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The Direct and Indirect Effects of Solar Ultraviolet Radiation in Boreal Lakes of the Experimental Lakes Area, Northwestern Ontario

by

William Fraser Donahue

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

Environmental Biology and Ecology

Department of Biological Sciences

Edmonton, Alberta

Spring 2000

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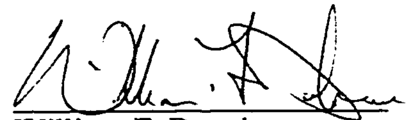

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"Because the universe was full of ignorance all around and the scientist panned through it like a prospector crouched over a mountain stream, looking for the gold of knowledge among the gravel of unreason, the sand of uncertainty and the little whiskering eight-legged swimming things of superstition."

Terry Pratchett 1991[1]

"Therefore I think that all things which evoke discipline: study, and our duties to men and to the commonwealth, war, and personal hardship, and even the need for subsistence, ought to be greeted by us with profound gratitude; for only through them can we attain to the least detachment; and only so can we know peace."

Robert J. Oppenheimer 1932[2]

"The preeminent transnational community in our culture is science."

Richard Rhodes 1986[3]

[1]Pratchett, T. 1991. *Witches Abroad*. London: Corgi books.

[2]Kimball Smith, A. and C. Weiner. 1980. *Robert Oppenheimer: Letters and Recollections*. Harvard University Press.

[3]Rhodes, R. 1986. *The Making of the Atomic Bomb*. New York: Touchstone.

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for the acceptance, a thesis entitled "The Direct and Indirect Effects of Solar Ultraviolet Radiation in Boreal Lakes of the Experimental Lakes Area, Northwestern Ontario" submitted by William Fraser Donahue in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the field of Environmental Biology and Ecology.

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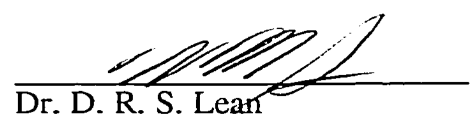

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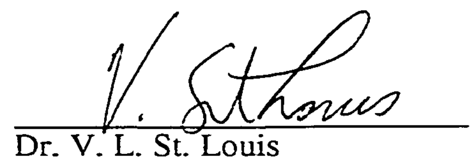

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DEDICATION

I dedicate this effort to my brother, Richard Sean Donahue, who was always my hero and whose too-early death shocked me into questioning everything I thought I knew.

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ABSTRACT

Solar ultraviolet radiation (UVR; 290-400 nm) is primarily attenuated by dissolved organic carbon (DOC) in boreal lakes. DOC is a mixture of organic molecules originating from in-lake metabolic processes (clear; autochthonous), or degradation of organic litter in a lake's catchment (brown; allochthonous). I have used mesocosm and whole-lake experiments at the Experimental Lakes Area (ELA) to study mechanisms of change in properties of DOC, and the effects of these changes on underwater UVR exposure.

UVR was the primary factor in DOC bleaching. Hydrogen peroxide (H_2O_2; 700 nM), photochemically produced when DOC absorbs UVR, also bleached DOC independently of UVR. Unlike UVR, H_2O_2 can bleach DOC throughout the mixed layer. DOC in H_2O_2-treated and UV-shielded enclosures became more autochthonous-like, and DOC in UV-exposed enclosures and surrounding Lake 239 remained primarily allochthonous. some important species of phytoplankton were negatively sensitive to UVR. However, chlorophyll and algal biomass were highest in UV-shielded, H_2O_2-treated enclosures, indicating that UVR and H_2O_2 had independent and opposite effects on phytoplankton. Increased algal nutrient availability may have occurred as a result of altered nutrient chemistry or differing trophic sensitivities to H_2O_2.

Whole-lake acidification also caused a switch in DOC quality from allochthonous to autochthonous-like in Lake 302S. This acid-induced change was due to increased chemical oxidation or precipitation of UV-absorbent aromatic portions of allochthonous DOC molecules, leaving relatively UV-transparent aliphatic chains behind. Acid-induced reduction of DOC concentration and colour increased UVR exposure within Lake 302S by up to ~3000%. Three- to 10-fold increases in photoprotective algal pigments were observed after acidification. A survey of 8 lakes suggests that increased UVR exposure plays an important role in structuring shallow benthic algal communities, especially after acidification, including changing assemblages, production of photoprotective pigments,

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and respiration. In contrast, invertebrate communities reflect the characteristics of the algal mats they inhabit, depending more on food quality and perhaps availability of shelter from UVR.

These physical, chemical, and biological interactions suggest that changes in the nature of DOC and increased UV-transmittance, that follow acidification, ozone depletion, and drought, may have important roles in biogeochemical processes within lakes.

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PREFACE

The structure of this thesis follows the paper format outlined by the Faculty of Graduate Studies and Research, University of Alberta, 1999. I present my research in five manuscripts, chapters 2 through 6. The introductory chapter is intended to outline my research and present rationales for experimental design and interpretation and presentation of results. The concluding chapter is intended to summarize my work on boreal lakes presented in the five research chapters, and to present possibilities for future research. Unavoidable repetition of common aspects between chapters is in keeping with the paper format, in that the same techniques underlie much of my work. For the same reason, and as a result of study designs and interpretation, similar threads of discussion are present in many of the chapters. Although I have used the first person singular in my writing here, I acknowledge the great role my co-investigators have played in the science described. Below is a list of manuscripts resulting from the five chapters as they have been or are expected to be published.

Chapter 2. William F. Donahue and David W. Schindler. Effects of solar ultraviolet radiation and mixed-layer depth on transparency and communities in mesocosms in boreal lakes.

Chapter 3. William F. Donahue. 1998. Interference in fluorometric hydrogen peroxide determination using scopoletin-horseradish peroxidase. *Environmental Toxicology and Chemistry* **17** (5): 783-787.

Chapter 4. William F. Donahue and David W. Schindler. The effect of solar ultraviolet radiation and dilute hydrogen peroxide on optical, chemical, and biological properties of L239, Experimental Lakes Area, northwestern Ontario.

Chapter 5. William F. Donahue, David W. Schindler, Stephen J. Page, and Michael P. Stainton. 1998. Acid-induced changes in DOC quality in an experimental whole-lake manipulation. *Environmental Science and Technology* **32** (19): 2954-2960.

Chapter 6. William F. Donahue, Michael A. Turner, David L. Findlay, Peter R. Leavitt, David W. Schindler, Michael P. Stainton, and Stephen J. Page. Analysis of physical, chemical, and biological interactions in the structuring of shallow epilithic communities in boreal lakes.

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ACKNOWLEDGEMENTS

I would first like to thank Dr. Dave Schindler, who, based on a short conversation and a recommendation, gave me a chance when others weren't so likely to. I would also like to thank Dave for providing me a glimpse into the politics of environmental science, and the opportunity to see his awesome intellect in action. Finally, I would like to thank him for the freedom I have been permitted to pursue that which I have found most interesting, and for putting up with the "off-scale tortuosity" of my manuscript drafts.

Thank you also to my family. Thanks to my parents, who have been supportive (or at least willing to hold their tongues!) throughout the twists and turns and apparent dead-ends of my life, and through the good and bad times. Thank you for teaching me about physical and mental health, and encouraging me to learn and question and experience. Thank you both for making nature such a big part of my early life. Thanks, Dad, for keeping me grounded in reality and instilling in me an unbending sense of right and wrong, and at the same time teaching me of the tactical approach. Thanks, Mom, for taking me to the library when I was little, for letting me take art class, for encouraging me to turn over rocks, and for having a sense of humour when I emptied out my pockets after my days in the woods. I'd also like to thank my sister, Sheelah, for her great sense of humour and late games of cribbage - I'm glad I was able to pursue grad school in Alberta and get to know you once again.

Graduate school can be stressful and frustrating, and my friends helped me through those times, wittingly or otherwise. My track friends kept me sane from day to day and tolerated my railing against whatever had set me off most on any particular day. Thanks to Joanne Johnston, Rose-Mary Ball, Jodi Forster-Molstad, Leeanne Harrison, Laurel Sharun, Sintra Lewis, and Kyle Marcotte for laughing at my jokes (sometimes!) and for making me laugh in return, and to Kevin Olson and Sean Forbes for sharing the gut-searing, head-splitting, muscle-screaming workouts and making it oddly fun, too. Thanks to my coach, Al Weicker, for his sweetly sado-masochistic workouts and for making me run faster, and for all the really bad jokes. And thank you SO much, Jodi, for keeping my body working this last year - even for the agony of pressure-point release! Many of my best memories from the last 5 years are of our trials and travails on and off the track, of trips, and of training and competing in search of that perfect race that is always just out of reach. The Night of Bernice and the my first West Coast Series will remain forever in my memory.

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these times when funding for environmental science is ignorantly lacking. Long live Duct Tape™ and Zap-Straps™!

I would like to thank the Natural Sciences and Engineering Council of Canada, the Canadian Circumpolar Institute, the University of Alberta, the province of Alberta, and Elsie Shostak for personal and research funding.

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Finally, I would like to acknowledge the existence and beauty of that rare creative genius that makes the truly exceptional appear almost effortlessly simple. Thank you to Michelagnolo Buonarroti, Ludwig von Beethoven, Ernest Rutherford, Niels Bohr, and Mikhail Baryshnikov for making discovery beautiful.

Heylin, M. 1999. Institutionalizing Interdisciplinarity. *Chemical & Engineering News*: September 13.

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TABLE OF CONTENTS

1. GENERAL INTRODUCTION **1**

EFFECTS OF SOLAR ULTRAVIOLET RADIATION IN AQUATIC SYSTEMS 1
INTERACTIONS BETWEEN DISSOLVED ORGANIC CARBON AND UV RADIATION 2
GENERAL OUTLINE OF RESEARCH AND OBJECTIVES 5
Objectives and Hypotheses *5*
Study Designs *6*
UVR, UV-b, and UV-a *7*
LITERATURE CITED 8

2. THE EFFECTS OF SOLAR ULTRAVIOLET RADIATION AND MIXED-LAYER DEPTH ON TRANSPARENCY AND COMMUNITIES IN MESOCOSMS IN BOREAL LAKES **15**

INTRODUCTION 15
METHODS 16
RESULTS 19
DOC concentration, fluorescence, and absorbance *19*
UV effects on phytoplankton and bacteria *20*
Zooplankton *22*
DISCUSSION 22
DOC quality and quantity and UV transmittance *22*
Phytoplankton *24*
Zooplankton *26*
Conclusions *27*
LITERATURE CITED 28

3. INTERFERENCE IN FLUOROMETRIC HYDROGEN PEROXIDE DETERMINATION USING SCOPOLETIN-HORSERADISH PEROXIDASE **45**

INTRODUCTION 45
MATERIALS AND METHODS 46
General *46*
Apparatus *46*
Reagents *47*
Modified Procedure *47*
RESULTS AND DISCUSSION 48
Complex Emission Response of Scopoletin *48*
Hydrogen Peroxide Determinations *50*
LITERATURE CITED 51

4. THE EFFECT OF SOLAR ULTRAVIOLET RADIATION AND DILUTE HYDROGEN PEROXIDE ON OPTICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES OF OLIGOTROPHIC BOREAL LAKES **59**

INTRODUCTION 59
METHODS 61
RESULTS 64
Effects on DOC *64*
Effects of hydrogen peroxide and UVR on DOC bleaching *65*
Effects of hydrogen peroxide and UVR on nutrient concentrations *66*
Effects on phytoplankton *66*
Chlorophyll a 66
Algal biomass 67

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Algal community changes 67
DISCUSSION 68
LITERATURE CITED 71

5. ACID-INDUCED CHANGES IN DOC QUALITY IN AN EXPERIMENTAL WHOLE-LAKE MANIPULATION 95

INTRODUCTION 95
METHODS 96
Apparatus 97
Technique 97
Properties of the Lakes 99
RESULTS 99
DISCUSSION 101
LITERATURE CITED 104

6. ANALYSIS OF PHYSICAL, CHEMICAL, AND BIOLOGICAL INTERACTIONS IN THE STRUCTURING OF SHALLOW EPILITHIC COMMUNITIES IN BOREAL LAKES 116

INTRODUCTION 116
METHODS 117
RESULTS 120
Structuring of algal communities 120
Structuring of invertebrate communities 121
DISCUSSION 122
DOC absorbance of ultraviolet radiation 122
Algal responses 123
Taxa and biomass 123
Photoprotection 125
Invertebrate responses 126
Direct and indirect effects of UVR 126
Invertebrate densities and potential quality of algae as food 127
CONCLUSIONS 129
LITERATURE CITED 130

7. GENERAL DISCUSSION 161

POSSIBLE AREAS OF FUTURE RESEARCH 164
LITERATURE CITED 165

APPENDIX A. ALGAL BIOVOLUME, DENSITY, AND BIOMASS ON DAY 35 IN 0.75 M UV-EXPOSED AND UV-SHIELDED ENCLOSURES, AND RESULTS OF T-TESTS **167**

APPENDIX B. ZOOPLANKTON DENSITIES (/L) IN 0.75 M UV-SHIELDED AND UV-EXPOSED ENCLOSURES AND P-VALUES FROM T-TESTS **169**

APPENDIX C 170

C-1. ALGAL TAXA AND BIOMASS IN EPILITHON AT 0.1, 0.3, 0.7, AND 1.5 M IN LAKES 223 AND 224 170
C-2. ALGAL TAXA AND BIOMASS IN EPILITHON AT 0.1, 0.3, 0.7, AND 1.5 M IN LAKES 239 AND 260 172
C-3. ALGAL TAXA AND BIOMASS IN EPILITHON AT 0.1, 0.3, 0.7, AND 1.5 M IN LAKES 373 AND 468 174
C-4. ALGAL TAXA AND BIOMASS IN EPILITHON AT 0.1, 0.3, 0.7, AND 1.5 M IN LAKES 979 AND 302S 176

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LIST OF TABLES

Table 2-1: Half-times for fluorescence loss in UV-shielded and UV-exposed enclosures of different depth (days)........ 33

Table 4-1. DOC concentrations (mg/L) during 2 enclosure experiments, from June 4 - July 9 and August 18 - 20 September, 1996, in Lake 239 at the Experimental Lakes Area (± S. D.; n=3)........ 76

Table 4-2. Experiment 1: Nutrient concentrations in enclosures exposed to and shielded from solar UVR, and in enclosures shielded from UVR and treated with a once-daily 700. nM H_2O_2 pulse ($P \leq 0.07$ for differences between * and **)........ 77

Table 4-3. Experiment 2: Nutrient concentrations in enclosures exposed to and shielded from solar UVR, and in enclosures shielded from UVR and treated with a once-daily 700 nM H_2O_2 pulse, on Day 33 (all concentrations are µg/L ± S. D.) ($P \leq 0.065$ for differences between * and **)........ 78

Table 6-1: Water chemistry from 8 lakes sampled at the Experimental Lakes Area (* previously experimentally acidified; ** currently experimentally acidified; *** experimentally flooded peat bog)........ 138

Table 6-2. DOC quality and UV flux (measured spectroradiometrically and modeled from [DOC]) in 8 lakes sampled at the Experimental Lakes Area. PAR meaurements were also taken spectroradiometrically (*calculated from vertical profiles of PAR flux measured with a Li-Cor light meter). Models used for comparative UV predictions: 1=(Scully and Lean, 1994); 2=(Schindler et al., 1996). DOC peak fluorescence was measured at an excitation wavelength of 354 nm and emission wavelength of 496 nm, and reported as quinine sulfate units (QSU). DOC ratio is the fluorescence emission ratio of 450 and 500 nm emission intensities, with excitation at 370 nm. Water colours were noted as green (gr), brown (br), or orange (or)........ 139

Table 6-3: Percent variance in inter-site, inter-lake epilithon algal species composition and biomass explained by environmental variables (step-wise forward redundancy analysis)........ 140

Table 6-4: Percent variance in inter-site, inter-lake epilithic invertebrate species data explained by environmental variables (step-wise forward selection redundancy analysis; Monte Carlo permutations, n=99, P=0.01)........ 141

Table 6-5. Densities of invertebrate taxa as a function of chl *a* concentrations in epilithon and UV-b exposure from 4 depths in 6 ELA lakes (July, 1996)........ 142

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LIST OF FIGURES

Figure 1-1. Energetic continuum of radiation wavelengths of the solar spectrum. UV-b wavelengths below 292 nm are filtered out by the stratospheric ozone layer........... 14

Figure 2-1. Transmission spectra of UV-impermeable (UF-3) and UV-permeable (OP-4) plexiglass. 34

Figure 2-2. DOC concentrations (± S. D.) in enclosures of varying depth and UVR exposure. Other than on day 26, there were no differences between treatments (** is greater than *; P<0.05).. 35

Figure 2-3. Decreases in fluorescence (excitation @ 354 nm, emission @ 496 nm; ± S. D.) in UV-shielded and UV-exposed enclosures of different depths. The greatest loss in fluorescence was in 0.75 m deep UV-exposed enclosures. 36

Figure 2-4. UVR penetration in UV-exposed and UV-shielded enclosures of different depths (± S. D.) between June 5 and July 9, 1996. The greatest increases in UVR penetration were in the shallowest UV-exposed enclosures.............................. 37

Figure 2-5. Carbon-specific fluorescence (CSF; ± S.D.) in UV-exposed and UV-shielded enclosures of different depth. Carbon in the shallowest UV-shielded enclosures was most bleached. 38

Figure 2-6. Carbon-specific absorbance (CSA; /mgC; 327 nm) in UV-shielded and UV-exposed enclosures of different depth over the last 10 days of a 5 week experiment. As a result of bleaching, DOC in the shallowest UV-exposed enclosures had the lowest CSA. 39

Figure 2-7. Chl a concentrations in UV-exposed and UV-shielded enclosures of different depths, from June 5 to July 9, 1996 (± S. D.; ** is greater than *; P<0.05). A pulse of tree pollen between days 13 and 18 appeared to cause the eventual rapid increase in chl a concentration from days 22 to 25.. 40

Figure 2-8a. Algal biomass on Day 1 in UV-exposed and UV-shielded enclosures of different depths. There were no differences in taxa or biomass between treatments (P>0.05).. 41

Figure 2-8b. Algal taxa and biomass in 0.75 m enclosures that were shielded from or exposed to solar UV radiation for 35 days (** is greater than * for each class; P<0.05). 42

Figure 2-9. Bacterial biomass in enclosures of different depth and exposed to or shielded from solar UV radiation. Other than a general increase in biomass after the pollen pulse, no discernible treatment effect occurred.. 43

Figure 2-10. Bacterial densities in enclosures of different depth and exposed to or shielded from solar UV radiation. Other than on Day 18, when densities were greater in the shallowest UV-exposed enclosures than in other treatments (P<0.05), no discernible treatment effect occurred over the course of the experiment.............................. 44

Figure 3-1. Predictable nature of emission response of a scopoletin solution after shielding from excitation radiation for 3 minutes (Runs 1, 4 and 6) versus after exposure to visible light immediately prior to excitation (Runs 2, 3 and 5) (Ex = 354 nm; Em = 496

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nm). Note also general decrease in initial emission intensity (Runs 1 to 6) as a result of photobleaching of scopoletin during consecutive runs.................................. 53

Figure 3-2. Decrease in emission intensity (496 nm) of scopoletin as a result of bleaching by excitation radiation (354 nm) and a typical calibration curve via standard addition on a fluorometer not equipped with a shutter. Each solid circle represents the injection of an aliquot of H_2O_2. 54

Figure 3-3. Decrease in fluorescent emission of scopoletin as a result of addition of HRP in the absence of H_2O_2. Volumes (µL) of scopoletin and HRP added are indicated.. 55

Figure 3-4. Unpredictable nature of emission response of scopoletin solution replicates after pre-run exposure to visible light (A and B) compared to response after shielding of scopoletin from all wavelengths for three minutes prior to excitation (C). 56

Figure 3-5. Conceptual interference during standard addition calibrations for H_2O_2 determination under constant excitation. (t_0) = addition of HRP; (t_2-t_1) = duration of calibration; (B-D) = fluorescence decrease due to HRP addition; (B-C) = bleaching of scopoletin during calibration; (B-E) = apparent fluorescence decrease due to H_2O_2 additions; [(D-E)-(B-C)] = actual fluorescence decrease due to H_2O_2 additions. None of this accounts for any unpredictable fluorescence changes in the scolopetin......... 57

Figure 3-6. Calibration using standard H_2O_2 solutions. Additions (µL) of scopoletin, HRP and H_2O_2, respectively, to 20 mL DDW are shown. The grey line is the emission of a test sample of known [H_2O_2] (46.9 µM); predicted [H_2O_2] according to calibration from this example is 48.1 µM.. 58

Figure 4-1. Decreases in QSU fluorescence in 0.75 m deep UV-exposed, UV-shielded, and No UV + H_2O_2 (700 nM once-daily addition; 1 enclosure was 1.125 m deep) enclosures from June 5 - July 9, 1996.. 79

Figure 4-2. Changes in DOC fluorescence in 0.75 m deep UV-shielded and UV-shielded + 700 nM H_2O_2, and in 1.15 m UV-exposed enclosures during 5 weeks, from August 18 - September 20, 1996. 80

Figure 4-3. Decrease in fluorescence loss coefficient with increasing depth of enclosure and UV-shielding. Enclosures shielded from solar UVR and treated once daily with a 700 nM pulse of H_2O_2, however, had loss coefficients midway between the UV-exposed and UV-shielded enclosures. Two H_2O_2-treated enclosures were 0.75 m deep, and one was 1.15 m deep. Comparison to a range of depths of UV-shielded and UV-exposed enclosures is made to account for the two depths of H_2O_2-treated enclosures.. 81

Figure 4-4. Mean fluorescence loss coefficients (± S. D.) over 34 days in 0.75 m UV-shielded and No UV + H_2O_2 enclosures, 1.125 m UV-exposed enclosures, and L239 at the Experimental Lakes Area ($P<0.05$; August 18 - September 20, 1996)........... 82

Figure 4-5. Increases in proportion of autochthonous DOC in UV-shielded and No UV + H_2O_2 enclosures. Fluorescence ratios in UV-shielded and No UV + H_2O_2 enclosures were significantly higher than in UV-exposed enclosures, and not significantly different from each other, from day 5 until day 34. Note that, despite bleaching, DOC in UV-exposed enclosures remained allochthonous throughout the 5-week experiment.83

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Figure 4-6. Increases in UVR penetration in enclosures shielded from and exposed to solar UVR, and shielded from UVR with daily 700 nM pulse additions of H_2O_2 during a 5-week experiment (June 5 - July 9, 1996). 84

Figure 4-7. Mean 1% UV depth in UV-shielded, UV-exposed, and No UV + H_2O_2 enclosures and L239, over 5 weeks in the autumn of 1996 (± S. D.). 85

Figure 4-8. Decreases in absorbance loss coefficients with increasing depth of enclosures and UV-shielding. Enclosures shielded from solar UVR and treated once daily with a 700 nM pulse of H_2O_2 had loss coefficients between those of the UV-exposed and -shielded enclosures. 86

Figure 4-9. Mean absorbance loss coefficients in 0.75 m No UV and No UV + H_2O_2 enclosures, 1.125 m UV-exposed enclosures, and L239 at the Experimental Lakes Area (August 18 - September 20, 1996). 87

Figure 4-10a. Chlorophyll a concentrations in UV-exposed, UV-shielded, and No UV + H_2O_2 enclosures over the course of a 5-week experiment (June 5- July 9, 1996). Unanticipated nutrient release from the pulse of pollen resulted in large increases in chlorophyll in all treatments. 88

Figure 4-10b. Chl a in UV-shielded, UV-exposed, and No UV + H_2O_2 enclosures from August 18 - September 20, 1996. Until day 13, chlorophyll in No UV + H_2O_2 enclosures was greater than in the other treatments ($P<0.05$). After this, a bloom over the next 10 days in one of the H_2O_2-treated enclosures was great enough to induce large variance in the treatment mean chlorophyll concentration, even though concentrations in the other H_2O_2-treated enclosures were higher than any in the other treatments (on each day, ** is greater than * by $P<0.05$). 89

Figure 4-11. Algal biomass in shallow enclosures on day 1 of the first experiment (June 5, 1996). Chrysophytes, dinoflagellates, and cryptophytes dominated in all 3 treatments. There were no differences between assemblages of the different treatments ($P>0.05$).90

Figure 4-12. Algal biomass in 0.75 m enclosures shielded from solar UVR and treated once daily with a 700 nM pulse of H_2O_2 for 4 weeks (June 5 - July 9, 1996). By day 13, communities had become dominated by diatoms, rather than chrysophytes. 91

Figure 4-13. Algal biomass in UV-shielded, UV-exposed, and No UV + H_2O_2 enclosures at the end of a 5-week experiment (June 5 - July 9, 1996). Phytoplankton in the UV-shielded and UV-exposed enclosures was dominated by chlorophytes and cyanophytes, and diatoms dominated in the H_2O_2-treated enclosures. In the UV-shielded enclosures there was greater biomass of all taxa except euglenophytes and chrysophytes, compared to the UV-exposed enclosures ($P<0.05$). 92

Figure 4-14. Algal communities in No UV, UV, and No UV + H_2O_2 enclosures (August 20 - September 20, 1996). In the H_2O_2-treated enclosures, on day 18 cyanophyte, diatom, and chlorophyte biomass, and total algal biomass were higher than in the other treatments. On day 30, diatom biomass was higher in the H_2O_2-treated enclosures ($P<0.05$). 93

Figure 4-15. Relationships between chl a and algal biomass in enclosures exposed to and shielded from solar UVR, and enclosures shielded from UVR and treated once daily with a pulse of H_2O_2 (700 nM) (Experiment 1: June 5 - July 9; Experiment 2: August

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18 - September 20, 1996). Concentrations of chl a per unit algal biovolume were higher in the autumn experiment than in the summer experiment........................94

Figure 5-1. Differences in carbon-specific fluorescence (CSF) emission scans (Ex: 370 nm) between allochthonous and autochthonous DOC. For allochthonous DOC, CSF is higher than autochthonous DOC, and the peak fluorescence is also shifted to a higher wavelength. The ratio of emissions at 450 and 500 nm indicates the quality or source of DOC. Suwanee River DOC has a ratio of approximately 1.4, and Lake Fryxell DOC has a ratio of 1.8-1.9 (McKnight et al., In review)................................108

Figure 5-2. History of pH change (± S. D.) in experimentally acidified (L223 and L302S) and reference lakes (L239 and L224) at the Experimental Lakes Area (1985-1996)..109

Figure 5-3. History of DOC concentration change (± S. D.) in acidified (L223 and L302S) and reference lakes (L239 and L224) at the Experimental Lakes Area (1985-1996)..110

Figure 5-4. Carbon-specific fluorescence (fluorescence/mg of C/L; ± S. D.) in acidified lakes (L223 and L302S) and reference lakes (1239 and L224) at the Experimental Lakes Area (1985-1996)...111

Figure 5-5. Patterns of DOC quality change (±S. D.) in acidified lakes (L223 and L302S) and reference lakes (L239 and L224) at the Experimental Lakes Area (1985-1997). As L302S became more acidic, DOC became more autochthonous-like. Recovery from acidification has resulted in shifts back to allochthonous DOC dominance in L223, and a similar shift is occurring in L302S..112

Figure 5-6. General trends in DOC quality shift related to pH change from experimental acidification. L302S has been predominantly autochthonous-like, relative to reference lakes L224 and L239, between 1985 and 1997. L223 has shifted from more autochthonous to very similar to reference lakes (L239 and L224) as its pH became circumneutral...113

Figure 5-7. Peak fluorescence of DOC from reference lakes (L224 and L239), an experimentally eutrophied lake (L227), and two experimentally acidified lakes (L223 and L302S) between 1985 and 1997, related to DOC quality. Unacidified lakes all appear to be dominated by allochthonous DOC, whereas acidified lakes have far more autochthonous or autochthonous-like DOC. With chemical recovery, DOC in L223 returned in quality to that of reference systems...114

Figure 5-8. Comparison of DOC quality and integrated UV transmittance (290-400 nm; 1 cm path length) in alpine and boreal lakes. More allochthonous boreal lakes have lower UV transmittance than do more autochthonous boreal and alpine lakes. A quadratic regression fitting the data is shown. 115

Figure 6-1. Eight lakes (marked in black) surveyed for biological, chemical, and physical parameters in July, 1996, at the Experimental Lakes Area, northwest Ontario, Canada. The star denotes the ELA field station...143

Figure 6-2. DOC concentrations and corresponding 1% depths for UV-a and UV-b in a suite of 8 lakes sampled at the ELA. Modeled values are from Scully and Lean (1994) and Schindler et al. (1996). In situ measurements were performed using a Licor Model LI-1800UM Underwater Spectroradiometer, and mid-July, cloudless solar fluxes were integrated over the ultraviolet spectra. Both UV-b models agree well with each other over the range of DOC concentrations covered by this survey. However, they both

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increasingly deviated from observed UV-b penetration for lakes with less than 4 mg/L DOC. Similarly, Scully and Lean's UV-a model underestimated UV-a penetration in ELA lakes with less than 5 mg/L DOC..144

Figure 6-3. Ordination plot of redundancy analysis (RDA) of algal species and environmental variables in epilithon of 8 lakes of the Experimental Lakes Area. Epilithon was sampled in triplicate at 0.1, 0.3, 0.7, and 1.5 m in two sites in each lake. The relative length of arrows indicates the relative strength of environmental factors in determining algal assemblages. The relative position of taxa along a line parallel to the direction of any environmental variable's arrow indicates alignment of the taxa along a gradient of that variable. The head of any arrow indicates the direction of a positive increase in that variable...145

Figure 6-4. Total algal biomass in epilithon at four depths (0.1, 0.3, 0.7, and 1.5 m) in 8 lakes at the Experimental Lakes Area and the flux of photosynthetically active radiation (PAR) to which the epilithic communities were exposed at solar noon on a typical cloudless day in mid-July, 1996 ($F=0.031$, $P=0.861$). Points are labeled with the number of the lake. 147

Figure 6-5. Dominance of green algae (Chlorophyta), as a proportion of the total algal biomass, in algal assemblages subject to high PAR exposure in epilithon from 4 depths of 8 ELA lakes. Sites exposed to high PAR fluxes were dominated by chlorophytes ($F=7.083$; $P=0.013$). Experimentally acidified L302S (circled), although allowed to recover to pH 5.6, had assemblages with higher proportions of chlorophytes than at sites with similar PAR exposure in unmanipulated lakes. 148

Figure 6-6. Chlorophyte dominance in epilithon subject to high in situ solar UV-b fluxes (% chlorophytes = 0.345(UVB)+0.110; $r^2 = 0.47$; $F = 24.6$, $P < 00001$). Many sites with very low UV-b insolation had low proportions of green algae, although experimentally acidified L302S (circled) had relatively high chlorophyte dominance despite comparable UV-b exposure..149

Figure 6-7. Filamentous green algae dominance in epilithon exposed to a gradient of solar UV-b fluxes in 8 ELA lakes. Depths are 0.1, 0.3, 0.7, and 1.5 m, and fluxes are typical of a cloudless day in mid-July, 1996 (proportion filamentous greens = 0.351(UV-b) + 0.100; $r^2 = 0.482$; $F = 26.1$, $P < 0.001$). The circled data points represent algal assemblages in experimentally acidified L302S, at pH 5.6 at the time of sampling. 150

Figure 6-8. Patterns of diatom dominance in epilithon from sites exposed to different incident solar UV-b fluxes in 8 ELA lakes (diatoms = $0.3307e^{(-1.022UV\text{-}b)}$; $r^2=0.300$; $F=9.41$, $P=0.0056$). 151

Figure 6-9. Dominance of the total pigment complement in epilithon by scytonemin, a photoprotective pigment. The epilithon from sites with the greatest PAR exposure in 8 ELA lakes contained scytonemin comprising 80-90 % of the total pigment mass (Proportion scytonemin=$0.0064e^{0.00258PAR}$; $r^2=0.709$; $F=68.27$, $P<0.0001$)..........152

Figure 6-10. Portion of scytonemin in the total pigment complement in epilithon from sites exposed to different solar UV-b fluxes in 8 ELA lakes (scytonemin = 0.200log(UV-b) + 0.672; $F=13.16$, $P=0.0015$)...153

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Figure 6-11. Ordination plot of the redundancy analysis (RDA) of epilithon invertebrate assemblages and environmental variables in 8 ELA lakes. Epilithon was sampled on south-facing shores at 0.1, 0.3, 0.7, and 1.5 m in each lake............................154

Figure 6-12. Trends of invertebrate densities along a chl a gradient in epilithon from 0.1, 0.3, 0.7, and 1.5 m in 8 ELA lakes: chironomid larvae ($F=107$, $P<0.0001$); oligochaetes ($F=63.2$, $P<0.0001$); and nematodes ($F=21.5$, $P=0.002$)...............155

Figure 6-13. Chironomid densities versus diatom biomass in littoral epilithon of 8 ELA lakes. Experimentally acidified L302S had a much higher chironomid density than any other lake, perhaps as a result of release from predation pressure due to the extirpation of minnow populations. For lakes excluding L302S, chironomid density = $2878(\text{diatom biomass})^{0.303}$ ($r^2=0.296$, $F=6.73$, $P=0.020$)...............................156

Figure 6-14. Trend in chironomid larvae densities in epilithon along a gradient of exposures of solar PAR in 6 ELA lakes. Those sites exposed to the greatest noon PAR flux on a typical July day had the lowest densities of chironomids (chironomid density $= 140200(e^{-0.00158PAR})$; $r^2=0.543$; $F=20.21$, $P=0.0003$).................................157

Figure 6-15. Chironomid larvae densities in epilithon at four depths in 6 ELA lakes along a gradient of solar UV-b exposure. Chironomid densities were greatest at those sites exposed to the lowest noon UV-b flux on a typical July day (chironomid density $= 20500e^{-0.912(UV\text{-}b)}$; $r^2=0.518$; $F=0.518$, $P=0.0017$). 158

Figure 6-16. Chironomid larvae per unit algal biomass in epilithon from four depths exposed to different solar PAR fluxes in littoral zones of 6 ELA lakes (with L302S: chironomids/μg algae $= -80.4\ln(PAR) + 614$, $r^2=0.420$, $F=12.29$, $P=0.0027$; without L302S: chironomids/μg algae $= 90.237e^{-0.00113PAR}$, $r^2=0.246$, $F=5.21$, $P=0.037$). At sites exposed to high fluxes of PAR, there were fewer chironomid larvae per unit algae...159

Figure 6-17. Differences in densities of chironomid larvae per total algal biomass (μg^{-1}) in epilithon exposed to a gradient of natural UV-b flux at different depths in 6 ELA lakes (no L302S: chironomids/algal biomass $= e^{(3.31-0.812/UVB)}$, $r^2=0.333$, $F=6.98$, $P=0.019$; all lakes: chironomids/algal biomass $= e^{(3.51-0.971/UVB)}$, $r^2=0.289$, $F=7.92$, $P=0.013$). There were fewer chironomid larvae per mass algal biomass at sites that were exposed to higher fluxes of UV-b than at those sites exposed to low fluxes....................160

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1. GENERAL INTRODUCTION

Effects of Solar Ultraviolet Radiation in Aquatic Systems

Ultraviolet radiation (UVR; 290-400 nm; Figure 1-1) is involved in many chemical, physical and biological processes and interactions in aquatic systems. The direct negative effects of UVR in aquatic systems can be seen at molecular, cellular, community and whole-lake levels. While long-wave UVR (310-400 nm) and short-wave visible radiation (400 - 480 nm) may be necessary for certain DNA photorepair processes in phytoplankton and fish (Karentz, 1994, Siebeck et al., 1994, Vincent and Roy, 1993), excess UVR absorption by nucleic acids can produce genetic mutations (Harm, 1980, Karentz et al., 1991, Vincent and Roy, 1993).

Stratospheric ozone preferentially absorbs shorter wavelengths in the ultraviolet spectrum, effectively shielding the surface of the Earth from high fluxes of UV-b (290-320 nm). Concern about increasing solar UV-b has stemmed from reports of decreased stratospheric ozone concentrations (Crutzen, 1992). As a result, ~10% increases in UV-b flux have been predicted. Documented cases of increased ground-level UV-b fluxes (Kerr and McElroy, 1993) support the theoretical predictions. As a result of reduced emission of chlorofluorocarbons, UV-b radiation may be near its predicted maximum, leading into an extended slow recovery period to pre-ozone depletion levels in the next half-century (Madronich et al., 1998).

The importance of UVR as an environmental factor has been emphasized even more by recent descriptions of the enhancement of underwater UVR environments by climate change and acidification (Yan et al., 1996). During a 20-year period of warm, dry weather at the Experimental Lakes Area, the number of days ephemeral streams did not flow increased, resulting in reduced DOC inputs into many ELA lakes (Schindler and Curtis, 1997). During the same period, large decreases in DOC concentration were observed in experimentally acidified lakes as a result of chemically-enhanced flocculation and precipitation (Schindler et al., 1996). The large increases in underwater UVR exposure in some lakes (2-3 orders of magnitude), due to increased UV penetration within the lakes as a result of decreases in allochthonous dissolved organic carbon, are likely more important than increased atmospheric transmittance of UV-b (~10% predicted). In addition, higher underwater UVR fluxes as a result of a combination of climate and anthropogenic factors are not expected to decrease with a reversal of ozone depletion. As a result, there has been growing interest in effects of changing UV exposure in aquatic systems.

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Historically, biological effects of UVR in aquatic systems have been studied by isolating particular organisms or classes of organisms in the laboratory or *in situ*, following metabolic or survival changes over short periods of time, from hours to days. For example, many investigators investigated effects of UVR on freshwater or marine phytoplankton (Davidson and Marchant, 1994, El-Sayed et al., 1990, Helbling et al., 1994, Karentz et al., 1991, Smith et al., 1992), invertebrates like corals (Gleason and Wellington, 1993) and zooplankton species (Hessen, 1994), or vertebrates such as amphibians (Blaustein, 1994). In experimental streams, freshwater diatom assemblages exhibited altered growth and complete species replacements as a result of extended UVR exposure (Bothwell et al., 1993). Many of these studies were designed to investigate direct effects of UVR on particular organisms, and indirect effects due to trophic interactions between organisms were often inferred from the results. A fundamental assumption in many of these studies was that lower trophic levels would drive the response of the community as a whole.

One of the first attempts to investigate effects of UV upon more than one trophic level yielded dramatic results, indicating a greater sensitivity of invertebrate grazers to UV-b than the algae they fed upon in experimental streams (Bothwell et al., 1994). This "solar cascade" (Williamson, 1995) involved a counter-intuitive accumulation of algal biomass in UV-exposed streams, due to the release of algal grazing pressure. These results showed that studying one trophic level in isolation would not have led to a recognition of differing sensitivities to UVR among trophic levels. In this instance, biological interactions were mediated by differing sensitivities to UVR. UVR has since been shown to affect a wide variety of organisms of different trophic levels, including decreasing the digestibility of algae by zooplankton grazers (van Donk and Hessen, 1995), marked sensitivities to UV-b in zooplankton themselves (Vinebrooke and Leavitt, 1999, Zagarese et al., 1994), initiating short-term emigration responses in stream invertebrates (Donahue and Schindler, 1998) and impacting selection of spawning habitat in freshwater fish (Williamson et al., 1996). Clearly, increases in the level of UV-b associated with documented and predicted winter stratospheric ozone depletion (Kerr and McElroy, 1993, Stolarski et al., 1992), acidification, and climate change have the potential to impact many basic biological processes and interactions in freshwater systems.

