, shielded lake water, lake water kept in the dark) following the 12 hour exposure period (Figure 3.7, Table 3.3). Nor were there significant differences between the corresponding treatments with additional CDOM (unshielded, shielded and dark).

Survival and growth responses to UVR and CDOM

When the heterotrophic flagellate *Paraphysomonas* sp. was exposed to UV-B radiation passing through 5.5 cm of oligotrophic lake water (1.17 mg C L^{-1} DOC), the population declined rapidly during the 12 hour exposure period and suffered 100% mortality within 24 hour post-exposure. Flagellates incubated in the higher CDOM water, but with only natural lake water in the shielding dish also were undetectable by 24 hour post-exposure (Figure 3.8). The presence of the layer of CDOM between the flagellates and the UV-B radiation source had a significant effect on survival and maximum specific growth rate ($p < 0.001$). Populations in treatments shielded by CDOM increased during the UV exposure period and maintained stable populations for >48 hour whether or not the flagellates were incubated in lake water with added CDOM (Figure 3.8 and 3.9). However, the deleterious effect of UV radiation on *Paraphysomonas* sp. was evident from the higher growth rates and greater abundances reached in the dark (Figure 3.8, Table 3.4.). *Paraphysomonas* sp. incubated in the dark had maximum specific growth rates of 2.08 and 2.17 d^{-1} in lake water and CDOM-amended lakewater, respectively, (Table 3.4) which shows that the CDOM was stimulatory to this simple two trophic-level system. This stimulatory effect was not evident when bacterial cells were exposed to UV radiation under identical conditions as the flagellates (Figure 3.10).

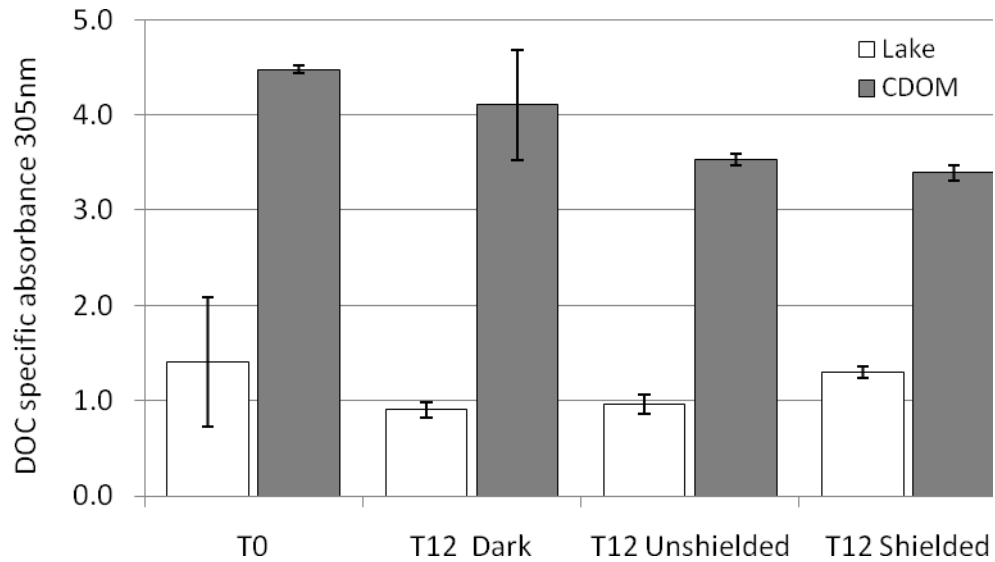


Figure 3.7 DOC-specific absorbance at 305 nm (mean \pm SE, n = 3) in laboratory CDOM-additions. Unshielded treatments were incubated under a dish of natural lake water from Lake Giles, Pennsylvania. Shielded treatments were incubated under CDOM-amended lake water (2.5 mg C L⁻¹ final concentration).

Treatment	Exposure (hours)	DOC (mg C L ⁻¹)	A _{d305} (m ⁻¹)	A _{d305} [DOC] ⁻¹
<u>Lake Treatments</u>				
Lake	12	1.62 (0.23)	1.55 (0.14)	0.96 (0.10)
Shielded Lake	12	1.68 (0.25)	1.99 (0.24)	1.30 (0.06)
Lake Dark	12	1.69 (0.14)	1.53 (0.18)	0.91 (0.08)
<u>DOC Treatments</u>				
DOC	12	2.89 (0.09)	10.24 (0.25)	3.54 (0.06)
Shielded DOC	12	2.95 (0.42)	9.98 (0.23)	3.39 (0.09)
DOC Dark	12	2.60 (0.35)	10.54 (0.09)	4.11 (0.58)

Table 3.3 DOC concentration, dissolved absorbance (A_{d305}) and DOC-specific absorbance (A_{d305}[DOC]⁻¹) of lake water and DOC amended lake water samples that were unshielded, shielded with CDOM, or not exposed to UV (dark). Average (SD) of three replicates per treatment.

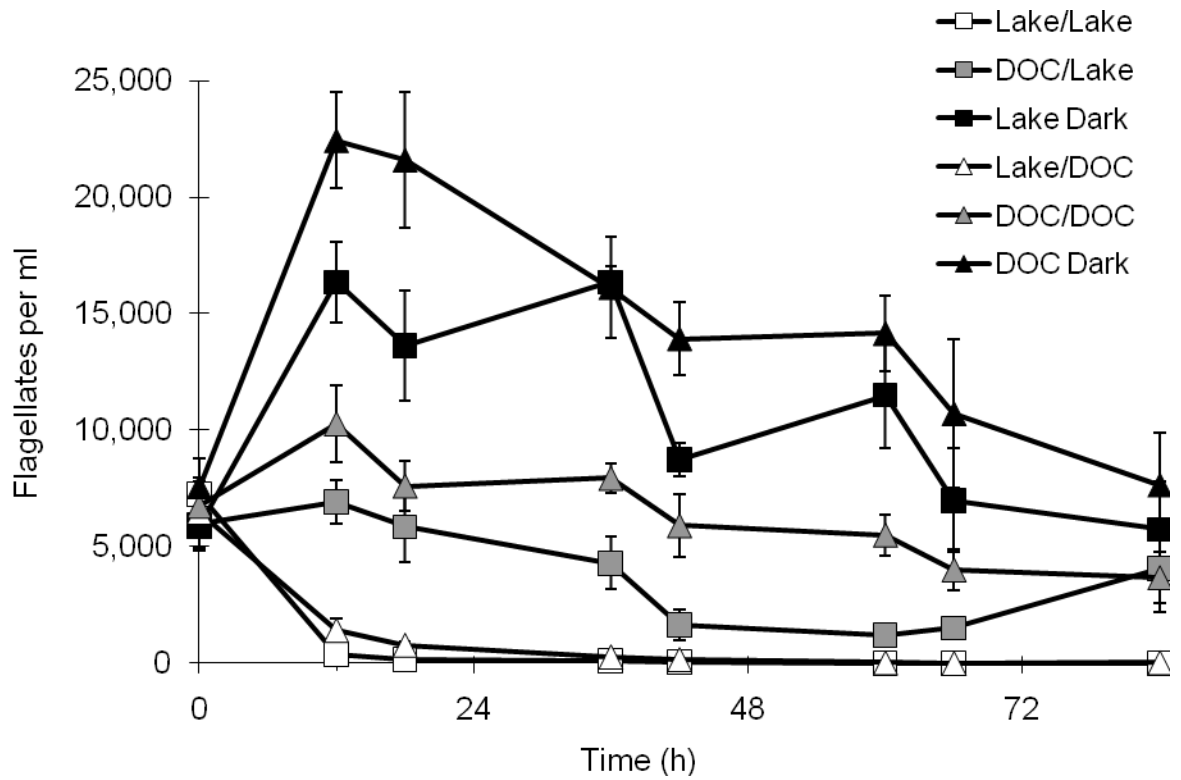


Figure 3.8 Abundance changes (mean \pm SE, $n = 3$) of *Paraphysomonas* sp. over a 4 day period following exposure to PRR plus UV radiation in treatments shielded with CDOM or unshielded (under natural lakewater). The first word in the legend indicates contents of shielding chamber and the second indicates contents of dishes containing flagellates. For example, for Lake/DOC, the flagellates were in water containing CDOM that was shielded by a dish of lake water. Dark controls were never exposed to the lamps.

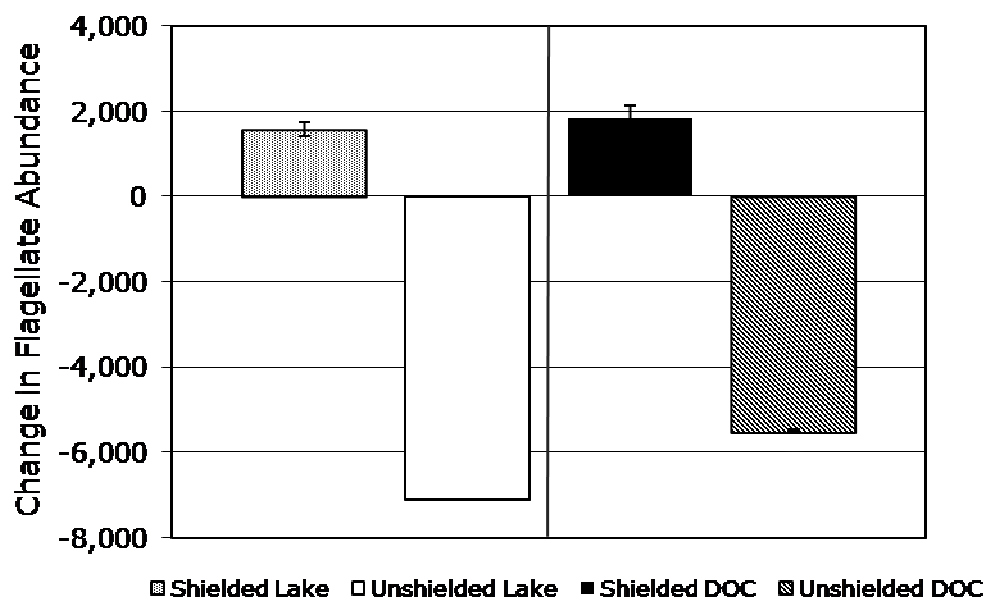


Figure 3.9 Changes in the abundance of *Paraphysomonas* sp. 12 hours after exposure to UV-B radiation plus PRR in treatments when shielded with CDOM or unshielded (under natural lakewater).