Interactions Between Dissolved Organic Carbon and UV Radiation

Dissolved organic matter (DOM), the primary attenuator of UVR in many freshwater systems, is a mixture of complex, low to high molecular weight organic molecules originating from either microbial and planktonic metabolism within a body of water

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(autochthonous) or from degradation of organic litter in the catchment (allochthonous). Dissolved organic carbon (DOC), the amount of carbon in DOM, is routinely measured and used as an indication of the amount of DOM in water. In addition to its varying molecular weight, general chemical structures of DOC vary according to the nature of the organic source (McKnight et al., 1994, Valla, 1992). Water high in allochthonous DOC can usually be recognized as brown or tea-coloured, due to relatively high aromaticity and resultant spectral absorbance properties. DOC concentrations tend to fluctuate on a daily and annual basis; they generally correlate with the amount of organic matter in the soil of a watershed, the area of wetlands, and the hydrologic flow of the system, peaking in the late spring, decreasing over the course of the summer, and remaining at fairly constant low levels during the winter (Schindler et al., 1992)

The potential for high molecular weight and greatly varied chemical structure contribute to the complexity of DOC and allow it to affect many physical and biological processes (Pospísil, 1992). For example, DOC is a major nutrient source for bacteria (Lindell et al., 1995) and is thus important to higher trophic levels (Vincent and Roy, 1993, Wetzel et al., 1995). Because allochthonous DOC colours water, DOC is a major factor in the attenuation of light in aquatic systems (Kirk, 1976). It also preferentially absorbs lower-wavelength radiation, often resulting in the absorption of UVR within the first few centimeters of water (Cooper and Lean, 1989). Increased DOC can lead to a decrease in the depth to which photosynthesis occurs, and might affect the establishment of thermal layers in water bodies by altering the water clarity (Mazumder and Taylor, 1994, Schindler, 1971). On the other hand, loss or reduced inputs of DOC can lead to large increases in UVR penetration, and increased depth of euphotic zones and epilimnia. DOC also binds metal complexes, and pollutants such as mercury, cadmium, copper, and agricultural pesticides (Kunc, 1992, Winner and Owen, 1991), effectively altering their availability to organisms, and has been shown to increase the permeability of phytoplankton cell membranes (Twiss et al., 1993). Any breakdown of DOC therefore has the potential to alter underwater optical environments, nutrient availability, plant growth, and bioavailability of pollutants. Thus it is important to understand the processes that effect quantitative and qualitative changes in DOC.

UVR can cause the photochemical breakdown, or photolysis, of DOC (Cooper et al., 1989, Cooper et al., 1988, Kieber et al., 1990, Lean et al., 1994). The production of reactive by-products, such as superoxides, hydroxyl radicals and hydrogen peroxide (H_2O_2), can occur as a result of UV irradiation of DOC. Of these three byproducts, H_2O_2 is the longest lived and may also lead to the production of OH^- (Cooper and Lean, 1989, Lean et al., 1994). As a result, it is most frequently the subject of study.

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Superoxide and H_2O_2 have extensive internal biochemical effects in both animal and plant cells, including the changing of enzymatic products used in the dark reactions of photosynthesis (Asami and Akazawa, 1977, Fridovich, 1986, Takabe et al., 1980). However, little is known of the environmental effects of these chemicals on aquatic food-chains. The production of H_2O_2 in lakes has been examined (Cooper and Lean, 1989, Cooper et al., 1988, Lean et al., 1994, Scully et al., 1995, Scully et al., 1996), and assumptions related to its toxicity to organisms have been made (Scully et al., 1996, Scully et al., 1997). Recent work investigated the short-term (~1 hour) effects of added H_2O_2 upon phytoplankton and bacterial production rates in 20 mL incubations of lake water (Xenopoulos and Bird, 1997). Photosynthetic uptake of $^{14}CO_2$ and bacterial incorporation of ^{3}H-leucine decreased when $[H_2O_2]$ was greater than 1000 nM. However, photosynthesis appeared to be stimulated by concentrations of 100 nM, and bacterial leucine uptake was inhibited by concentrations as low as 3.8 nM. Important ecological questions such as community effects of H_2O_2 have not been studied extensively beyond short-term, test-tube-scale metabolic incubations.

There is a possibility of differing UV-sensitivities at different trophic levels. As a result, fundamental approaches to investigations of UV impacts have changed to try to incorporate potential effects of UV radiation on trophic interactions (DeNicola and Hoagland, 1996, Williamson, 1995) and life histories or animal behaviour in aquatic systems (Donahue and Schindler, 1998, Williamson et al., 1996). However, to assume that every organism or every system is equally sensitive to UV, or any other environmental variable, based solely on trophic levels or functional interactions, would be incorrect (Donahue and Clare, 1999, Hill et al., 1997, Hill and McNamara, 1999).

I am interested in the direct and indirect effects UVR has in aquatic ecosystems. Absorbance and transmittance properties of DOC vary greatly among lake types, thereby affecting the underwater optical environment. It is likely that UVR affects the optical properties of DOC, and it is possible that reactive photochemical by-products also lead to qualitative and quantitative changes in DOC in lakes. In addition, UVR affects organisms directly through its energetic potential to cause cellular damage. It also may affect organisms indirectly as a result of photochemical production of radicals like H_2O_2, superoxides and hydroxyl radicals, and through biological interactions due to differing direct sensitivities that organisms at different levels in the food-web may have for it or its chemical by-products. I am also interested in the physical and chemical interactions of UVR and DOC, and their role in the structuring of communities through direct and indirect organismal responses.

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General Outline of Research and Objectives

My research was conducted at the Experimental Lakes Area, located in northwestern Ontario, approximately 100 km southeast of Kenora and Lake of the Woods (49°40'N, 93°44'W). Lakes at the ELA are typical oligotrophic, softwater Canadian Shield lakes with a wide range of allochthonous DOC (Schindler et al., 1992). Many of the ELA lakes are surrounded by a predominantly jack pine (*Pinus banksiana*) forest that has regenerated since an extensive fire in the area in 1980 (Bayley et al., 1992).

Objectives and Hypotheses

Two enclosure experiments were performed to investigate DOC-UV interactions and their effects on planktonic communities. The first experiment was designed to investigate how optical properties and/or removal rates of DOC change as a result of UVR exposure and depth of mixed layer, whether changes in DOC quality and quantity result in altered UVR environments in lakes, and how these may affect planktonic communities. I hypothesized that UVR would have a dominant role in the photobleaching of DOC and a strong negative impact on the planktonic community. I further hypothesized that increased mixing depth would both decrease the rate of this bleaching and reduce the biological impact of UVR.

In the second experiment, I hypothesized that H_2O_2, normally produced as a result of UV-DOC reactions, would have deleterious effects on the planktonic communities and also perhaps lead to bleaching and losses of DOC, independent of the impact of UVR.

A collaborative project was also undertaken, with the assistance of Dr. Michael Turner and Mr. Dave Findlay of the Freshwater Institute, and Dr. Peter Leavitt of the University of Regina, to answer general questions about the potential role of UVR, in the structuring of epilithic communities in lakes at the ELA, compared to other chemical, biological and physical factors. The purpose of this descriptive survey was to identify patterns of epilithic community structure in lakes, and contrast them to gradients of physical and chemical variables among the sites and lakes, including exposure to solar UVR. We hypothesized that UVR would be one of the primary environmental factors affecting the structure of shallow-water, attached communities, as a result of their obligate exposure to it.

Finally, the goal of a spatio-temporal survey of archived water samples was to determine whether DOC quality changed as a result of experimental acidification, and also to test whether there is enough sensitivity in fluorometric techniques to discern changing DOC patterns that may have occurred as a result of changes in climate and eutrophication.

Chapter 3 includes a description of analytical problems that I encountered in, and suggestions for improving, determinations of nanomolar concentrations of H_2O_2 using the

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scopoletin-horseradish peroxidase technique. This is the modified technique that I used in all H_2O_2 determinations.

Study Designs

In both enclosure experiments, polyethylene enclosures, 1 m^2 in cross-section and of varying depths, were suspended from a floating foam and wood frame anchored in the east bay of L239, in close proximity to the ELA camp. Exposure of enclosure water to UVR was controlled with plexiglass covers: OP-4 was used for full-spectrum exposure, and UF-3 was used to filter out UVR (50% cutoff at 380 nm). Experiments were performed in triplicate, and enclosure positions were randomly selected before the apparatus was assembled. All enclosures were assembled in an extended line in an east-west direction, resulting in a dock-like structure that enabled me to move about the enclosures for sampling and H_2O_2 additions.

In the first experiment, three depths (0.9, 1.65, and 2.4 m) of enclosure were used, representing three different mixed-layer depths. Triplicate enclosures of UV-exposed and UV-shielded treatments at each of the depths were used, as well as three additional shallow enclosures shielded from UVR into which were added daily aliquots of H_2O_2, to a total concentration of 700 nM. These additional enclosures were used as a pilot study for the second experiment, used to test the independent effects of the photochemical products of DOC-UV interactions (H_2O_2) and UVR on DOC and plankton. In this second experiment, nine shallow enclosures were used: three exposed to UVR; three shielded from UVR; and three shielded from UVR and injected daily with H_2O_2 to a final concentration of 700 nM.

In both enclosure experiments, absorbance and fluorescence properties and concentration of DOC were used to infer qualitative and quantitative changes in DOC as a result of chemical, biological, and photochemical processes. Measures of biological parameters (invertebrate, algal, and bacterial densities, and chl *a* concentrations) were used to infer changes in community structure, as well as sensitivities of taxa to UVR and H_2O_2.

In the survey of attached communities, we sampled epilithon from four depths at two different south-facing sites in eight lakes. The lakes were selected along a gradient of DOC concentrations and UV-transparencies, including an experimentally acidified lake. In addition, I completed a spatio-temporal survey of 6 different lakes at ELA, with the use of water samples archived at the Freshwater Institute, beginning in 1985 and continuing through 1997 (when I analyzed the samples).

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UVR, UV-b, and UV-a

For logistical reasons, I did not investigate the independent effects of UV-a and UV-b in my enclosure experiments. Had I incorporated another treatment, for example using Mylar® plastic film to filter out UV-b, resulting in a (PAR + UV-a) treatment, the number of enclosures in my first experiment would have increased from twenty-one to thirty-three, and from nine to twelve in the second experiment. In the absence of assistance, this was not possible. For this reason, I did not differentiate between UV-b and UV-a in my experiments, and here I refer simply to UVR in the introductions to these experiments and the interpretation of their results.

In addition, I am most interested in chemical and optical interactions involving DOC and UVR. In the ultraviolet spectrum, DOC most readily absorbs the shorter wavelengths of UV-b, and absorbs the longer wavelengths of high-UV-a the least. As a result, the underwater spectrum varies according to the concentration of DOC and/or the depth at which the spectrum is measured. In lakes, inter-lake differences or intra-lake changes in concentrations and absorbance qualities of DOC thus are more important than ozone-related changes in determining the transmittance of UVR (Schindler et al., 1996) or portions of the spectrum - including ratios of UV-b and UV-a. For this reason, I have minimized discussion of ozone-related increases in UV-b flux in the context of exposure of aquatic systems.

It could also be argued that the nature of the energetic continuum of radiation (Figure 1-1) renders arbitrary the distinction between discrete portions of the ultraviolet spectrum as "a" and "b" in discussions of chemical or physical effects. The chemical or physical effect of particular wavelengths or portions of the spectrum depends solely on the radiative flux and the photon energy. General differentiations have been made between UV-b and UV-a as a result of this continuum of quantal differences, however this is most often in the context of their effects on organisms. In differentiating between large portions of the solar spectrum, one must acknowledge that solar radiation at 330 nm ("UV-a") is more similar to that at 318 nm ("UV-b"), in terms of flux and energetic potential, than to that at 390 nm, which is still grouped according to popular nomenclature under the general umbrella of the title "UV-a".

However, different portions of the ultraviolet spectrum affect organisms to different degrees. Organisms have evolved a number of protective mechanisms for dealing with high-energy UVR. Pigments produced for protection from or detection of solar radiation have distinct absorbance properties, often effective for only particular band-widths. In addition, biological weighting functions (Neale et al., 1998, Neale et al., 1994) and biologically active dosage rates or action spectra (Diaz et al., 1994, Madronich, 1994) have

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been developed that incorporate combinations of solar flux, photon energy, and wavelength-dependent organismal sensitivity. Similarly, chemical action spectra, or apparent quantum yields or formation efficiencies, have been described in determining relative efficacy of wavelength-specific solar energy fluxes in chemical processes (Scully et al., 1996, Scully et al., 1997).

In describing my experiments and discussing their implications (Chapters 2 and 4), I intentionally refer only to UVR because of the treatments I used and the resulting inability to differentiate between UV-b and UV-a. However, decisions made in describing the effects of UV-b and PAR on epilithic communities (Chapter 6) were based on mathematical and modeling constraints, rather than physical, chemical, or biological assumptions. In non-linear correspondence analysis, variables are eliminated according to certain criteria, including colinearity. UV-a flux was colinear to both UV-b and PAR, and as a result it was excluded from further inclusion in the modeling process. UV-b and PAR, however, were not colinear to the degree that would dictate the exclusion of one or the other, and thus they were considered by the model. For this reason only, in Chapter 6, I describe community patterns relative to PAR and UV-b exposure and not UV-a exposure, despite the growing body of evidence of the biological and chemical importance of wavelengths in the UV-a region of the spectrum. UV-a's collinearity to both UV-b and PAR, and the similarity of relationships of both integrated UV-b and PAR fluxes with many biological variables, suggest that similar relationships would exist between integrated UV-a fluxes and the same biological variables in the survey.

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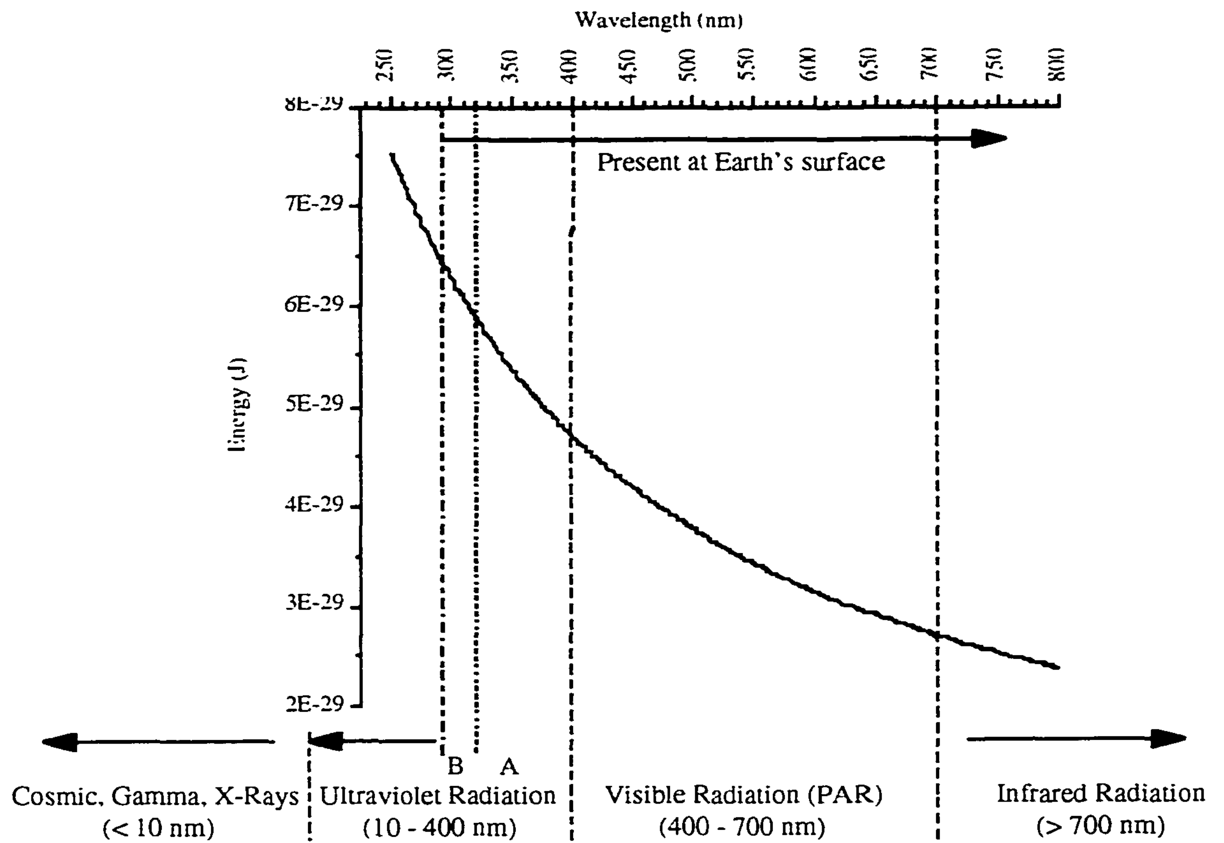

Figure 1-1. Energetic continuum of radiation wavelengths of the solar spectrum. UV-b wavelengths below 292 nm are filtered out by the stratospheric ozone layer.

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2. THE EFFECTS OF SOLAR ULTRAVIOLET RADIATION AND MIXED-LAYER DEPTH ON TRANSPARENCY AND COMMUNITIES IN MESOCOSMS IN BOREAL LAKES

Introduction

There has been increasing evidence of the synergistic effects of climate change, acidification and ozone depletion on chemical and physical environments within boreal lakes (Gorham, 1996, Schindler et al., 1996b, Yan et al., 1996). One important consequence of acidification is the loss of dissolved organic matter (DOM), usually measured as dissolved organic carbon (DOC) (Dillon and Molot, 1997, Schindler and Curtis, 1997), which is the primary attenuator of solar radiation. In addition, warming trends have caused lower DOC inputs to lakes, as a result of decreased streamflow (Schindler et al., 1996a, Schindler et al., 1997). These lower DOC concentrations have caused increases in lake thermocline depths and water transparency (Mazumder and Taylor, 1994, Schindler et al., 1996a) and exposure of aquatic organisms to solar ultraviolet radiation (UVR) (Schindler et al., 1996b). DOC is therefore the critical component that ties together climate change, acidification, and ozone depletion in the enhancement of UVR fluxes in lakes (Schindler et al., 1996b).

Many aquatic organisms are sensitive to UVR. Algae exposed to high UVR can exhibit decreased growth or photosynthetic rates (Davidson and Marchant, 1994, Jokiel and York, 1984, Moeller, 1994, Neale et al., 1994, Ryan and Beaglehole, 1994, Vincent and Quesada, 1994). Similarly, zooplankton, including rotifers (Vinebrooke and Leavitt, 1999, Williamson et al., 1994), copepods (Ringelberg et al., 1984, Williamson et al., 1994), and *Daphnia* spp. (Hessen, 1994, Williamson et al., 1994, Zagarese et al., 1994) may display decreased survival, growth, or reproduction in the presence of high UVR. In addition, benthic invertebrates such as chironomid larvae have been shown to be sensitive to UVR, colonizing UVR-exposed substrates less than those shielded from UVR over a period of weeks (Bothwell et al., 1994). However, organisms inhabiting natural, high-UVR systems often have compensatory behavioural or protective physiological mechanisms that allow them to cope with high fluxes of UVR, such as the selection by yellow perch of deeper spawning sites in lakes with low attenuation of UVR (Williamson et al., 1996), increased drift of stream invertebrates when exposed to high UVR (Donahue and Schindler, 1998, Kiffney et al., 1997), or the development of protective pigmentation or vertical migration strategies in some planktonic crustaceans and algae (Ringelberg et al., 1984, Siebeck and Böhm, 1994).

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There is some evidence of the possibility of differing sensitivities to solar UVR at different trophic levels (Bothwell et al., 1994, Cabrera and Pizarro, 1994). As a result, investigations of UVR impacts have changed to incorporate studies of potential effects of UVR on trophic interactions (DeNicola and Hoagland, 1996, Williamson, 1995), life histories, or animal behaviour in aquatic systems (Donahue and Schindler, 1998, Kiffney et al., 1997, Williamson et al., 1996). It is already clear that different trophic interactions and ecosystems are not equally sensitive to UVR (Donahue and Clare, 1999, Hill et al., 1997, Hill and McNamara, 1999).

Models have been developed to predict UVR attenuation in lakes and have been based on the concentration of DOC (Morris et al., 1995, Schindler et al., 1996b, Scully and Lean, 1994). However, DOC can be bleached by solar radiation (Curtis and Adams, 1995, Molot and Dillon, 1997a, Molot and Dillon, 1997b, Morris and Hargreaves, 1997) or chemically altered by acidification (Donahue et al., 1998), resulting in decreased absorbance of UVR, likely due to a reduction in the UVR-absorbent aromatic portions of DOC molecules (McKnight et al., 1994). Because UVR is preferentially attenuated by DOC (Scully and Lean, 1994), anything that decreases the concentration of DOC in lakes or its optical absorbance has the potential to increase UV penetration and exposure of aquatic organisms. As a result, predictive UVR models based on concentrations of DOC alone may be restricted in their applicability to some systems.

By using enclosures of different depths to simulate different mixed-layer depths, hence different exposures to UVR, I investigated the role of UVR in changing the properties of DOC in boreal lakes. I determined whether mixed-layer depth affects the rates of degradation, bleaching, or mineralization, and quality and quantity of DOC, and describe changes in optical environments and biological communities resulting from interactions between mixed-layer depth and UVR exposure.

Methods

An enclosure experiment was carried out in the East Bay of L239 at the ELA, which is well mixed with the main body of the lake. Triplicate enclosures, 1 m X 1 m in cross-section and 0.75, 1.5, and 2.25 m deep, were collapsed vertically, sunk, and filled with lake water by pulling the tops to the surface, as described by Curtis and Schindler (1997). Two optical treatments of each set of enclosures of different depth were used:, UV-exposed and UV-shielded. UVR exposure was controlled by covering each enclosure with either UVR-impermeable (UF-3) or UVR-permeable (OP-4) plexiglass (Figure 2-1). The experiment began with the filling of the enclosures on 4 June 1996, and sampling began on 5 June 1996. The placements of the treatment replicates were randomized, and enclosures

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were attached to a floating wooden frame, thus forming a long raft for ease of sampling. All enclosures were aligned in an East-West orientation to ensure exposure to equal diurnal solar regimes. Enclosures were stirred with a paddle each morning, and also before each enclosure was sampled.

Every 4 - 5 days, for the 5 week duration of the experiment, enclosures were sampled for analyses of DOC absorbance and fluorescence. Samples were also taken for chlorophyll *a* (chl *a*) concentration, and phytoplankton, zooplankton, and bacterial identification and enumeration. DOC concentrations were measured on Day 26 (1 July), and again on Days 30 and 34. Determinations of nutrient concentrations were also done periodically during the experiment. Throughout the experiment, integrated vertical profiles of unfiltered water from the enclosures were collected using a 1-inch diameter, 2.5 m long tygon tube. Water was collected in 6 L Nalgene containers, pre-rinsed with sample water, and sub-samples were used for all analyses.

Optical absorbance (328 nm) was measured spectrophotometrically relative to distilled, deionized water (DDW; Milli-Q System, Millipore, Bedford, MA, USA) in a 10 cm quartz cell, from which 1% UV penetration depth was calculated (Wetzel and Likens, 1991). Carbon-specific absorbance (CSA; $(mgC/L)^{-1}$) was used to determine whether absorbance changes occurred when normalized for the mass of DOC, as described previously (Blough et al., 1993, Molot and Dillon, 1997a). By normalizing for mass of carbon, one can identify qualitative changes in absorbance properties of DOC that may result from biological or chemical activity.

Uncorrected fluorescence scans of DOC were performed on a Shimadzu RF-1501 scanning spectrofluorometer with a xenon lamp (concave, non-aberration excitation/emission monochromators, with blazed holographic grating, F/2.4, 900 grooves/mm; dynode feedback light source compensation system with monochromatic light monitoring function; and photomultiplier tubes for both excitation and emission side detection; Mandel Scientific, Guelph, ON, Canada) and using optically clear quartz cuvettes (pathlength = 1 cm). Variations in lamp spectral intensities were corrected using scans of a standard 1 μg/L quinine sulfate solution in 0.1 N H_2SO_4 (Scully and Lean, 1994) and absolute fluorescence values are reported in quinine sulfate units, where: 1 QSU = fluorescence at a given excitation and emission wavelength of the standard quinine sulfate solution.

DOC fluorescence was measured at an excitation wavelength of 354 nm and emission wavelength of 496 nm according to Scully and Lean (1994) and used here as a proxy for the amount of coloured dissolved organic matter. In addition to absolute fluorescence, carbon-specific fluorescence (CSF; QSU/mgC) was used to determine whether DOC

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fluorescence changed when normalized for mass of carbon in a liter of water. In this way, I could determine whether changes in peak fluorescence were a result of either DOC loss or qualitative changes, for example as a result of bleaching.

Spectrofluorometric analyses of DOC were performed on the samples taken 36 days after the end of the experiment, to test whether relative differences in DOC quality (autochthonous versus allochthonous) developed in enclosures of differing depth and UVR exposure (McKnight et al., In review). Spectrofluorometric analyses of quality were not done throughout the experiment because the description of the technique only became available at the end of the experiment. For this analysis, scans of sample blanks of DDW were performed to remove effects of Raman scattering. Excitation radiation was fixed at 370 nm, and scans of emission intensities were performed from 370 nm to 650 nm. After subtraction of DDW blank fluorescence values from those of the acidified samples, the ratio of the emission intensity at 450 nm (1 QSU = 16.72 arbitrary fluorescence units) to that at 500 nm (1 QSU = 8.01 arbitrary fluorescence units) was calculated. A range of values from approximately 1.4 to 1.9 has been used elsewhere to indicate qualitatively the type of DOC present; a ratio of 1.4 is typical of terrestrially-derived DOC, such as in a peat bog or low-order stream or lake, and a ratio of 1.8-1.9 is typical of microbially-derived DOC, such as in Antarctic lakes (McKnight et al., In review) which have little terrestrial vegetation or organic soil in their catchments. Similar high values have been found in acidified lakes, in which acidification has caused chemical oxidation or precipitation of the aromatic portions of DOC molecules, leaving behind "autochthonous-like" (AUDOC) DOC of low aromaticity (Donahue et al., 1998).

Phytoplankton chl *a* was extracted using 95% ethanol, and filtered using pre-combusted Whatman GF/F glass filters. The concentration of chl *a* was measured fluorometrically according to a standard procedure (Welschmeyer, 1994). Unfiltered samples for phytoplankton enumeration were preserved in 4% Lugol's preservative. Counts and identification were performed on an inverted microscope at magnifications of 125X and 400X with phase contrast illumination. All counts were done by a modified Utermöhl technique (Nauwerck, 1963), and wet biomass estimates were made from approximations of cell volumes of each species, according to best-fit formulae for different taxa (Vollenweider, 1974).

Unfiltered samples were preserved in 5% formalin for bacterial enumeration. Bacteria were counted using the DAPI fluorescence technique, and counted at 400X magnification (Porter and Feig, 1980).

For zooplankton samples, 6 L of water from the shallowest (0.75 m) enclosures were filtered through a 200 μm plankton net, and 12 L samples were taken from the 1.5 and

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2.25 m enclosures. More than 6 - 12 L were not filtered for zooplankton, to prevent disruption of the community structure.

Repeated-measures ANOVA was used to determine whether quantitative measures of optical and biological factors varied with UV treatment, depth, and time. For DOC and DIC concentrations, fluorescence, 1% UV depth, and carbon-specific fluorescence and absorbance, RM-ANOVAS were run for the entire sampling periods covered, normalizing for the effect of time on experimental results. For chl *a*, RM-ANOVAS were performed for both the entire experimental period and for the first 22 days, after which a large pulse of spring pine pollen entered mesocosms, resulting in algal blooms in all enclosures. In addition to RM-ANOVAs, one-way ANOVAs and *post-hoc* analyses using the Modified Least-Square Difference (Bonferroni) test were performed with a significance level of 0.05 to determine whether statistical differences existed between the 6 combinations of UV (2) and depth (3) treatments on any given day during the experiment (5 d.f. within groups; 12 d.f. between groups). SPSS 6.1.1 (1995) was used for all statistics, including best-fit regressions for calculation of loss coefficients for fluorescence data and depth:bleaching rate curves.

Results

DOC concentration, fluorescence, and absorbance

DOC concentrations were not significantly different between days 26 and 34 as a result of depth of enclosure or UV-exposure for any of the treatments (RM-ANOVA; $0.585 \geq P \geq 0.182$) (Fig 2-2). The only significant difference in [DOC] between treatment combinations on the three sampling days was on Day 26, between the 0.75 m UV-exposed enclosures (7.56 mg/L) and the 2.25 m UV-exposed enclosures (7.05 mg/L) (1-way ANOVA; Bonferroni test; $P<0.05$). On both days 30 ($P=0.230$)and 34 ($P=0.902$), DOC concentrations were not significantly different between any two treatment groups (1-way ANOVA). For DIC (not shown), no two groups on any of the three sampling days were different ($0.879 \geq P \geq 0.601$; 1-way ANOVA; Bonferroni test).

Although significant decreases in DOC peak fluorescence occurred in all enclosures as a result of time ($P<0.001$), depth ($P<0.001$), light ($P<0.001$), and combinations of all three ($P=0.037$; RM-ANOVA), the rates of decrease in fluorescence were much greater in the UV exposed mesocosms (Fig 2-3). The greatest decrease in fluorescence occurred in 0.75 m enclosures and the lowest decrease occurred in 2.25 m enclosures. The decrease in fluorescence was rapid enough in the shallowest UV-exposed enclosures that after the 24-hour period between the setup of the enclosures and first sampling, DOC fluorescence

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(19.9) was already lower than in the 2.25 m enclosures (1-way ANOVA; Bonferroni test; P=0.006). During the remainder of the experiment, differences in DOC fluorescence between treatment groups increased. Loss of fluorescence over the course of the experiment was most rapid in the shallowest UV-exposed enclosures, and slowest in the deepest UV-shielded enclosures. Half-times of exponential loss coefficients are listed in Table 2-1.

UVR penetration (327 nm) increased significantly in the enclosures during the 5-week experiment (Fig. 2-4). There were significant interactions between depth and time, UV treatment and time, and all three factors (RM-ANOVA; P<0.01). By the end of the experiment, the 0.75 m UV-exposed enclosures had a 1% UVR depth that was at least ~1.5x that in the UV-protected treatments. Similarly, penetration of UVR in the 0.75 m UV-exposed enclosures was ~1.5x that in the 2.25 m UV-exposed enclosures. 1% UVR depth increased by 56% in the 0.75 m, 33% in the 1.5 m, and 23% in the 2.25 m UVR-exposed enclosures. Increases in UVR penetration in the UV-shielded enclosures were 21% (0.75 m), 13% (1.5 m), and 8% (2.25 m).

UVR-induced decreases in carbon-specific fluorescence (CSF) were observed, and inversely related to the depth of enclosures (Figs. 2-5). CSF differed significantly between treatment groups as a result of both depth and light treatment (P<0.001; RM-ANOVA). However, due to the limited period covered by DOC sampling, changes within treatment groups over the 9 days were not significant as a result of either depth or light, normalized for time. CSF in UV-exposed enclosures was less than in UV-shielded, and was proportional to enclosure depth. By the end of the experiment, CSF in the UV-exposed enclosures was 22-36 % less than in the UV-shielded enclosures of the same depth.

Similar to CSF, carbon-specific absorbance (CSA; 327 nm) was lowest in UV-exposed enclosures and was proportional to enclosure depth (P<0.001; 1-way ANOVA; Fig. 2-6). At the end of the experiment, CSA in UV-exposed enclosures was 11-24 % less than in the UV-shielded enclosures of the same depth.

UV effects on phytoplankton and bacteria

Over the full course of the experiment, there was no significant treatment effect or interaction between time, depth, or UV exposure on chl *a* concentration (RM-ANOVA; 0.149≤P≤0.714). Interpretation of changes in chl *a* concentrations was complicated by a pulse of pollen from the surrounding jack pine forest that lasted several days between Days 13 and 17, resulting in algal blooms in all enclosures on Day 26 (Fig. 2-7) This was likely a result of increased phosphorus inputs (Lee et al., 1996). However, if analysis is limited to the 22 days before the pollen-induced algal blooms in all enclosures (Fig. 2-7), UV

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treatment had a significant interaction with time in changing chl *a* in the enclosures (RM-ANOVA; $P<0.05$). By Day 18, chl *a* concentrations in the UV-exposed treatments were less than in the 2 shallower UV-shielded treatments ($P<0.05$; Fig. 2-7).

Algal biomass decreased as a result of UVR exposure, although taxa and biomass were not different in any of the treatment groups on Day 1 ($P>0.05$; Fig 2-8a). On Day 35, however, algal biomass was ~140% higher in the 0.75 m UV-shielded enclosures than in the 0.75 UV-exposed enclosures (Fig 2-8b). Densities of most classes of algae were also significantly higher in the shallow UV-shielded enclosures than in the shallow UV-exposed enclosures, especially the euglenophytes, cyanophytes, diatoms and cryptophytes (Fig. 2-8b). In addition, algal biomass in the shallowest UV-shielded enclosures was ~120% higher on Day 35 than in the intermediate and deep enclosures of both optical treatments, consistent with patterns of chl *a* concentrations.

The total biomass per unit volume, mean cell volume, and densities of the majority of algal species did not differ between 0.75 m UV-exposed and -shielded enclosures (Appendix A). However, of the 69 species observed in the shallow UV-shielded enclosures 24 were absent from the UV-exposed enclosures, including 6 of the 7 *Staurastrum* species found. *Tabellaria fenestrata* biomass was 85% lower in the shallow UV-exposed enclosures, accounting for the majority of the difference in diatom biomass between the two 0.75 m treatments ($P=0.038$; Figure 2-8b). In addition, the large relative differences in biomass of UV-sensitive *Coelosphaerium* spp. (-90% in UV-exposed enclosures) and *Radiocystis geminata* Skuja (-80% in UV-exposed enclosures) accounted for the majority of difference in cyanophyte biomass between the shallow treatments. Biomasses of *Coelosphaerium minutissimum*, *Chlorella vulgaris*, and *Trachelomonas armata* were higher in the shallow UV-shielded enclosures ($P\leq0.05$), and unidentified large chlorophytes had lower biomass ($P=0.067$). *Mougeotia* spp. also had lower biomass in the UV-shielded enclosures, whereas 9 other taxa had higher biomass ($0.097\leq P\leq0.149$). Three algae, *Sphaerozosma granulatum*, *Scenedesmus* spp., and *Zygnema* spp., were present in the shallow UV-exposed enclosures but absent from the UV-shielded. Based on these differences, taxa that did exhibit differences between UV treatments generally responded negatively to the presence of solar UVR, except for large chlorophytes and *Mougeotia* spp. In addition, the negative response to UVR of a relatively small number of species resulted in large differences in total algal biomass. Thus, these UV-sensitive species, and especially *Tabellaria fenestrata*, *Coelosphaerium* spp., and *Radiocystis geminata* Skuja, were very important in determining the overall structure - and perhaps function - of the phytoplankton communities in my enclosures.

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For mean cell volume, *Chlorella vulgaris* cells were smaller and *Staurastrum pentacerum* cells were larger in the UV-exposed enclosures ($P<0.05$). Five other taxa also had larger average cell sizes in the shallow UV-exposed enclosures ($0.116 \leq P \leq 0.149$). Conversely, *Cosmarium depressum* cells were smaller ($P=0.116$) in the UV-exposed enclosures. In terms of cell density, cells of *Coelosphaerium minutissimum*, *Microcystis aeruginosa*, *Radiocystis geminata*, and *Trachelomonas armata* were more numerous in the UV-shielded enclosures ($P<0.05$), and unidentified large chlorophyte cells were more numerous in the UV-exposed enclosures ($P=0.067$). Nine other taxa were also more dense in the UV-shielded enclosures ($0.097<P<0.158$), and *Mougeotia* spp. filamentous green algae were less dense ($P=0.133$).

Bacterial densities and biomass were highly variable. There was no treatment effect on either, other than time ($P<0.05$; Figs. 2-9 and 2-10). On Day 18, after the pollen pulse, bacterial biomass in the 0.75 m UV-exposed enclosures was higher than in all treatment groups ($P<0.05$), other than the 2.25 m UV-shielded enclosures. Bacterial densities were also higher in the 0.75 m UV-exposed enclosures than the 2.25 m UV-exposed enclosures ($P<0.05$). However, despite the differences between treatments on other sampling days, there were no discernible patterns of change in bacterial biomass or density as a result of either UVR exposure or enclosure depth.

Zooplankton

Zooplankton densities were low in general, and there appeared to be little treatment effect (Appendix B). However, there were lower densities of male *Diaptomus minutus* and *Epischura lacustris* (calanoid copepods) in the 0.75 m UV-exposed enclosures ($P=0.061$ and 0.001, respectively). In addition, *Holopedium gibberum* was less dense in UVR-exposed enclosures ($P=0.131$). Contrary to these negative sensitivities to UV exposure, *Acroperus* cf. *harpae* (a benthic chydorid) were much more dense in the 0.75 m UV-exposed enclosures than in the shielded ones ($P=0.007$) and not present at all in the 2.25 m enclosures, suggesting development of a benthic periphyton community and perhaps some resistance to UVR.

Discussion

DOC quality and quantity and UV transmittance

Decreases in DOC fluorescence and absorbance in the UV spectrum in enclosures exposed to solar UVR were greater than in enclosures shielded from UVR, indicating that DOC was being bleached as a result of UVR. Loss of DOC in lake water as a result of

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photooxidation has been shown to be much slower than in stream water, suggesting that DOC in lake water may be more subject to bleaching than mass losses (Molot and Dillon, 1997b). All of the enclosures had similar DOC concentrations, indicating that UV did not enhance mineralization of DOC to DIC. It is also possible that photolytic oxidation of coloured DOC into a more refractory uncoloured component was occurring, or that removal of DOC was balanced by coincident increases in autochthonous DOC (Kieber et al., 1990). However, fluorescence analysis of DOC quality indicated that AUDOC production was greater in the UV-shielded enclosures than in the UV-exposed ones, and greater in the shallowest enclosures than in the deeper ones (unpubl. data; Chapter 4; Donahue et al., 1998). Increased UVR penetration in the UV-shielded enclosures may have been due to a combination of bleaching of allochthonous DOC and increased proportions of low-aromaticity AUDOC. In the UV-exposed enclosures, CSF and CSA decreases did not coincide with an increase in the DOC fluorescence ratio, as they did in the UV-shielded enclosures, and thus colour changes likely were not associated with an increase in the proportion of AUDOC. Instead, the greater decreases in CSF and CSA coincident with continuous allochthonous DOC signals in UV-exposed enclosures suggest that losses in colour were due to bleaching by UVR.

Fluorescence loss in the UV-exposed enclosures was faster than colour losses observed in previous enclosure experiments at the ELA (Curtis and Schindler, 1997). However, their unshielded enclosures were 3.0 m deep, compared to the range of shallower enclosures that I used. An extrapolation of the half-times calculated for fluorescence loss in my UV-exposed enclosures to one 3 m deep yields a predicted half-time for fluorescence loss of 120 days, very close to the 122 day half-time for colour found by Curtis and Schindler for uncovered enclosures. The similarity between Curtis and Schindler's observations and mine suggests that the majority of colour loss in DOC in enclosures was due to UVR, at least over time scales on the order of weeks or months. It also confirms that it is possible to compare my results to much longer experiments, that the portion of the solar spectrum absorbed by UV-transparent plexiglass was negligible, and that my UV-exposed treatments were very similar to full solar exposure.

Without a strict mechanistic description achieved through a variety of chemical analyses, I have relied on changes in fluorescence and absorbance characteristics of the DOC to infer the changes in source of DOC, or resistance to change in the case of the UV-exposed enclosures. As elsewhere (Donahue et al., 1998), I have used the term "autochthonous-like" to describe apparently low-molecular-weight, less aromatic DOC in the UV-shielded enclosures. I hesitate to identify it as autochthonous because I am not certain of the mechanism(s) behind its production. With the use of radio-labeled humic

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substances or carbon isotopes, one would be better able to identify potential mechanisms resulting in these sorts of DOC quality change, including autochthonous carbon fixation or microbial metabolism of allochthonous DOC (Wetzel et al., 1995), or perhaps other photochemical processes such as those that include interactions between reactive oxygen species and DOC.

The differences in optical qualities of allochthonous, AUDOC, and photo-bleached DOC observed in my enclosures indicate that underestimation of levels of UVR in aquatic environments may occur when using conventional DOC-UVR predictive models. In lakes such as saline lakes (Curtis and Adams, 1995), or lakes that have been subjected to decreased allochthonous DOC inputs due to climate warming (Schindler et al., 1990) or increased removal of aromatics due to acidification (Donahue et al., 1998), these models will likely underestimate UVR penetration as a result of increased mass-weighted transparency of DOC to UVR. Without incorporating the optical quality of DOC, these models may be limited in their ability to infer UVR penetration for lake types that were not included in their calibration sets.

Phytoplankton

Differences in algal biomass, cell sizes, densities, and taxonomy as a result of depth and UVR treatments indicate species-dependent sensitivity to UVR that ranged from reduction (negative sensitivity) to stimulation (positive sensitivity), as found also by Xiong et al. (1996). In my experiment, large reductions in biomass of a few species accounted for the majority of the differences in diatom and cyanophyte biomass observed between UV treatments. Similar sensitivity of diatoms to UV radiation also has been observed in UV-exposed experimental streams, where periphytic diatom production (*Tabellaria fenestrata*, *T. flocculosa*, *Fragilaria crotonensis*, and *F. vaucheriae*) was 30-40% less than in UV-shielded streams (Bothwell et al., 1993). The absence from my shallow UV-exposed enclosures of 35% of the algal species observed in the UV-shielded enclosures suggests that phytoplankton assemblages from L239 exhibit a broad negative sensitivity to UVR. More importantly, the large reductions in total biomass observed as a result of a few specific sensitivities to UVR indicate that sensitivities of only a few critical species can still lead to large changes in phytoplankton standing crop, with potential negative trophic consequences.

In the case of light limitation of photosynthesis, one would expect higher algal biomass in shallower enclosures and, when combined with negative UV-sensitivity, highest biomass in shallow, UV-shielded enclosures. My observations of higher chl *a* concentrations and total algal biomass in the shallowest NO UV treatments suggest that

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phytoplankton were stimulated by high levels of PAR and removal of apparent UV-suppression. Conversely, the similarity in biomass among all the remaining treatments suggests that there was a balance between UVR sensitivity and PAR-dependence of phytoplankton. In the shallow UV-exposed enclosures, any potential benefits of increased PAR likely were negated by the direct limitation by UVR, or perhaps indirectly via its photochemical byproducts.

The unforeseen occurrence of a wind-driven pollen pulse in the middle of my enclosure experiment provided serendipitous support for the idea that a trade-off existed between nutrient limitation and that by PAR and/or UVR. A large increase in bioavailable P occurs after a pollen pulse (Lee et al., 1996). If the communities in the enclosures were P-limited, like in most ELA lakes (Schindler, 1971), one would expect increases in algal production with increases in bioavailable P. Contrary to Bothwell et al.'s (1993) findings that degree of UVR inhibition was independent of P limitation, the lack of difference between optical treatments after pollen-induced increases in algal standing crop in all treatments suggests that algae were less limited by solar radiation than by nutrients in my enclosures. In addition, it suggests that nutrient limitation in my enclosures may have increased the degree of UV inhibition of phytoplankton.

The increases in algal biomass and chl *a* concentration that occurred soon after the pulse allude to the importance of nutrient limitation relative to UV-inhibition. However, inference of interactions between environmental changes and algal communities that are based in part on chl *a* concentrations can be misleading. For example, in high-light conditions, light saturation of chlorophyll can occur, and lower concentrations of chl *a* are needed for carbon fixation (Vincent et al., 1984). Similarly, the chlorophyll complement can increase in response to low-light conditions to prevent pigment saturation and ensure maximal photosynthetic efficiency. Although biomass and chl *a* in all enclosures increased after the pollen pulse, the lack of consistent differences in algal biomass or chl *a* between depth treatments suggests that interactions and physiological processes other than differential sensitivities to UVR or nutrient availability were in effect.

Certain phytoplankton may be very sensitive to UVR damage when constrained to shallow, high-exposure strata within a lake during windless periods in midsummer. During extended periods when mass movement of water in the mixed-layer is minimal, phytoplankton in surface layers, sinking only centimeters per day (Fisher et al., 1996), could suffer extreme UVR exposure. While diatoms like *Tabellaria fenestrata* may not comprise a large part of pelagic phytoplankton populations, they are a significant part of littoral communities. Thus, increased UVR-exposure in littoral zones of lakes also may

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have the capacity to change algal community structure and productivity in periphyton over relatively short time periods as a result of differing sensitivity among taxa.

Zooplankton

The majority of UVR-zooplankton studies have been on *Daphnia* species, and under laboratory conditions with artificial lights. Different response mechanisms have been observed, including avoidance of UV-B by *D. galeata* and clones of *D. pulex* via vertical migration (Hessen, 1994, Siebeck and Böhm, 1994). In addition, *D. pulex obtusa*, a non-migrating cladoceran from a shallow mountain pond, has been shown to have greater recovery capacities for reducing lasting UVB damage (Siebeck and Böhm, 1994). Photoprotective compounds like cuticular melanin (Hessen, 1994) and antioxidants like carotenoid pigments (Hessen, 1994, Ringelberg et al., 1984) also protect zooplankton from UVR-induced damage in alpine and arctic lakes, although the latter generally contain much higher concentrations in copepods than in cladocerans. However, there have been few experimental examinations of impacts of solar UVR on natural zooplankton communities.

In one experiment, natural zooplankton communities in 3 L bags were exposed for 3 days to natural radiation at two different depths in an oligotrophic and a eutrophic lake (Williamson et al., 1994). Some zooplankton exhibited substantial UV-induced mortality in the oligotrophic lake, but none was observed in the eutrophic one, even at the shallowest depth of 0.2 m. Contrary to Williamson et al.'s findings that survival of both male and female *Diaptomus* spp. was reduced in the presence of UV-B to a depth of 6 m, I observed greater densities of *Diaptomus minutus* males in my shallow UV-exposed enclosures than in UV-shielded. However, total numbers of *D. minutus* did not differ between treatments in my experiment, similar to Williamson et. al.'s results from the eutrophic lake. In addition, *Diaphanosoma brachyurum* survival was reduced in Williamson et al.'s shielded and unshielded bags at shallower depths, indicating sensitivity to longer-wavelength UVR.

In general, densities of zooplankton in my shallowest enclosures were higher than in the deep enclosures, but this may have been a result of "bag effect". Substantial periphyton developed on the inner sides of enclosures - and bottoms of shallow enclosures - over the course of the experiment. In my experiment, the development of periphyton was accompanied by a shift of the community from a planktonic one to one more similar to a littoral community, especially in the shallow enclosures. For example, *Acroperus* cf. *harpae*, a benthic chydorid, was much more dense in the UV-exposed 0.75 m enclosures than in the UV-shielded, and not present at all in the 2.25 m enclosures. It is possible that the littoral zooplankton assemblages that developed in my enclosures are better adapted to high UVR fluxes than planktonic ones. The general lack of treatment effect on zooplankton

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in my study also may be a result of a longer generation time than for bacteria or algae. In the absence of direct mortality, UVR-related impacts that could include reduced reproductive output might take longer to develop and be observed in a community of longer-lived organisms than permitted in a short-term 5-week experiment. It is also possible that the zooplankton, many of which are capable of migrating vertically within the enclosures, were able to seek refuge from UVR within the bags and thus did not exhibit significant effects of UVR on densities. Many of the zooplankton taxa in a eutrophic lake did not exhibit the sensitivities seen in an oligotrophic lake (Williamson et al., 1994). It is possible that UVB fluxes were similarly sufficiently attenuated by DOC in my enclosures to reduce the exposure of zooplankton to sub-critical levels. It is also possible that, as a result of limiting myself to sampling only 6 - 12 L of water, I did not achieve the resolution needed to discern treatment effects in relatively low density zooplankton assemblages. Finally, it is possible that the zooplankton in my enclosures were simply resistant to UVR.

Conclusions

My experiments show that UVR effects on many organisms may depend on whether they can migrate or circulate deep enough to escape high UV exposure. Calm, sunny conditions that allow microstratification to isolate surface waters could result in significant reductions in a few key species of phytoplankton. Although the results of my 5-week experiments were inconclusive for effects of UVR on zooplankton, it appears that benthic fauna may be relatively resistant to UVR, or at least better able to find refuge from high fluxes.

Increases in UVR penetration can occur independently from losses of DOC, as shown here and elsewhere (Curtis and Schindler, 1997, Donahue et al., 1998, Molot and Dillon, 1997a). In this experiment the primary mechanism for increasing UVR exposure was the bleaching of DOC by high-energy wavelengths in the UVR spectrum, rather than simply DOC removal.

In the event of predicted stratospheric ozone reductions, clear lakes would experience a greater photochemical quantum yield than under current conditions (Scully et al., 1997). Greater water transparency due to either acidification or climate warming would result in greater mixed-layer depths, and also increase the exposure of planktonic organisms to solar UVR (Mazumder and Taylor, 1994, Schindler et al., 1996b). In addition to the increased UVR penetration caused by bleaching and photochemical alteration of DOC, clearer lakes could be more susceptible to UVR as a result of an increase in surficial and subsurficial UVB:UVA, both biologically and photochemically. With decreased DOC inputs and increased residence times projected by climate warming scenarios, greater water clarity and

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deeper mixed layers as a result of increased bleaching will result in increased UVR penetration and exposure of biota in boreal lakes. This could have dramatic effects on the photochemical and biological processes within these lakes.

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Table 2-1: Half-times for fluorescence loss in UV-shielded and UV-exposed enclosures of different depth (days)

Depth (m)	No UV	UV
0.75	114	38.5
1.5	181	56.3
2.25	354	84.1

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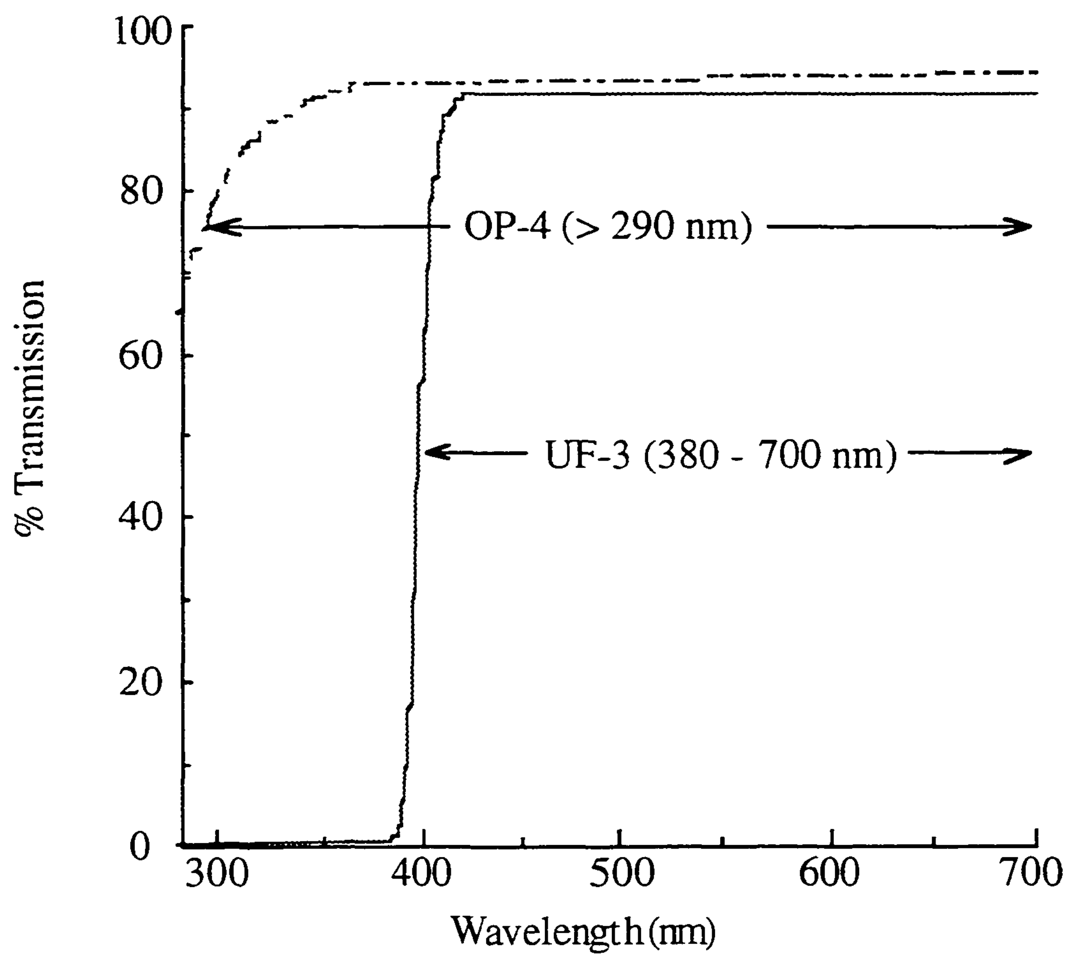

Figure 2-1. Transmission spectra of UV-impermeable (UF-3) and UV-permeable (OP-4) plexiglass.

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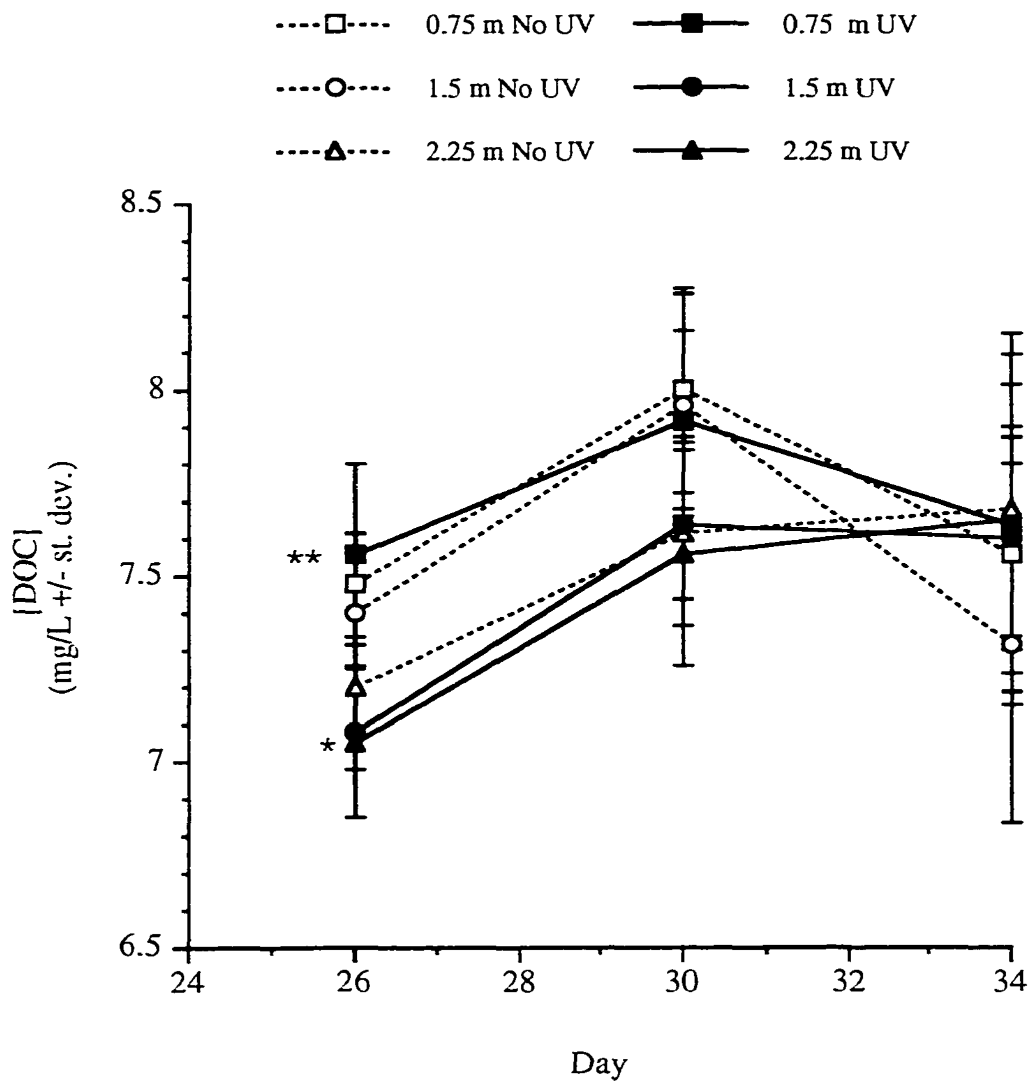

*Figure 2-2. DOC concentrations (± S. D.) in enclosures of varying depth and UVR exposure. Other than on day 26, there were no differences between treatments (** is greater than *; P<0.05).*

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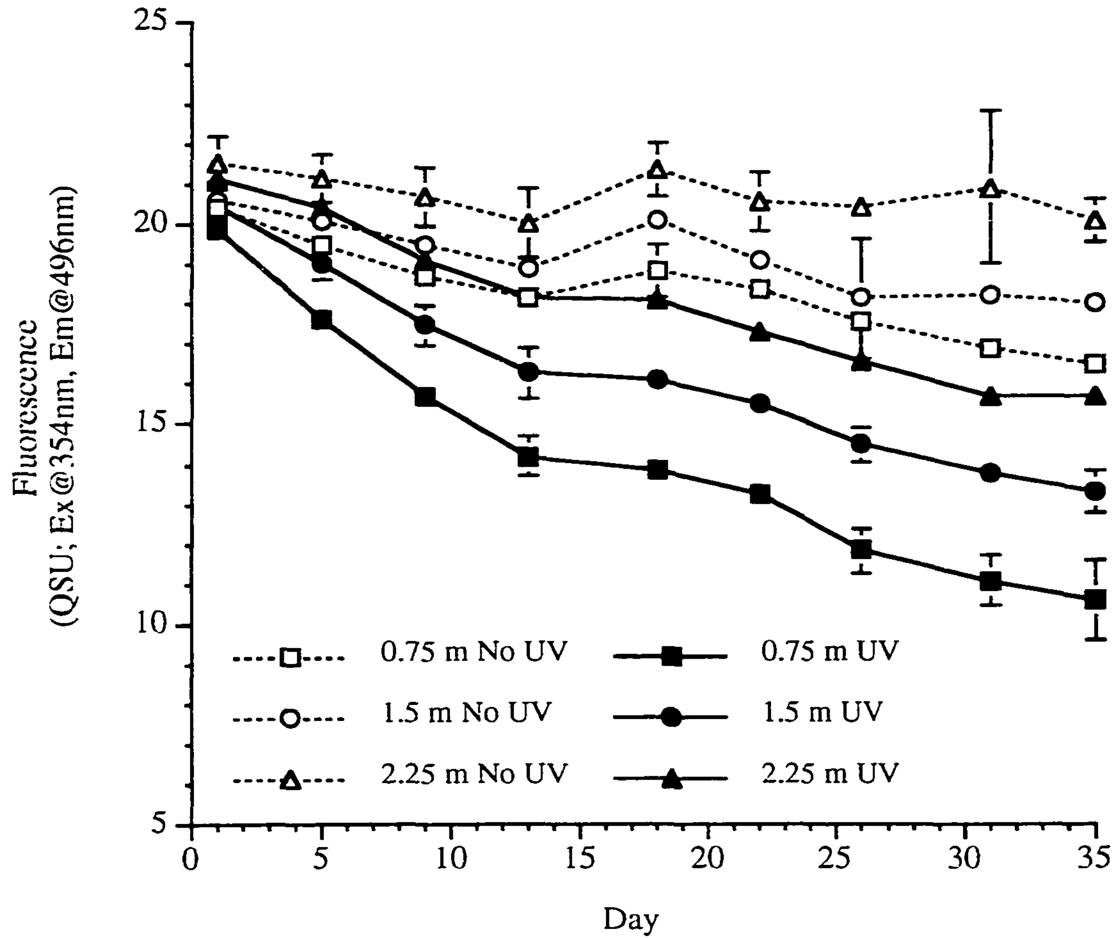

Figure 2-3. Decreases in fluorescence (excitation @ 354 nm, emission @ 496 nm; ± S. D.) in UV-shielded and UV-exposed enclosures of different depths. The greatest loss in fluorescence was in 0.75 m deep UV-exposed enclosures.

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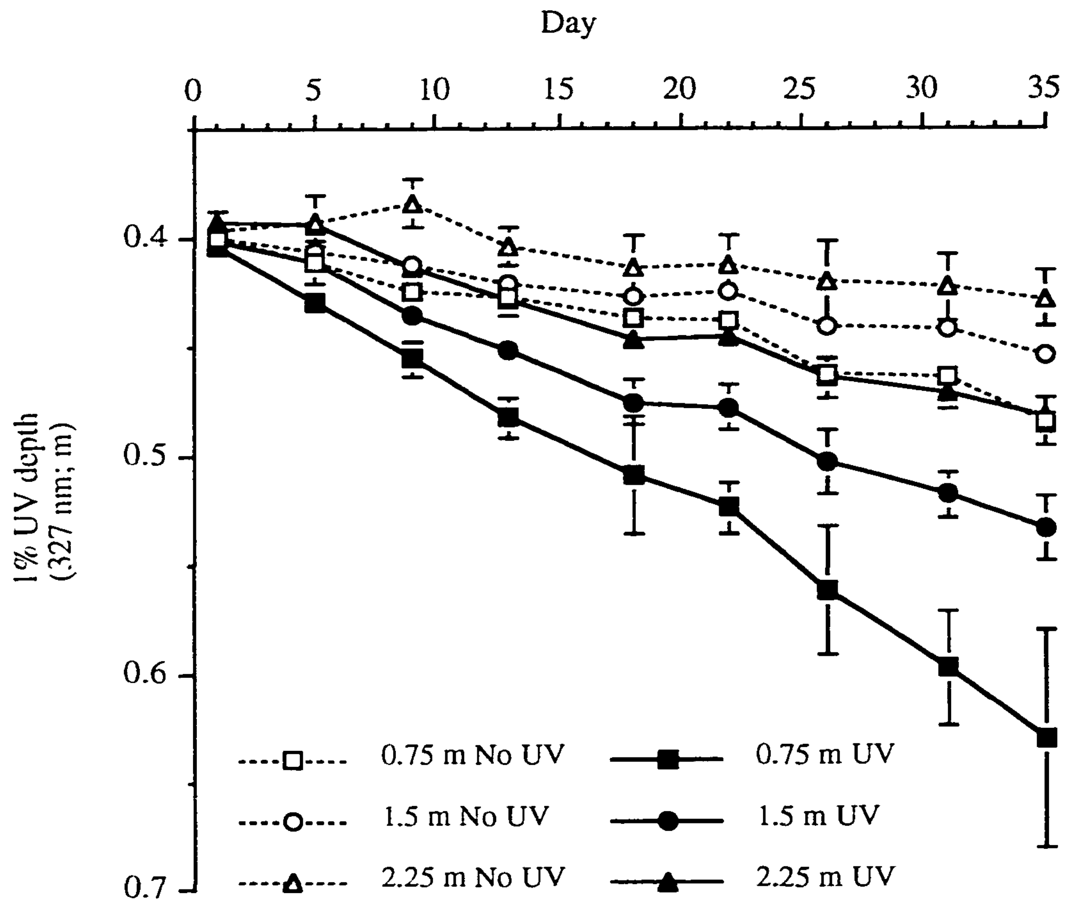

Figure 2-4. UVR penetration in UV-exposed and UV-shielded enclosures of different depths (± S. D.) between June 5 and July 9, 1996. The greatest increases in UVR penetration were in the shallowest UV-exposed enclosures.

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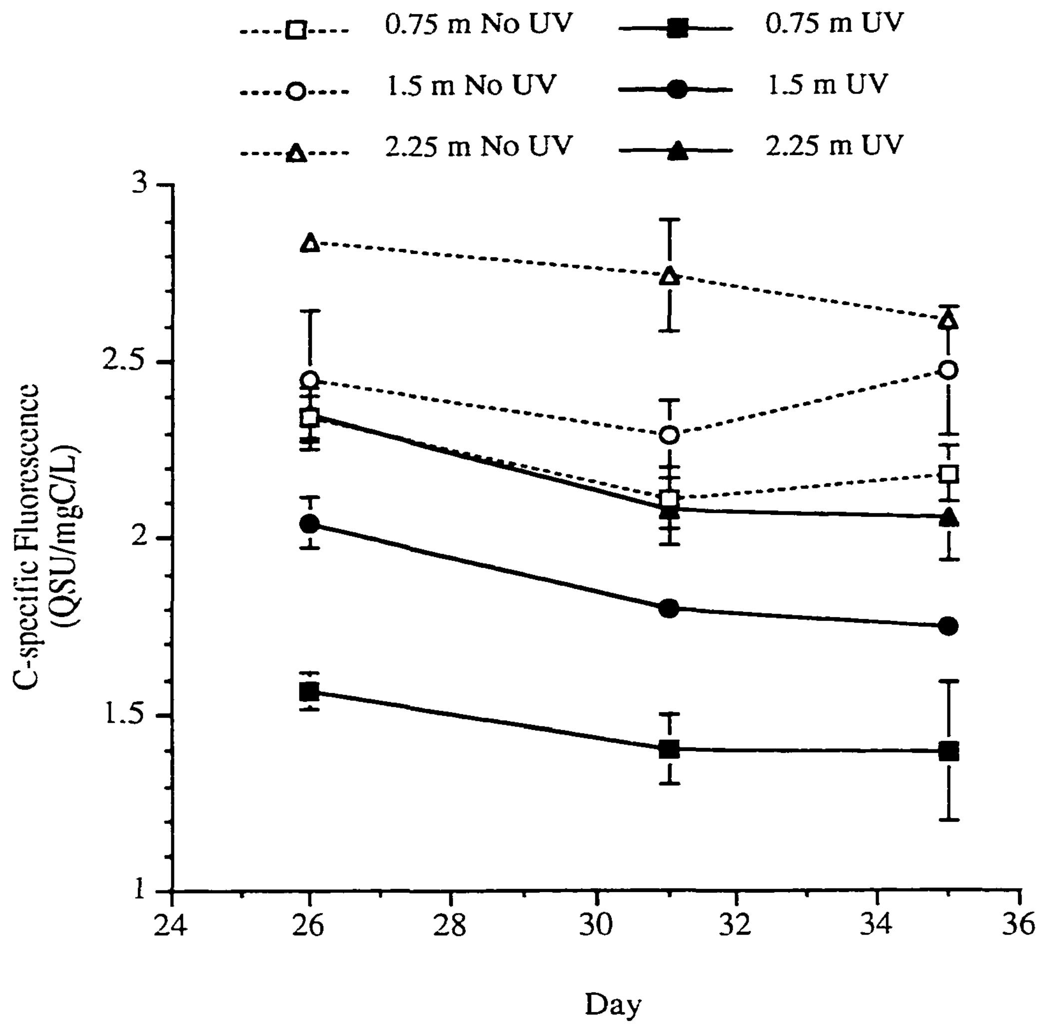

Figure 2-5. Carbon-specific fluorescence (CSF; ± S.D.) in UV-exposed and UV-shielded enclosures of different depth. Carbon in the shallowest UV-shielded enclosures was most bleached.

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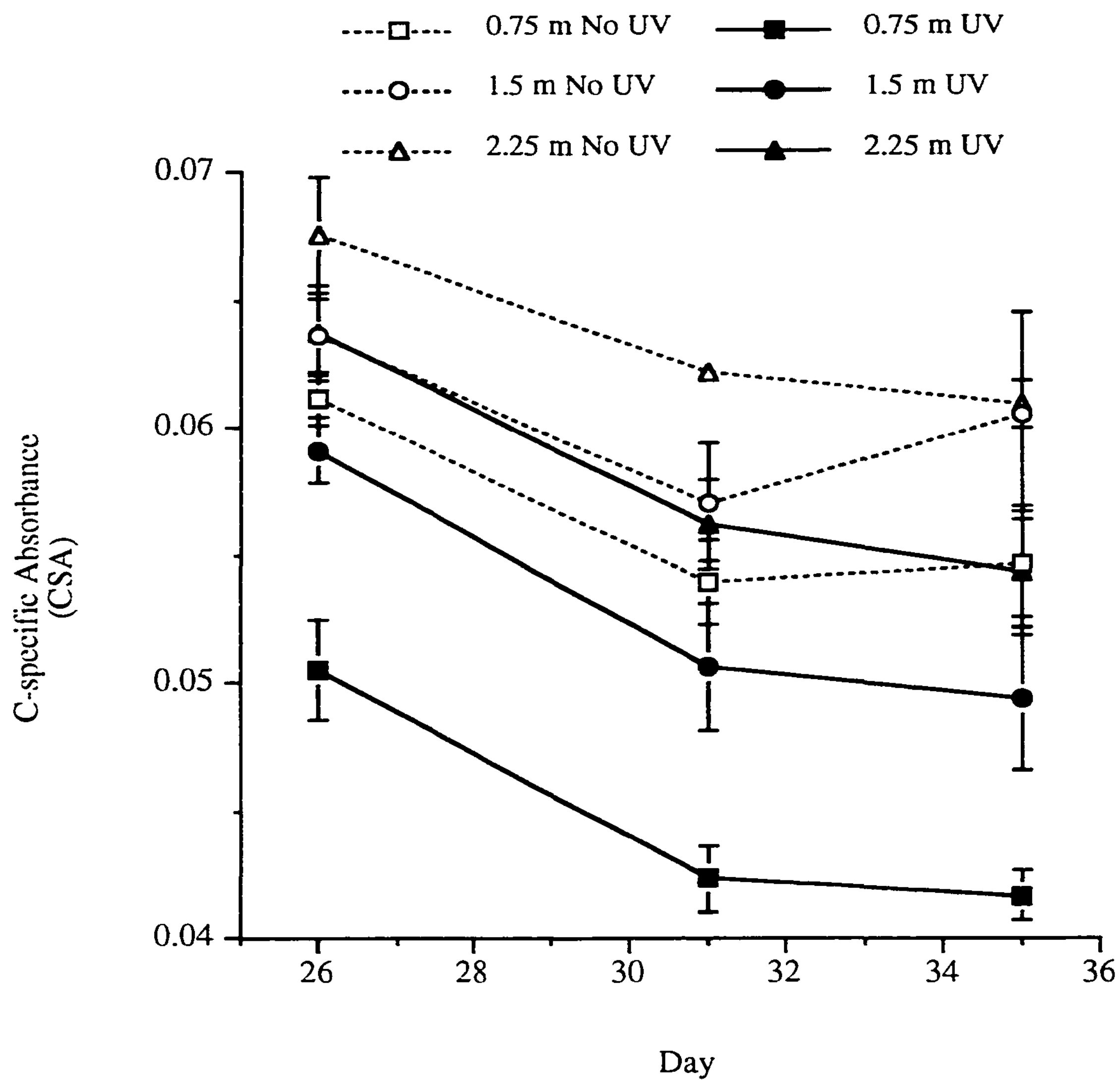

Figure 2-6. Carbon-specific absorbance (CSA; /mgC; 327 nm) in UV-shielded and UV-exposed enclosures of different depth over the last 10 days of a 5 week experiment. As a result of bleaching, DOC in the shallowest UV-exposed enclosures had the lowest CSA.

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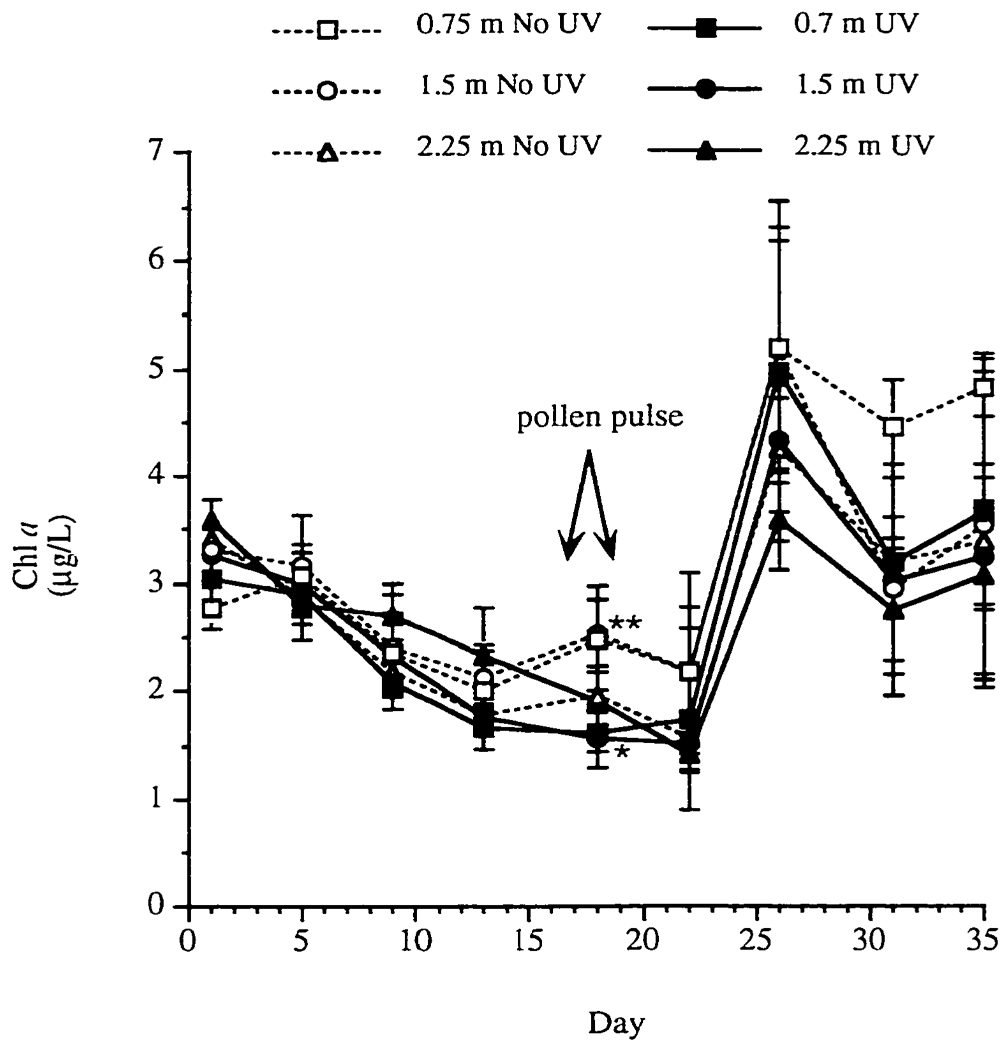

Figure 2-7. Chl a *concentrations in UV-exposed and UV-shielded enclosures of different depths, from June 5 to July 9, 1996 (± S. D.; ** is greater than *; P<0.05). A pulse of tree pollen between days 13 and 18 appeared to cause the eventual rapid increase in chl* a *concentration from days 22 to 25.*

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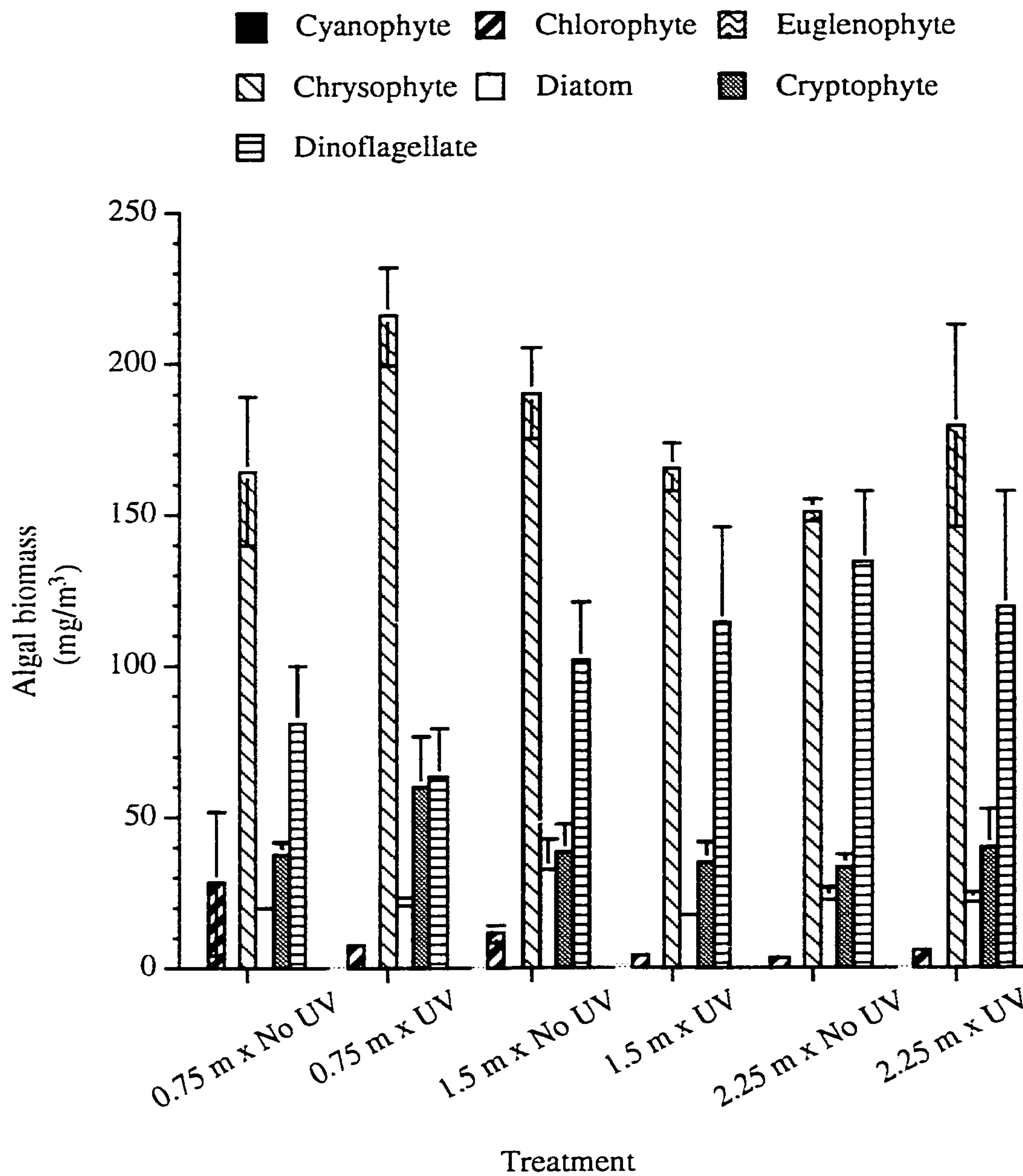

Figure 2-8a. Algal biomass on Day 1 in UV-exposed and UV-shielded enclosures of different depths. There were no differences in taxa or biomass between treatments (P>0.05).