<u>UV Treatment</u>	<u>Growth Rate (d⁻¹)</u>		
	<u>Dark</u>	<u>Unshielded</u>	<u>CDOM Shielded</u>
Lake water	2.08 ± 0.52	-6.76 ± 1.07	0.33 ± 0.66
Lake water + CDOM	2.17 ± 0.19	-3.21 ± 0.45	0.84 ± 0.50

Table 3.4 Maximum specific growth rates of *Paraphysomonas* sp. (mean ± SE, n = 3) in a laboratory shielding experiment when kept in the dark or exposed to 58 kJ m⁻² of UV-B with (+) or without added CDOM, and with or without a 5.5 cm UV layer of CDOM (shield).

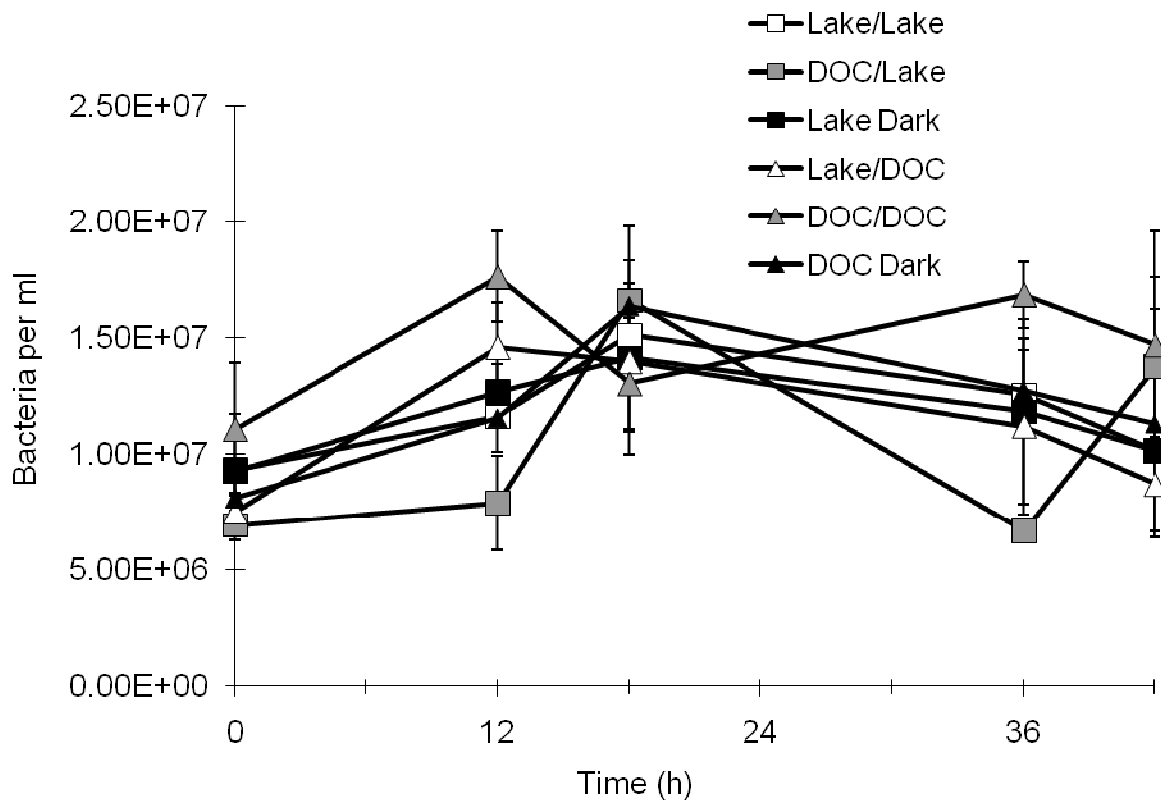


Figure 3.10 Abundance changes (mean \pm SE, $n = 3$) of a mixed bacterial community over 42 hours following exposure to PRR plus UV radiation in treatments shielded with CDOM or unshielded (under natural lakewater). The first word in the legend indicates contents of shielding chamber and the second indicates contents of dishes containing flagellates. For example, for Lake/DOC, the flagellates were in water containing CDOM that was shielded by a dish of lake water. Dark controls were never exposed to the lamps.

Potential DNA damage measured with dosimeters

DNA dosimeters indicated that CDOM shielding reduced DNA damage. The percentages of CPDs [mB DNA]⁻¹ in dosimeters were significantly fewer ($p < 0.001$) in the two shielded treatments in which a 5.5 cm layer of CDOM enriched lake water was between the DNA dosimeters and the UVR source than when lake water alone served as a “filtering” layer (Figure 3.11). Dimer formation in the DNA dosimeters was not significantly different between the two shielded treatments or between the two unshielded treatments, suggesting that the CDOM in the shallow dishes did not add substantially to UV-B protection. However ANOVA indicated a significant interaction effect between incubating water type and whether or not the dosimeter was shielded ($p < 0.001$).

Lake Experiment: CDOM and UV radiation effects on a natural microbial food web

Temperature, oxygen and DOC changes

Temperature and oxygen profiles of Lake Giles varied little over the experimental period with epilimnetic temperatures ranging between 20.7°C and 21.9°C and dissolved oxygen between 8.4 and 9.0 ppm. Depth profiles from June 2004 show that the epilimnion (mixed layer) extended to a depth of 5.5 m, consistently for the length of the experiment (Figure 3.12). There were no significant differences in temperature or oxygen concentration between replicates of a given incubation (Table 3.5), with temperatures ranging between 20.7°C and 22.4°C and dissolved oxygen between 8.2 and 9.0 ppm. A significant difference in oxygen concentration was detected between treatments, but oxygen saturation was 93% or greater for the lake and all treatments over the course of the experiment.

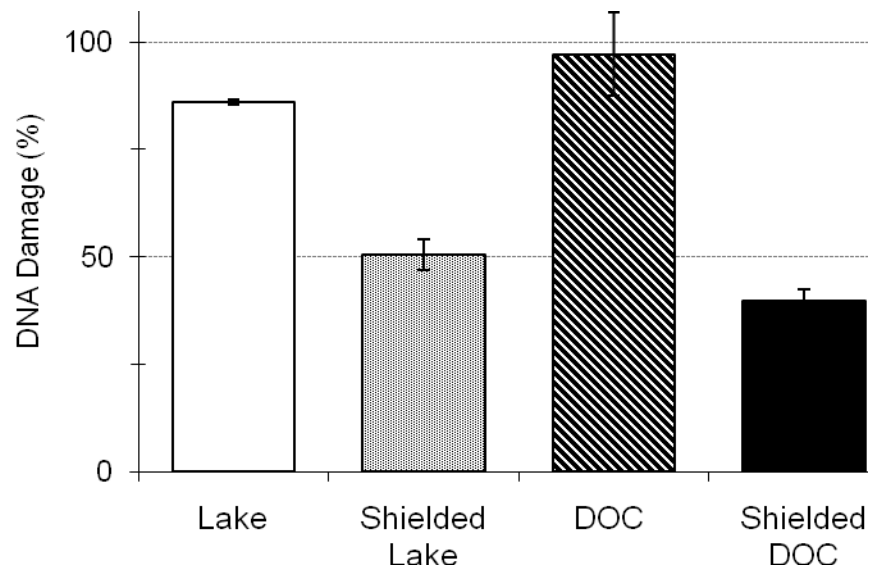


Figure 3.11 Percentage of DNA damage in dosimeters exposed to UVR and PRR with or without a “shielding layer” of CDOM as compared to those dosimeters exposed in lake water with no shielding layer above. Error bars indicate standard error. Percentage of DNA damage in dosimeters kept in the dark was less than 0.2%.

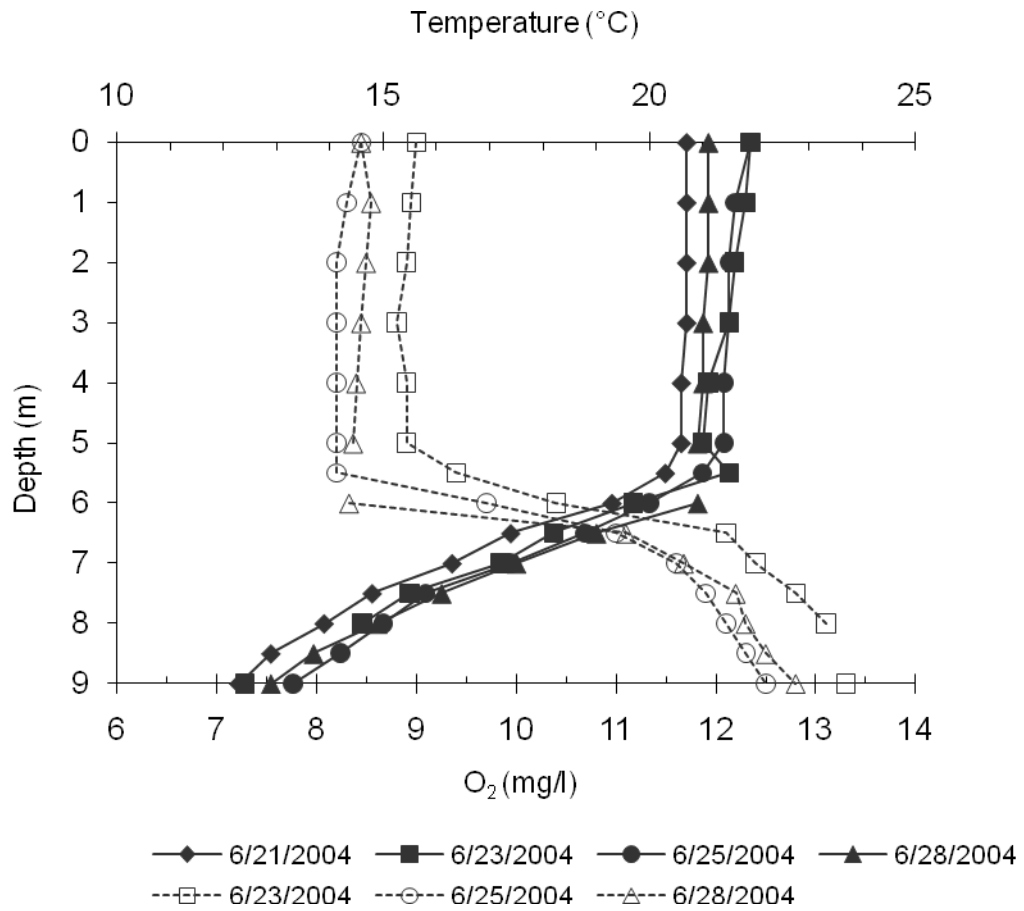


Figure 3.12 Temperature (filled symbols) and oxygen (open symbols) profiles for Lake Giles taken late June 2004.