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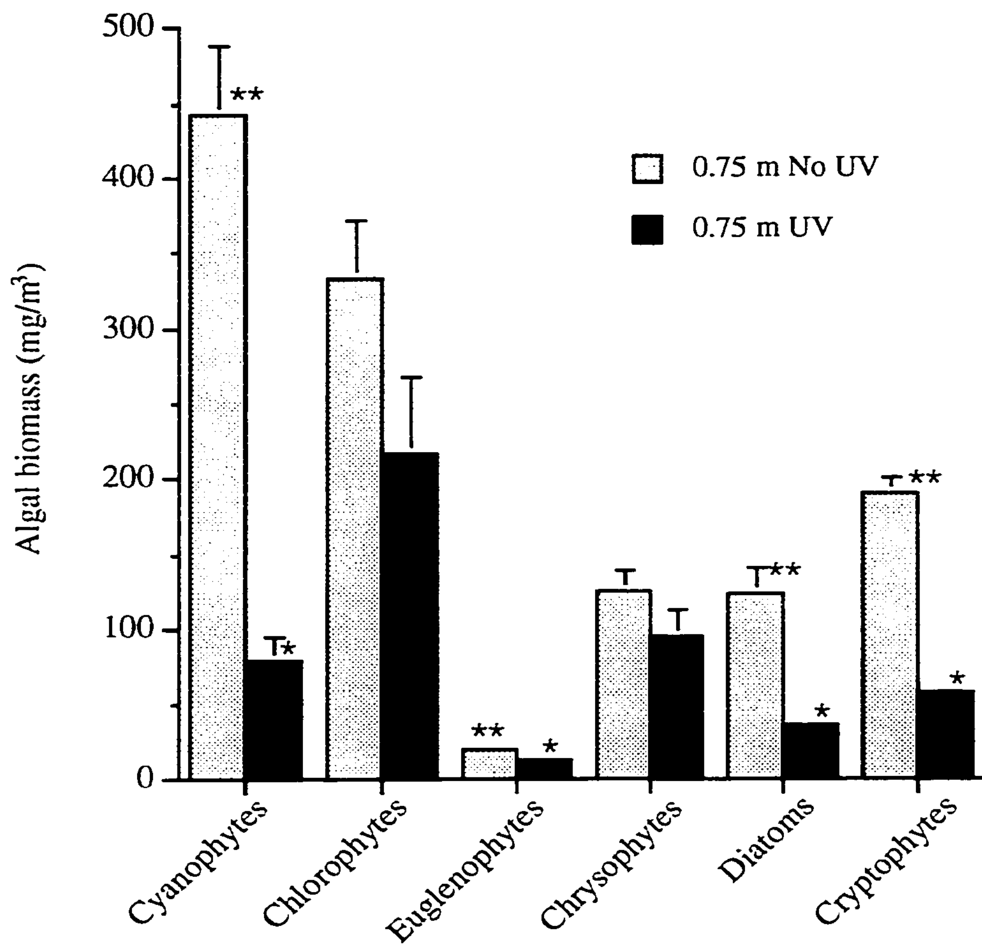

*Figure 2-8b. Algal taxa and biomass in 0.75 m enclosures that were shielded from or exposed to solar UV radiation for 35 days (** is greater than * for each class; P<0.05).*

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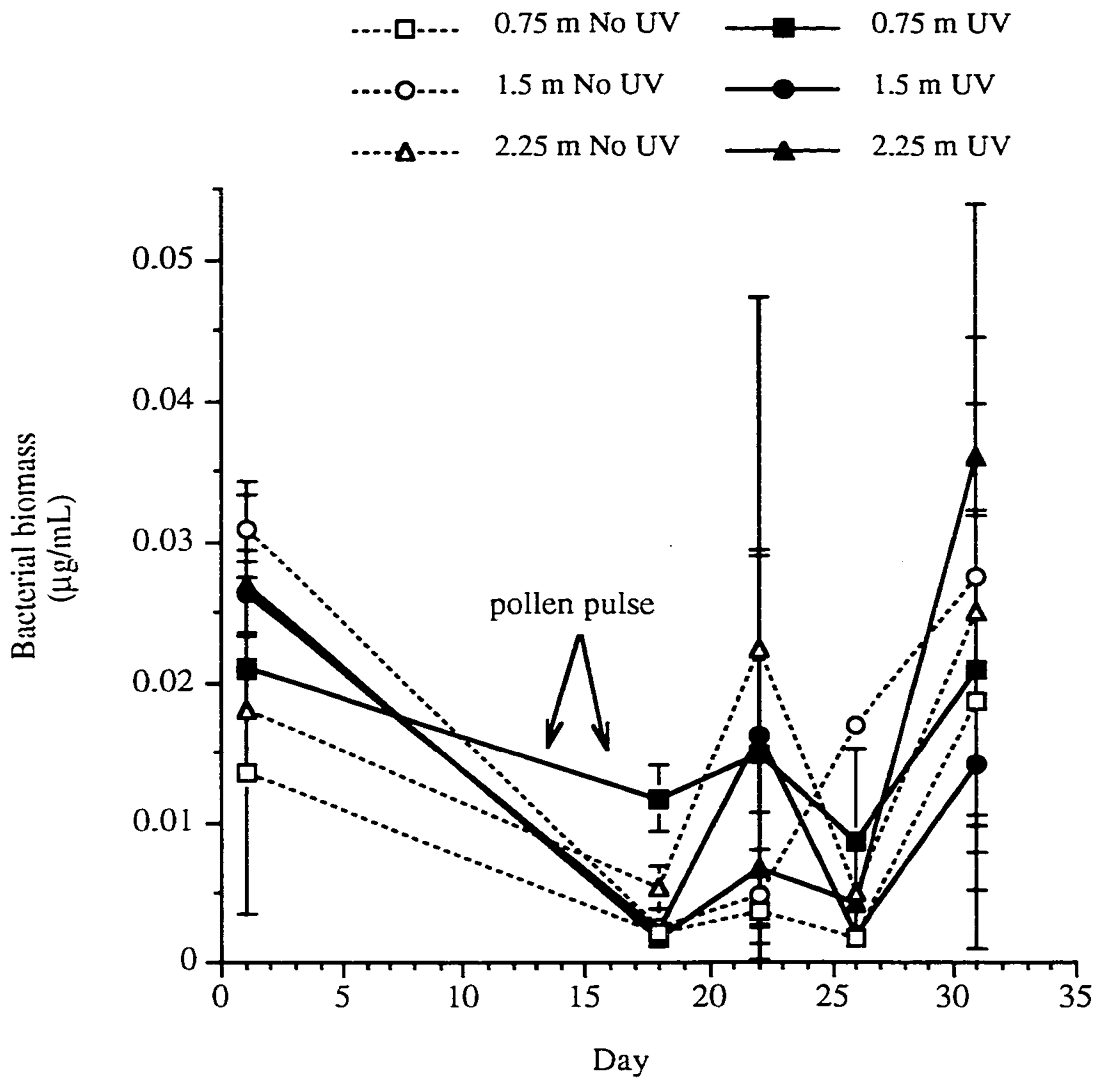

Figure 2-9. Bacterial biomass in enclosures of different depth and exposed to or shielded from solar UV radiation. Other than a general increase in biomass after the pollen pulse, no discernible treatment effect occurred.

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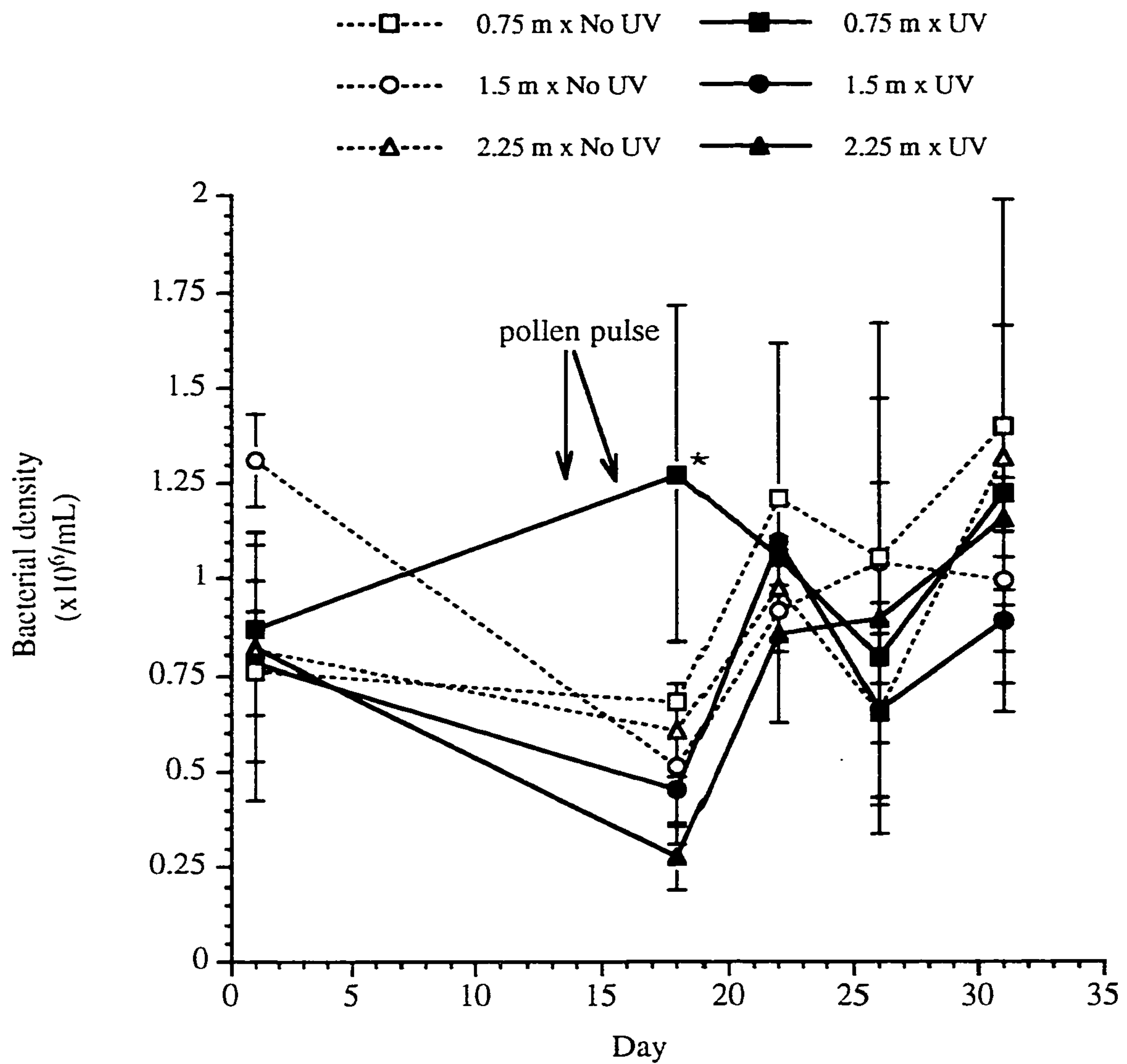

Figure 2-10. Bacterial densities in enclosures of different depth and exposed to or shielded from solar UV radiation. Other than on Day 18, when densities were greater in the shallowest UV-exposed enclosures than in other treatments (P<0.05), no discernible treatment effect occurred over the course of the experiment.

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3. INTERFERENCE IN FLUOROMETRIC HYDROGEN PEROXIDE DETERMINATION USING SCOPOLETIN-HORSERADISH PEROXIDASE[1]

Introduction

There is considerable interest in the chemistry and measurement of reactive oxygen species produced in aquatic systems by photochemical interactions between ultraviolet radiation and dissolved organic matter (Cooper and Lean, 1992, Lean et al., 1994, Scully et al., 1996). Hydrogen peroxide (H_2O_2) is the most commonly measured of these, because it is less reactive than other oxygen radicals and has relatively low decay rates *in situ*. These properties enable it to accumulate to much higher, more easily measured, concentrations than other more reactive species like superoxide or hydroxyl radicals. Fluorometric techniques (Helz and Kieber, 1985, Holm et al., 1987, Kieber and Helz, 1986, Lazrus et al., 1985, Miller and Kester, 1988) are common methods for determinations of naturally low H_2O_2 concentrations in water (Cooper and Lean, 1992, Cooper et al., 1988, Helz and Kieber, 1985, Kieber and Helz, 1986, Lean et al., 1994, Scully et al., 1996) .

The scopoletin-horseradish peroxidase technique for determining low H_2O_2 concentrations in water is a simple one, in theory, and is widely used. An enzyme, horseradish peroxidase (HRP), is used to form an enzyme-substrate complex with H_2O_2, and this complex reacts with a fluorescent tracer, scopoletin. Reductions in gross fluorescence of this tracer are used to quantify H_2O_2. In these techniques (Holm et al., 1987, Kieber and Helz, 1986, Lazrus et al., 1985), presence of naturally occurring H_2O_2 has been assumed to be indicated by decreases in fluorescence of scopoletin, after the addition of HRP. The degree of decrease in fluorescence is auto-correlated to a curve generated by the addition of standard amounts of H_2O_2 to the same sample and measuring further amounts of decrease in fluorescence of scopoletin. One must be certain that no reactions are occurring that can alter the fundamental relationship between fluorescence and concentration, otherwise the assumption of correlation is an incorrect one and estimates of concentration will be erroneous.

In this paper the interference in the fluorometric determination of H_2O_2 by prior exposure of scopoletin to visible wavelengths is described. Also described are

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[1]*A version of this chapter has been published.* William F. Donahue. 1998. *Environmental Toxicology and Chemistry 17 (5): 783-787.*

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changes that occur to scopoletin fluorescence as a result of photobleaching by constant exposure to excitation wavelengths in these techniques, as well as fluorescence decreases in the presence of HRP. These modes of interference can introduce imprecision and inaccuracy into the measurement of H_2O_2 concentrations in water with the fluorometric scopoletin-HRP technique. A modification of the scopoletin-HRP technique is reported, using calibration by standard solutions to solve the problem of potential interference in fluorometric determinations of nanomolar concentrations of H_2O_2.

It is possible that other emission processes are initiated by exposure of scopoletin to visible light, such as phosphorescence or *E*-type delayed fluorescence (Badley, 1976, Banwell, 1983, Beens and Weller, 1969), and this may be what alters its fluorometric response after exposure to this visible light. This paper does not attempt to quantify interactions of this sort, but rather to identify and provide a solution to a problem associated with previously described techniques.

Materials and Methods

General

Identification of complex emission-response interference with fluorescence spectra was completed using distilled, deionized water (DDW) at the University of Alberta from 27 July - 2 August, 1995. Assays of natural samples of H_2O_2, collected from Lakes 239 and 470 at the Experimental Lakes Area, in northwestern Ontario, Canada, were completed between 10 August and 13 September, 1995. Lake samples for H_2O_2 determinations were collected using a 2 m long, 2.5 cm diameter Tygon tube, and stored in opaque plastic bottles until analysis was completed.

Apparatus

Time-series fluorometric analyses of scopoletin fluorescence (Figures 3-1, 3-2, 3-4 and 3-5) were carried out with a Shimadzu Spectrofluorometer, Model RF-1501 (Mandel Scientific Company, Guelph, Ontario, Canada), with a 3 mL quartz cell. The instrument was computer-driven using Shimadzu PC-1501 Personal Fluorescence Software for Windows. Effects of interference by visible wavelengths in excitation and emission response, and time-series photobleaching of scopoletin (Figure 3-3) were corroborated on a Turner Model 10-005 filter fluorometer. All excitation wavelengths were 365 nm and emission wavelengths were 496 nm (Holm et al., 1987). Standard solution assays and calibrations were done using the shutter-equipped Shimadzu Model RF-1501 Spectrofluorometer.

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Reagents

Water for all reagents and standard solutions used in identification of complex emission responses was from a distillation/deionization/activated carbon adsorption system (Milli-Q System, Millipore), and stored in Nalgene polyethylene bottles. A hydrogen peroxide standard (1×10^{-5} M) was prepared immediately before use using 30% reagent grade stock solution (BDH Chemical). A 0.5 M phosphate buffer (pH 7) was prepared by dissolving 13.34 g KH_2PO_4 and 21.58 g Na_2HPO_4 in 500 mL of distilled deionized water (DDW). Scopoletin (7-Hydroxy-6-methoxy-2H-1-benzopyran-2-one) and horseradish peroxidase (type II; 170 purpogallin units/mg solid) were both from Sigma Chemical. Reagent solutions of scopoletin (5×10^{-5} M) and horseradish peroxidase (HRP) (20 mg solid in 5 mL of DDW, with 100 µL buffer) were also prepared immediately before use, and were stored on ice and in the dark during analyses. Water used in standard solution calibration for determination of photochemically-produced H_2O_2 in lake water was from the water body to be tested, collected before sunrise and stored in dark glass bottles with Teflon-lined caps for at least 24 hours before use, to ensure natural breakdown of all H_2O_2 (Lean et al., 1994). Lake water samples were collected and analyzed for H_2O_2 as soon as possible.

Modified Procedure

In preparation of standard solutions, 100 µL of buffer is added to 30 mL of lake water, previously collected from the lake to be sampled for H_2O_2 and allowed to sit in the dark for at least 24 hours. 60 µL of 5×10^{-5} M scopoletin solution is then added and a standard addition of H_2O_2. After vortexing, 30 µL of HRP solution is added, and the sample is vortexed again. The filled fluorometer cell is immediately inserted into the spectrofluorometer equipped with a shutter apparatus, allowing isolated exposure of the cuvette to excitation radiation and then subsequent shielding. The standard is then allowed to sit inside the spectrofluorometer for 3 minutes, with the shutter closed to exclude incident excitation radiation. After 3 minutes, fluorescence is measured via shuttered, instantaneous exposure of the sample. During the 3 minutes the standard is sitting shielded from excitation radiation in the spectrofluorometer, the next standard can be prepared, minus the HRP. The enzyme is added as soon as the previous standard is run and one is prepared to rinse the cell. Amounts of the scopoletin stock solution and the concentrations of H_2O_2 standard solutions are previously determined according to the instrument sensitivity and natural concentrations of H_2O_2 in lake water samples. Scopoletin additions need to be low enough for fluorescence to remain on-scale, and high enough to ensure that

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scopoletin fluorescence is not quenched entirely by the highest concentration of H_2O_2 in the standard solutions. Typically, six different standard solutions of H_2O_2 (0, 200, 400, 600, 800, 1200 & 1600 µL of a total standard solution volume of 30 mL) were used in the calibration curve for samples from a humic lake with H_2O_2 concentrations from 100-450 nM.

Assays of samples of natural H_2O_2 proceed in the same fashion and according to the same timing as those of the standard solutions used in producing the calibration curve, and normally initiated within 10 minutes of sampling. It is important to note the time when each sample is taken. Replicates of the same sample can be assayed sequentially, or, if one has a large number of samples one may prefer to run them all once and then again in the same order, with time of determination recorded for each sample. In this way, one can back-calculate initial H_2O_2 concentrations at the time of sampling based upon logarithmic decay of H_2O_2 in the samples themselves, inferred from multiple-point curves for each sample. This may or may not be necessary, depending on natural rates of decay of H_2O_2 in the samples, which may vary from lake to lake and correlate somewhat with dissolved organic carbon content (Norm M. Scully: personal communication) and bacterial densities (Lean et al., 1994). Natural waters were not filtered to remove microorganisms and possibly stabilize H_2O_2 concentrations because of time limitations; if one or a few samples are to be run one might filter the sample. However, the time involved in filtering large numbers of samples before analysis will introduce the opportunity for undetected H_2O_2 decay.

As in any quenching interaction, the timing of mixture and incubation becomes very important as a result of changes in scopoletin fluorescence after HRP addition. It is therefore necessary to record the time after addition of the enzyme, and to keep it constant between standards and samples.

Results and Discussion

Complex Emission Response of Scopoletin

Scopoletin is a highly fluorescent chemical and is excited in both the ultraviolet and visible radiation spectra. Like many other optically reactive chemicals, scopoletin exhibits decreases in fluorescence emission intensities with prolonged or repeated excitation (operational definition used here for "bleaching"). For example, in six sequential time-series analyses of scopoletin fluorescence during which excitation radiation exposure totaled 17 minutes, initial fluorescence decreased by 35% (Figure 3-1). Recent descriptions of this technique do not account for decreases in absolute fluorescence of

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scopoletin as a result of continual exposure of samples to excitation wavelength, as occurs during standard additions calibrations. Bleaching of scopoletin can occur over the short time-period needed to measure initial decreases in emission intensity, which are attributed to the presence of natural concentrations of H_2O_2, and the extended time-period required for calibration using standard additions of H_2O_2 (Figure 3-2). Because it takes longer to perform calibrations on a sample (minutes) than to measure the initial change in scopoletin fluorescence (seconds), emission decreases as a result of bleaching are likely to lead to an overall underestimation of $[H_2O_2]$ by contributing to a greater inferred decrease in fluorescence per aliquot of H_2O_2 added than is actually the case. The degree to which scopoletin bleaching occurs will be dependent upon length of exposure to excitation radiation and lamp intensity; the use of a shutter-equipped fluorometer helps to limit bleaching during determinations.

The assumption that initial additions of HRP to a sample spiked with scopoletin lead to decreases in fluorescence only in the presence of H_2O_2 (Holm et al., 1987, Kieber and Helz, 1986, Zika et al., 1982) is also faulty. Addition of the enzyme to H_2O_2-free water/scopoletin solutions can lead to dramatic decreases in fluorescence (Figure 3-3). This decrease in fluorescence independent of H_2O_2 indicates either a change in the fluorometric properties of the scopoletin or a decrease in the concentration of scopoletin itself, possibly as a result of oxidation by the HRP. This decrease also suggests that decreases in fluorescence after additions of H_2O_2 must be compared to the initial fluorescence of the scopoletin in the presence of HRP alone, as suggested when the technique was first described by Perschke and Broda (1961). If one were to compare this enzyme-dependent decrease to a calibration curve associated with later additions of H_2O_2 aliquots to the same sample, one might overestimate the $[H_2O_2]$ in the initial sample by not taking into account the decrease in initial fluorescence attributable to the addition of the enzyme.

Complicating the above problems, scopoletin seems to exhibit a "dynamic" fluorescence equilibrium. Upon initial excitation, emission is high - then emission drops sharply over approximately 30 seconds to achieve an excited-state equilibrium. How it behaves after that depends on the recent excitation history of the sample. Emission responses of scopoletin over 3-minute excitation periods were unpredictable when samples were shielded from radiation for up to 2-1/2 minutes before excitation (author's unpublished data). However, responses are very predictable in form, exhibiting the development of an excited-state equilibrium after being allowed to sit for 3 minutes unexposed to any excitation wavelengths, including visible light (Figure 3-1, runs 1, 4 and

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6; Figure 3-4, run C). Assays of replicates using the same scopoletin stock solution illustrate the similarity in initial emission intensity (Figure 3-4).

In those samples not previously shielded from all excitation wavelengths, emission response was highly irregular and unpredictable (Figures 3-1 and 3-4). Fluorescence of scopoletin decreased steadily in one example (Figure 3-1, run 2), coincidentally similar to normal calibration curves using standard additions of H_2O_2 in a fluorometer not equipped with a shutter apparatus (Holm et al., 1987, Zika et al., 1982), and fluctuated wildly in others (Figure 3-1, runs 3 and 5; Figure 3-4, runs A and B). Fluorescence behaviour of scopoletin in unshielded samples would likely contribute to error in estimation of $[H_2O_2]$, and it would be hard to factor this out simply because of its unpredictability; whether fluorescence will increase or decrease at a given time is unknown. For the same reason, it is difficult to quantify error associated with these fluctuations in emission intensity.

Figure 3-5 is a conceptualization of how HRP addition and bleaching of scopoletin during H_2O_2 determination can interfere with interpretations when using standard additions of H_2O_2 aliquots. The unpredictable nature of scopoletin fluorescence fluctuation under constant excitation and after previous exposure to visible light makes it difficult to incorporate into this. Figure 3-2 gives one an idea of the degree to which scopoletin fluorescence fluctuations and bleaching, when combined, can obscure a calibration attempt using a standard addition technique. Comparison of scopoletin bleaching and the H_2O_2 calibration in Figure 3-2 indicate that up to 40% of the fluorescence decrease ascribed to H_2O_2 additions may be the result of bleaching of scopoletin. This could result in an equivalent 40% underestimation of $[H_2O_2]$ in the original sample, in this example.

Hydrogen Peroxide Determinations

The need to control for fluorometric interactions between scopoletin and HRP suggests one must perform calibrations with independent solutions covering a range of H_2O_2 concentrations. In this way, one can also avoid deceptive, complex and time-dependent changes in scopoletin fluorescence. When samples with scopoletin and HRP are shielded for a period of three minutes before excitation, predictable and repeatable emission responses occur that seem to be independent of the length of time the sample is exposed to excitation radiation. Use of independent standards of varying $[H_2O_2]$ for calibration yield predictable and expected emission responses (Figure 3-6). The relationship between decreased emission intensity and $[H_2O_2]$ was highly significant ($r^2 > 0.99$) in 11 different calibration trials from 10 August - 13 September 1995, and replicate measurements of samples (e.g., n=7 and n=3) were very similar (coefficient of variation = 2.8% and 0.8%, respectively). The high degree of linearity in these trials also supports the efficacy of

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allowing naturally low levels of H_2O_2 present in lake waters collected early in the morning to continue to decay in the dark for a minimum of 24 hours; the zero-point for calibration curves would deviate from regression lines if initial concentrations of H_2O_2 were significant. Some previous descriptions of this technique suggest the use of a bovine catalase enzyme to remove all H_2O_2 from standard waters (Kieber and Helz, 1986). However this may lead to further complications as a result of competitive inhibition between the catalase and HRP when it comes to later enzyme-substrate complexation with H_2O_2 (Holm et al., 1987). As a result, this might result in underestimation of $[H_2O_2]$ in the sample.

It is essential in fluorometric analyses that individual and interactive responses of constituent chemicals to excitation wavelengths be identified to reduce error in the results of the techniques. These modifications using standard solutions of H_2O_2 permit very precise measurement of $[H_2O_2]$, and there is no reason to believe that levels of sensitivity previously reported (Holm et al., 1987) are compromised. Equally important, this technique eliminates any possible confounding complex emission response as a result of exposure of scopoletin to visible light prior to ultraviolet excitation. In addition, one avoids problems associated with underestimation of natural H_2O_2 concentrations as a result of decreased fluorescence of scopoletin during extended exposure to excitation wavelengths during calibration.

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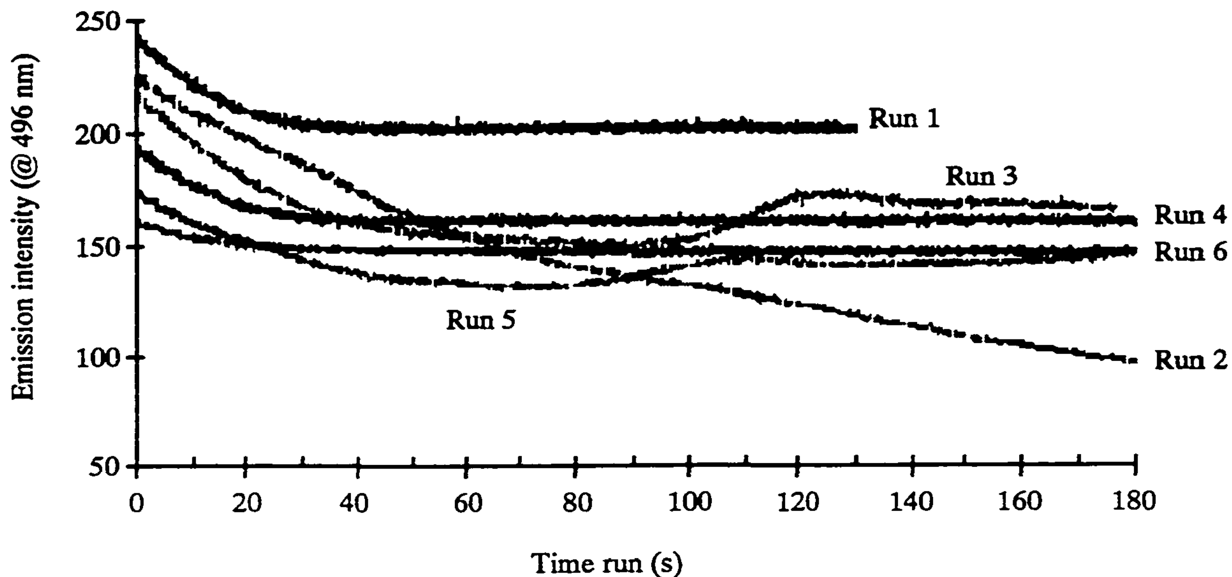

Figure 3-1. Predictable nature of emission response of a scopoletin solution after shielding from excitation radiation for 3 minutes (Runs 1, 4 and 6) versus after exposure to visible light immediately prior to excitation (Runs 2, 3 and 5) (Ex = 354 nm; Em = 496 nm). Note also general decrease in initial emission intensity (Runs 1 to 6) as a result of photobleaching of scopoletin during consecutive runs.

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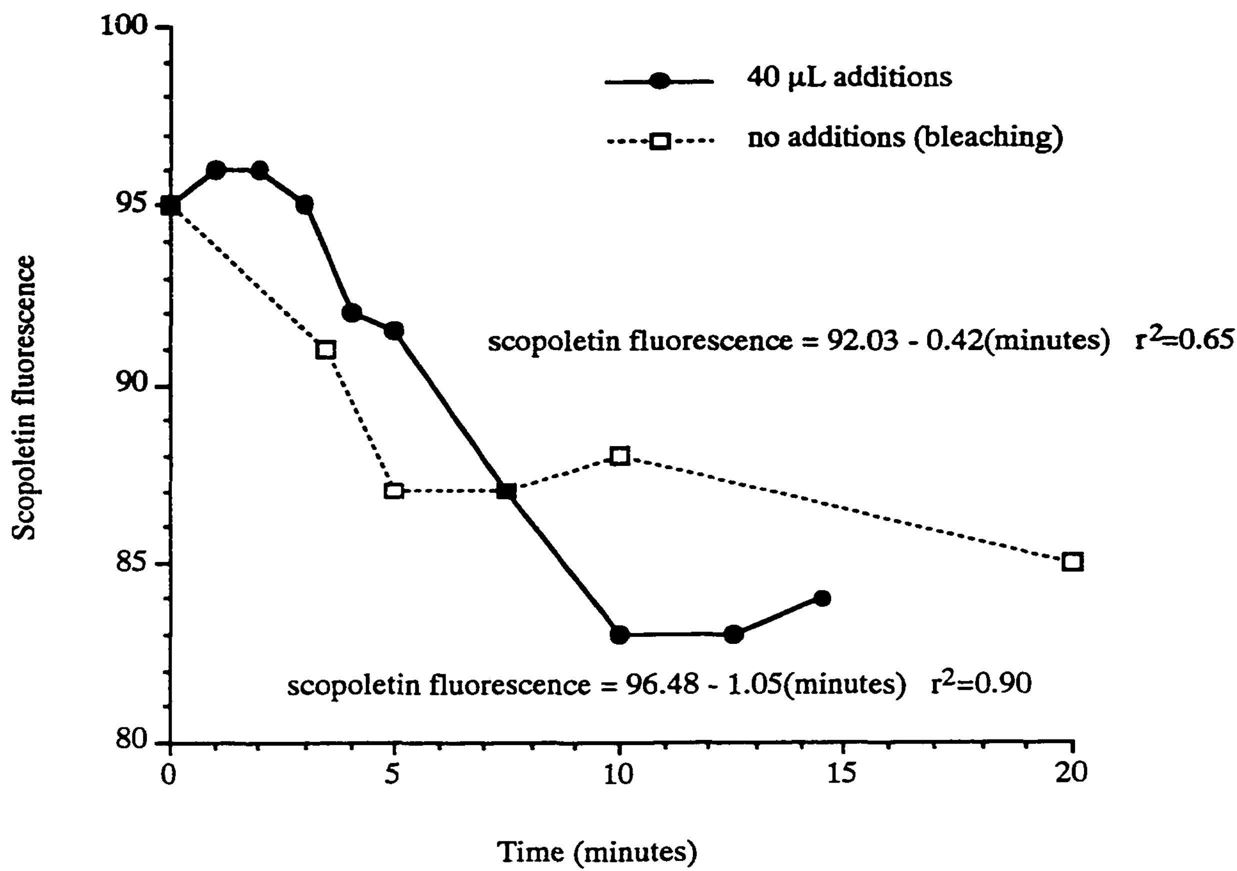

Figure 3-2. Decrease in emission intensity (496 nm) of scopoletin as a result of bleaching by excitation radiation (354 nm) and a typical calibration curve via standard addition on a fluorometer not equipped with a shutter. Each solid circle represents the injection of an aliquot of H_2O_2.

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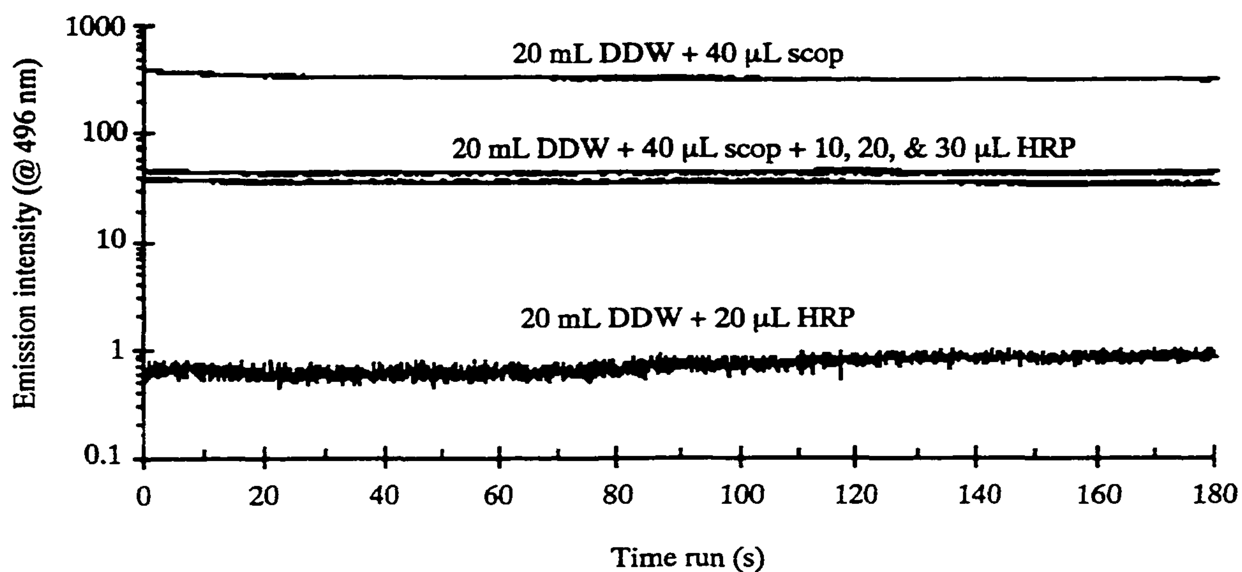

Figure 3-3. Decrease in fluorescent emission of scopoletin as a result of addition of HRP in the absence of H_2O_2. Volumes (μL) of scopoletin and HRP added are indicated.

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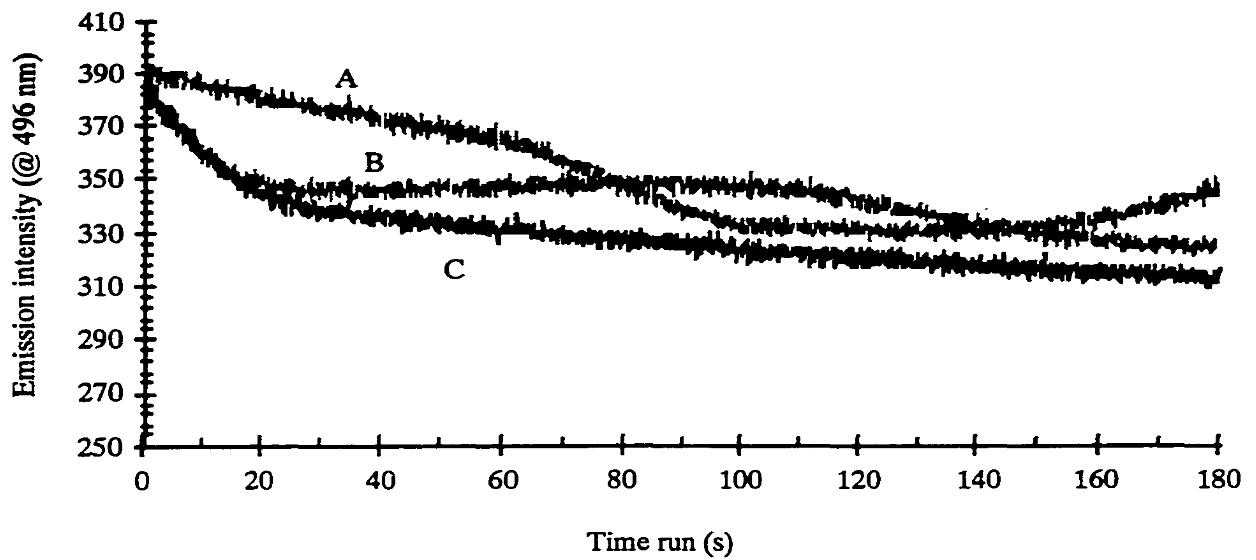

Figure 3-4. Unpredictable nature of emission response of scopoletin solution replicates after pre-run exposure to visible light (A and B) compared to response after shielding of scopoletin from all wavelengths for three minutes prior to excitation (C).

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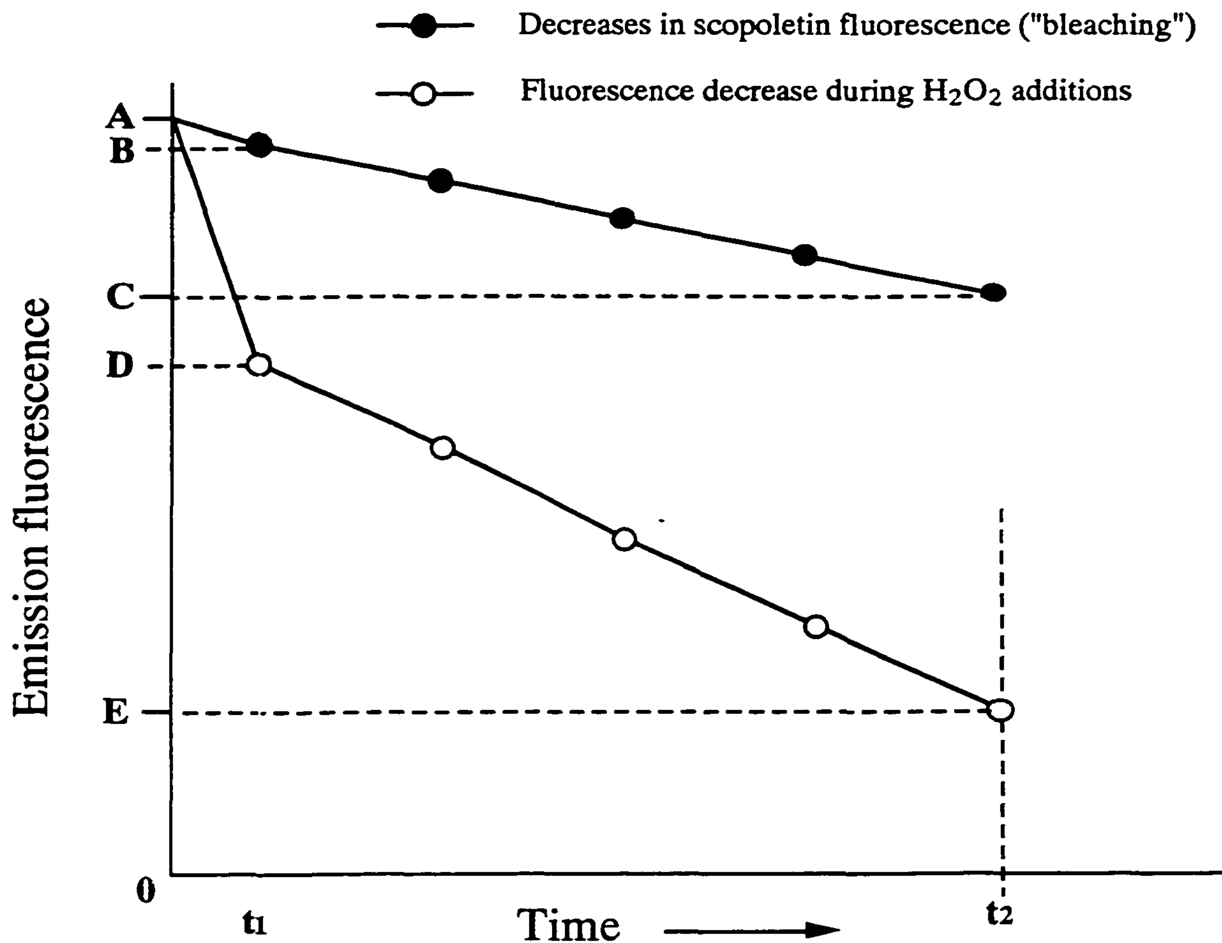

Figure 3-5. Conceptual interference during standard addition calibrations for H_2O_2 determination under constant excitation. (t_0) = addition of HRP; (t_2-t_1) = duration of calibration; (B-D) = fluorescence decrease due to HRP addition; (B-C) = bleaching of scopoletin during calibration; (B-E) = apparent fluorescence decrease due to H_2O_2 additions; [(D-E)-(B-C)] = actual fluorescence decrease due to H_2O_2 additions. None of this accounts for any unpredictable fluorescence changes in the scolopetin.

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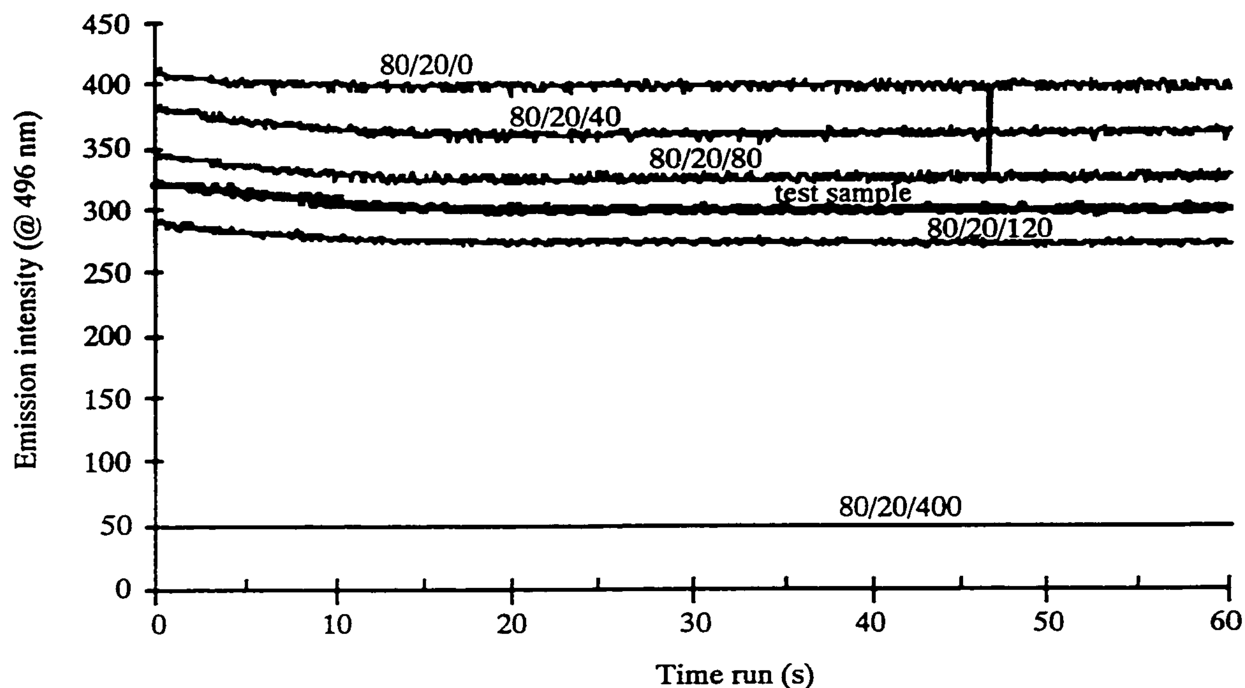

Figure 3-6. Calibration using standard H_2O_2 solutions. Additions (µL) of scopoletin, HRP and H_2O_2, respectively, to 20 mL DDW are shown. The grey line is the emission of a test sample of known [H_2O_2] (46.9 µM); predicted [H_2O_2] according to calibration from this example is 48.1 µM.

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4. THE EFFECT OF SOLAR ULTRAVIOLET RADIATION AND DILUTE HYDROGEN PEROXIDE ON OPTICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES OF OLIGOTROPHIC BOREAL LAKES

Introduction

Ultraviolet radiation (UVR) can cause photochemical breakdown and decreases in fluorescence and absorbance ("bleaching") of dissolved organic carbon (DOC) (De Haan, 1993, Zepp, 1988). In addition, the production of reactive by-products, such as superoxides ($O_2^{-\bullet}$), hydroxyl radicals ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2), can occur as a result of UV irradiation of DOC (Cooper et al., 1989, Cooper et al., 1988, Lean et al., 1994). It has been suggested that the presence of reactive oxygen species can induce the oxidation of DOC (Stumm and Morgan, 1996). However, no attempts have been made to separate the effects of these photochemical oxidizing agents on absorbance and fluorescence properties of DOC from direct effects of UVR. The objective of this study was to determine whether photochemical by-products like H_2O_2 affect DOC or pelagic communities in boreal lakes, independent of UV-R exposure.

DOC consists of a mixture of complex, low to high molecular weight organic molecules originating metabolically within a body of water (autochthonous DOC) or from degradation of organic litter in the catchment (allochthonous DOC). The chemical structure of DOC also varies according to the nature of the organic source (McKnight et al., In review, Valla, 1992). DOC concentrations tend to fluctuate on a daily and annual basis. They generally correlate with the amount of organic matter in the soil (e.g., uplands versus wetlands), and the hydrologic flow of the system, peaking in the late spring, decreasing over the course of the summer, and remaining at fairly constant levels during the winter (Urban et al., 1989).

Both its greatly varied molecular weight and chemical structure contribute to the complexity of DOC and allow it to affect many physical and biological processes (Pospísil, 1992). For example, DOC is a major nutrient source for bacteria (Vincent and Roy, 1993, Wetzel et al., 1995). Because allochthonous DOC colours water, DOC is a major factor in the attenuation of light in aquatic systems (Kirk, 1976, Schindler, 1971). It also preferentially absorbs low-wavelength radiation, often resulting in the absorption of UVR within the first few centimeters of water (Cooper and Lean, 1989). Increased DOC can lead to a decrease in the depth to which photosynthesis occurs, and might affect the establishment of different thermal layers in water bodies by altering the water clarity (Mazumder and Taylor, 1994, Schindler et al., 1996). DOC has been shown to increase the permeability of phytoplankton cell membranes (Twiss et al., 1993) and root cell

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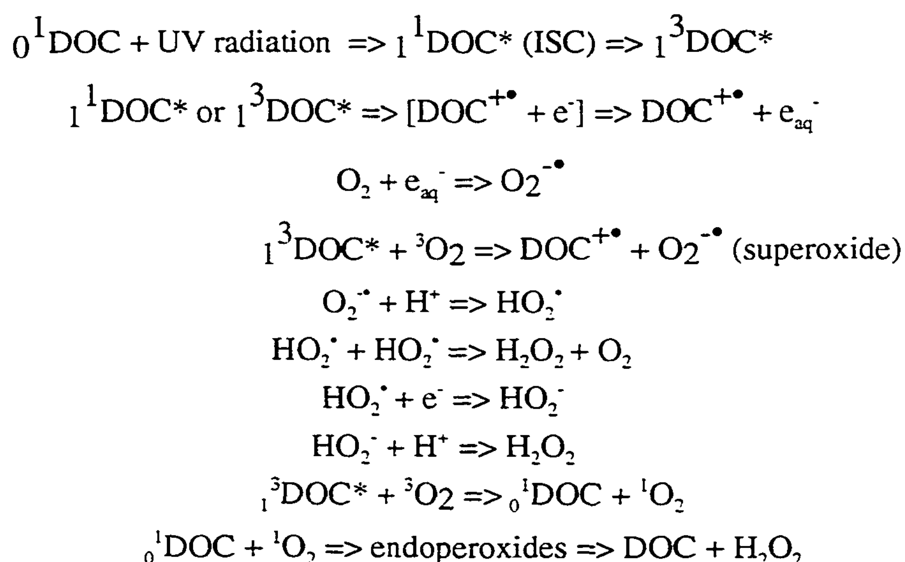
membranes in certain plants and to increase the activity level of ATPase (Maggioni et al., 1992). DOC also binds metal complexes, and pollutants such as mercury, cadmium, copper, and agricultural pesticides (Jackson et al., 1980, Kunc, 1992, Winner and Owen, 1991), effectively altering their availability to organisms. Any breakdown of DOC therefore has the potential to alter underwater optical environments, nutrient availability, plant growth, and bioavailability of pollutants. Thus it is important to understand the processes that effect quantitative and qualitative changes in DOC.

In addition to direct changes in DOC as a result of photolysis, reactive photochemical by-products have the capacity to effect changes in DOC. The following mechanisms for photochemical production of reactive oxygen species are some of those that have been suggested (Cooper et al., 1994, Stumm and Morgan, 1996):



$${}_0{}^1DOC + \text{UV radiation} \Rightarrow {}_1{}^1DOC^* \text{ (ISC)} \Rightarrow {}_1{}^3DOC^*$$

$${}_1{}^1DOC^* \text{ or } {}_1{}^3DOC^* \Rightarrow [DOC^{+\bullet} + e^-] \Rightarrow DOC^{+\bullet} + e_{aq}^-$$

$$O_2 + e_{aq}^- \Rightarrow O_2^{-\bullet}$$

$${}_1{}^3DOC^* + {}^3O_2 \Rightarrow DOC^{+\bullet} + O_2^{-\bullet} \text{ (superoxide)}$$

$$O_2^{-\bullet} + H^+ \Rightarrow HO_2^{\bullet}$$

$$HO_2^{\bullet} + HO_2^{\bullet} \Rightarrow H_2O_2 + O_2$$

$$HO_2^{\bullet} + e^- \Rightarrow HO_2^-$$

$$HO_2^- + H^+ \Rightarrow H_2O_2$$

$${}_1{}^3DOC^* + {}^3O_2 \Rightarrow {}_0{}^1DOC + {}^1O_2$$

$${}_0{}^1DOC + {}^1O_2 \Rightarrow \text{endoperoxides} \Rightarrow DOC + H_2O_2$$

where ${}_0{}^1DOC$ is the generalized electronic ground state of DOC, ${}_1{}^1DOC^*$ and ${}_1{}^3DOC^*$ are the electronically excited singlet and triplet states, respectively, and ISC is intersystem crossing. Of the byproducts, H_2O_2 is the longest lived and accumulates to concentrations of up to 2000 nM in lakes (Scully et al., 1995). Reduction of H_2O_2 also can lead to the production of highly reactive $OH^{\bullet}$ (Cooper and Lean, 1989, Lean et al., 1994, Stumm and Morgan, 1996). For these reasons and because H_2O_2 is easily measured, it is most frequently the subject of study.

In addition to their reactions with DOM, superoxide and H_2O_2 have extensive internal biochemical effects in both animal and plant cells, including the changing of enzymatic products used in the dark reactions of photosynthesis (Asami and Akazawa, 1977,

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Fridovich, 1986, Takabe et al., 1980). The production of H_2O_2 in lakes has been examined (Cooper and Lean, 1989, Cooper et al., 1988, Lean et al., 1994, Scully et al., 1995, Scully et al., 1996) and assumptions related to its toxicity to organisms have been made (Scully et al., 1996). Recent work investigated the short-term (~1 hour) effects of added H_2O_2 upon phytoplankton and bacterial production rates in 20 mL incubations of lake water (Xenopoulos and Bird, 1997). However, important ecological questions such as community effects of H_2O_2 have not been studied extensively beyond short-term, test-tube-scale metabolic incubations. Little is known of the environmental effects of these chemicals on aquatic food-chains.

Methods

Two enclosure experiments were conducted in the East Bay of Lake 239 (L239) at the Experimental Lakes Area (ELA), northwestern Ontario (49°40'N, 93°44'W), from June 4 - July 9 and August 18 - September 20 1996. Lakes of the ELA are typical oligotrophic, softwater Canadian Shield lakes dominated by allochthonous DOC (Schindler et al., 1992). L239 is surrounded by a predominantly jack pine (*Pinus banksiana*) forest that has regenerated since an extensive fire in the area in 1980 (Bayley et al., 1992).

Triplicate polyethylene enclosures, 1 m^2 in cross-section and 0.75 or 1.125 m deep, were suspended from a floating foam and wood frame anchored in the east bay of L239. Three treatments (UV-exposed, UV-shielded, and UV-shielded with H_2O_2 additions) were used to investigate the effects of H_2O_2 on in-lake processes. UV exposure was controlled by covering each enclosure with either UV-impermeable (UF-3 or GE Lexan©) or UV-permeable (OP-4) plexiglass. In the summer experiment one of the UV-exposed enclosures was 1.125 m deep, and in the fall experiment all three UV-exposed enclosures were 1.125 m deep; all other enclosures were 0.75 m deep. Treatment positions were randomly selected before the apparatus was assembled. Enclosures were filled at the beginning of the experiments by lowering them several meters below the surface and raising them (Curtis and Schindler, 1997). In conjuction with the first experiment, another experiment was performed consisting of enclosures of varying depth (Chapter 1), and some of the data from that experiment were included here to address the difference in depth of some of the UV-exposed enclosures from the other treatments.

Beginning the day after enclosures were installed, three of the UV-shielded enclosures received a pulse of H_2O_2 each morning, to a final concentration of 700 nM H_2O_2 in the enclosures. All enclosures were also stirred vigorously with a paddle rinsed in Lake 239 each morning. Natural H_2O_2 concentrations can range from 50 - 3200 nM in lakes (Cooper and Lean, 1992). In addition, modeled areal decay-adjusted daily H_2O_2 production for

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Canadian lakes ranged from 7 $mg/m^2/day$ for arctic Char Lake to 33 $mg/m^2/day$ for Hamilton Harbour in Lake Ontario (16.4 $mg/m^2/day$ in western Lake Ontario) (Scully et al., 1995). In addition, temperate Canadian lakes' areal decay-adjusted daily H_2O_2 production ranged from 12.7 - 27.7 $mg/m^2/day$ (Scully et al., 1995) and ~50-60 $mg/m^2/day$ on days without rain (Lean et al., 1994). H_2O_2 concentrations of up to ~450 nM were determined previously for shallow enclosures similar to those I used here under mid-summer, sunny conditions (author's unpublished data), and a once-daily 700 nM H_2O_2 injection is equivalent to 17.9 mg/m^2. Thus, the H_2O_2 concentrations used here were realistic when compared to both the amount of H_2O_2 produced and the maximum concentrations achieved in lakes. Samples of water were taken, post-stirring, at 4-5 day intervals throughout the experimental periods and analyzed for absorbance and fluorescence, DOC quantity and quality, chl *a*, and phytoplankton and zooplankton densities. In the second experiment, nutrient concentrations were also measured at the beginning and end of the experiment.

H_2O_2 and chl *a* determinations, and DOC fluorescence analyses were performed on a Shimadzu RF-1501 scanning spectrofluorometer with a xenon lamp (concave, non-aberration excitation/emission monochromators, with blazed holographic grating, F/2.4, 900 grooves/mm; dynode feedback light source compensation system with monochromatic light monitoring function; and photomultiplier tubes for both excitation and emission side detection; Mandel Scientific, Guelph, ON, Canada) and using optically clear quartz cuvettes (pathlength = 1.000 cm). The instrument was computer-driven using Shimadzu PC-1501 Personal Fluorescence Software for Windows.

Chl *a* analyses were performed according to standard procedures after filtering with combusted Whatman GF/F filters and extraction with 95% ethanol (Welschmeyer, 1994). Routine fluorometric H_2O_2 determinations were performed according to a modified scopoletin-horseradish peroxidase technique (Donahue, 1998). The major summer pollen pulse occurred at approximately Day 22 of the first experiment. Each morning, plexiglass shields were wiped clear of condensation, which ran back into the enclosures. In this way, pollen that was deposited on the plexiglass during the previous 24 hours eventually entered the enclosures beneath the plexiglass. This pollen

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pulse resulted in approximate doubling of [chl *a*] in all enclosures, and coincident increases in bacterial and phytoplankton densities. Pollen also contains large amounts of readily available phosphorus (J. Kennedy, C. Fraser; unpublished data; Lee et al., 1996). Due to this unexpected event, I contrast changes in biological factors over the course of the experiment with changes occurring during the first 22 days. Optical and DOC properties are described for the 5-week duration of this experiment.

As a result of financial limitation, a limited number of counts and identification of phytoplankton were done. I present the patterns that were evident, and compare inferred biomass to chl *a* concentrations to establish general relationships between the two for both experiments. In this manner, biomass and chl *a* data are used to complement each other in the interpretation of experimental results. Unfiltered samples for phytoplankton enumeration were preserved in 4% Lugol's preservative. Counts and identification were performed on an inverted microscope at magnifications of 125X and 400X with phase contrast illumination. All counts were done by a modified Utermöhl technique (Nauwerck, 1963), and wet biomass estimates were made from approximations of cell volumes of each species, according to best-fit formulae for different taxa (Vollenweider, 1974).

Spectrofluorometric analyses of DOC origin (allochthonous versus autochthonous) were performed on the samples according to McKnight et al. (In review). Scans of sample blanks of distilled deionized water (DDW) were also performed to remove effects of Raman scattering. Variations in lamp spectral intensities were corrected using scans of a standard 1 µg/L quinine sulfate solution in 0.1 N H_2SO_4 (Scully and Lean, 1994), and absolute fluorescence values are reported in quinine sulfate units, where: 1 QSU = fluorescence at a given excitation and emission wavelength of the standard quinine sulfate solution.

Fluorescence of samples that were acidified to pH 2 with concentrated HCl was measured, as well as for unacidified samples. Excitation radiation was fixed at 370 nm, and scans of emission intensities were performed from 370 nm to 650 nm. After subtraction of DDW blank fluorescence values from those of the acidified samples, the ratio of the emission intensity at 450 nm to that at 500 nm was calculated. A range of values from approximately 1.4 to 1.8-1.9 was used to indicate qualitatively the type of DOC; a ratio of 1.4 is typical of terrestrially-derived DOC, such as in a peat bog or low-order stream or lake, and a ratio of 1.9 is typical of microbially-derived DOC, such as in Antarctic lakes (McKnight et al., In review), which have little terrestrial vegetation or organic soil in their catchments, or acidified lakes, in which loss of aromatic portions of DOC molecules likely has occurred due to oxidation or precipitation (Donahue et al., 1998). Absolute peak fluorescence values, when combined with [DOC] and the DOC

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quality ratio, were used to corroborate the occurrence of potential changes in optical properties of DOC, as a result of either a change in the dominant source of DOC or solar bleaching.

DOC determinations were performed via high-temperature combustion (Ogawa and Ogura, 1992) in an Ionics (HTCO) carbon analyzer at the University of Alberta, or by persulfate digestion at the Freshwater Institute in Winnipeg, MB. Spectroscopic measurements of absorbance were performed at 327 nm as an indication of UV attenuation.

Repeated-measures (RM-) ANOVA was used to determine whether biological and chemical factors varied with treatment and time throughout the experiments. RM-ANOVAs were only run for the entire sampling period, normalizing for any significant effect of time. Mean values of factors such as [chl *a*], absolute fluorescence, DOC fluorescence ratio, and 1% UV transmittance (328 nm) on the same and different sampling days were compared using one-way ANOVA, and Bonferroni's modified LSD (least-squares distance) test. Cochran's and Bartlett-Box univariate homogeneity of variance tests and Levene's Test for equality of variance were used to determine similarity of variance among means. Coefficients of loss in DOC fluorescence and absorbance were calculated, and compared using ANOVA. All statistics were performed using SPSS 6.1.1 (1995). I consider a significance level of 0.05 to be statistically significant, however acknowledge that levels > 0.05 do not necessarily indicate that important changes were not occurring, due to the complex biological, chemical, and physical system being described, or that statistically "non-significant" changes may not have had some biological relevance.

Results

Effects on DOC

In the first experiment, there were no significant differences in DOC concentration between treatments on any of days 27, 31, and 35 (P=0.19, 0.94, and 0.23 respectively). There were also no significant treatment effects through time (P=0.48; Table 4-1). Based on similarities in absorbance and fluorescence at the beginning of the experiment, and on the lack of difference in initial DOC concentrations in similar enclosures previously placed in Lake 239 (P. J. Curtis; unpublished data), I am assuming that the concentrations of DOC at the beginning of this experiment were not significantly different between treatments. In the second experiment, DOC concentrations were the same at the beginning of the experiment, and had increased slightly in all three treatments by the final day (RM-ANOVA; P=0.22; Table 4-1). Thus, there were no significant differences in carbon loss rates for the No UV and No UV + H_2O_2 treatments. However, at the end of the summer

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experiment, DOC concentrations in the No UV + H_2O_2 enclosures were higher than in the UV-exposed enclosures (Table 4.1).

All treatments showed evidence of bleaching over the course of the experiments. UV-exposed enclosures had the greatest losses in fluorescence (Figures 4-1 and 4-2; RM-ANOVA; P<0.001). Enclosures shielded from UV and treated with H_2O_2 had fluorescence loss rates (k_{fluor}) intermediate between the enclosures exposed to and shielded from UVR (Figures 4-3 and 4-4). Enclosure depth was also important, with the highest k_{fluor} occurring in the shallowest enclosures (Figure 4-3).

Enclosures of the first experiment were left undisturbed in L239 for an additional 36 days after the end of the first experiment in early July, and sampled a final time. On August 14, in the 0.75 m enclosures, DOC in UV-exposed waters appeared to be largely allochthonous (DOC fluorescence ratio = 1.45±0.01; n=3), and UV-shielded waters relatively more autochthonous (ratio = 1.60±0.00; n=3; P<0.0001) (not shown). DOC concentrations in the UV-exposed and UV-shielded enclosures were not different, indicating that net loss rates of DOC were not increased by UV-induced bleaching. It does, however, suggest that allochthonous DOC in the UV-shielded enclosures was replaced in part by autochthonous DOC, or "autochthonous-like" DOC, the production of which was not related to either bleaching of allochthonous material or biological activity.

At the beginning of the second experiment, all treatments had similar allochthonous DOC (P=0.087), but by Day 5 both the UV-shielded and H_2O_2 treatments had increased proportions of autochthonous DOC relative to UV-exposed enclosures and L239 (P=0.0001; Figure 4-5). The degree of difference in DOC quality between these two groups increased during the remainder of the experiment; the UV-exposed enclosures and L239 contained apparently unchanging allochthonous DOC throughout the experiment, whereas the enclosures of the other two treatments experienced a continued shift towards more autochthonous DOC (RM-ANOVA; P<0.001).

Effects of hydrogen peroxide and UVR on DOC bleaching

Bleaching of DOC in enclosures also led to increased UVR penetration transmittance in the first (P=0.001) and second (P=0.06) experiments (Figures 4-6 and 4-7). During the June-July experiment, 1% UV-depth increased from 0.40 to 0.63 m (+57.0%) in the UV-exposed enclosures (Figure 4-6). In the H_2O_2-treated enclosures, 1% UV-depth increased to 0.52 m (+30.1%), and in the UV-shielded enclosures it increased to 0.48 m (+21.2%). In the second experiment, during late-August and September, 1% UV depth increased in the UV-exposed enclosures from 0.33 m to 0.40 m (+20.2%), from 0.31 m to 0.37 m (+17.0%) in the No UV + H_2O_2 enclosures, and from 0.35 m to 0.39 m (+13.3%) in the

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UV-shielded enclosures (Figure 4-7). In Lake 239, 1% UV depth was highly variable throughout the experiment, and by the end of the 33 days had decreased by 3.7%.

Absorbance loss coefficients (k_{abs}) from the UV-exposed enclosures in the first experiment were much greater than from the UV-shielded enclosures, and the loss coefficients from the No UV + H_2O_2 treatment were intermediate (Figure 4-8). In the second experiment, not only was k_{abs} for each treatment lower than in the first experiment, but the differences between them were also less (P=0.076; Figure 4-9). However, k_{abs} from the No UV + H_2O_2 treatment was still between those for the UV-shielded and -exposed enclosures.

Effects of hydrogen peroxide and UVR on nutrient concentrations

At the end of both experiments, TDN concentrations were highest in the H_2O_2-treated enclosures. In the June experiment, TDN concentrations in the UV and H_2O_2-treated enclosures were higher than in the No UV enclosures ($P \leq 0.05$; Table 4-2). However, in the September experiment, TDN concentrations were higher in the H_2O_2-treated enclosures than in the UV-exposed enclosures (P=0.065; Table 4-3). Also in the September experiment, NO_3 concentrations were lower in the UV-exposed enclosures than in both of the other treatments (P=0.007), and NH_4^+ was lower in the UV-exposed enclosures than in the H_2O_2-treated enclosures (P=0.179). Generally, high variation between replicates reduced the significance of differences in nutrient concentrations in the second experiment. TDN:TDP was higher in L239 than in the UV-exposed enclosures, because of decreased TDN in the enclosures and lower TDP in the lake. Particulate N:P and C:P ratios in L239 were less than in the UV-exposed enclosures because of lower particulate P in the UV-exposed enclosures and lower particulate N in the lake.

Effects on phytoplankton

Chlorophyll a

In both experiments, H_2O_2 additions to UV-shielded enclosures initially resulted in greater chl *a* concentrations than in the UV-shielded and UV-exposed enclosures. Between days 9 and 18 of the June experiment, when the first biological effects of the pollen pulse appeared, chl *a* concentrations in the H_2O_2 treatments was 29-66% higher than in the UV-shielded enclosures and 48-99% higher than in the UV-exposed treatments (LSD test; $P<0.05$) (Figure 4-10a). By day 18, chl *a* concentrations had decreased in the UV-exposed enclosures by 47%, in the UV-shielded enclosures by 11%, and in the H_2O_2-treated enclosures by 5%.

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Before day 18 of the second experiment, chl *a* concentrations in the No UV, H_2O_2-treated enclosures were 28-36% higher than in the UV-shielded enclosures, and 30-43% higher than in the UV-exposed treatments ($P<0.05$; Figure 4-10b). On day 18 of the second experiment, chl *a* concentrations in all H_2O_2-treated enclosures were higher than in all other enclosures, at 11.6 µg/L, 6.0, and 6.9 µg/L (8.2±3.0 µg/L), compared to 4.7±0.4 and 4.9±0.9 µg/L, respectively in the UV-shielded and UV-exposed enclosures. The bloom in the one H_2O_2-treated enclosure reached a peak on Day 22, with a chl *a* concentration of 13.7 µg/L. Had the concentration of chl *a* in the one H_2O_2-treated enclosure not been so high and induced such high variance in the mean, chlorophyll for the H_2O_2 treatment may have been significantly different than in the other treatments on days 18 or 22. After Day 22 in the second experiment, the concentration of chl *a* in the H_2O_2-treated enclosures declined to levels similar to those of the other treatments ($P\geq0.20$).

Algal biomass

At the beginning of the first experiment algal biomass was the same in all treatments (Figure 4-11). Biomass had approximately doubled by day 13 in the H_2O_2-treated enclosures and, although it may have decreased between Days 13 and 26, was still higher after 26 days than at the beginning of the experiment (Figure 4-12). Despite these increases in algal biomass in the H_2O_2-treated enclosures at the end of the experiment, enclosures shielded from UV had the highest algal biomass, and biomass estimates in the H_2O_2-treated and UV-exposed enclosures were not different (Figure 4-13). In the second experiment, algal biomass in the H_2O_2-treated enclosures was higher than in the other treatments on day 18, and similar to the other treatments on the final day (Figure 4-14).

Patterns of difference in algal biomass were similar to changes observed in chl *a* concentrations. A positive relationship existed between algal biomass and chl *a* for both experiments (Figure 4-15) Because the ranges of chl *a* concentrations observed during the experiments are covered by the comparisons, I have inferred that other concentrations of chl *a* and levels of biomass for phytoplankton during the experiments were likely similarly correlated. However, the biomass:chl *a* relationships did differ seasonally. Algae in the September experiment generally had more chl *a* per unit volume than in the June experiment (Figure 4-15).

Algal community changes

At the beginning of the first experiment, chrysophytes accounted for more than 50% of the total biomass of the phytoplankton community (Figure 4-11). Dinoflagellates, cryptophytes, and diatoms comprised the majority of that which remained, and

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chlorophytes generally accounted for a small portion (Figure 4-11). On Day 13, diatoms dominated the community in the H_2O_2-treated enclosures , whereas chrysophytes had become much less abundant (Figure 4-12). However, unlike the diatom domination in the H_2O_2-treated enclosures at the end of the experiment, the algal assemblages in UV-shielded and UV-exposed enclosures were dominated by chlorophytes and cyanophytes. In the UV-shielded enclosures, all taxa except euglenophytes and chrysophytes had greater biomass than in the UV-exposed enclosures at the end of the first experiment (Figure 4-13).

At the beginning of the second experiment, algal assemblages were similar among treatments and dominated by diatoms and cyanophytes (Figure 4-14). On day 18 of the second experiment, cyanophyte, diatom, and chlorophyte biomass were higher in the H_2O_2-treated enclosures than the other treatments, and diatom biomass was higher on day 30 (Figure 4-14).

Discussion

My experiments showed that the primary mechanism for increasing UV exposure was the bleaching of DOC by high-energy wavelengths in the UV spectrum, rather than simply DOC removal. Fluorescence and absorbance in the UV declined while DOC concentrations did not decrease. UV-exposed waters had the highest loss rates for fluorescence and absorbance, and UV-shielded waters had the lowest loss rates. The UV-shielded H_2O_2-treated enclosures had loss rates midway between the two optical treatments, indicating that some of the loss of fluorescence and UV absorbance was caused by H_2O_2, independent of UVR exposure. Although the loss rates for the shallow UV-exposed enclosures were quite high, extrapolation results in a predicted half-time for fluorescence of 120 days, very close to the 122 day half-time for colour found by Curtis and Schindler (1997) in similarly constructed, uncovered 3 m deep enclosures. Loss rates of the second experiment were lower than the first, and the differences among the treatments were not as great. This was likely because photochemical rates, including bleaching and production of oxygen radicals through the Fenton reactions, are greater in June than in September due to higher solar flux intensity and the longer days.

Combined, changes in fluorescence:absorbance relationships and the fluorescence ratios suggest that there was a shift toward a greater proportion of autochthonous or "autochthonous-like" (i.e., smaller molecular weight and lower aromaticity) DOC in both UV-shielded treatments. Low molecular weight DOC can fluoresce more per unit absorbance than high-molecular-weight DOC (Stewart and Wetzel, 1980). In both experiments, k_{abs}:k_{fluor} in the UV-shielded and H_2O_2-treated enclosures (respectively, 0.93

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and 0.95 in the June experiment, and 1.23 and 1.50 in the September experiment) was greater than in the UV-exposed enclosures (0.73 and 0.69 in Experiments 1 and 2). This suggests that the ratio of low to high molecular weight DOC within the UV-shielded and H_2O_2-treated enclosures increased, relative to the UV-exposed enclosures, throughout the experiments. This is contrary to what one might expect, if one assumed that UV exposure of DOC in the enclosures for a 5-week period would result in significantly greater photolysis of DOC in the UV-exposed enclosures than in the UV-shielded treatments (Wetzel et al., 1995). One could conclude that the proportion of autochthonous-like DOC in UV-shielded and H_2O_2 treatments increased, similar to the shifts in the quality of DOC observed during experimental acidification of ELA lakes (Donahue et al., 1998). However, changes observed as a result of experimental acidification were likely a result of chemical oxidation or precipitation of aromatic portions of DOC molecules. In the enclosure experiments described here, observed qualitative differences in DOC between both No UV and H_2O_2 treatments and UV-exposed enclosures, as identified by altered optical properties, can be explained by either enhanced carbon fixation or microbial modification of DOC in the UV-shielded treatments. It is possible that H_2O_2 may have increased the microbial bioavailability of DOC, in a similar manner to UV photolysis (Lindell et al., 1995, Wetzel et al., 1995). Ultimately, however, it appears that autochthonous production of DOC was enhanced in the UV-shielded and H_2O_2-treated enclosures, and was not in the UV-exposed enclosures.

The seasonal difference in the algal biomass:chl *a* may have been as a result of the need to maximize photosynthetic potential in lower-light conditions in September, or conversely achievement of photosynthetic saturation in June. It is also possible that pigments were bleached more during the June experiment by the greater intensity of solar radiation at this time of year. The difference observed in the enclosures emphasizes the need to calibrate biomass and pigment relationships because of their ability to change, temporally and spatially.

The algal blooms observed in the H_2O_2-treated enclosures on Day 18 suggest that most major taxa, and particularly diatoms, were stimulated, either as a result of direct stimulation or perhaps decreased microbial competition (Xenopoulos and Bird, 1997). Diatoms can be considered as rapid colonizers or early successional species, responding quickly when presented with an open niche (Oemke and Burton, 1986). At the same time, the lower relative diatom biomass observed in the UV-exposed enclosure was consistent with other studies that have identified negative sensitivities of diatom communities to UV radiation (Bothwell et al., 1993, Buma et al., 1995, Mostajir et al., 1999, Vinebrooke and Leavitt, 1999).

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It is also possible that algae were stimulated as a result of H_2O_2-aided chemical transformation of nutrients from organic to inorganic forms. Differences observed in dissolved nitrogen species concentrations and stoichiometric ratios between the treatments suggest there may have been enhanced photochemical nutrient transformation in the H_2O_2-treated enclosures. However, higher ammonium concentrations in the H_2O_2-treated enclosures also could indicate reduced NH_4-uptake, similar to that reported previously as a result of UVR-induced photoinhibition of marine phytoplankton (Behrenfeld et al., 1995). It may also be a result of inhibition of nitrification by certain dissolved organic compounds and their decomposition products, as observed in soils (Rice and Pancholy, 1972, Rice and Pancholy, 1973).

Despite lower phytoplankton biomass in the UV-shielded enclosures than in the H_2O_2-treated enclosures during much of the two experiments (perhaps suggestive of lower algal production), coincident shifts toward a more autochthonous DOC signal seen in the UV-shielded enclosures could have been a result of greater relative bacterial production. Mostajir et al. (1999) found that carbon fixation was the same in enclosures exposed to different fluxes of UVR, but partitioning between phytoplankton and bacterial production varied with UV exposure. Recent work investigated the short-term (~1 hour) effects of added H_2O_2 upon phytoplankton and bacterial production rates in 20 mL incubations of lake water (Xenopoulos and Bird, 1997). Photosynthetic uptake of $^{14}CO_2$ and bacterial incorporation of ^3H-leucine decreased when $[H_2O_2]$ was greater than 1000 nM. However, photosynthesis appeared to be stimulated by concentrations of 100 nM, and bacterial leucine uptake was inhibited by concentrations as low as 3.8 nM (Xenopoulos and Bird, 1997). In my experiments, with the daily pulse additions of H_2O_2 to a concentration of 700 nM, bacterial production could have been greatly reduced in the absence of UVR without a significant inhibition of photosynthetic production. In the presence of such a H_2O_2-induced decrease in bacterial production, phytoplankton production may have increased as a result of removal of competition for nutrients. In addition, in the UV-exposed enclosures, any increased availability of nutritive break-down products of UV-induced photolysis of DOC that otherwise have been found to enhance bacterial production, such as small fatty acids like acetic, formic, citric, pyruvic, and levulinic (Wetzel et al., 1995), might not have any great effect on bacterial production as a result of H_2O_2 and UVR inhibition. Likewise, direct inhibition of phytoplankton by UVR could have negated any potential increase in nutrient availability.

Enhanced UVB may lead to a trophic shift of matter and energy away from an herbivorous food web and into microbial food webs as a result of differing trophic sensitivities to UVB and suppression of ciliate predators of heterotrophic flagellates

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(Mostajir et al., 1999, Vinebrooke and Leavitt, 1999) . My results indicate that oxygen radicals like H_2O_2 may exert pressure in the opposite direction, leading to trophic shifts away from a microbial food web and toward an herbivorous one. Phytoplankton production in many lakes is nutrient limited. However planktonic chl *a* had a general positive relationship with DOC and negative relationships with PAR and UVB flux in 8 lakes surveyed at the Experimental Lakes Area covering a range of DOC concentrations (unpublished data; Chapter 5). In the absence of a strong UVB flux, such as in waters with higher concentrations of DOC, pelagic communities might shift toward an herbivorous food web as a result of greater shielding from UVR and generation of higher concentrations of reactive oxygen species that are more harmful to bacteria.

Reactive oxygen species are not necessarily isolated to the shallow strata exposed to high UVR. Indirect effects of UV radiation, in the form of biological and chemical changes brought about by reactive oxygen radical production, may thus be observed at depths beyond which UV radiation penetrates in boreal lakes. Dilute H_2O_2 enhances the bleaching of DOC by UVR, and may have the capacity to alter DOC qualitatively throughout a mixed layer. My study also suggests that solar ultraviolet radiation and its photochemicals can have very different effects on planktonic communities. However, differential sensitivities of the microbial community to photolytic changes in nutrient and reactive oxygen species concentrations and UV radiation need to be examined in more detail to determine what balance exists between them under natural conditions, and what roles they play in the structuring of planktonic food webs.

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Table 4-1. DOC concentrations (mg/L) during 2 enclosure experiments, from June 4 - July 9 and August 18 - 20 September, 1996, in Lake 239 at the Experimental Lakes Area (± S. D.; n=3).