Temperature

RSquare	0.982
Root Mean Square Error	0.105
Mean of Response	21.56
Observations	48

Source of variation	df	SS	Fs	p
Treatment	3	0.23	6.918	0.0016*
Bag Number[Treatment]	8	0.07	0.774	0.6294
Day	3	14.3	431.5	<0.001*
Treatment*Day	9	0.18	1.769	0.1274

Oxygen

RSquare	0.976
Root Mean Square Error	0.057
Mean of Response	8.554
Observations	48

Source of variation	df	SS	Fs	p
Treatment	3	0.15	15.74	<0.001*
Bag Number[Treatment]	8	0.02	0.913	0.5225
Day	3	2.88	300.8	<0.001*
Treatment*Day	9	0.11	3.681	0.0051*

Table 3.5 Repeated measures ANOVA table for temperature and oxygen concentration between sample bags incubated at the surface of Lake Giles in June of 2004. Treatments include exposed to or shielded from ultraviolet radiation in combination with or without additional CDOM.

Total DOC concentrations increased slightly in all treatment bags by the end of the experiment (Figure 3.13). Exposure to ultraviolet radiation (UVR) had no significant effect on DOC concentration over the course of the experiment in either CDOM treatment (Table 3.6). DOC-specific absorbance at 305 nm decreased by approximately 43% in the +CDOM treatment exposed to UVR, while a decrease of approximately 20% was seen in the +CDOM treatment in which UVR was blocked (Figure 3.13).

Survival and growth responses of microorganisms to UVR and CDOM

Bacterial populations doubled in all treatments peaking in abundance on day 2 (Figure 3.14). Populations in treatments that did not receive additional CDOM had maximum specific growth rates (MSGR) of 0.56 d^{-1} and 0.32 d^{-1} for UVR-shielded and UVR exposed treatments, respectively. Cells in treatments that received additional CDOM had MSGR of 1.2 d^{-1} for UVR-shielded, and 1.1 d^{-1} for UVR exposed (Table 3.7). The addition of CDOM had a significant effect on the MSGR of bacterial populations while effects of UVR exposure were not significant. Abundances of bacteria in all treatments dropped to approximately initial densities by day 3 (Figure 3.14)

Heterotrophic flagellate community abundances doubled in treatments with additional CDOM regardless of UVR exposure, with a peak in abundance occurring on day 3 (Figure 3.15), one day after bacterial abundances peaked (Figure 3.14). Flagellates in treatments that did not receive additional CDOM exhibited initial declines, and only those shielded from UVR reached a population growth size above starting densities with a peak abundance on day 4 (Figure 3.15). Community abundances in treatments that did not receive additional CDOM had MSGR of 0.62 d^{-1} for UVR-shielded (Table 3.7). MSGR of the heterotrophic flagellate community were significantly affected

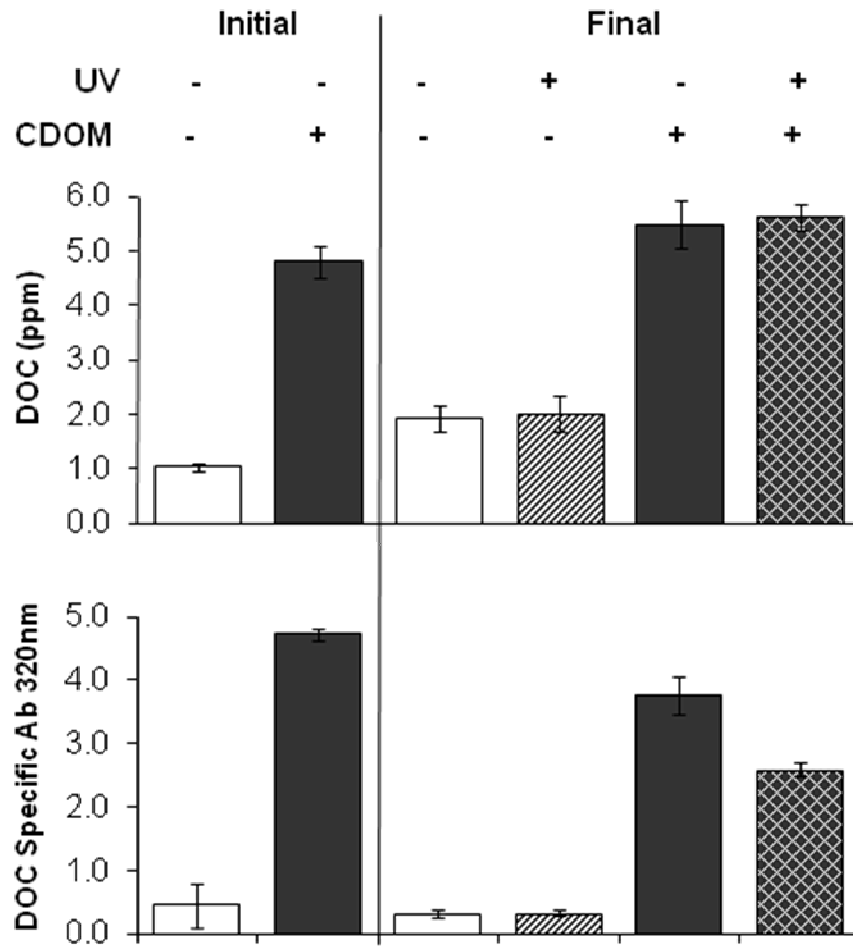


Figure 3.13 Initial and final DOC concentrations and DOC-specific absorbance at 305 nm during a 10-day UVR- and CDOM-manipulated field experiment.

Source of variation	df	MS	Fs	p
Subgroups	3	21.88		
CDOM (columns)	1	65.58	3571.3	0.001
UV(rows)	1	0.05	2.94	
INTERACTION	1	0.01	0.27	
Within (Error)	44	0.02		
Total	47			

Table 3.6 ANOVA table for final DOC concentration between treatments incubated at the surface of Lake Giles in June of 2004.

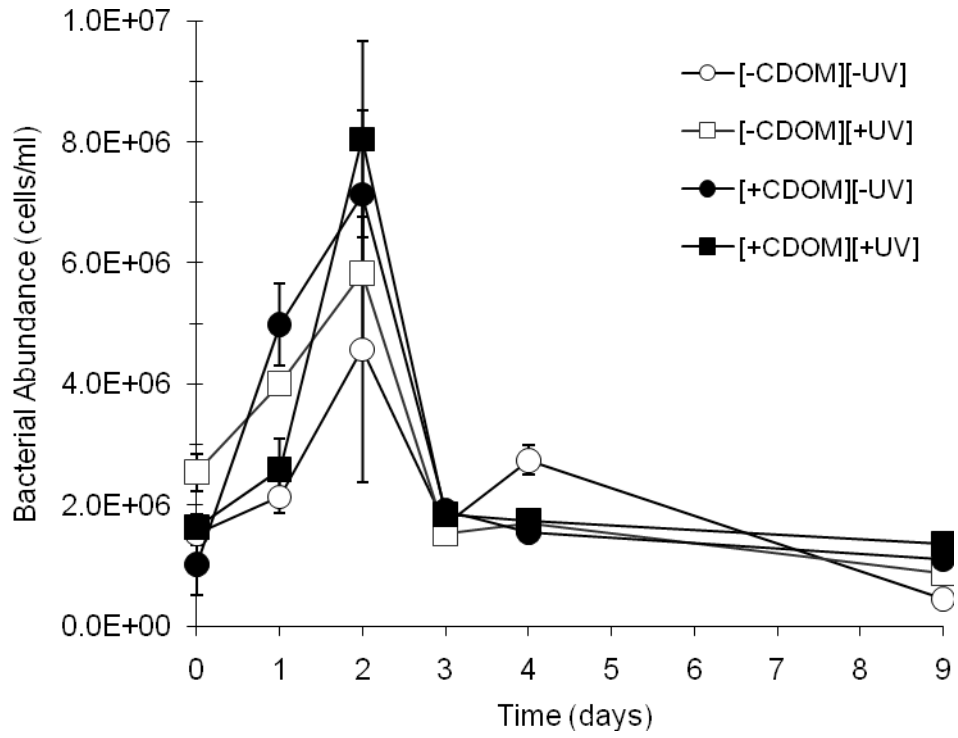


Figure 3.14 Abundance changes (mean \pm SE, $n = 3$) of bacteria over the duration of a 10-day UVR- and CDOM-manipulated lake experiment.