	Experiment 1			Experiment 2	
	Day 27	Day 31	Day 35	Day 2	Day 33
No UV	7.48 ± 0.14	8 ± 0.28	7.56 ± 0.32	6.42 ± 0	6.71 ± 0.26
No UV + H_2O_2	7.24 ± 0.18	7.96 ± 0.30	7.16 ± 0.07	6.56 ± 0.26	7.16 ± 0.26
UV	7.56 ± 0.24	7.92 ± 0.24	7.64 ± 0.45	6.27 ± 0.29	6.56 ± 0.26
P (1-way ANOVA)	0.19	0.94	0.23	0.30	0.07

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Table 4-2. Experiment 1: Nutrient concentrations in enclosures exposed to and shielded from solar UVR, and in enclosures shielded from UVR and treated with a once-daily 700. nM H_2O_2 pulse ($P \leq 0.07$ for differences between * and **)

Experiment 1	TDN (µg/L ± S. D.)		TDP (µg/L ± S. D.)	
	Day 14	Day 35	Day 14	Day 35
No UV	301.7±24.7	178.3±25.2*	2.33±0.58	2.00±1.00
H_2O_2	290.0±30.4	236.7±12.6**	2.00±0.00	1.67±0.56
UV	298.3±38.2	228.3±7.6**	2.33±0.58	1.33±0.56

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Table 4-3. Experiment 2: Nutrient concentrations in enclosures exposed to and shielded from solar UVR, and in enclosures shielded from UVR and treated with a once-daily 700 nM H_2O_2 pulse, on Day 33 (all concentrations are µg/L ± S. D.) (P≤0.065 for differences between * and **).

Expt 2	Dissolved					Particulate		
(Day 33)	NO3	NH4+	TDN	TDP	N:P	N:P	C:N	C:P
No UV	11.7±4.2**	39.3±13.1	293.3±32.2	2.0±1.0	173±85	23±9	12±2	270±65
H_2O_2	12.7±3.1**	55.0±10.4	328.3±24.7**	1.7±0.6	214±74	24±8	12±0	276±91
UV	1.3±1.4*	34.0±13.5	250.0±21.2*	3.0±1.4	92±36	29±4	11±1	334±35
L239	0.5	23.0	305.0	1.0	305	20	12	240

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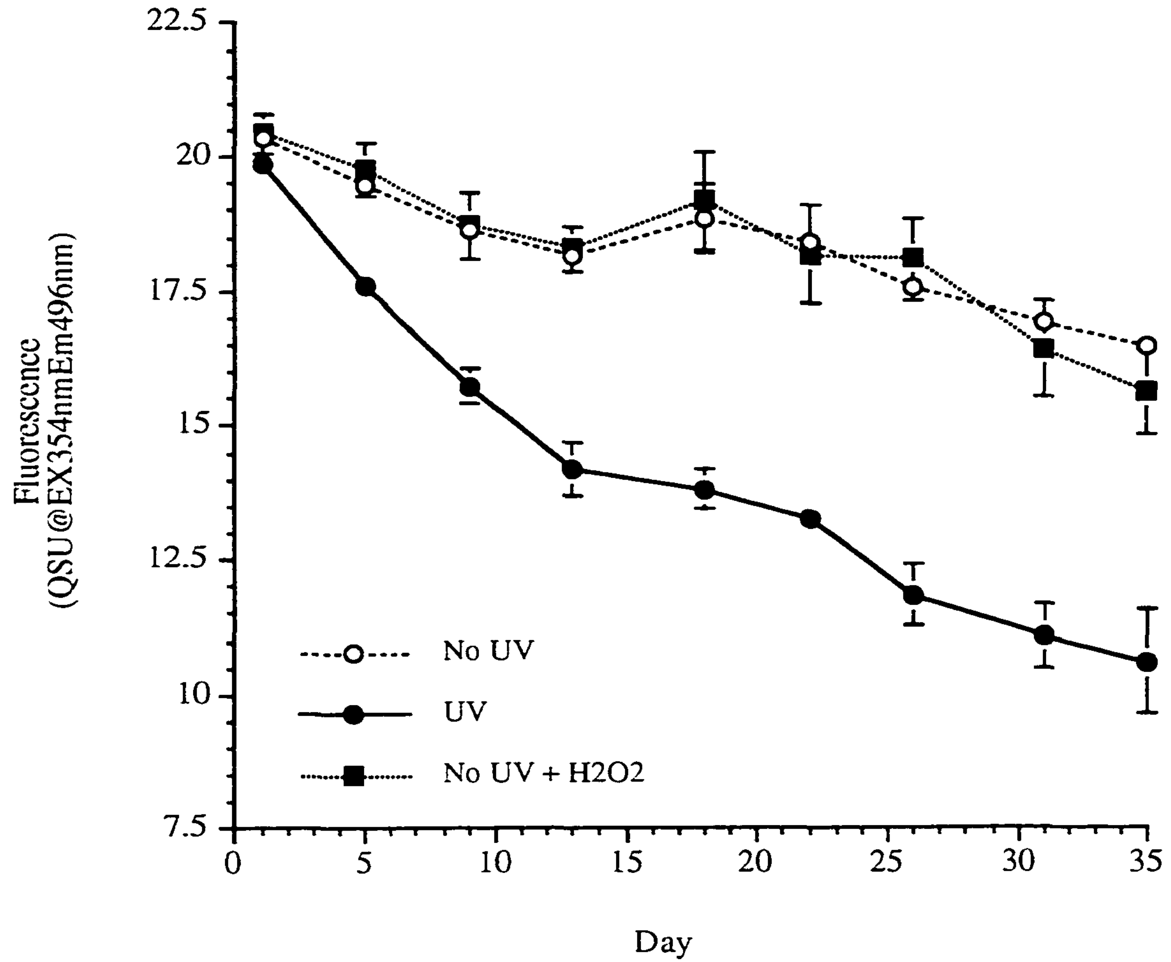

Figure 4-1. Decreases in QSU fluorescence in 0.75 m deep UV-exposed, UV-shielded, and No UV + H_2O_2 (700 nM once-daily addition; 1 enclosure was 1.125 m deep) enclosures from June 5 - July 9, 1996.

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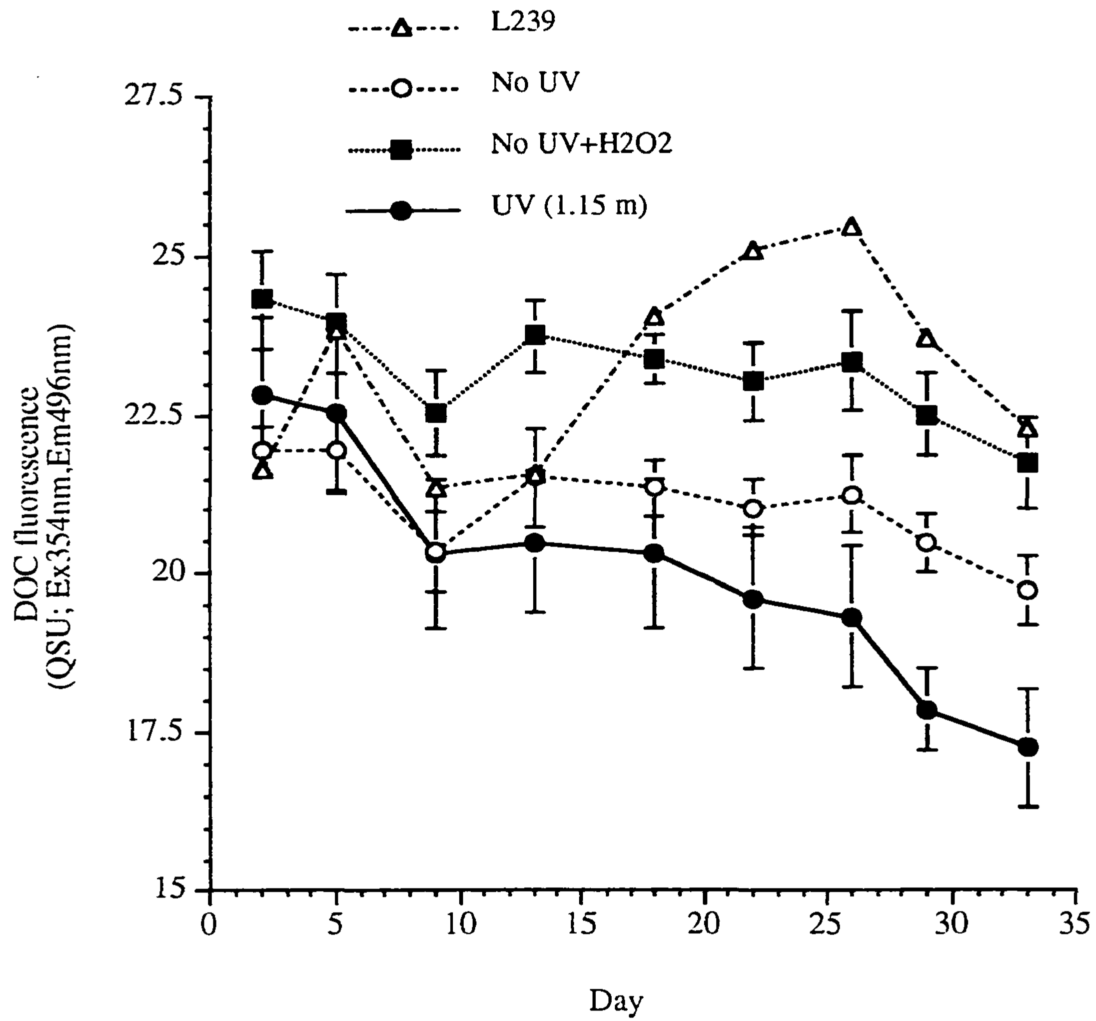

Figure 4-2. Changes in DOC fluorescence in 0.75 m deep UV-shielded and UV-shielded + 700 nM H_2O_2, and in 1.15 m UV-exposed enclosures during 5 weeks, from August 18 - September 20, 1996.

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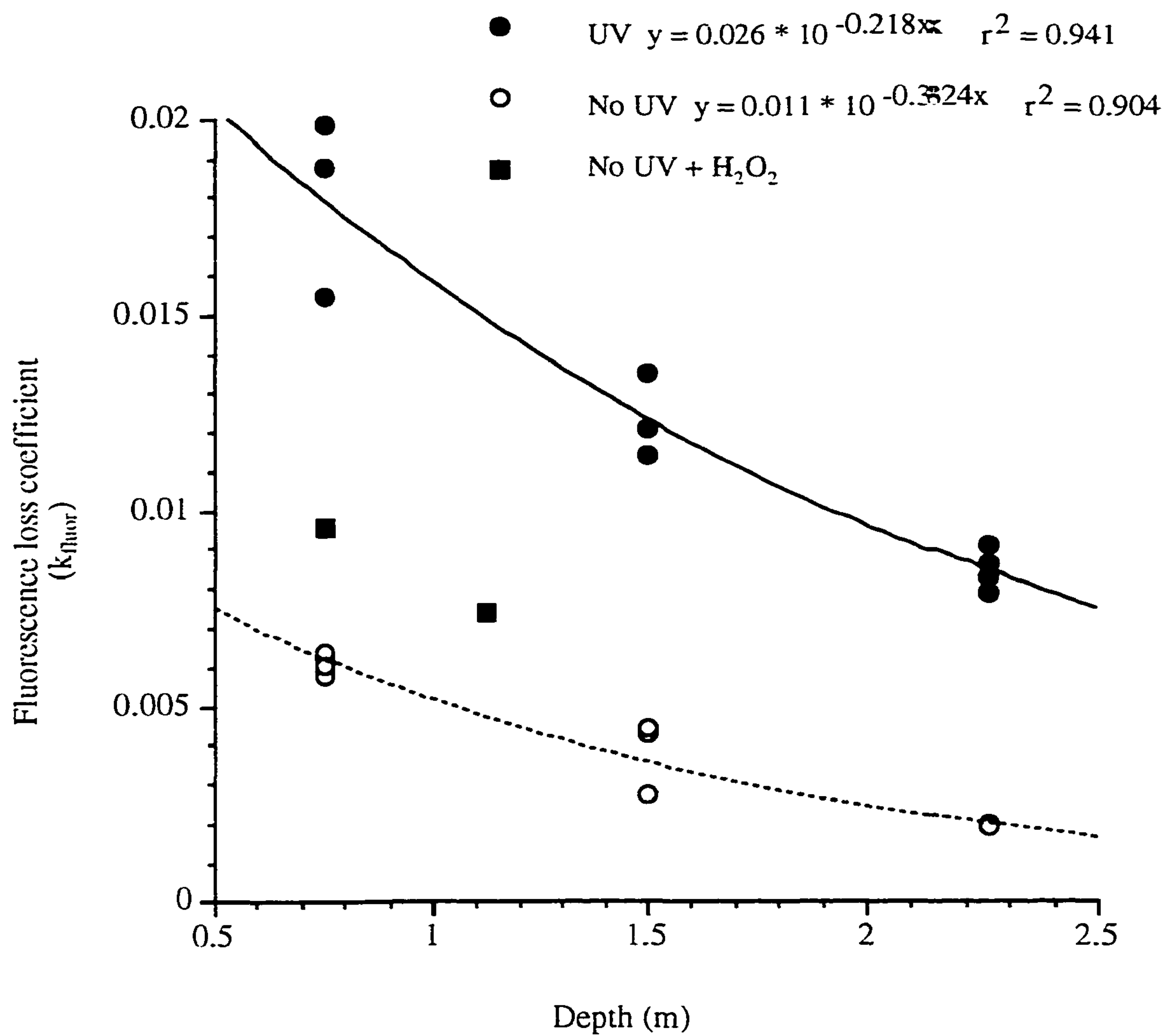

Figure 4-3. Decrease in fluorescence loss coefficient with increasing depth of enclosure and UV-shielding. Enclosures shielded from solar UVR and treated once daily with a 700 nM pulse of H_2O_2, however, had loss coefficients midway between the UV-exposed and UV-shielded enclosures. Two H_2O_2-treated enclosures were 0.75 m deep, and one was 1.15 m deep. Comparison to a range of depths of UV-shielded and UV-exposed enclosures is made to account for the two depths of H_2O_2-treated enclosures.

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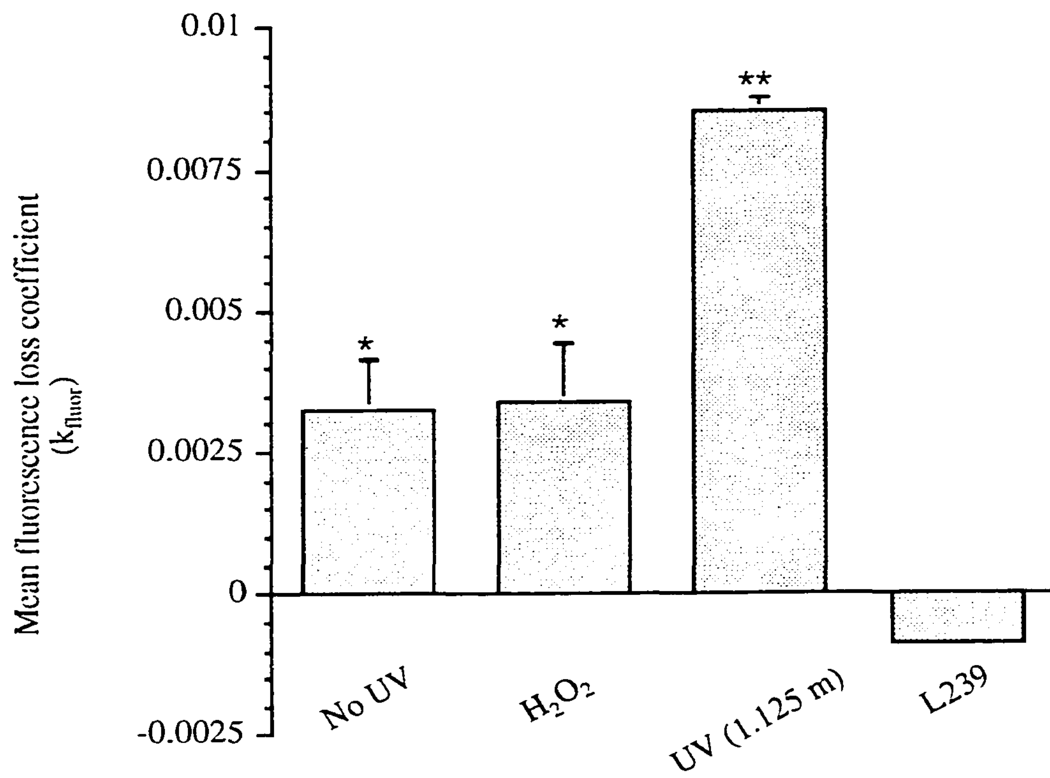

Figure 4-4. Mean fluorescence loss coefficients (± S. D.) over 34 days in 0.75 m UV-shielded and No UV + H_2O_2 enclosures, 1.125 m UV-exposed enclosures, and L239 at the Experimental Lakes Area ($P<0.05$; August 18 - September 20, 1996).

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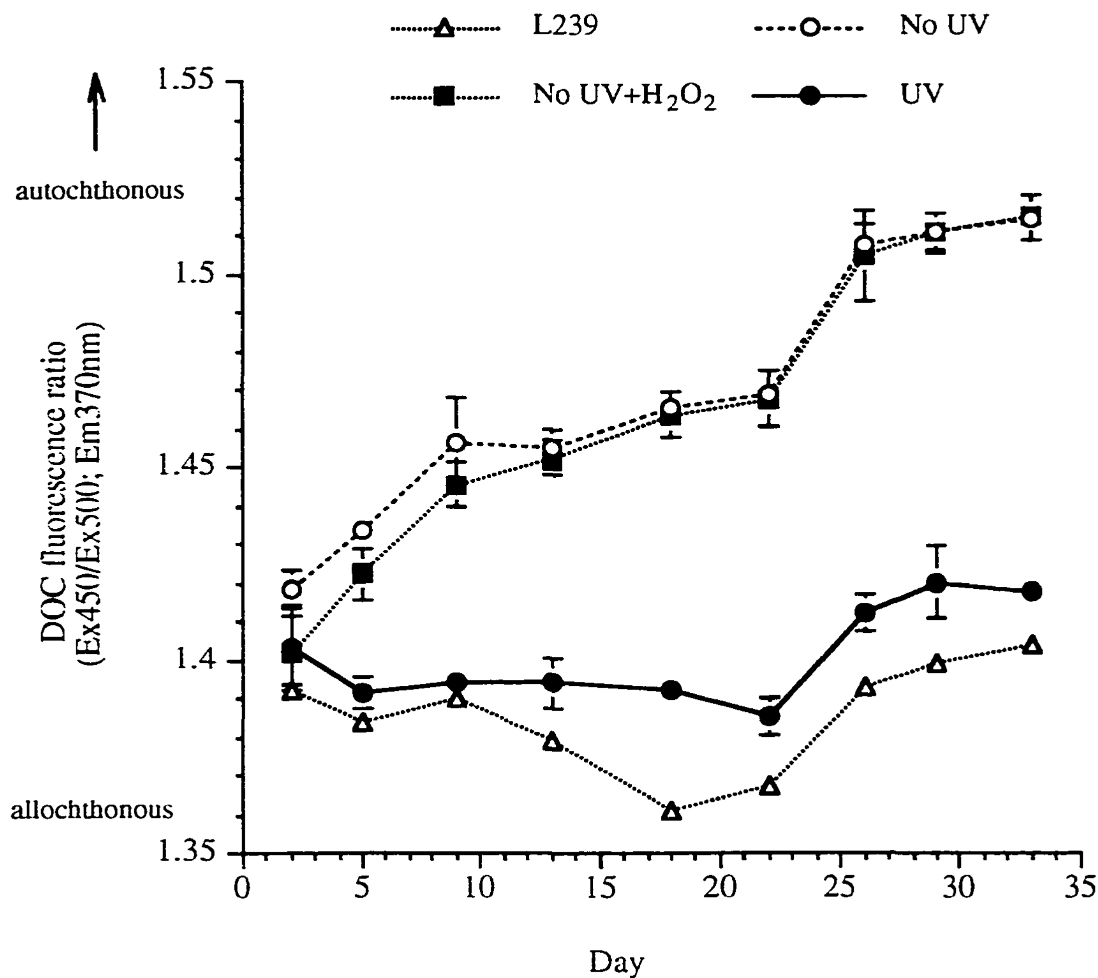

Figure 4-5. Increases in proportion of autochthonous DOC in UV-shielded and No UV + H_2O_2 enclosures. Fluorescence ratios in UV-shielded and No UV + H_2O_2 enclosures were significantly higher than in UV-exposed enclosures, and not significantly different from each other, from day 5 until day 34. Note that, despite bleaching, DOC in UV-exposed enclosures remained allochthonous throughout the 5-week experiment.

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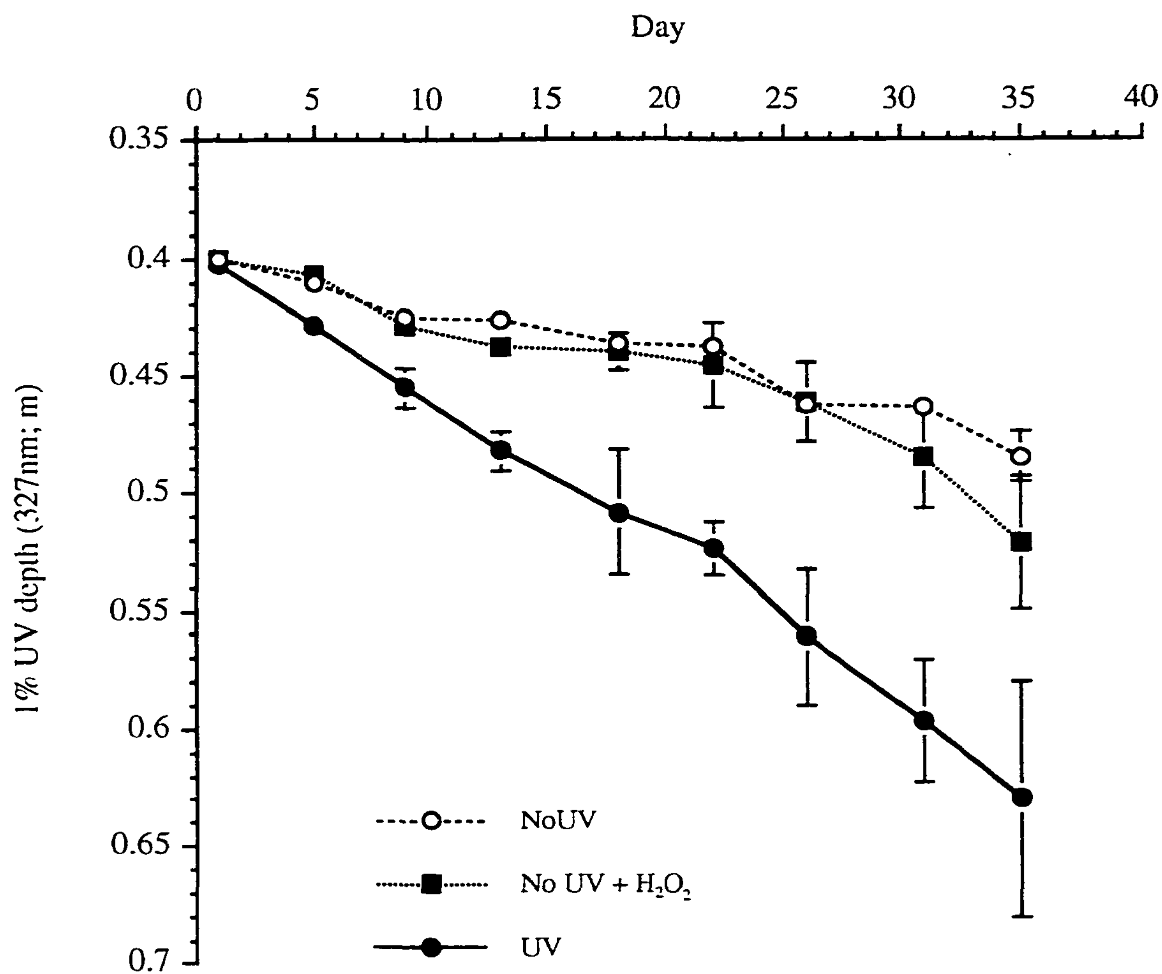

Figure 4-6. Increases in UVR penetration in enclosures shielded from and exposed to solar UVR, and shielded from UVR with daily 700 nM pulse additions of H_2O_2 during a 5-week experiment (June 5 - July 9, 1996).

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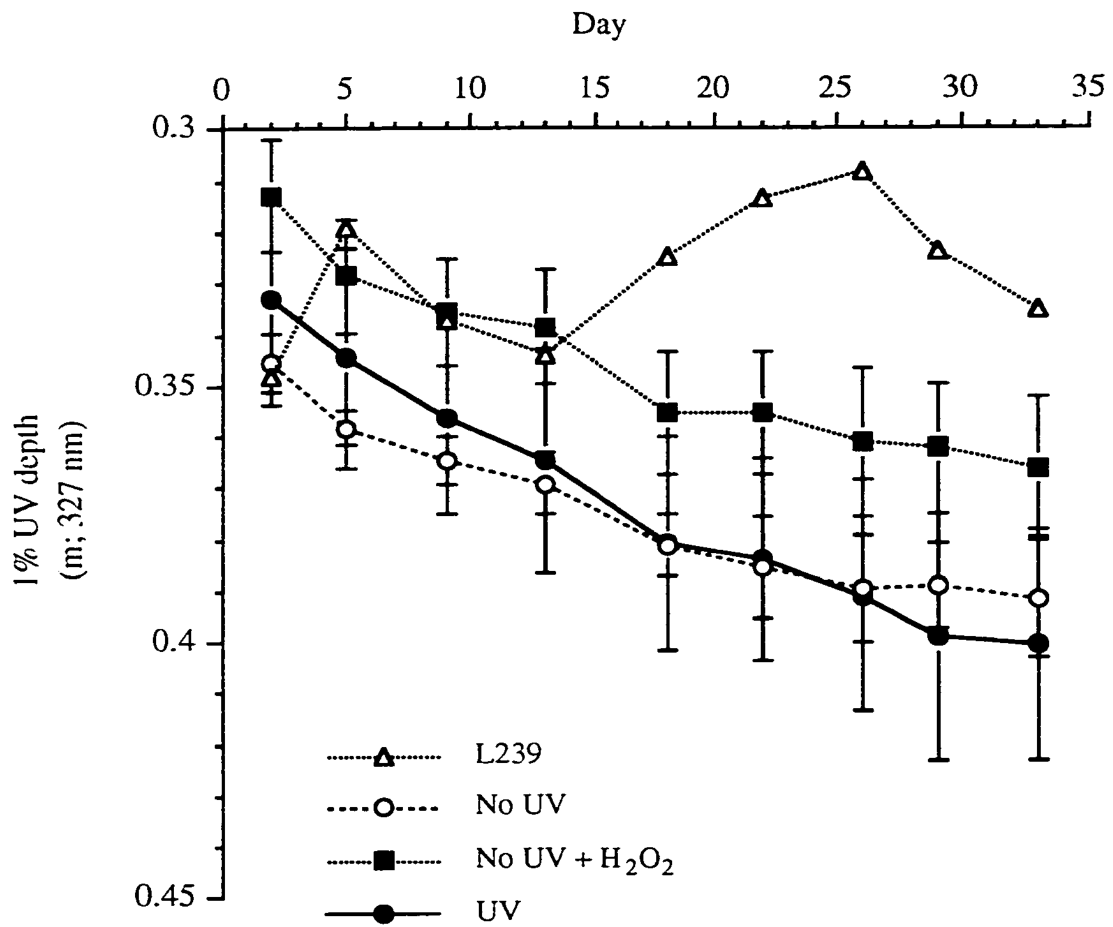

Figure 4-7. Mean 1% UV depth in UV-shielded, UV-exposed, and No UV + H_2O_2 enclosures and L239, over 5 weeks in the autumn of 1996 (± S. D.).

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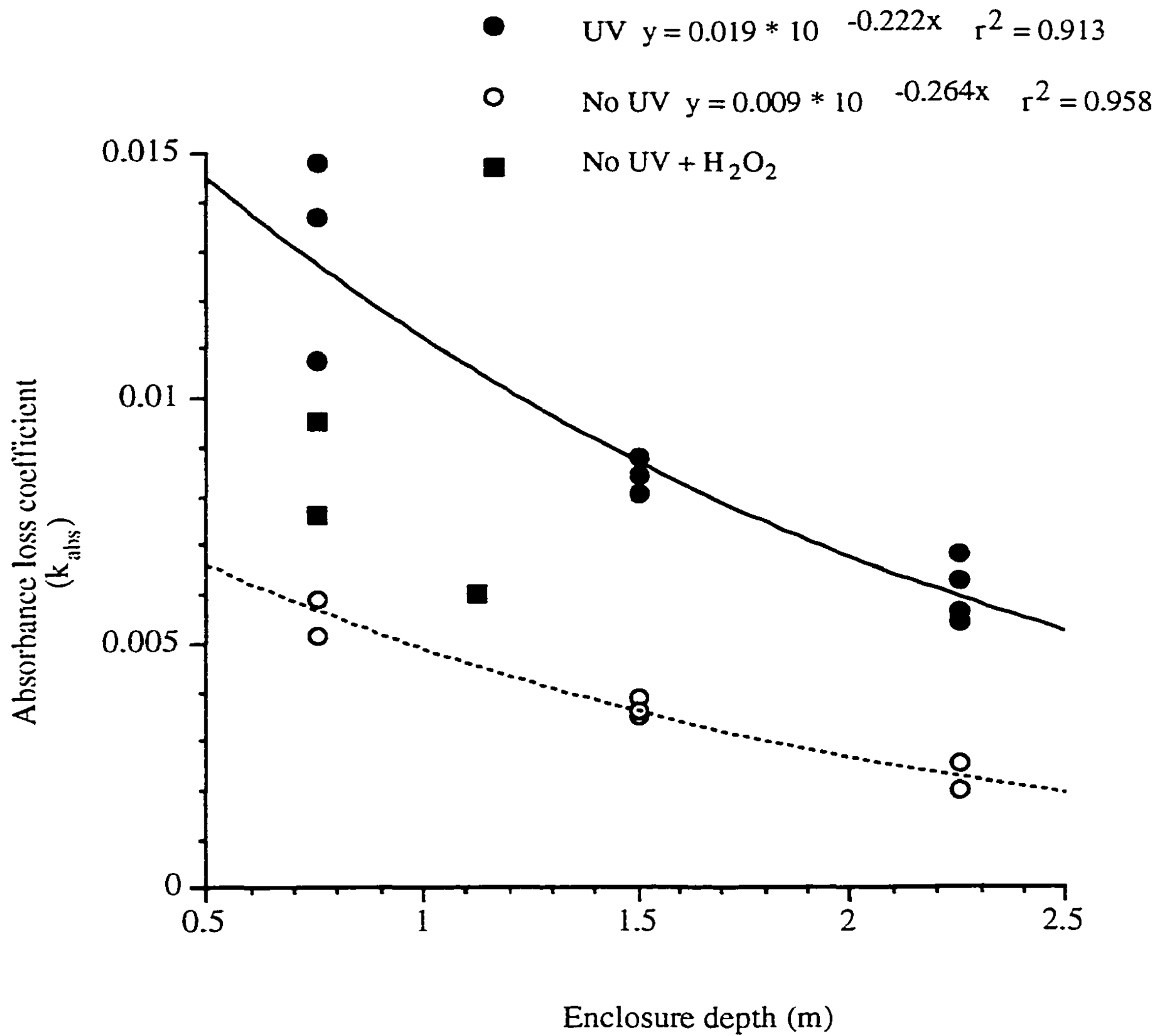

Figure 4-8. Decreases in absorbance loss coefficients with increasing depth of enclosures and UV-shielding. Enclosures shielded from solar UVR and treated once daily with a 700 nM pulse of H_2O_2 had loss coefficients between those of the UV-exposed and -shielded enclosures.

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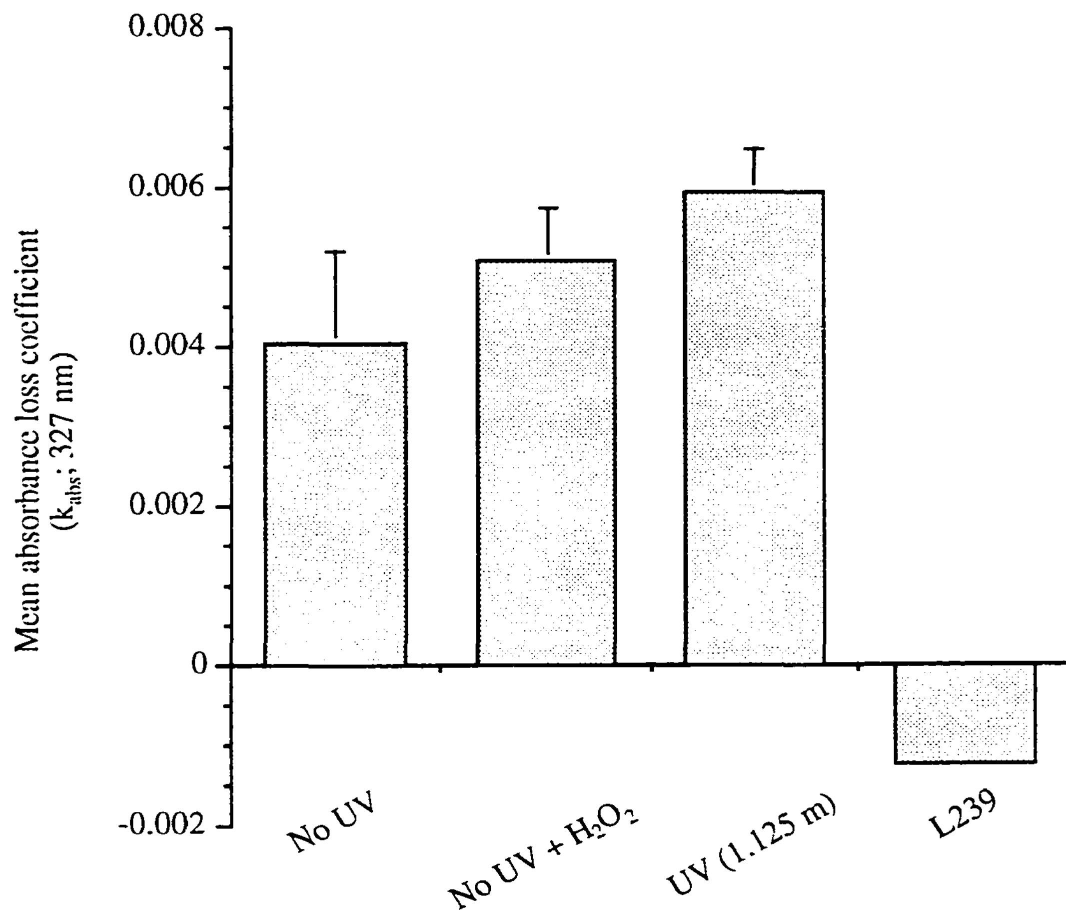

Figure 4-9. Mean absorbance loss coefficients in 0.75 m No UV and No UV + H_2O_2 enclosures, 1.125 m UV-exposed enclosures, and L239 at the Experimental Lakes Area (August 18 - September 20, 1996).

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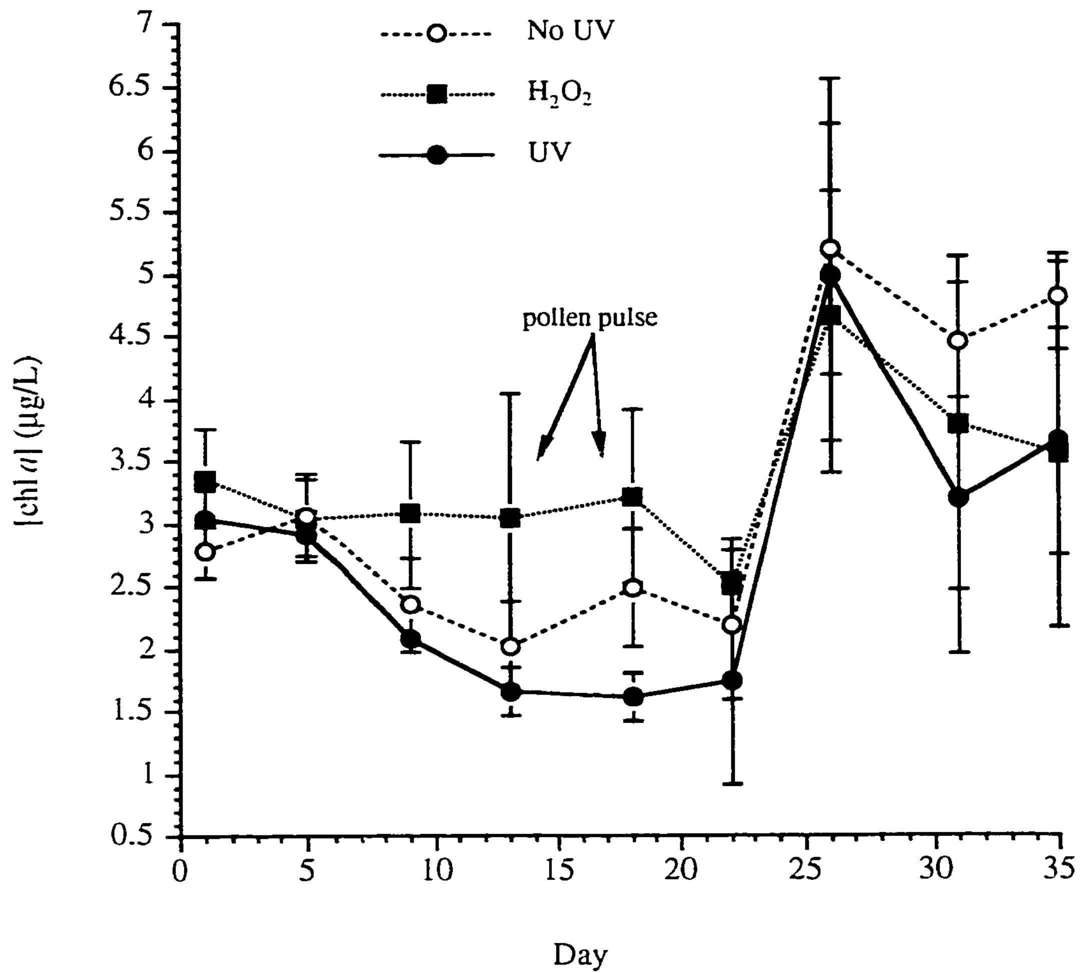

Figure 4-10a. Chlorophyll a *concentrations in UV-exposed, UV-shielded, and No UV + H_2O_2 enclosures over the course of a 5-week experiment (June 5- July 9, 1996). Unanticipated nutrient release from the pulse of pollen resulted in large increases in chlorophyll in all treatments.*

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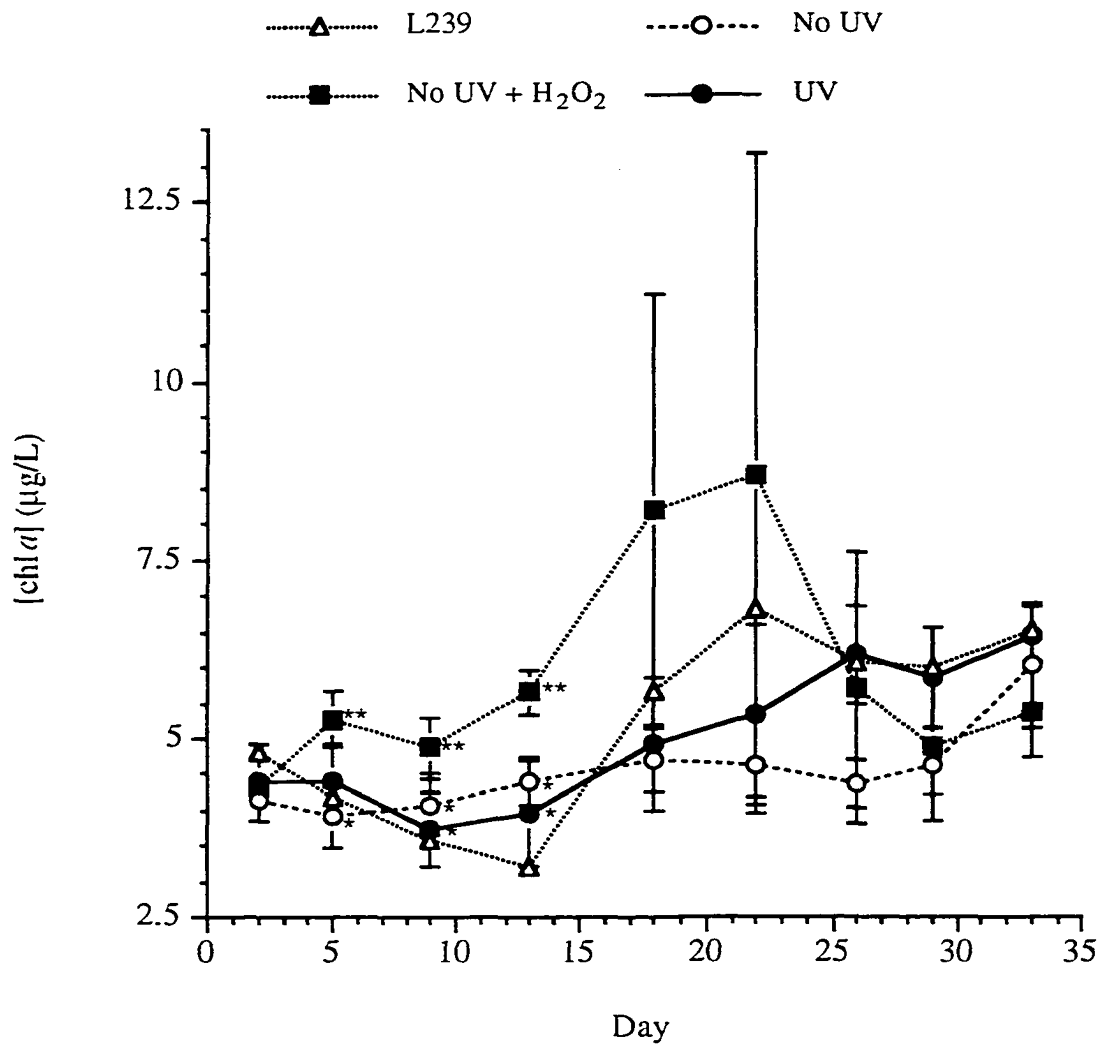

*Figure 4-10b. Chl a in UV-shielded, UV-exposed, and No UV + H_2O_2 enclosures from August 18 - September 20, 1996. Until day 13, chlorophyll in No UV + H_2O_2 enclosures was greater than in the other treatments ($P<0.05$). After this, a bloom over the next 10 days in one of the H_2O_2-treated enclosures was great enough to induce large variance in the treatment mean chlorophyll concentration, even though concentrations in the other H_2O_2-treated enclosures were higher than any in the other treatments (on each day, ** is greater than * by $P<0.05$).*

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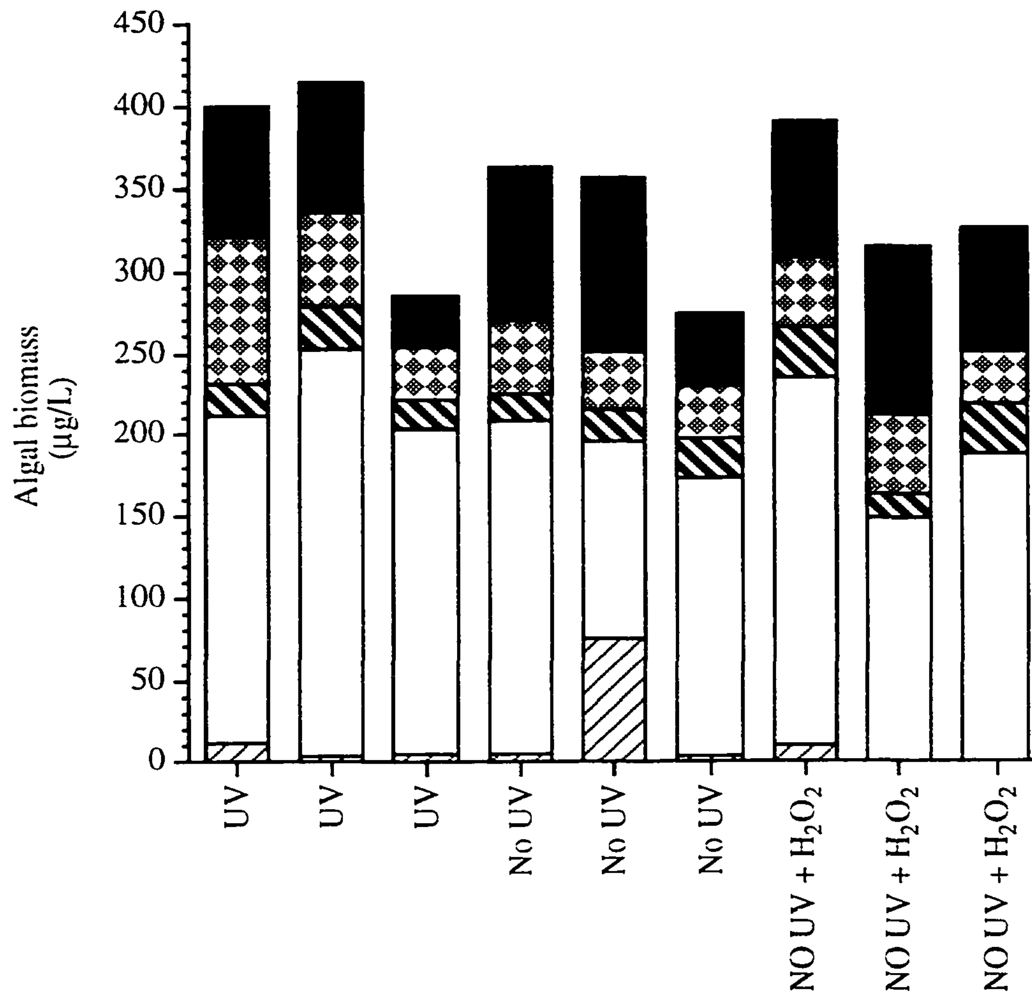

Figure 4-11. Algal biomass in shallow enclosures on day 1 of the first experiment (June 5, 1996). Chrysophytes, dinoflagellates, and cryptophytes dominated in all 3 treatments. There were no differences between assemblages of the different treatments (P>0.05).

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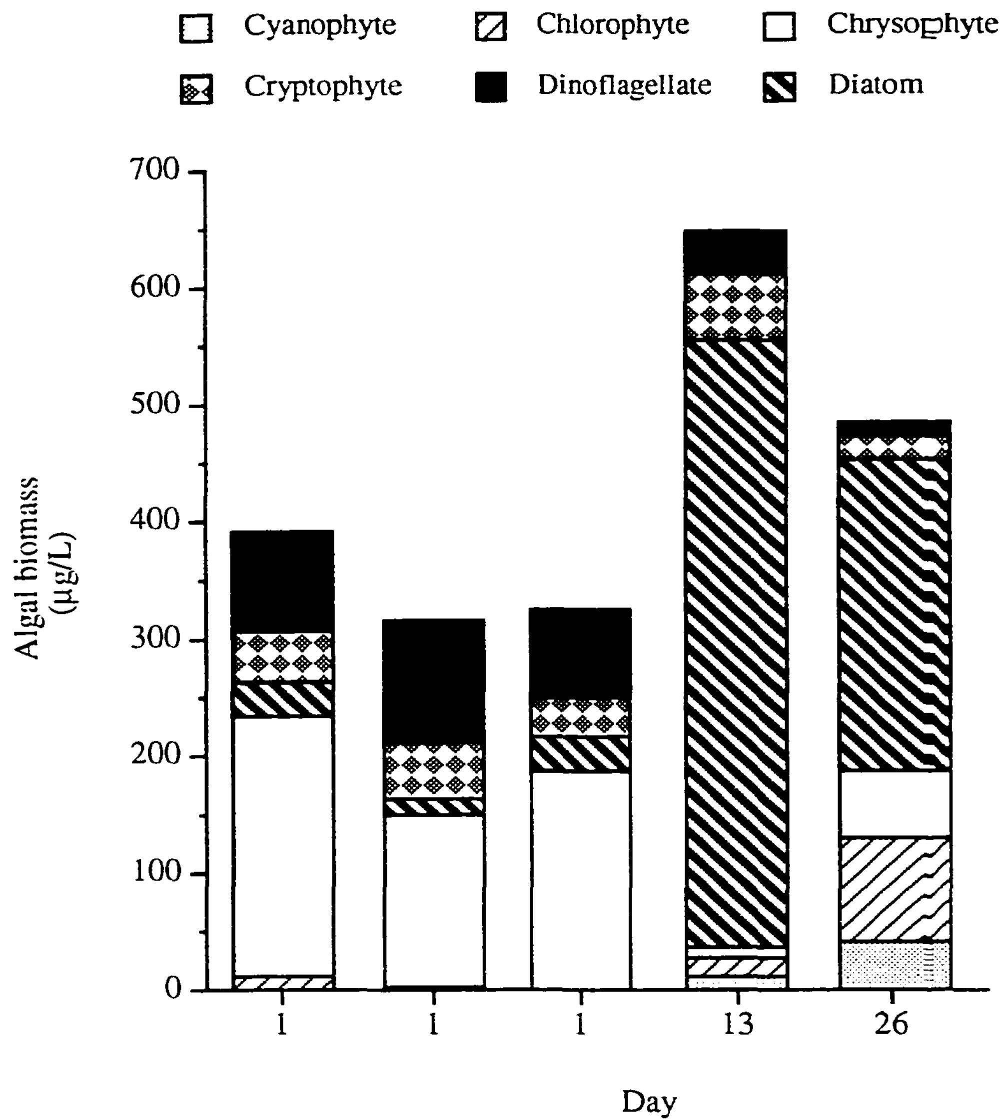

Figure 4-12. Algal biomass in 0.75 m enclosures shielded from solar UVR and treated once daily with a 700 nM pulse of H_2O_2 for 4 weeks (June 5 - July 9, 1996). By day 13, communities had become dominated by diatoms, rather than chrysophytes.

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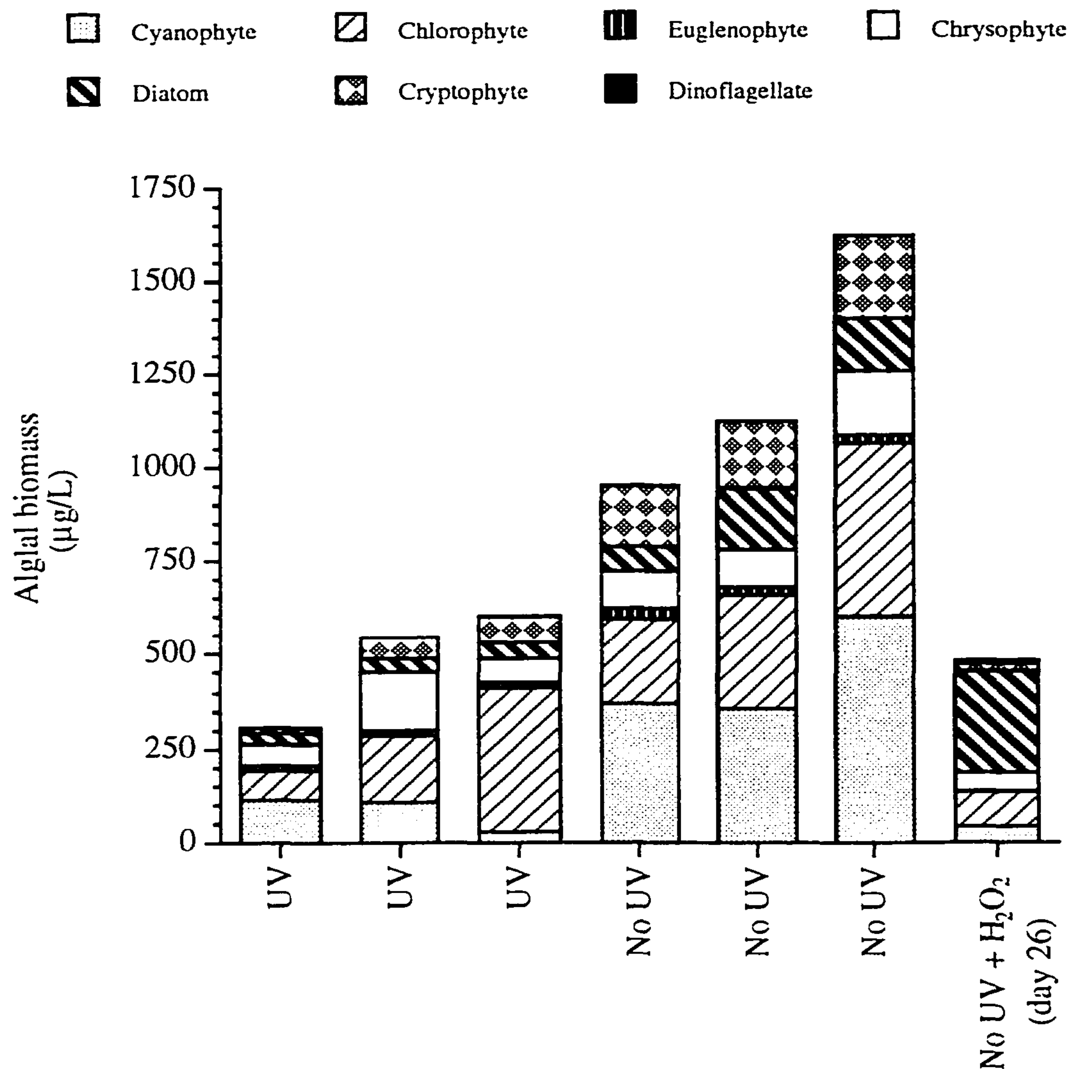

Figure 4-13. Algal biomass in UV-shielded, UV-exposed, and No UV + H_2O_2 enclosures at the end of a 5-week experiment (June 5 - July 9, 1996). Phytoplankton in the UV-shielded and UV-exposed enclosures was dominated by chlorophytes and cyanophytes, and diatoms dominated in the H_2O_2-treated enclosures. In the UV-shielded enclosures there was greater biomass of all taxa except euglenophytes and chrysophytes, compared to the UV-exposed enclosures ($P<0.05$).

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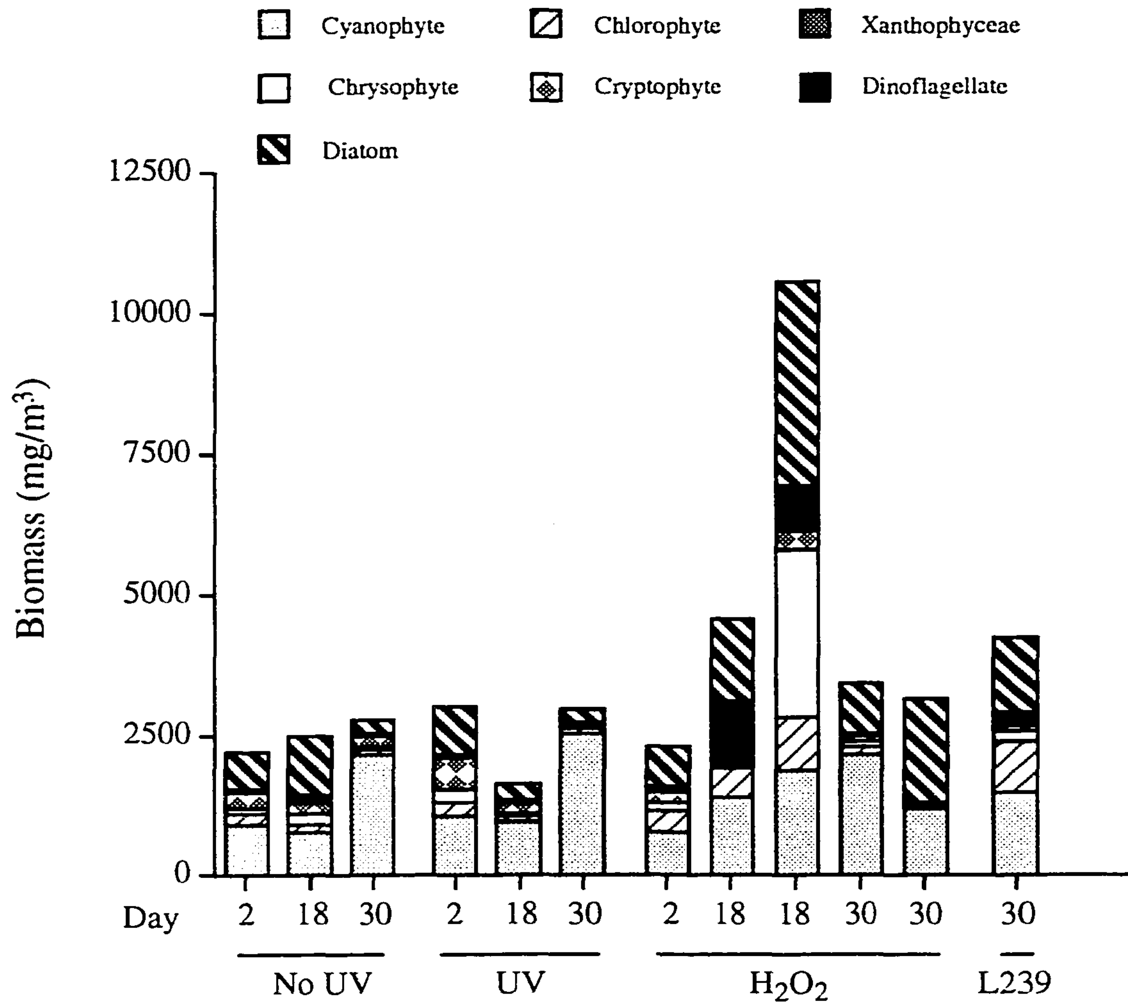

Figure 4-14. Algal communities in No UV, UV, and No UV + H_2O_2 enclosures (August 20 - September 20, 1996). In the H_2O_2-treated enclosures, on day 18 cyanophyte, diatom, and chlorophyte biomass, and total algal biomass were higher than in the other treatments. On day 30, diatom biomass was higher in the H_2O_2-treated enclosures ($P<0.05$).

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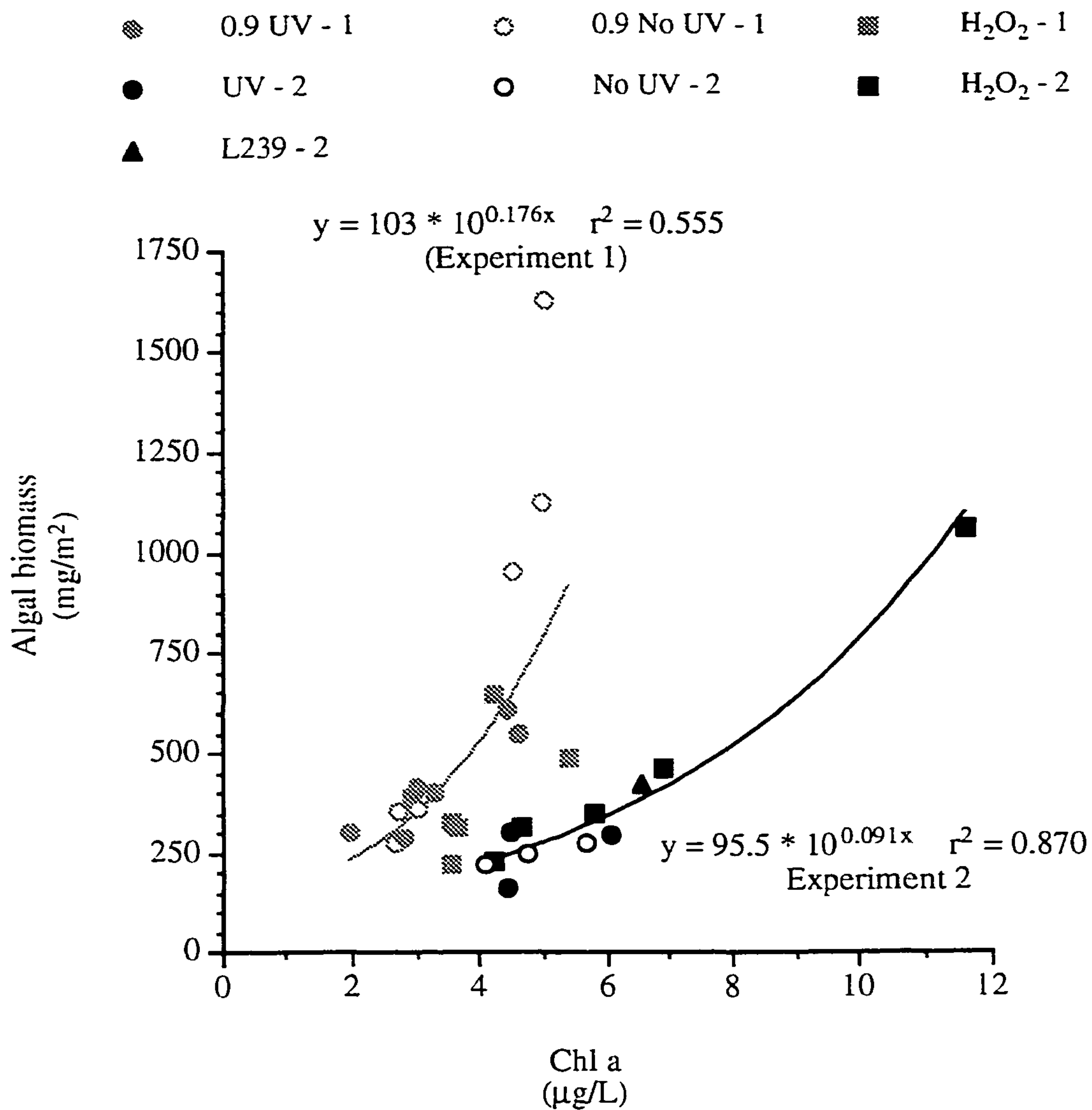

Figure 4-15. Relationships between chl a *and algal biomass in enclosures exposed to and shielded from solar UVR, and enclosures shielded from UVR and treated once daily with a pulse of H_2O_2 (700 nM) (Experiment 1: June 5 - July 9; Experiment 2: August 18 - September 20, 1996). Concentrations of chl* a *per unit algal biovolume were higher in the autumn experiment than in the summer experiment.*

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5. ACID-INDUCED CHANGES IN DOC QUALITY IN AN EXPERIMENTAL WHOLE-LAKE MANIPULATION[1]

Introduction

Climate warming and lake acidification can increase the exposure of aquatic organisms to UV radiation by causing decreases in dissolved organic matter (usually measured as dissolved organic carbon, DOC), which attenuates UV (Schindler and Curtis, 1997, Schindler et al., 1996b, Yan et al., 1996). Both climate warming and acidification are therefore expected to exacerbate the effects of increasing incident UV-B radiation caused by stratospheric ozone depletion, and as a result, in the words of Eville Gorham (1996), lakes are under a "three-pronged attack."

The declines in DOC caused by climate warming are due largely to decreased watershed runoff and therefore inputs of DOC from terrestrial catchments (Schindler et al., 1996a, Schindler et al., 1997), although increased in-lake removal also appears to be important (Dillon and Molot, 1997, Schindler et al., 1997). At the Experimental Lakes Area (ELA), northwestern Ontario, from 1970 to 1990, 15-25% declines in [DOC] were seen in reference lakes, leading to increased depth of lake epilimnia and euphotic zones (Fee et al., 1996, Schindler et al., 1996a) in addition to higher inferred penetration of UV radiation (Schindler et al., 1996b)

In two experimentally acidified lakes at the ELA (Lakes 223 and 302S) (Schindler and Curtis, 1997, Schindler et al., 1996b), and in an anthropogenically acidified lake near Sudbury, Ontario (Yan et al., 1996), dissolved organic carbon (DOC) concentrations decreased dramatically as pH decreased. Paleolimnological reconstructions of organic carbon concentrations also suggest that lakes in Norway underwent a loss of total organic carbon during acidification (Davis et al., 1985). Both precipitation of DOC and increased photolytic breakdown of DOC to CO_2 have been held responsible for the decreases caused by acidification (Effler et al., 1985, Molot and Dillon, 1996, Molot and Dillon, 1997).

Carbon budgets indicate that large quantities of DOC in lakes can be lost to sediments (Schindler et al., 1997). Coagulation and precipitation of chemical substances increase in the presence of divalent cations (Weilenmann et al., 1989), and aluminum has been shown experimentally to be highly effective in increasing coagulation of humic acid at environmentally realistic pHs (3.8, 4.8, and 7) (Schnitzer and Khan, 1972). Because

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humic fractions of DOC contain the coloured aromatic portions (Chen et al., 1978, Choudry, 1984), preferential and persistent removal of humic fractions during lake acidification could cause large changes in optical properties of lakes.

Models of historical increases in UV penetration have simply predicted UV penetration from [DOC], based on recent calibrations in lakes (Morris and Hargreaves, 1997, Scully and Lean, 1994, Williamson et al., 1996). However, recent work suggests that these models are restricted in their usefulness to the type of lake for which they are calibrated. For example, in closed-basin lakes of the Canadian prairies, DOC concentrations are high (20-330 mg/L), but waters are very transparent to all wavelengths of solar radiation, for the DOC has been highly bleached by decades or even centuries in the lakes (Curtis and Adams, 1995). UV radiation also has been implicated in the reduction of carbon-specific UV attenuation by DOC through bleaching processes, in addition to [DOC] reductions through photomineralization (Morris and Hargreaves, 1997). The specific absorbance of DOC can also vary greatly between systems from different geographic areas (Laurion et al., 1997, Morris et al., 1995), possibly as a result of either bleaching (e.g., saline lakes) or variations in the source of DOC (e.g., alpine and arctic lakes, which have poorly-developed soils in their catchments, versus boreal lakes). We hypothesized that there could be similar temporal changes in specific absorbance in lakes caused by acidification and climate warming.

With spectrofluorometric analysis, it has become possible to measure qualitatively whether DOC is derived within a body of water (autochthonous), or is from the terrestrial catchment as a result of decomposition of organic litter (allochthonous) (McKnight et al., In review). Autochthonous and allochthonous DOC have very different optical properties (McKnight et al., 1994), with allochthonous DOC absorbing more visible and ultraviolet radiation than autochthonous. With this in mind, it becomes important to account not only for concentrations of DOC, but also for the type of DOC. This is likely especially important when the absolute concentration of DOC is < 1μg/L, where subtle changes in the optical properties of DOC could radically alter the underwater optical environment.

In this paper, we describe the effects of experimental lake acidification on both the quality and quantity of DOC in an attempt to improve on recent optical models that ignore DOC quality. We also examine the utility of spectrofluorometric analysis in determining the properties of DOC, and the value of long-term sample archiving programs.

Methods

Routine sampling of experimental and reference systems at the ELA continues yearound, and is most intense during the open-water season from March to October. Small

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water samples have been archived in low-temperature (4.0 +/- 0.5 °C; chart-recorded), high-humidity (near saturation), climate-controlled environments since 1985. Samples are stored in the dark in new Nalgene bottles, pre-rinsed with sample water. Evaporative loss was documented according to weight changes, and was minimal. During July 1997, archived samples of epilimnetic waters ($z = 1$ m) were used to reconstruct the DOC histories of several lakes at ELA.

Apparatus

Uncorrected fluorescence scans were performed on a Shimadzu RF-1501 scanning spectrofluorometer with a xenon lamp (concave, non-aberration excitation/emission monochromators, with blazed holographic grating, F/2.4, 900 grooves/mm; dynode feedback light source compensation system with monochromatic light monitoring function; and photomultiplier tubes for both excitation and emission side detection; Mandel Scientific, Guelph, ON, Canada) and using optically clear quartz cuvettes (pathlength = 1.000 cm). The instrument was computer-driven using Shimadzu PC-1501 Personal Fluorescence Software for Windows.

Technique

Spectrofluorometric analyses were performed on the samples according to McKnight et al. (In review). Scans of sample blanks of distilled, deionized water (DDW; distillation/deionization/activated carbon adsorption system; Milli-Q System, Millipore) were also performed to remove effects of Raman scattering. Variations in lamp spectral intensities were corrected using scans of a standard 1 µg/L quinine sulfate solution in 0.1 N H_2SO_4 (Scully and Lean, 1994), and absolute fluorescence values are reported in quinine sulfate units, where: 1 QSU = fluorescence at a given excitation and emission wavelength of the standard quinine sulfate solution.

Fluorescence of samples was measured minutes after acidification to pH 2 with concentrated HCl, as well as for unacidified samples. Excitation radiation was fixed at 370 nm, and scans of emission intensities were performed from 370 nm to 650 nm. After subtraction of DDW blank fluorescence values from those of the acidified samples, the ratio of the emission intensity at 450 nm to that at 500 nm was calculated. A range of values from approximately 1.4 to 1.9 was used to indicate qualitatively the type of DOC; a ratio of 1.4 is typical of terrestrially-derived DOC, such as in a peat bog or low-order stream or lake, and a ratio of 1.8-1.9 is typical of microbially-derived DOC, such as in Antarctic lakes (McKnight et al., In review) that have little terrestrial vegetation or organic soil in their catchments. Variation of fluorescence ratios for acidified and unacidified samples was

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low (within 1% of each other) for the ELA lakes (WFD's unpublished data), so we do not consider that acidification of samples before fluorometric determinations significantly affected our interpretations.

Figure 5-1 illustrates general differences between autochthonous and allochthonous DOC (DOC courtesy of D. McKnight). Absolute peak fluorescence intensity is lower per unit carbon for autochthonous DOC, and the peak center is also shifted to a lower wavelength. An in-lake enclosure experiment also showed that bleaching of DOC by solar UV does not greatly alter the fluorescence ratio, suggesting little change in the quality or source of DOC (Chapter 2). However, carbon-specific fluorescence (CSF) and carbon-specific absorbance (CSA) decreased with bleaching. Despite a >22% reduction in fluorescence in UV-exposed enclosures during the 33-day experiment, DOC quality changed very little (Chapter 2). This DOC bleaching corresponded to a >20% increase in 1% UV depth (328 nm) in the UV-exposed enclosures. Despite this bleaching, DOC in the UV-exposed enclosures remained very similar in quality to that of Lake 239 (L239), in which the enclosures were located and in which DOC fluorescence had changed very little after 33 days. It should also be noted that DOC fluorescence in L239 was highly variable, but the DOC fluorescence ratio changed very little. In UV-shielded enclosures, however, DOC fluorescence ratio gradually increased over the duration of the experiment, correlating with increases in phytoplankton density and [chl *a*] (Chapter 2), and suggesting that, in this case, increased DOC fluorescence ratio may have been a result of increased production of autochthonous DOC. By using changes in absolute fluorescence and transmittance, and DOC fluorescence ratio, one can separate the effects of bleaching from a switch in the nature of DOC from allochthonous to autochthonous.

Here we have approximated peak fluorescence by measuring fluorescence at 454 nm emission (370 nm excitation), midway between peaks for autochthonous and allochthonous DOC, in order that waters from different sources be easily compared. Differences between peak fluorescence and fluorescence at 454 nm were negligible for all samples relative to long-term changes in fluorescence described here. In this paper, fluorescence ratios and fluorescence values (excitation @ 370 nm; emission @ 454 nm; referred to as "peak fluorescence") were combined with [DOC] to distinguish whether changes in the optical properties of water were the result of changes in the dominant nature of DOC or bleaching by solar radiation.

Variable numbers of archived samples were analyzed for each of the lakes. Means and standard deviations presented in Figures 5-2 to 5-5, inclusive, represent different numbers of samples for any given year, ranging from 1 or 2 (where no standard deviation bars are present) to 8, depending on availability. Where possible, samples chosen were taken at

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approximate 4-week intervals throughout the open-water season, generally from March until October. In some cases, intra-annual trends related to factors such as hydrology or photobleaching contribute to increasing the presented annual standard deviation.

Integrated UV transmittance (290 - 400 nm) has been used as a representative index of total transmission in the UV spectrum in boreal and alpine lakes. Absorbance of lake samples from surveys completed in 1996 were measured spectrophotometrically, and transmittance values calculated at 1-nm intervals (pathlength = 1.000 cm). Typical surface solar UV flux values (measured spectroradiometrically at 1-nm intervals during a cloudless summer day and angle-corrected; 290 - 400 nm) were applied to calculated transmittance values, yielding % of remaining initial solar flux through a 1-cm pathlength, at 1-nm intervals. The resulting curve of remaining solar UV flux was integrated, and total integrated UV transmittance calculated for a pathlength of 1 cm (used in Figure 5-8).

Properties of the Lakes

Whole-lake acidification experiments were performed on two lakes at the ELA, starting in 1976, and long-term monitoring of hydrological, meteorological, chemical, physical and biological processes was performed in these as well as in reference systems. Experimental acidification of L302S with sulfuric acid began in 1982. The pH was decreased from 6.75 in 1981 to 5.31 in 1985, when archiving of samples began. It was decreased to 4.5 by 1989, where it was held until 1991 (Cruikshank, 1994, Schindler et al., 1991) (Figure 5-2). Starting in 1991, lake recovery was initiated and pH was allowed to recover to 6.0 by early 1997. L223 was experimentally acidified to pH 5 between 1976 and 1981, held at pH 5 until 1983, then allowed to recover slowly by adding less acid each year (Findlay and Kasian, 1990, Schindler et al., 1991, Schindler et al., 1985). In 1995, acid additions ceased and circumneutral pH was reached (Figure 5-2). Samples from L223 were used to examine DOC fluorescence trends during the final stages of pH recovery, from 5.5 to 7.0. During the period 1985-1996, the pH values in two reference lakes, L239 and L224, increased very slightly, then decreased. [DOC] gradually decreased in the late 1980s, due to prolonged drought and warm temperatures (Schindler et al., 1996a), but increased again in the wetter 1990s. By 1995, pH values in L223, L224, and L239 were very similar.

Results

DOC concentrations in the experimentally acidified lakes decreased with decreasing pH (Schindler et al., 1996b). In L302S, [DOC] declined rapidly between 1981 and 1985 as pH decreased to 5.3. DOC concentrations was reduced to 4.5 mg/L with pH values of 5.0 (Schindler et al., 1992), then decreased to 1.5 - 1.7 mg/L between 1989 and 1991, at pH =

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4.5. During pH recovery, [DOC] has also recovered in L302S (Figure 5-3). In L223, slight declines in [DOC] were seen during acidification, and increases in [DOC] accompanied increases of pH to circumneutral levels during recovery, although the degree of DOC change was more muted than in L302S (Figure 5-3), probably because acidification was only to pH 5.0.

There were also changes in the properties of the DOC. In the two acidified lakes carbon-specific fluorescence (CSF), or the peak fluorescence (QSU) per unit of carbon, was much lower than in L239, but similar to L224 (Figure 5-4). CSF followed the trends in pH change in Lakes 302S and 223, decreasing by approximately 25% in L302S between 1985 and 1989, the period of acidification covered by the archived samples, and increased approximately 100% between 1991 and 1996, as the lake became less acidic. In L223, there was also a trend of increasing CSF as the lake approached circumneutral pH. In L239, CSF decreased during the warm, dry 1980s, then increased again during the wetter 1990s. CSF of DOC in L224 followed the same temporal trends as L239, although changes were less pronounced. However, CSF in L224 remained relatively low even during the wetter 1990s, perhaps a function of the small catchment of L224, while it increased in the recovering acid lakes.

Fluorometric analysis of DOC shows that a shift in dominance from allochthonous to autochthonous or "autochthonous-like" (AUDOC) carbon occurred as L302S was acidified (Figure 5-5). Although there were no samples from the early years of acidification, large increases in the DOC fluorescence ratio during years of acidification after 1985 indicate a change in the properties of DOC in L302S. The proportion of AUDOC in reference lakes 239 and 224 also increased in the 1980s, although to a lesser degree, however, it declined again in the wetter 1990s when DOC inputs from catchments increased. As pH increased in the acidified lakes during recovery, the DOC fluorescence ratio decreased (Figure 5-5). Similar, but less pronounced changes occurred in reference lakes, possibly as the result of climate warming and drought.

Overall, a negative relationship between DOC fluorescence ratio and pH was observed (Figure 5-6). Although L302S has not yet fully recovered to circumneutral pH, the recovery of pH in L223 is complete. As pH increased in both recovering lakes, the DOC fluorescence ratios slowly decreased, approaching values typical of the two reference systems, L239 and L224. In fully-recovered L223, DOC fluorescence ratios are now well within the values from the reference lakes.

Figure 5-7 illustrates the general nature of fluorescence of DOC in several ELA lakes, including reference systems, acidified lakes, and an experimentally eutrophied lake, between 1985 and 1997. Peak fluorescence has been used as an indication of [DOC] in the

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past (Scully and Lean, 1994), and is compared, as a measure of the amount of colored DOC, to DOC quality in ELA lakes. The DOC fluorescence ratio is similar in all reference lakes and in experimentally-eutrophied L227, indicating that allochthonous sources were dominant. However, the quality of DOC in L239 changed only slightly during the period, whereas in L224 and L227 a greater range in DOC fluorescence ratio was observed during the 13-year period. The DOC ratio indicated that, in acidified lakes L302S and L223, either autochthonous or autochthonous-like DOC was dominant. Again, recovering L223 spanned the gap between acidified and reference systems.

Peak fluorescence, as a proxy for amount of coloured DOC, decreased in the acidified systems at the same time that the DOC fluorescence ratio increased (Figure 5-7), indicating that total coloured organic matter was decreasing. Large fluctuations in peak fluorescence occurred in L224 and L239 over the 13-year period. Eutrophied L227 showed the highest values and the greatest variation in peak fluorescence, although the type of DOC did not vary greatly.

Analysis of DOC from 56 boreal lakes and streams at ELA and from 24 montane and alpine lakes in the Canadian Rocky Mountains of Banff and Jasper National Parks, where catchments may contain little vegetation, confirms that integrated UV transmittance (290-400 nm) is also strongly affected by the nature, as well as the amount, of DOC (Figure 5-8). The more autochthonous the DOC, as measured by fluorescence ratio, the greater the UV transmittance. In an unacidified boreal lake of DOC ratio 1.4, approximately 85% of integrated UV photon flux (290 - 400 nm) would remain (cm^{-1}), whereas in a lake with a ratio ~ 1.6, approximately 93% of UV would remain. This decrease in UV absorbance as DOC quality changes from allochthonous to autochthonous represents a 2.2-fold increase in 1% UV depth, despite no change in [DOC].

Discussion

Contemporary DOC-UV calibrations and long-term [DOC] measurements predicted that the depth of 1% UV-B penetration in L302S increased by more than 900% during acidification (Schindler et al., 1996b). Our analysis suggests that the magnitude of the increase in UV penetration may have been underestimated, due to simultaneous changes in optical properties as a result of bleaching and selective oxidation (Chen et al., 1978) or precipitation of allochthonous DOC (ALDOC).

The increasing proportion of AUDOC as pH decreased cannot be explained by increasing production of autochthonous DOC. Phytoplankton biomass increased slightly in all lakes during the period (Findlay and Kasian, 1990) but there were no consistent changes

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in productivity (Schindler et al., 1996a). In contrast, production of littoral algae declined (Turner et al., 1995).