Treatment	Growth Rate (d⁻¹)		
	Bacteria	Heterotrophic Flagellates	Phototrophic Flagellates
- CDOM / -UV	0.56±0.32	0.62±0.22	1.0±0.12
- CDOM / +UV	0.32±0.15	0.53±0.13	0.31±0.07
+CDOM / -UV	1.2±0.08	1.1± 0.10	0.67±0.08
+CDOM / +UV	1.1±0.31	2.1± 0.01	0.34±0.07

Table 3.7 Maximum specific growth rates of bacteria and flagellate assemblages incubated at the surface of Lake Giles June 2004.

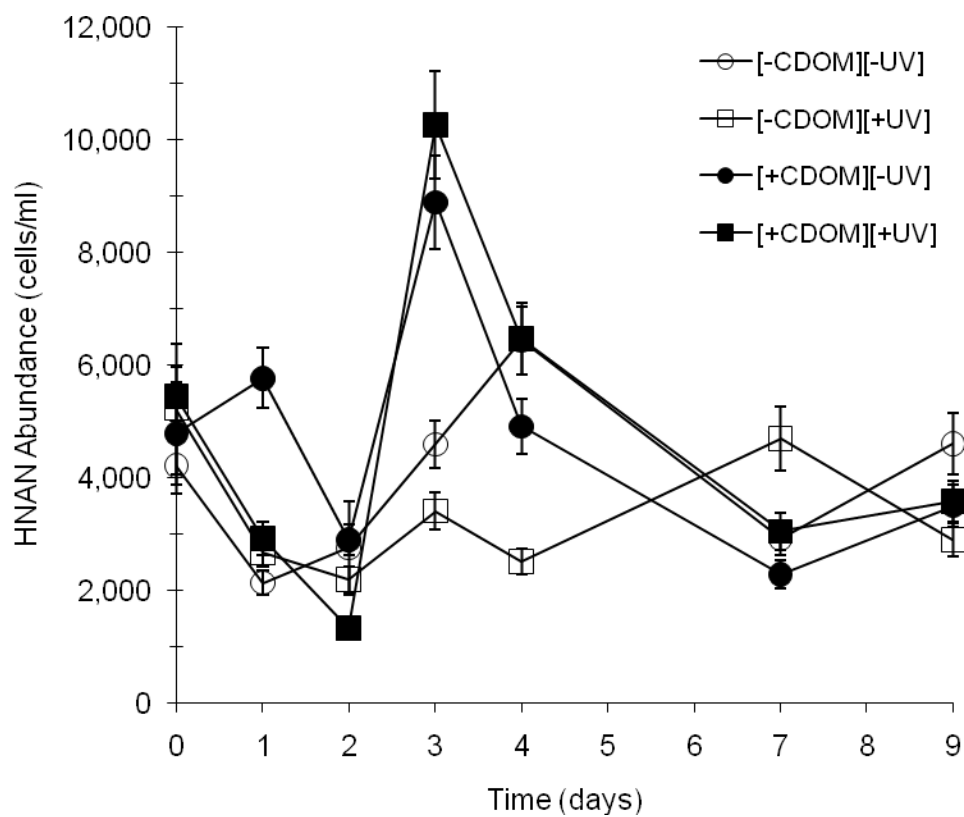


Figure 3.15 Abundance changes (mean \pm SE, n = 3) of heterotrophic nanoflagellates (HNAN) over the duration of a 10-day UVR- and CDOM-manipulated lake experiment.

by UVR ($p < 0.001$) and CDOM ($p < 0.001$), and a significant interactive effect also was noted. In all treatments, densities of heterotrophic flagellates stabilized at or just below initial abundances by day 7 (Figure 3.15).

Phototrophic flagellate community abundances remained low in all treatments until approximately day 9 when those shielded from UVR with and without additional CDOM increased (Figure 3.16). Communities exposed to UVR had MSGR of 0.31 d^{-1} and 0.34 d^{-1} for -CDOM and +CDOM treatments, respectively, but not until the last two days of the experiment. Phototrophic flagellates in treatments that were shielded from UVR by acrylic had MSGR of 1.0 d^{-1} for -CDOM and 0.67 d^{-1} for +CDOM treatments (Table 3.7). MSGR of the phototrophic flagellate community were significantly affected by UVR ($p < 0.001$), but no significant effects of CDOM were detected.

Dosimeter DNA damage

In treatments where UVR was blocked by OP-4 acrylic, there was no significant change in UVR induced DNA damage, as measured in DNA dosimeters (CPD mb^{-1}), in the presence or absence of additional CDOM ($40.4 \pm 1.62 \text{ CPD mb}^{-1}$ and $40.9 \pm 1.93 \text{ CPD mb}^{-1}$, respectively) (Figure 3.17). DNA damage was 8 times greater in treatments exposed to UVR with additional CDOM ($118.2 \pm 11.0 \text{ CPD mb}^{-1}$) and 23 times greater in lake water treatments without additional CDOM ($948.7 \pm 64.0 \text{ CPD mb}^{-1}$). DNA damage (CPD mb^{-1}) in DNA dosimeters was significantly affected by UVR ($p < 0.001$) and CDOM ($p < 0.001$), as well as exhibiting a significant interactive effect ($p < 0.001$).

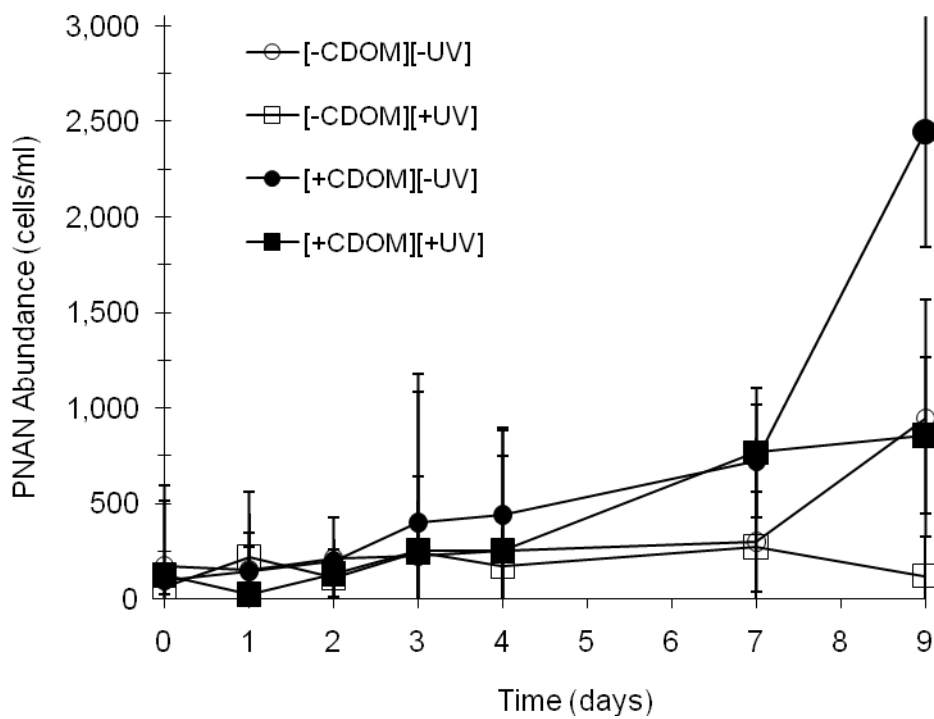


Figure 3.16 Abundance changes (mean \pm SE, $n = 3$) of photosynthetic nanoflagellates (PNAN) abundance over the duration of a 10-day UVR- and CDOM-manipulated lake experiment.

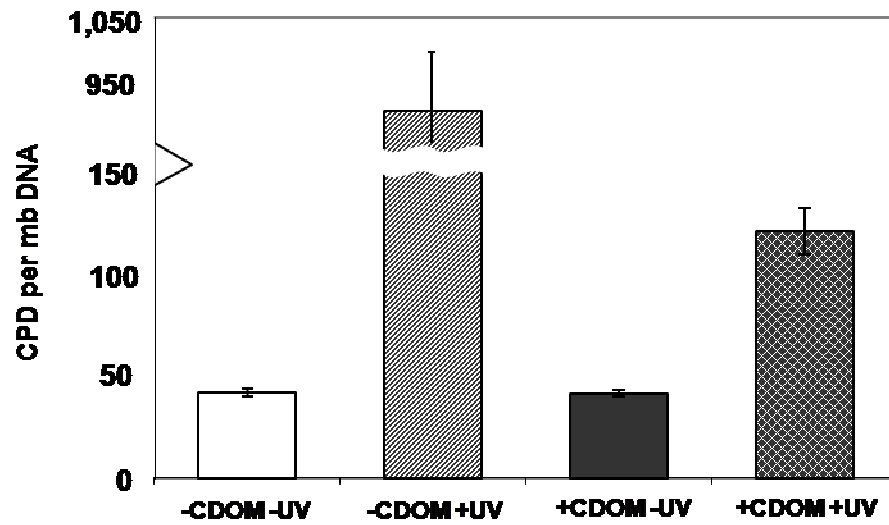


Figure 3.17 DNA damage as CPD [Mb DNA]⁻¹ for dosimeters incubated under natural solar radiation in Lake Giles, PA with or without added CDOM and shielded or unshielded from UVR.

CHAPTER 4

DISCUSSION

Direct effects of UV-B radiation on the heterotrophic flagellate *Paraphysomonas*

Potential responses to UV-B exposure

Depending on water clarity, organisms living in the surface waters of aquatic systems can encounter injurious levels of ultraviolet radiation (UVR) and consequently must adapt mechanisms to minimize its negative effects. Behavioral and biochemical responses to UVR exposure, which are not mutually exclusive, are known for a broad range of species, and include avoidance, the use of photoprotective compounds, and various enzymatic repairs of UVR-induced damage.

Phototaxis, the movement toward or away from a light source, is of obvious importance to photosynthetic protists, but some heterotrophic protists also alter swimming direction or speed in response to changes in light direction or intensity (Häder 1984). Specific UVR-elicited negative phototaxis was observed in the ciliate *Ophryoglena flava* and two *Parauronema* clones, which suggests avoidance of damaging radiation (Marangoni et al. 2004). However, weakened photorientation and/or reduced motility in the presence of UVR was determined for the flagellates *Chlamydomonas nivalis*, *Euglena gracilis*, *Astasia longa*, *Bodo saltans* and *Goniomonas* sp. (Häder & Häder 1989, Sommaruga & Buma 2000). Sommaruga & Buma (2000) also noted that motility was unaffected in the presence of UVR for several other protists, including the ciliate *Cyclidium* sp. and two marine *Paraphysomonas* species. Clearly, avoidance of natural levels of UVR is not a universal response in protists.

Another mechanism that can moderate the effects of UVR is photoprotection (Tartarotti et al. 2001, Banaszak 2003). Pigmentation can modify the intracellular intensity and wavelength distribution of light, as can colorless UV-absorbing compounds. Relatively well-studied photoprotective compounds found in protists include carotenoids and a suite of mycosporine-like amino acids (MAAs). Carotenoids absorb in the UV-A part of the spectrum, and though their role in passive screening of UV is unclear, they do act to quench oxygen radicals produced during UVR exposure (White & Jahnke 2002, Zagarese et al. 2003). MAAs appear to have a relatively limited taxonomic distribution (Banaszak 2003). Various yeasts, some cyanobacteria and green algae, and several photosynthetic dinoflagellates are known to produce MAAs in response to UVR exposure (Neale et al. 1998, Groniger et al. 2000). These compounds do accumulate through dietary acquisition in zooplankton (Newman et al. 2000, Tartarotti et al. 2001, Moeller et al. 2005). Dietary accumulation, for example by ciliates such as *Favella ehrenbergii* that feed preferentially on dinoflagellates (Stoecker et al. 1981), is a potential mechanism for heterotrophic protists to gain photoprotection from these compounds. MAAs also are found in at least one ciliate due to its association with symbiotic dinoflagellates (Sommaruga et al. 2006). In addition, other pigments that absorb in the far-UV are known for some ciliates, and these may convey some level of resistance to UVR (Modenutti et al. 1998) or stimulate avoidance of bright light (Lenci et al. 1997).

Even when an organism uses photoprotection and/or evasion of UVR, these mechanisms are likely to be only partly effective in eliminating UVR exposure. Cellular DNA strongly absorbs UV-B radiation, and even a relatively small exposure can result in the formation of different types of lesions between adjacent pyrimidine bases. Cyclobutane pyrimidine dimers (CPDs) account for a majority of these UVB-induced

photoproducts, but pyrimidine [6-4] pyrimidinone dimer ([6-4] photoproduct) are also produced in measurable amounts (Mitchell & Nairn 1989). Both types of these bulky lesions inhibit DNA replication and transcription, and if not repaired can lead to inaccurate transmission of genetic information or cell death (Sinha & Hader 2002). Repair mechanisms that have evolved to correct UVB-induced DNA damage fall into two general categories: dark repair mechanisms, of which nucleotide excision repair (NER) is the most important for UVR damage in eukaryotes; and photoenzymatic repair (PER) [see recent reviews in Sinha and Hader (2002), Mitchell (2004) and Zagarese et al. (2003)]. NER is a light-independent repair mechanism that cleaves the damaged DNA strand before and after the lesion, resulting in the removal of an oligonucleotide containing the dimer. DNA polymerase then fills in the gap with new nucleotides using the opposite DNA strand as a template. In PER a photolyase enzyme binds to the UV-generated lesion, and uses the energy of light to directly cleave the bond formed between pyrimidine bases (i.e., the dimer). Photolyase enzymes require absorption of photoreactivating radiation (PRR) in the visible (400-700 nm) and UV-A (320-400 nm) parts of the spectrum, with wavelength for maximum PER efficiency determined to be in the 380 – 440 nm range for some bacteria, yeast and *Euglena* (Mitchell 2004).

Although, PER is widespread taxonomically (Mitchell 2004), its importance relative to dark repair is highly variable for different organisms. PER has been shown to be a particularly important mechanism of DNA repair in several species of amphibians (Blaustein et al. 1994), zooplankton (Malloy et al. 1997, Grad et al. 2001), protists (Smith-Sonneborn 1979, Sanders et al. 2005) and bacteria (Peccia & Hernandez 2002). (Zenoff et al. 2006) found several strains of aquatic bacteria with varying degrees of reliance on PER and no obvious use of NER, although *Cytophaga* sp. had efficient CPD

removal in both the dark and under PRR. The freshwater crustacean *Daphnia pulicaria* utilized NER to a greater extent than PER (MacFadyen et al. 2004), and five species of temperate freshwater larval fish all used NER while only two (yellow perch and bluegill) demonstrated PER abilities (Olson & Mitchell 2006).

The dominance of PER in UV-B challenged Paraphysomonas sp.

In several heterotrophic protists PER seemed to be a major part of the response to a UV-B challenge. Likewise, the experiments described here indicated that *Paraphysomonas* sp. was heavily dependent on PER for survival and growth after exposure to UV-B. There was 100% mortality in the absence of photorepair radiation (PRR) at all except the lowest UV-B exposure tested (Figures 3.1 – 3.5). The large role of PRR for *Paraphysomonas* sp. exposed to UV-B was revealed by the reduction of CPDs in DNA from flagellate cells exposed to PRR; this decrease in CPDs was not observed in the absence of PRR (Table 3.2). While the PER treatment did not exclude NER from occurring, the treatments moved to the dark (no PER) after UV-B exposure gave no indication of dimer repair (Table 3.2). This dominant role of PER in *Paraphysomonas* sp. was consistent with observations of the ciliates *Paramecium tetraurelia* and *Glaucoma* sp. (Smith-Sonneborn 1979, Sanders et al. 2005). *P. tetraurelia* had greater clonal longevity if it received PRR when exposed to UV-B, while populations of *Glaucoma* sp. accumulated lethal amounts of DNA damage in absence of PRR, but had fewer CPDs and grew in the presence of PRR (Smith-Sonneborn 1979, Sanders et al. 2005). NER is a metabolically costly and relatively slow DNA repair mechanism requiring ATP and multiple enzymes as compared to PER (Vetter et al. 1999, Sinha & Hader 2002), and it may be that the rapid population growth of protists selects for PER over NER.

Yet not all heterotrophic protists use PER, and its importance may vary even within the same genus. One clone of the ciliate *Cyclidium* sp. was found to have very little tolerance of UV-B, whether or not PRR was present during UV-B exposure (Sanders et al. 2005). Another strain of *Cyclidium* did not accumulate any measurable DNA damage or show reduced motility when exposed to UV-B with PRR (Sommaruga & Buma 2000). While PER was important for the freshwater *Paraphysomonas* sp. examined here, similar experiments gave little suggestion of PER in the marine *P. imperforata* (R.W. Sanders, personal communication).

Since NER is slow and energetically costly relative to PER, which uses one enzyme that requires light for energy, it is possible that PER quickly removes most dimers and NER then works over a longer time frame to remove the remaining damage. Heat-killed bacteria (HKB) were grazed to low levels once *Paraphysomonas* sp. reached peak abundances, which lead to decreases in flagellate population size within 24 hours, these experiments might not demonstrate a time and energy demanding process such as NER. However, given the rapid and total mortality observed in the absence of photorepair radiation, it seems most likely that *Paraphysomonas* sp. is reliant on PER for sustained survival of UV-B exposed populations.

UV-B Intensity, food and temperature effects on

UVR challenged Paraphysomonas sp.

There was a clear effect of UV-B intensity on survival and growth rate on *Paraphysomonas* sp. with higher UV-B intensity resulting in greater inhibition and/or damage. This is a general phenomenon and is seen with dosimeters (Figure 3.6) as well as with organisms. The role of intensity is an important component of the response of an aquatic organism because of the differential absorption of solar energy by water and

dissolved substances. Organisms that move or are mixed into deeper water will be less affected by UV-B than those remaining directly at the surface, especially since attenuation for PRR is less than for UV-B.

The effects of UVR on population growth of *Paraphysomonas* sp. in these experiments can be ascribed to direct effects on the flagellates rather than an effect on their food since the quality of HKB as food was not affected by UV-B exposure. HKB that were irradiated prior to being used as food at the highest level of UV-B used in the experiments still led to growth rates by *Paraphysomonas* sp. that were statistically indistinguishable from those fed non-irradiated HKB. In all treatments where there was growth of *Paraphysomonas* sp., including controls, a notable decline in flagellate abundance occurred within 24 hours of reaching peak abundance. This is typical of batch culture experiments, and also in field experiments where predators are removed, because heterotrophic protists grow rapidly and graze their prey below a threshold level, which can no longer support the flagellate population at high abundance (Fenchel 1987, Caron 1990, Seitzinger & Sanders 1997). Fed-batch experiments, in which additional food was added as HKB declined, would not have changed our interpretation of the experiments. Heterotrophic protist cultures can be resurrected from UV-exposed treatments (if they received PRR) by adding more food (R.W. Sanders, personal communication). This suggests that the PER was highly effective in these organisms, and that unless UVR caused 100% of a population to die, even a single individual could establish a new protist population via asexual reproduction.

Temperature has strong effects on feeding, respiration and growth in heterotrophic protists (Rose & Caron 2007), and at least one ciliate had a temperature optimum for survival after exposure to UV-B (Sanders et al. 2005). Populations of *Paraphysomonas*

sp. maintained at two temperatures also showed differential abilities of recovery after identical UV-B exposures, with increased survival and growth at 20°C relative to 15°C (Figures 3.1 – 3.5). The interaction of temperature with DNA repair is known for several other taxa exposed to ecologically relevant levels of UV-B and temperature, including a red alga, a rotifer and several crustacean zooplankton species (Pakker et al. 2000, Williamson et al. 2002, MacFadyen et al. 2004) . The variation of sensitivity to UV-B with temperature is an important aspect for organisms in temperate regions. Solar radiation, including UV-B flux, increases rapidly in early spring when water temperature remains below the observed optima for DNA repair in various of the laboratory experiments noted above. Thus there is potential for aquatic communities to be shaped by species tolerance to seasonal changes that include shifts in the ratio of UV-B exposure to temperature. Temperature alone is considered to have a role in altering aquatic community composition (Strecker et al. 2004), but the effect of increasing UV levels during a period of relatively slow seasonal change in temperature is not known. Trends of increasing global temperature may alter community structure by shifting local temperature out of organisms' tolerance ranges, and thus rendering them more susceptible to UVR-induced DNA damage.

Organic Matter and Its Influence on UV Penetration in Freshwater Systems

Organic matter is categorized into nonhumic or humic substances. Nonhumic substances are comprised of low molecular weight compounds such as carbohydrates, proteins, peptides and amino acids. Generally these are easily utilized and degraded by microorganisms, resulting in their low concentrations in natural waters. Humic substances, which are found in greater concentrations in fresh waters, are a mixture of naturally occurring organic substances with high molecular weights and tend to be

recalcitrant to biological degradation. These compounds are often referred to as colored- or chromophoric- dissolved organic matter (CDOM), and can cause some natural waters to be dark in color. These organic substances can be further described based on their origin. Autochthonous organic matter is produced within the system (e.g., lake) by the breakdown of algal cells as well as the feeding byproducts of aquatic heterotrophs, and tends to be of nonhumic character. Allochthonous organic matter enters the lake system from the outside in the form of aquatic (marsh) or terrestrial vegetation and tends to have a greater concentration of humic substances. It is this allochthonous organic matter that greatly influences CDOM content and therefore lake water color. Changes in DOC and color have been shown to be related to the period when most of the surface ice is gone in lakes that freeze seasonally (ice-out dates) and to seasonal precipitation, such that years with late ice-out dates and high spring rain were associated with high DOC concentrations and darker water color in the spring (Pace & Cole 2002).

Effects of UV Radiation Shielding by CDOM

The ability of CDOM to absorb UV radiation has been well established. Morris et al. (Morris et al. 1995) demonstrated that UV transparency varies widely among lakes, and that absorbance by dissolved material (a_d), principally from DOC, was important in determining the penetration of the UVR wavelengths. Studies conducted at my study site, Lake Giles, revealed that the attenuation coefficients of UV and PAR (K_{dUV} & K_{dPAR}) in the epilimnion varied seasonally, with minimum values (maximum penetration) occurring near the summer solstice (Morris & Hargreaves 1997). The seasonal variation observed for epilimnial K_{d320} suggest significant variations in epilimnial exposure to solar UVR (Morris & Hargreaves 1997).

As expected, DOC-specific absorbance of UV radiation was much greater in lake water that was amended with naturally-derived CDOM to reach concentrations between 2 and 4 times greater than that of Lake Giles (Table 3.3). Lake Giles water for laboratory experiments had an initial DOC level of 1.17 mg C L^{-1} , while the surface water during the lake experiment was 1.04 mg C L^{-1} . Since changes in climate could alter the CDOM input into oligotrophic lakes, experiments were designed to test changes in microbial interactions if DOC levels in Lake Giles were to increase to that of numerous lakes in the Pocono Mountains and elsewhere that currently have more CDOM than Lake Giles.

The biological benefits of reduced UV exposure due to absorbance by CDOM can be measured in various ways. The reduction of UV-induced DNA damage is a major benefit of CDOM shielding. DNA dosimeters in both the laboratory and lake experiments indicate that CDOM shielding significantly reduced DNA damage. As expected, the presence of a “filtering” layer of CDOM in the laboratory experiments reduced DNA damage by 50 to 60 percent as compared to the damage incurred in low DOC lake water with no “filtering” layer above (Figure 3.11). When low DOC water was used as the “filtering” layer DNA damage was only reduced by 3 to 14 percent. In Lake Giles experiments, treatments without UV-shielding acrylic, but supplemented with CDOM (4.8 mg C L^{-1} DOC) had DNA damage in dosimeters of $118 \pm 11 \text{ CPD mb}^{-1}$ compared to $949 \pm 64 \text{ CPD mb}^{-1}$ in similar treatments without CDOM supplements (Figure 3.17). In lake experiments the Beartooth Mountains of western Montana, increasing the DOC concentration from $1.02 \pm 0.02 \text{ mg L}^{-1}$ to $4.01 \pm 0.01 \text{ mg L}^{-1}$ reduced DNA dosimeter damage from approximately 700 CPD mb^{-1} to approximately 120 CPD mb^{-1} (Cooke et al. 2006). Clearly, higher CDOM levels in lake water reduces DNA damage.

Another metric for examining the benefit of CDOM in the absence of DNA damage estimates is population growth, by either increases in abundance or reproductive effort. For example, incubation of the copepod *Leptodiaptomus ashlandi* in the presence of an increased DOC concentration enhanced reproduction by increasing the number of young per female and the proportion of females producing eggs. UVR shielding may be particularly important for those zooplankton, which live in colder waters and may be dependent upon photoenzymatic repair of DNA damage (Cooke et al. 2006).

Nielsen and Ekelund (1993) described increased growth rates in the dinoflagellate *Gyrodinium aureolum* when shielded from UV-B by humic substances. However, with bacteria and their predators it is difficult to separate the effects of shielding from the effects of additional nutritional resources in experiments that supplement with CDOM. Shielding from UV-B definitely leads to increased bacterial production (Bailey et al. 1983, Lindell et al. 1996) but bacterial populations also are expected to grow faster when an additional nutrient source is added. Likewise, heterotrophic flagellates are inhibited by UV exposure (Figure 3.15), but responded with increased growth when bacterial prey increase.

Laboratory experiments that I performed with *Paraphysomonas* sp. and a mixed bacterial community were intended to separate the factors of 1) UV-shielding verses 2) increased food resources, both expected with CDOM additions. Maximum specific growth rates of *Paraphysomonas* sp. increased significantly when a layer of high CDOM water was used to shield them from UV-B radiation (Table 3.4). But, growth was significantly less than in CDOM treatments kept in the dark, indicating that damage from

UV-B still occurred in the presence of shielding CDOM. The dosimeter data confirms that shielding with 5.5 cm of 2.5 mg C L⁻¹ DOC reduces DNA damage, but does not eliminate it. Without the shielding, flagellate populations rapidly declined (Figure 3.11), and did not recover. Growth rates were still stimulated in all treatments to which CDOM was added relative to the same UV-B manipulation without CDOM. But the percent stimulation in the dark was only 4% above lake water, while in the shielded treatment the growth rate of *Paraphysomonas* sp. was 155% greater than in lake water alone (Table 3.4). This suggests that shielding from UV-B damage can be a major component of the response of the microbial food web to allochthonous CDOM input into lakes. The bacterial community used showed no significant differences between any treatments over the course of the experiment indicating no effects of UV-B or enhanced food web stimulation under these experimental conditions (Figure 3.10).

In experiments conducted at the surface of Lake Giles, assemblages of bacteria, heterotrophic nanoflagellates, and phototrophic nanoflagellates also grew fastest in water amended to a higher DOC concentration. However, as noted above, it is difficult with bacteria and their predators to separate the effects of shielding from the effects of nutritional resources in this type of experiment. In treatments without CDOM additions, shielding with a UV-blocking plastic led to higher growth rates in bacteria and flagellates (Table 3.7). Addition of CDOM to the lake water led to higher growth rates relative to lake water alone for bacteria and heterotrophic flagellates, but not phototrophic flagellates. Interestingly, incubation under UV-blocking acrylic led to apparent increases in growth rate of the bacteria and phototrophic flagellates, but not the heterotrophic flagellates (Table 3.7). I hypothesize that in the +CDOM/+UV treatment, the presence of UV-B released additional labile DOC relative to the +CDOM/-UV treatment, which led to

increased bacterial growth rates. But, our method of determining growth (change in abundance) underestimated bacterial growth significantly in the +CDOM/+UV treatment because grazing by the rapidly growing population of heterotrophic flagellates kept bacteria abundance from increasing. The grazing-balanced growth by the bacteria allowed heterotrophic flagellates to have a significantly higher growth rate in the +CDOM/+UV treatment than in any other (Table 3.7).

In any case, additions of CDOM of a level meant to simulate allochthonous inputs may lead to relatively short-term changes in microbial population sizes. In both laboratory experiments and in field experiments at Lake Giles, the community of heterotrophic flagellates (and bacteria) tended to grow rapidly after supplements with CDOM. However, population sizes typically fell to initial levels within a period of several days to a week (Figures 3.8, 3.10, 3.14 and 3.15). In enclosure experiments conducted in Pipit Lake (Alberta, Canada), Vinebrooke and Leavitt (1998) found that amendments of dissolved organic matter similar to predicted allochthonous inputs did not significantly affect the final abundances of heterotrophic bacteria and protists. Likewise, in mesocosm experiments in Lake Giles, bacterial production increased after CDOM addition, but dropped to background levels after a few days, as did flagellate abundance (Vinebrooke & Leavitt 1998, Sanders et al. 2008).

Seasonal changes in the source and lability of dissolved organic matter, as well as seasonal changes in solar intensity, have been shown to be associated with shifts in bacterioplankton community composition. For example, Crump et al. (2003) observed changes in bacterial community composition in Toolik Lake, Alaska that were associated with an influx of labile terrestrial DOM as snow meltwater, followed by another shift as

the phytoplankton community developed. These researchers attributed the shift in bacterial community structure in Toolik Lake to differences in the DOC quality, but UVR has also been shown to alter bacterial community structure (Langenheder et al. 2006). These data confirm the importance of CDOM as a UV shield but suggest that the biologically available organic matter may not be transferred to higher trophic levels during periods of UV stress.

CHAPTER 5

CONCLUSIONS AND FUTURE STUDIES

Implications for aquatic systems

Quantifying the limits of tolerance to ultraviolet radiation (UVR) for individual species is an important aspect of elucidating ecological impacts of solar radiation in aquatic environments. Understanding how UVR affects heterotrophic protists may be of special interest since they are recognized as having substantial effects on major ecosystem processes including nutrient recycling and transfer of carbon from bacteria and picophytoplankton through the food web (Sanders et al. 1992, Elser & Frees 1995, Baudoux et al. 2008).

A major goal of the experiments described here was to determine whether UVR could limit the distribution of a heterotrophic flagellate in an environment that received moderate to high UV influx. For this reason, we used a strain of *Paraphysomonas* isolated from Lake Giles, which was optically clear, and used levels of UVR that were ecologically relevant for that lake in our laboratory experiments. The maximum calculated UV-B flux in our experiments was similar to that determined for Lake Giles at a latitude of 41.2°N (Williamson et al. 2001) and in Pisa, Italy at a latitude of 43.7°N (Marangoni et al. 2004). Furthermore, the levels of photoproducts produced in DNA dosimeters in our experiments are consistent with UV-B exposure in temperate latitudes. Boelen et al. (2000) used similar dosimeters in marine systems and determined a conversion factor of CPDs produced in DNA dosimeters to scalar irradiance (1 CPD megabase⁻¹ of nucleotides per 3.1 J m⁻²). Using that conversion factor, the biological effective dose (BED) of our highest intensity was 496 J m⁻². This agrees well with the

BEDs of 592 and 456 J m⁻² calculated for the ocean surface at 34°N by (Boelen et al. 2000) and indirectly confirms the relevance of the UV-B exposures used in our laboratory experiments.

However, a direct comparison of the cumulative UVR dose from our experiments to field conditions may lead to inaccurate predictions of population change. This is because models predicting such effects typically assume that a cumulative dose has the same effect regardless of the dose rate (i.e., reciprocity). In other words, the same DNA damage determined here for *Paraphysomonas* sp. exposed to 56 kJ m⁻² over a 12 hour period would occur if it received 56 kJ m⁻² over a different time course if there were reciprocity. When photoenzymatic repair is relevant, as it is with *Paraphysomonas* sp., then reciprocity typically does not hold and applying the laboratory results to lakes with dissimilar radiation regimes could be misleading (Cullen & Neale 1997, Grad et al. 2001). Nevertheless, these experiments demonstrate that a species belonging to the most commonly isolated group of heterotrophic flagellates (Finlay & Clarke 1999) can survive and reproduce at the highest levels of UVR that it is currently likely to encounter in a temperate environment. Although higher fluxes of UV-B resulting from reduced atmospheric ozone might reduce the rate of population growth, we expect indirect effects on its predators and prey are more likely to alter population growth of *Paraphysomonas* than direct effects of UVR. It has been hypothesized that alterations in DOC concentrations within lakes resulting from changes in climate, lake hydrology, acid deposition and other environmental factors may be more important in controlling future UV environments in lakes than stratospheric ozone depletion (Williamson et al. 1996).

REFERENCES CITED

- Aldredge AL (1977) House morphology and mechanisms of feeding in the Oikopleuridae (Tunicata, Appendicularia). *J. Zool., Lond.* 181:175-188
- Bailey CA, Neihof RA, Tabor PS (1983) Inhibitory Effect of Solar Radiation on Amino Acid Uptake in Chesapeake Bay Bacteria. *Applied and Environmental Microbiology* 46:44-49
- Banaszak AT (2003) Photoprotective physiological and biochemical responses of aquatic organisms. In: Helbling EW, Zagarese H (eds) *UV effects in Aquatic Organisms and Ecosystems*. The Royal Society of Chemistry, p 329-356
- Banaszak AT, Trench RK (2001) Ultraviolet sunscreens in dinoflagellates. *Protist* 152:92-101
- Baudoux A, Veldhuis M, Noordeloos A, Noort Gv, Brussaard C (2008) Estimates of virus- vs. grazing-induced mortality of picophytoplankton in the North Sea during summer. *Aquatic Microbial Ecology* 52:69-82
- Blaustein AR, Hoffman PD, Hokit DG, Kiesecker JM, Walls SC, Hays JB (1994) UV repair and resistance to solar UV-B in amphibian eggs: A link to population declines? *Proc. Natl. Acad. Sci. USA* 91:1791-1795
- Boelen P, Boer MKd, Kraay GW, Veldhuis MJW, J.Buma AG (2000) UVBR-induced DNA damage in natural marine picoplankton assemblages in the tropical Atlantic Ocean. *Marine Ecology Progress Series* 193
- Brugerolle G, Adoutte A (1988) Probing protist phylogenies with an anti-tubulin antibody. *BioSystems* 21:255-268
- Caron DA (1990) Growth of two species of bacterivorous nanoflagellates in batch and continuous culture, and implications for their planktonic existence. *Marine Microbial Food Webs* 4:143-159
- Caron DA, Lim EL, Dennett MR, Gast RJ, Kosman C, DeLong EF (1999) Molecular phylogenetic analysis of the heterotrophic chrysophyte genus *Paraphysomonas* (Chrysophyceae), and the design of rRNA-targeted oligonucleotide probes for two species. *J. Phycol.* 35:824-837
- Carrick HJ, Fahnenstiel GL, Stoermer EF, Wetzel RG (1991) The importance of zooplankton-protozoan trophic couplings in Lake Michigan. *Limnol. Oceanogr.* 36:1335-1345
- Cooke SL, Williamson CE, Saros JE (2006) How do temperature, dissolved organic matter and nutrients influence the response of *Leptodiptomus ashlandi* to UV radiation in a subalpine lake? *Freshwater Biology* 51:1827-1837

- Crump BC, Kling GW, Bahr M, Hobbie JE (2003) Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. *Applied and Environmental Microbiology* 69:2253-2268
- Cullen JJ, Neale PJ (1997) Biological weighting functions for describing the effects of ultraviolet radiation on aquatic systems. In: Häder D-P (ed) *The effects of ozone depletion on aquatic ecosystems*. R.G. Landes, Austin, p 97-118
- Elser JJ, Frees DL (1995) Microconsumer grazing and sources of limiting nutrients for phytoplankton growth: Application and complications of a nutrient-depletion/dilution-gradient technique. *Limnol. Oceanogr.* 40:1-16
- Fenchel T (1987) *Ecology of Protozoa*. Science Tech/Springer-Verlag, Madison
- Finlay BJ, Clarke KJ (1999) Apparent global ubiquity of species in the protist Genus *Paraphysomonas*. *Protist* 150:419-430
- Grad G, Williamson CE, Karapelou DM (2001) Zooplankton survival and reproduction responses to damaging UV radiation: A test of reciprocity and photoenzymatic repair. *Limnol. Oceanogr.* 46:584–591
- Groniger A, Sinha RP, Klisch M, Hader D-P (2000) Photoprotective compounds in cyanobacteria, phytoplankton and macroalgae - a database. *Journal of Photochemistry and Photobiology B: Biology* 58:115-122
- Häder DP (1984) Photomovement. In: Senger H (ed) *Blue Light Effects in Biological Systems*. Springer-Verlag, New York, p 435-443
- Häder DP, Häder M (1989) Effects of solar UVB irradiation on photomovements and motility in photosynthetic and colorless flagellates. *Environ. Exp. Bot* 29:273-282
- Herman JR (2010) Global increases in UV irradiance during the past 30 years (1979-2008) estimated from satellite data. *Journal of Geophysical Research* 115
- Hoyer K, Karsten U, Wiencke C (2002) Induction of sunscreen compounds in Antarctic macroalgae by different radiation conditions. *Marine Biology*
- Kerr JB, McElroy CT (1993) Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. *Science* 262:1032-1034
- Langenheder S, Sobek S, Tranvik LJ (2006) Changes in bacterial community composition along a solar radiation gradient in humic waters. *Aquatic Sciences* 68 415-424
- Leech DM, Williamson CE (2001) In situ exposure to ultraviolet radiation alters the depth distribution of *Daphnia*. *Limnol. Oceanogr.* 46:416-420

- Leibold MA, West CT (1993) Experimental methods for measuring the effect of light acclimation on vertical migration by *Daphnia* in the field. *Limnol. Oceanogr.* 38:638-643
- Lenci F, Checcucci G, Ghetti F, Giofrè D, Sgarbossa A (1997) Sensory perception and transduction of UV-B radiation by the ciliate *Blepharisma japonicum*. *Biochim. Biophys. Acta* 1336:23-27
- Lindell MJ, Graneli HW, Tranvik LJ (1996) Effects of sunlight on bacterial growth in lakes of different humic content. *Aquatic Microbial Ecology* 11:135-141
- Lindell MJ, Granéli W, Tranvik LJ (1995) Enhanced bacterial growth in response to photochemical transformation of dissolved organic matter. *Limnol. Oceanogr.* 40:195-199
- MacFadyen EJ, Williamson CE, Grad G, Lowery M, Jeffrey WH, Mitchell DL (2004) Molecular response to climate change: temperature dependence of UV-induced DNA damage and repair in the freshwater crustacean *Daphnia pulicaria*. *Global Change Biology* 10:408-416
- Madronich S, McKenzie RL, Bjorn LO, Caldwell MM (1998) Changes in biologically active ultraviolet radiation reaching the Earth's surface. *Journal of Photochemistry and Photobiology B: Biology* 46:5-19
- Malloy KD, Holman MA, Mitchell D, III HWD (1997) Solar UVB-induced DNA damage and photoenzymatic DNA repair in antarctic zooplankton. *Proc. Natl. Acad. Sci. USA* 94:1259-1263
- Marangoni R, Marroni F, Giofrè D, Ghetti F, Colombetti G (2004) Biological weighting function of the UVB-induced impairment of phototaxis in the freshwater ciliate *Ophryoglena flava*. *Photochem. Photobiol.* 80:408-411
- Mitchell DL (2004) DNA damage induced by ultraviolet radiation. In: Meyers RA (ed) *Encyclopedia of Molecular Cell Biology and Molecular Medicine*, Vol 3. Wiley Publishers, Indianapolis
- Mitchell DL, Haipek CA, Clarkson JM (1985) (6-4)Photoproducts are removed from the DNA of UV-irradiated mammalian cells more efficiently than cyclobutane pyrimidine dimers. *Mutation Research* 143:109-112
- Mitchell DL, Nairn RS (1989) The biology of the (6-4) PD photoproduct. *Photochemistry and Photobiology* 49:805-819
- Modenutti BE, Balseiro EG, Moeller R (1998) Vertical distribution and resistance to ultraviolet radiation of a planktonic ciliate, *Stentor araucanus*. *Verh. Internat. Verein. Limnol.* 26:1636-1640

- Moeller RE, Gilroy S, Williamson CE, Grad G, Sommaruga R (2005) Dietary acquisition of photoprotective compounds (mycosporine-like amino acids, carotenoids) and acclimation to ultraviolet radiation in a freshwater copepod. *Limnology and Oceanography* 50:427-439
- Morris DP, Hargreaves BR (1997) The role of photochemical degradation of dissolved organic carbon in regulating the UV transparency of three lakes on the Pocono Plateau. *Limnol. Oceanogr.* 42:239-249
- Morris DP, Zagarese H, Williamson CE, Balseiro EG, Hargreaves BR, Modenutti B, Moeller R, Queimalinos C (1995) The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. *Limnol. Oceanogr.* 40:1381-1391
- Neale PJ, Banaszak AT, Jarriel CR (1998) Ultraviolet sunscreens in *Gymnodinium sanguineum* (Dinophyceae): mycosporine-like amino acids protect against inhibition of photosynthesis. *Journal of Phycology* 34:928-938
- Newman SJ, Dunlap WC, Nicol S, Ritz D (2000) Antarctic krill (*Euphausia superba*) acquire a UV-absorbing mycosporine-like amino acid from dietary algae. *Journal of Experimental Marine Biology and Ecology* 255:93-110
- Nielsen T, Ekelund NGA (1993) Effect of UV-B radiation and humic substances on growth and motility of *Gyrodinium aureolum*. *Limnol. Oceanogr.* 38:1570-1575
- Noel C, Gerbod D, Delgado-Viscogliosi P, Fast NM, Younes AB, Chose O, Roseto A, Capron M, Viscogliosi E (2003) Morphogenesis during division and griseofulvin-induced changes of the microtubular cytoskeleton in the parasitic protist, *Trichomonas vaginalis*. *Parasitology Research* 89:487-494
- Olson MH, Mitchell DL (2006) Interspecific variation in UV defense mechanisms among temperate freshwater fishes. *Photochemistry and Photobiology* 82:606-610
- Pace ML, Cole JJ (2002) Synchronous variation of dissolved organic carbon and color in lakes. *Limnol. Oceanogr.* 47:333-342
- Pakker H, Martins RST, Boelen P, Buma AGJ (2000) Effects of temperature on the photoreactivation of ultraviolet-B-induced DNA damage in *Palmaria palmata* (Rhodophyta). *J. Phycol.* 36:334-341
- Peccia J, Hernandez M (2002) Rapid immunoassays for detection of UV-induced cyclobutane pyrimidine dimers in whole bacterial cells. *Applied and Environmental Microbiology* 68:2542-2549
- Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* 25:943-948
- Rose JM, Caron DA (2007) Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. *Limnology and Oceanography* 52:886-895

- Sanders RW, Caron DA, Berninger U-G (1992) Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-ecosystem comparison. *Marine Ecology Progress Series* 86:1-14
- Sanders RW, Cooke SL, Fischer JM, Jeffrey WH, Macaluso AL, Moeller RE, Morris DP, Pakulski JD, Porter JA, Schoener DM, Williamson CE (2008) Dissolved Organic Carbon (DOC) Forcing of Plankton Dynamics in a Lake Ecosystem AGU Chapman Conference on "Lakes and Reservoirs as Sentinels, Integrators, and Regulators of Climate Change", Lake Tahoe, NV
- Sanders RW, Macaluso AL, Sardina TJ, Mitchell DL (2005) Photoreactivation in freshwater ciliates: differential responses to variations in UV-B flux and temperature. *Aquat. Microb. Ecol.* 40:283-292
- Sanders RW, Porter KG (1986) Use of metabolic inhibitors to estimate protozooplankton grazing and bacterial production in a monomictic eutrophic lake with an anaerobic hypolimnion. *Applied and Environmental Microbiology* 52:101-107
- Sanders RW, Porter KG, Caron DA (1990) Relationship between phototrophy and phagotrophy in the mixotrophic chrysophyte *Poterioochromonas malhamensis*. *Microbial Ecology* 19:97-109
- Schindler DW, Curtis PJ, Parker BR, Stainton MP (1996) Consequences of climate warming and lake acidification for UV-B penetration in North American boreal lakes. *Nature* 379:705-708
- Seitzinger SP, Sanders RW (1997) Contribution of dissolved organic nitrogen from rivers to estuarine eutrophication. *Marine Ecology Progress Series* 159:1-12
- Sharp JH, Peltzer ET, Alperin MJ, Cauwet G, Farrington JW, Fry B, Karl DM, Martin JH, Spitzzy A, Tugrul S, Carlson CA (1993) Procedures subgroup report. *Marine Chemistry* 41:37-49
- Sherr B, Sherr E (1989) Trophic impacts of phagotrophic protozoa in pelagic foodwebs. *Proceedings of the 5th International Symposium on Microbial Ecology*:388-393
- Sherr BF, Sherr EB (1984) Role of heterotrophic protozoa in carbon and energy flow in aquatic ecosystems. In: Klug MJ, Reddy CA (eds) *Current perspectives in microbial ecology*. American Society of Microbiology, p 412-423
- Sinha RP, Hader D-P (2002) UV-induced DNA damage and repair: a review. *Photochemical & Photobiological Sciences* 1:225-236
- Sinha RP, Häder D-P (2002) UV-induced DNA damage and repair: a review. *Photochemical and Photobiological Sciences* 1:225-236
- Smith-Sonneborn J (1979) DNA repair and longevity assurance in *Paramecium tetraurelia*. *Science* 203

- Sommaruga R (2003) UVR and its effect on species interactions. In: Helbling EW, Zagarese H (eds) UV Effects in Aquatic Organisms and Ecosystems, Vol 1. Royal Society of Chemistry, Cambridge, p 487-508
- Sommaruga R, Buma AGJ (2000) UV-induced cell damage is species-specific among aquatic phagotrophic protists. *Journal of Eukaryotic Microbiology* 47:450-455
- Sommaruga R, Whitehead K, Shick JM, Lobban CS (2006) Mycosporine-like amino acids in the zooanthea-ciliate symbiosis *Maristentor dinofereus*. *Protist* 157:185-191
- Stoecker DK, Guillard RRL, Kavee RM (1981) Selective predation by *Favella ehrenbergii* (Tintinnia) on and among dinoflagellates. *Biol. Bull.* 160:136-145
- Strecker AL, Cobb TP, Vinebrooke RD (2004) Effects of experimental greenhouse warming on phytoplankton and zooplankton communities in fishless alpine ponds. *Limnol. Oceanogr.* 49:1182-1190
- Tartarotti B, Laurion I, Sommaruga R (2001) Large variability in the concentration of mycosporine-like amino acids among zooplankton from lakes located across an altitude gradient. *Limnol. Oceanogr.* 46:1546-1552
- Vetter RD, Kurtzman A, Mori T (1999) Diel cycles of DNA damage and repair in eggs and larvae of Northern Anchovy, *Engraulis mordax*, exposed to solar ultraviolet radiation. *Photochemistry and Photobiology* 69:27-33
- Vinebrooke RD, Leavitt PR (1998) Direct and interactive effects of allochthonous dissolved organic matter, inorganic nutrients, and ultraviolet radiation on an alpine littoral food web. *Limnol. Oceanogr.* 43:1065-1081
- White AL, Jahnke LS (2002) Contrasting Effects of UV-A and UV-B on Photosynthesis and Photoprotection of β -carotene in two *Dunaliella* spp. . *Plant Cell Physiology* 43:877-884
- Williamson CE (1996) Effects of UV radiation on freshwater ecosystems. *Intern. J. Environmental Studies* 51:245-256
- Williamson CE, Grad G, De Lange HJ, Gilroy S, Karapelou DM (2002) Temperature-dependent ultraviolet responses in zooplankton: implications of climate change. *Limnol. Oceanogr.* 47:1844-1848
- Williamson CE, Neale PJ, Grad G, De Lange HJ, Hargreaves BR (2001) Beneficial and detrimental effects of UV on aquatic organisms: implications of spectral variation. *Ecol. Appl.* 11:1843-1857
- Williamson CE, Stemberger RS, Morris DP, Frost TM, Paulsen SG (1996) Ultraviolet radiation in North American lakes: attenuation estimates from DOC measurements and implications for plankton communities. *Limnol. Oceanogr.* 41:1024-1034

- Yan ND, Keller W, Scully NM, Lean DRS, Dillon P (1996) Increased UV-B penetration in a lake owing to drought-induced acidification. *Nature* 381:141-143
- Zagarese HE, Tartarotti B, Anon Suarez DA (2003) The significance of ultraviolet radiation for aquatic animals. In: Ambasht RS, Ambasht NK (eds) *Modern Trends in Aquatic Ecology*. Kluwer Academic/Plenum Publishers, New York, p 173-200
- Zenoff VF, Sineriz F, Farias ME (2006) Diverse Responses to UV-B Radiation and Repair Mechanisms of Bacteria Isolated from High-Altitude Aquatic Environments. *Applied and Environmental Microbiology* 72:7857-7863
- Zepp RG, Erickson DJ, Paul ND, Sulzberger B (2007) Interactive effects of solar UV radiation and climate change on biogeochemical cycling. *Photochemical & Photobiological Sciences* 6:286-300