It is more likely that the shift in proportion of AUDOC resulted from removal of ALDOC from the water column in acidified lakes, possibly as a result of Al-mediated coagulation and flocculation (Effler et al., 1985, Schindler et al., 1992, Schnitzer and Khan, 1972) rather than from compensatory increased autochthonous DOC production. Alternatively, it is possible that the aromatic portions of the DOC molecules were oxidized to H_2O and CO_2 in the presence of acid, which can result in losses of up to 90% of C (Chen et al., 1978), leaving the less UV-absorbent aliphatic portions of the molecules. It is for this reason that we have termed the product "autochthonous-like" DOC, as it is not the product of in-lake metabolism, but rather in-lake chemical alterations. Any acid-induced changes in the nature of DOC may also have important implications in the cycling of carbon and DOC-bound metals and nutrients like nitrogen, as well as its availability as a microbial substrate (Moran and Zepp, 1997, Wetzel et al., 1995).

Other authors have noted such changes in UV-absorbing properties among different types of DOC and also with acidification. With a decrease in pH from 7 to 4, associated changes in aromaticity of DOC indicated by E4/E6 ratio led to a increase in C-specific transmittance by a factor of approximately 2.8 (extrapolated from extinction coefficient changes @320 nm) (Chen et al., 1978), consistent with our predictions of 2- to 3-fold increases based on intersystem comparisons of DOC fluorescence ratios. In another recent study, an acidic lake (Lake Giles; pH 5.35) was shown to be more susceptible to UV-induced photochemical reductions of DOC absorbance than circumneutral lakes from the same region (Morris and Hargreaves, 1997). The authors went on to hypothesize that differences might have been a result of difference in DOC quality (allochthonous versus autochthonous) and that prior acidification might have altered DOC composition, thus affecting its optical properties.

Recovery of L223 from acidification was accompanied by a shift in the proportion of DOC back to ALDOC. Recovering L302S is also shifting back to ALDOC and becoming more similar to lakes in the area with circumneutral pH, however recovery is not complete. This confirms that the changes in the quality of DOC were linked to acidification, and further suggests that lakes recovering from acidification will also rapidly regain their "protective shield" from UV radiation. Unfortunately, the entire experimental period can not be investigated here, because archiving of L302S samples was not begun before the initial stages of acidification, and recovery is not yet complete. Only with complete recovery of L302S will we be able to confirm that the quality of DOC in L302S is similar to surrounding reference lakes.

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The greater differences in the quality of DOC in L224 relative to L239, indicated by fluorescence ratio, may be due to the different nature of the catchments. L224 has the smallest terrestrial catchment and the lowest DOC concentration among unperturbed ELA lakes (Schindler et al., 1992). Even subtle changes in DOC inputs to L224 as a result of climate change might therefore have a greater potential to result in changes in DOC fluorescence properties. Much of the DOC entering L224 comes from a small bog lake, L225, in the basin. The outflow of this system, which is highly colored, flows only following large or frequent precipitation events. However, CSF of DOC in L224 changed little between 1985 and 1996, and was lower than in the recovering acidified lakes by 1996 as a result of their increased CSF between 1990 and 1996. The difference in trends in CSF between L224 and the acidified lakes after 1990 suggests that changes in L302S and L223 were related to acidity and that DOC in L224, while optically different from L239, is generally stable in its optical properties, perhaps as result of its small catchment size and low DOC inputs.

A limitation of using fluorescence to interpret qualitative changes in DOC becomes apparent when dealing with systems with very low [DOC], including highly acidified lakes. The steep decline in DOC fluorescence ratio in L302 and high variability in fluorescence ratios after 1988 are most likely a result of the reduced fluorescence signal of the water - caused by both decreased [DOC] and decreased CSF. Background fluorescence of water increased, as a proportion of DOC fluorescence, to nearly 20% in samples taken during peak acidification, and small fluctuations in the fluorescence of either water blanks or DOC samples consequently resulted in greater variation in the fluorescence ratio. The higher values of fluorescence ratio than reported by McKnight et al. (In review) may also be a result of this increased signal variation during peak acidification and coincident with lowest [DOC]. Alternatively, the apparent shift in quality may be a result of increased allochthonous DOC inputs due to increased precipitation after 1988, however this is unlikely because peak fluorescence did not increase coincidentally. As absolute fluorescence of DOC from the recovering lakes increased, the variance in the fluorescence ratio decreased - likely a result of reduced effects of noise in the fluorescence signals. In this case, historical chemical and hydrologic data such as [DOC] and precipitation trends could be used to complement the fluorescence data. Without complementary data that provide additional explanations for changes within or among lakes, caution may be necessary in the interpretation of apparent shifts in DOC quality in low [DOC] lakes, as indicated by fluorescence ratios.

It is also possible that DOC in archived samples may change in quality and quantity during long-term cold storage. For example, rates of microbial and chemical processing

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are unknown, and may have changed the fluorescence properties of DOC. However, long-term stability of carbon-specific fluorescence and fluorescence ratios in reference lakes suggest that changes are very small.

Acidified lakes at the ELA contained DOC more similar to that from alpine or polar lakes than surrounding boreal lakes. As a result, they may have had higher UV penetration than predicted from [DOC] alone. Only after recovery to circumneutral pH do optical properties of DOC return to normal, and persistent increased UV transmittance may continue to be of concern in acidified lakes during recovering because of relative transparence of the DOC. In addition, acid-induced changes in DOC quality may affect nutrient cycling and microbial substrate availability within acidified lakes. This study emphasizes the importance of archiving water samples, so that *post hoc* analyses can be performed with the advent of technological improvement. This study also highlights the value of fluorescence analyses of DOC in interpreting chemical changes for describing physical properties within aquatic environments.

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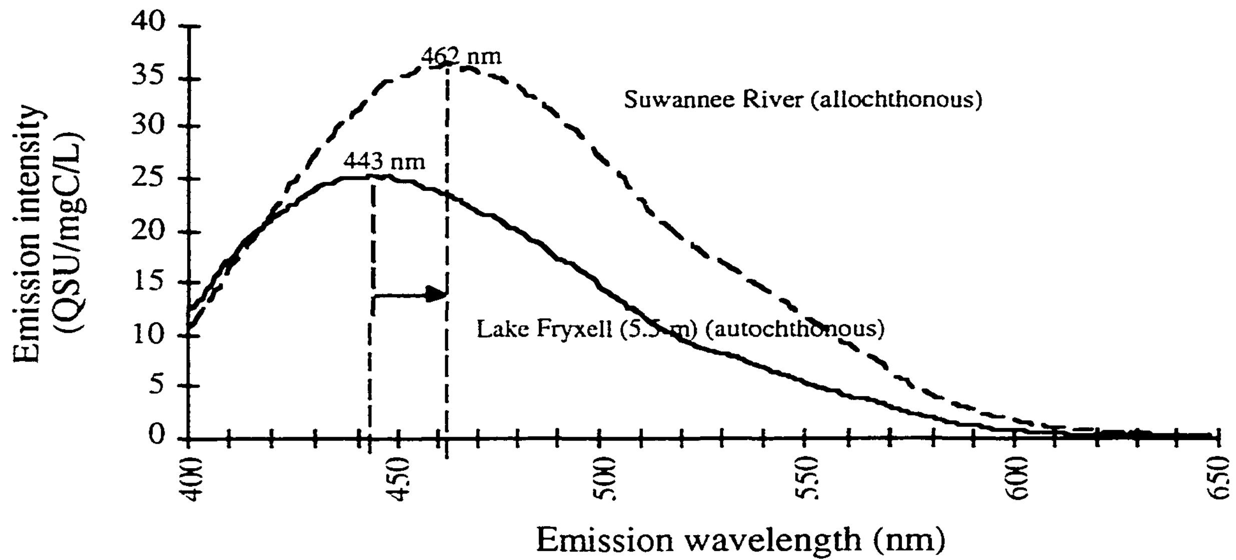

Figure 5-1. Differences in carbon-specific fluorescence (CSF) emission scans (Ex: 370 nm) between allochthonous and autochthonous DOC. For allochthonous DOC, CSF is higher than autochthonous DOC, and the peak fluorescence is also shifted to a higher wavelength. The ratio of emissions at 450 and 500 nm indicates the quality or source of DOC. Suwanee River DOC has a ratio of approximately 1.4, and Lake Fryxell DOC has a ratio of 1.8-1.9 (McKnight et al., In review).

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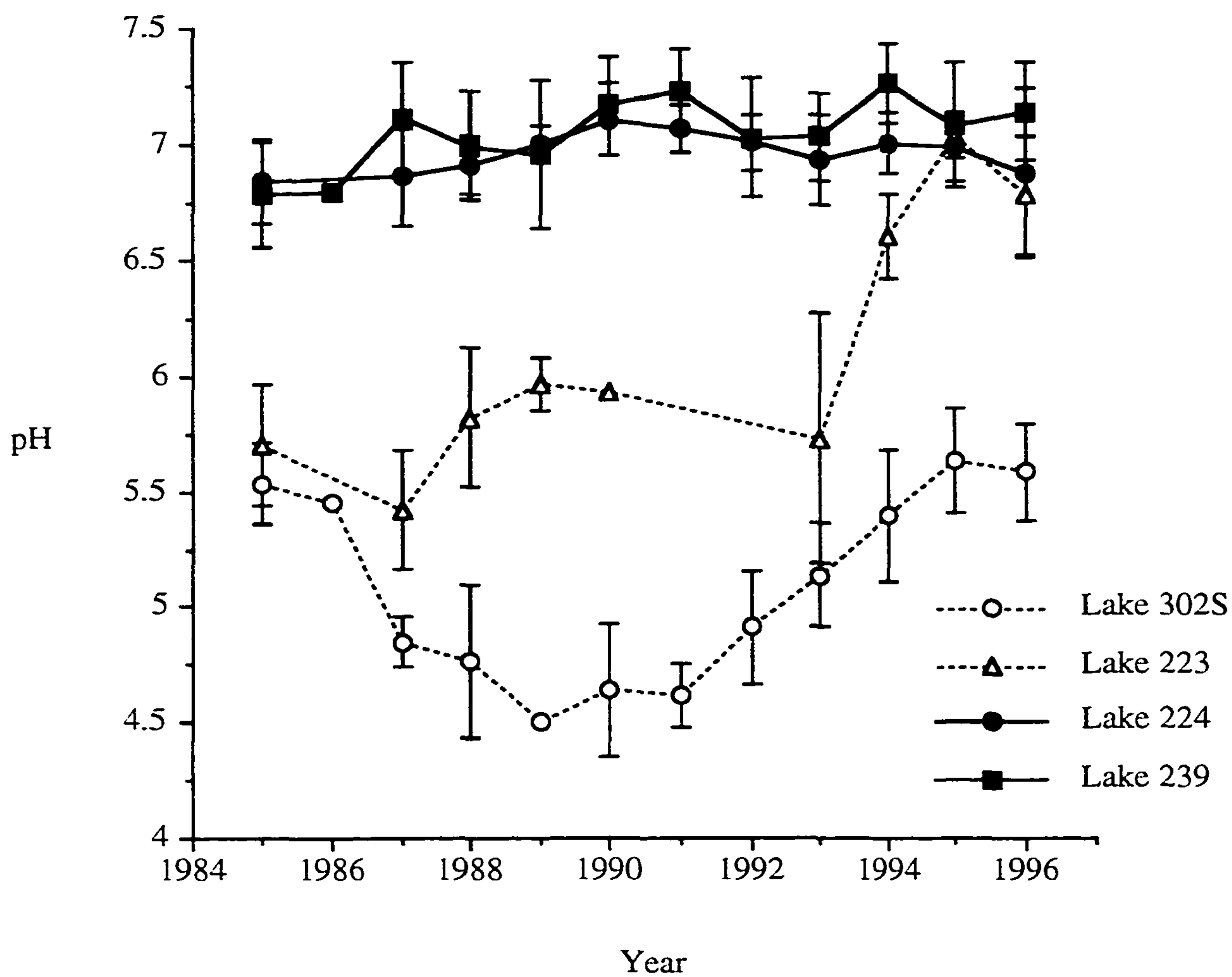

Figure 5-2. History of pH change (± S. D.) in experimentally acidified (L223 and L302S) and reference lakes (L239 and L224) at the Experimental Lakes Area (1985-1996).

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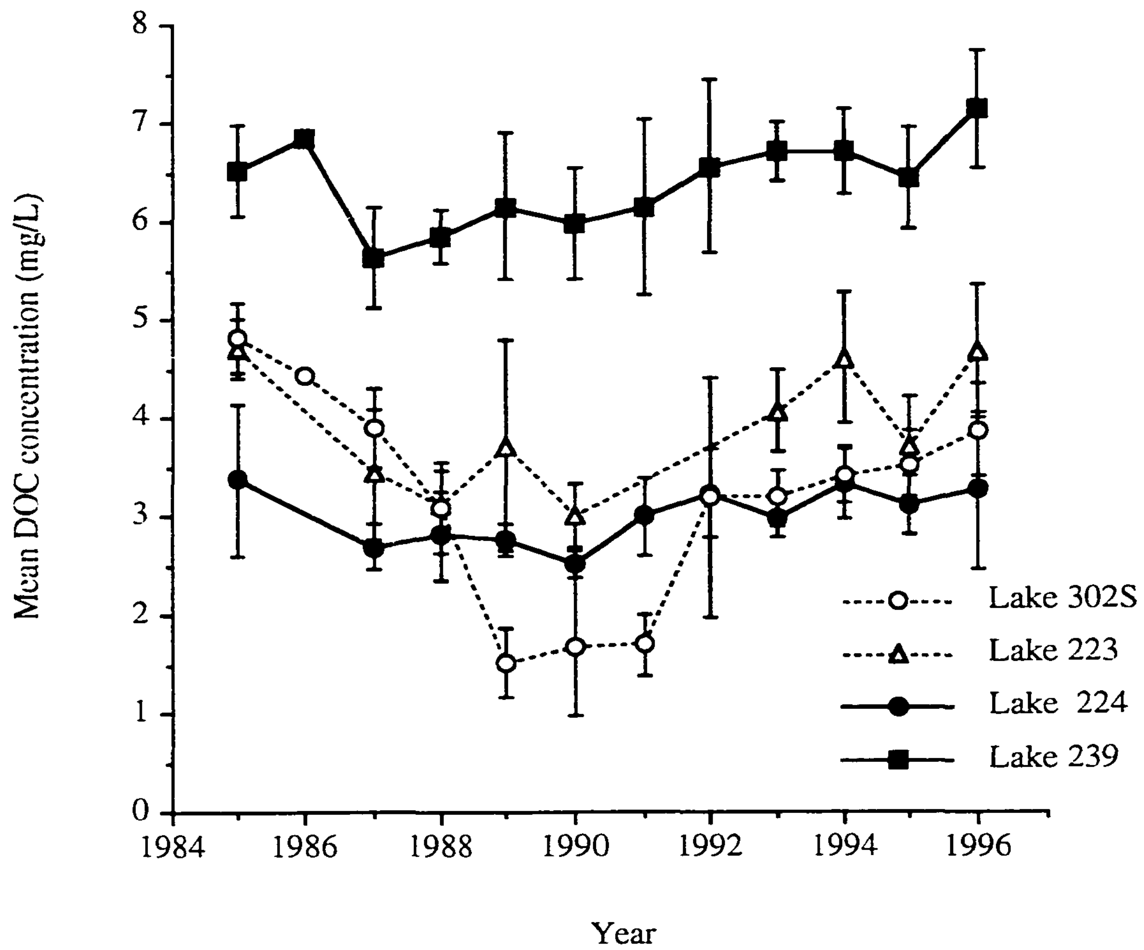

Figure 5-3. History of DOC concentration change (± S. D.) in acidified (L223 and L302S) and reference lakes (L239 and L224) at the Experimental Lakes Area (1985-1996).

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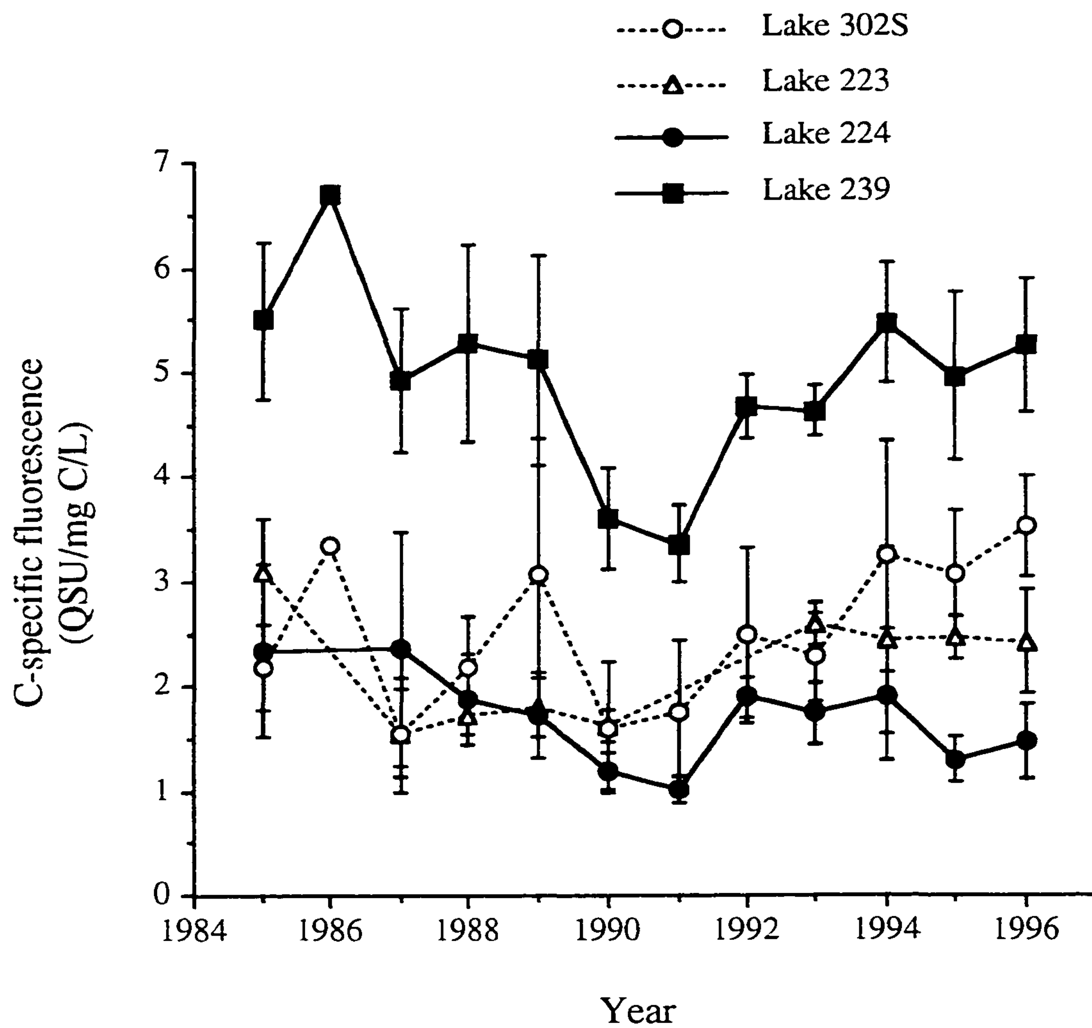

Figure 5-4. Carbon-specific fluorescence (fluorescence/mg of C/L; ± S. D.) in acidified lakes (L223 and L302S) and reference lakes (l239 and L224) at the Experimental Lakes Area (1985-1996).

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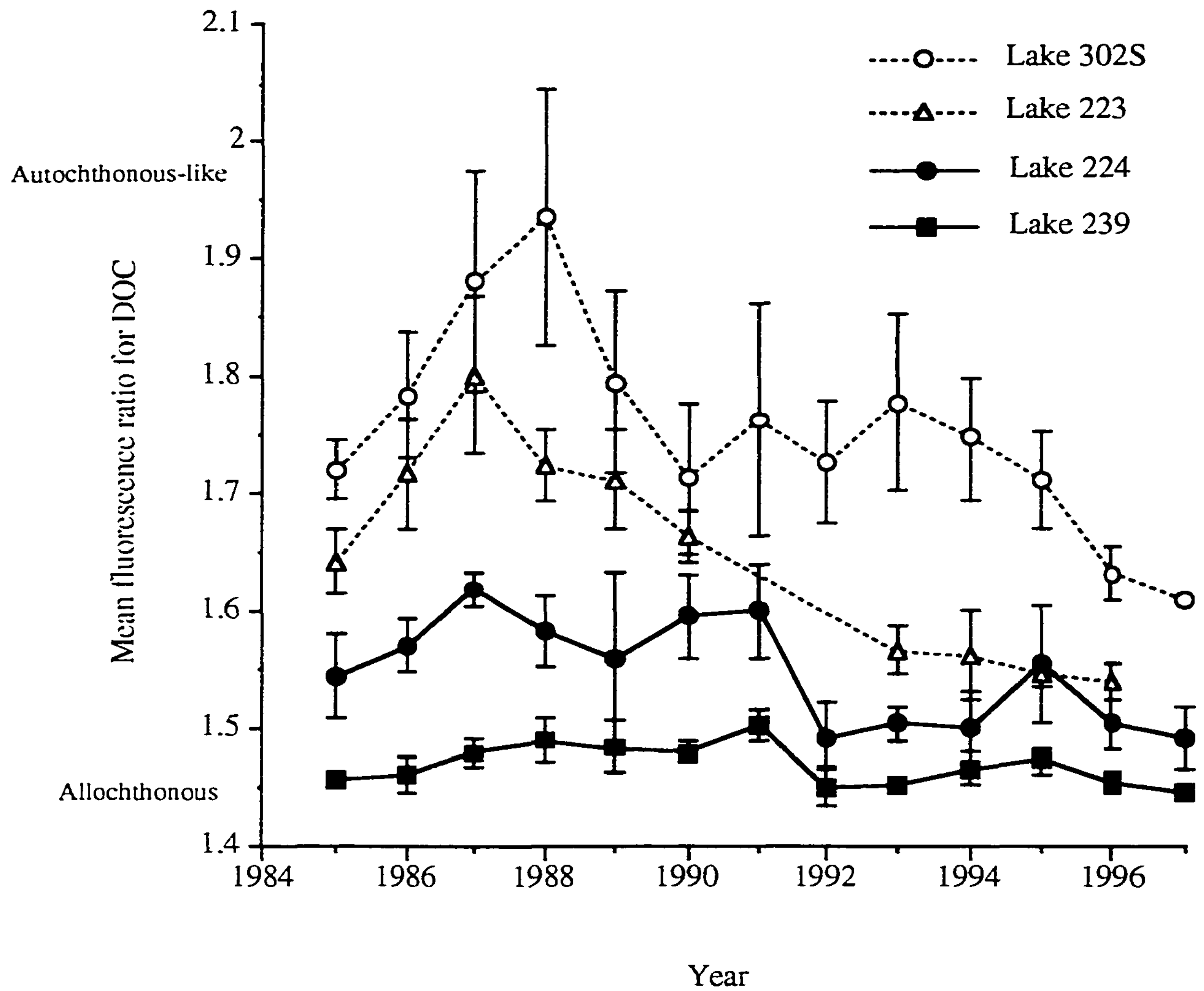

Figure 5-5. Patterns of DOC quality change (±S. D.) in acidified lakes (L223 and L302S) and reference lakes (L239 and L224) at the Experimental Lakes Area (1985-1997). As L302S became more acidic, DOC became more autochthonous-like. Recovery from acidification has resulted in shifts back to allochthonous DOC dominance in L223, and a similar shift is occurring in L302S.

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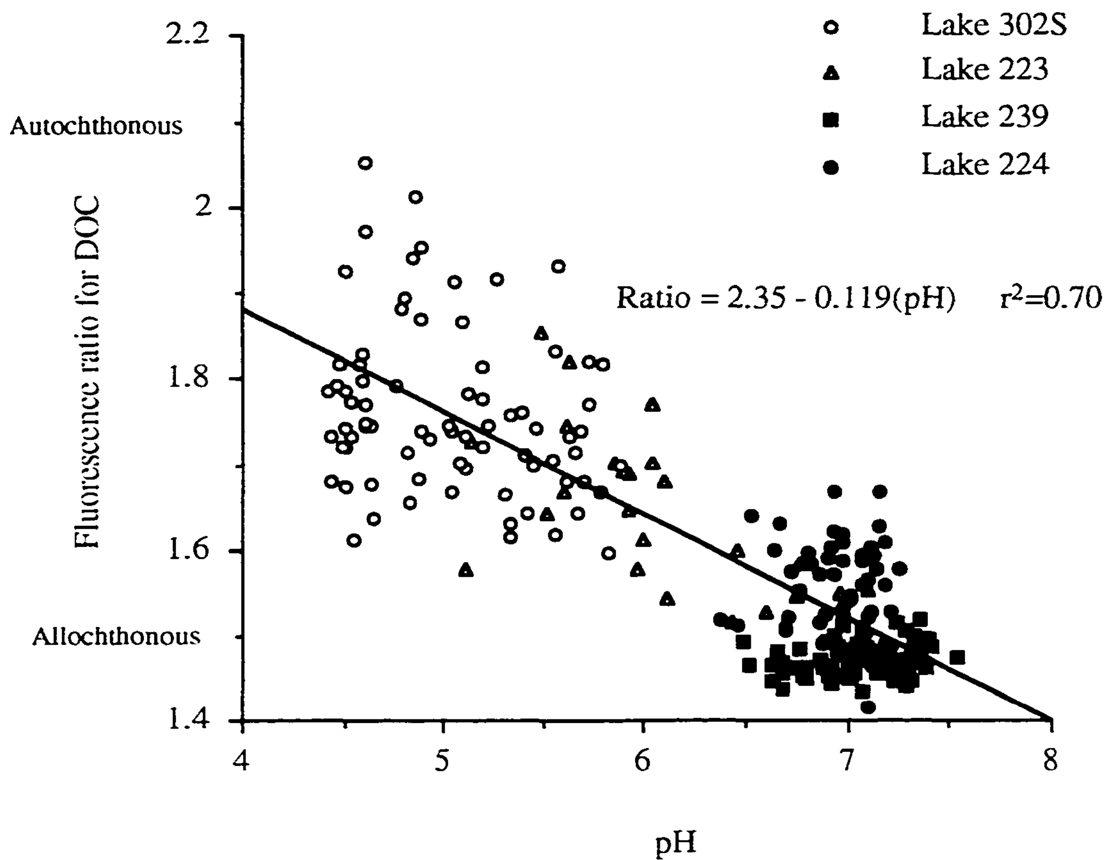

Figure 5-6. General trends in DOC quality shift related to pH change from experimental acidification. L302S has been predominantly autochthonous-like, relative to reference lakes L224 and L239, between 1985 and 1997. L223 has shifted from more autochthonous to very similar to reference lakes (L239 and L224) as its pH became circumneutral.

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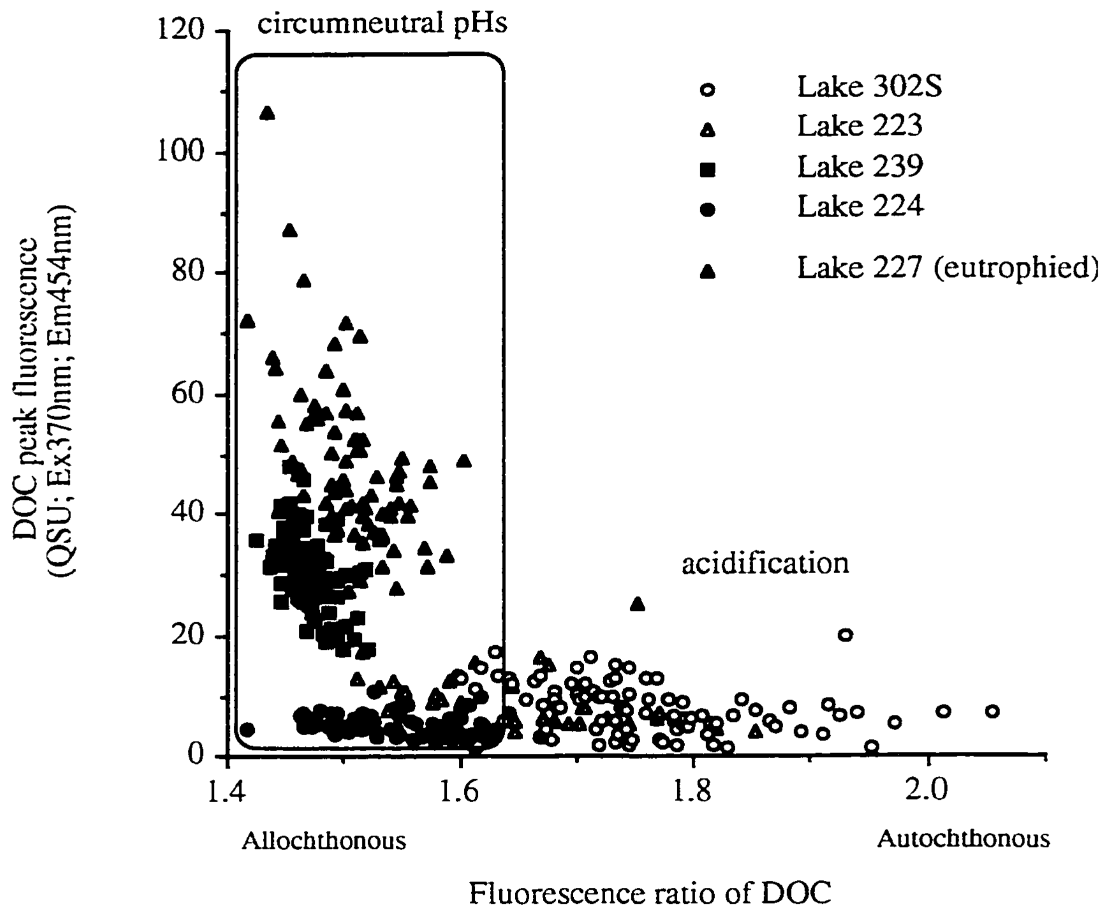

Figure 5-7. Peak fluorescence of DOC from reference lakes (L224 and L239), an experimentally eutrophied lake (L227), and two experimentally acidified lakes (L223 and L302S) between 1985 and 1997, related to DOC quality. Unacidified lakes all appear to be dominated by allcchthonous DOC, whereas acidified lakes have far more autochthonous or autochthonous-like DOC. With chemical recovery, DOC in L223 returned in quality to that of reference systems.

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Figure 5-8. Comparison of DOC quality and integrated UV transmittance (290-400 nm; 1 cm path length) in alpine and boreal lakes. More allochthonous boreal lakes have lower UV transmittance than do more autochthonous boreal and alpine lakes. A quadratic regression fitting the data is shown.

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6. ANALYSIS OF PHYSICAL, CHEMICAL, AND BIOLOGICAL INTERACTIONS IN THE STRUCTURING OF SHALLOW EPILITHIC COMMUNITIES IN BOREAL LAKES

Introduction

During experimental acidification of Lakes 302S and 223 at the Experimental Lakes Area (ELA), northwestern Ontario, there was a dramatic shift in benthic algal dominance from diatoms or filamentous cyanophytes to proliferation of metaphyton, a "new" form of detached growth in upper littoral zones dominated by filamentous green algae like *Spirogyra*, *Mougeotia*, and *Zygogonium* spp. (Turner et al., 1995b). They were often present in the epilithon, but usually did not proliferate there (Turner et al., 1987). At the same time, acidification caused decreased concentrations of dissolved organic carbon (DOC). DOC is the primary attenuator of solar radiation in many lakes (Kirk, 1976, Scully and Lean, 1994), and acidification hence led to large increases in UVR fluxes, especially in the shallows (Schindler et al., 1996). Some of the changes observed in benthic algal associations in L302S could have been a result of increased UVR or decreased grazer densities, as "hidden" treatments coincident with acidification (Schindler et al., 1996, Turner et al., 1987). More recently, analysis of sedimentary deposits in L302S has revealed that large increases in the algal concentrations of degradation products of scytonemin, a photo-protective pigment, accompanied acidification (Leavitt et al., 1997). However, there was no evidence of whether the pigment was pelagic or benthic in origin. Although pelagic communities have been studied a great deal, little attention has been paid to factors that structure benthic algal associations such as epilithon. Few studies have included both producers and grazers, or incorporated physical, chemical, and biological interactions. Here I attempt to dissect the roles of chemical, physical, and biological factors in the structuring of epilithon in the shallow littoral zone of boreal lakes at the ELA.

Discussions of ecological limitations in lakes usually refer to nutrient limitation (Schindler et al., 1971), and biological or trophic limitation (competition/predation) (Carpenter et al., 1985). Studies of physical limitation of aquatic communities often have focused on the roles of temperature (Cloern, 1977, Graham et al., 1996, Hamilton, 1971, Lewis and Bennett, 1975, Robarts et al., 1992, Waters, 1968) and solar radiation (Graham and Turner, 1987, Haney et al., 1983, Hudon and Bourget, 1983, Ibelings and Maberly, 1998, Lin-Ki, 1975, Roos, 1983, Schindler and Fee, 1975, Shearer et al., 1987, Smith et al., 1980, Stockner and Armstrong, 1971, Vincent et al., 1984). More recently, there has been a recognition of the importance of ultraviolet radiation (UVR) as both a direct and

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indirect mediator of change in aquatic communities (Blaustein et al., 1994, Bothwell et al., 1994, Bühlmann et al., 1987, Cabrera and Pizarro, 1994, Donahue and Schindler, 1998, Harm, 1980, Vinebrooke and Leavitt, 1999). However studies usually have been limited to small-scale experiments, often utilizing monocultures or very simple communities. The role of UVR in the structuring of lentic communities has been hypothesized by piecing together the results of many studies (Karentz et al., 1994, Schindler and Curtis, 1997, Schindler et al., 1996, Williamson, 1995), however few experimental or descriptive investigations have been performed to address these hypotheses in whole lakes.

Efforts to compare epilithic algal and invertebrate communities between and within lakes have been limited, and no attempt to connect large-scale, natural epilithic structure with environmental physical, chemical, and biological conditions has been made. The objective of this investigation was to identify the potential roles of UVR and other physical, chemical, and biological factors in the structuring of shallow-water epilithic communities in boreal lakes.

Methods

Between 24 July and 1 August 1996, 8 lakes at the ELA (49°40'N, 93°44'W; Figure 6-1) were sampled for water chemistry and optics (Tables 6-1 and 6-2). Lakes were selected to represent a range of DOC concentrations and UV transmittance (Figure 6-2), and included both reference systems and lakes that were previously experimentally acidified (L223 and L302S) (Schindler, 1994, Schindler et al., 1985), as well as an experimentally flooded peat bog (L979) (Kelly et al., 1997). In these lakes, epilithic algal and invertebrate assemblages were sampled.

On the same cloudless summer days that epilithon sampling was performed, ultraviolet (UV-b, 300-320 nm; and UV-a, 320-400 nm) and photosynthetically active radiation (PAR) spectra were measured spectroradiometrically at 1-nm intervals using a Licor Model LI-1800UM Underwater Spectroradiometer which was angle-corrected (Table 6-2). These spectra were measured in the 2 hours bracketing solar noon. The instrument has a response range from 300 to 850 nm with a half power band-width of 8 nm and a total band-width of 16 nm. The instrument uses a standard cosine receptor allowing for the measurement of flux densities in diffuse light (at depth) or at low sun angles. Multiple scans at the surface and at multiple depths were collected and averaged to produce a mean of "n" measurements, which was often essential when there were any surface waves. The instrument uses two calibration files to process raw data, one for measurements made in air, and one for those made in water. The instrument has been calibrated annually in air

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and water by Licor. Temperature profiles and Secchi depth were also measured and water colour described.

Uncorrected fluorescence scans of DOC were performed on a Shimadzu RF-1501 scanning spectrofluorometer with a xenon lamp using optically clear quartz cuvettes (pathlength = 1 cm). (Specifications included: concave, non-aberration excitation/emission monochromators, with blazed holographic grating, F/2.4, 900 grooves/mm; dynode feedback light source compensation system with monochromatic light monitoring function; and photomultiplier tubes for both excitation and emission side detection; Mandel Scientific, Guelph, ON, Canada).

Spectrofluorometric analyses of DOC quality were performed according to McKnight et al. (In review). Scans of sample blanks of distilled, deionized water (DDW; distillation/deionization/activated carbon adsorption system; Milli-Q System, Millipore) were also performed to remove effects of Raman scattering in water (Skoog, 1985). Variations in lamp spectral intensities were corrected using scans of a standard 1 µg/L quinine sulfate solution in 0.1 N H_2SO_4 (Scully and Lean, 1994). Absolute fluorescence values are reported in quinine sulfate units, where: 1 QSU = fluorescence at a given excitation and emission wavelength of the standard quinine sulfate solution. Excitation radiation was fixed at 370 nm, and scans of emission intensities were performed from 370 nm to 650 nm. A range of the ratio of the emission intensities at 450 nm and 500 nm was used to indicate qualitative characteristics of DOC. A ratio of 1.4 is typical of terrestrially-derived DOC, such as in a peat bog or low-order stream or lake, and a ratio of 1.8-1.9 is typical of microbially-derived DOC, such as in Antarctic lakes (McKnight et al., In review) that have little terrestrial vegetation or organic soil in their catchments.

Biological sampling sites in the 8 lakes were selected on south-facing, rocky (bedrock or boulder) shorelines. Two sites were chosen for each lake sampled, and 4 depths were sampled at each site: 0.1, 0.3, 0.7, and 1.5 m. At each depth, 4 epilithon samples were taken from either bedrock or large boulder substrate using a scraper (Turner et al., 1991). Distance of epilithon sampling sites from shore, approximate slope of substrate, and water depth were all measured at each site, and biofilm thickness was measured for each sample. In addition, the appearance of algal communities was described. Samples from each depth within a particular site were pooled immediately after sampling, and stored on ice and in the dark until returned to the lab for resuspension and subsampling for algal and invertebrate enumeration and identification, and pigment analyses (Turner et al., 1991, Turner et al., 1987, Turner et al., 1995b). In this way, assemblages specific to each depth within each lake were identified. Subsamples to be analyzed for pigments were frozen immediately; subsamples for algal enumeration were preserved in acid Lugol's and FAA solution (4%

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final volume); and subsamples for invertebrate enumeration were preserved with 80% ethanol.

Algal pigments, including the photo-protective pigment scytonemin (Garcia-Pichel and Castenhoz, 1991, Leavitt et al., 1997, Vincent et al., 1993), were extracted, isolated, identified, and quantified using standard procedures, including high-performance liquid chromatography (HPLC) (Leavitt and Findlay, 1994, Mantoura and Llewellyn, 1983). Extractions were done using acetone, methanol, and water (80:15:5, by volume) for 24 hours in darkness at 10°C. Extracts were then filtered through 0.2 µm Acropore membrane filters, and dried and stored under nitrogen gas in the dark at -20°C. Dried extracts were dissolved in a precise volume of injection solvent (70% acetone, 25% ion-pairing reagent, 5% methanol), in which Sudan II dye (3.2 mg/L) served as an internal reference. Pigments were separated on a Hewlett-Packard 1050 HPLC with a Rainin 200 C-18 column (5 µm particle) and were detected with in-line Hewlett-Packard 1046A fluorometer and 1050PDA spectrophotometer detectors. Pigment concentrations were quantified using HPLC calibration equations that were determined using authentic standards supplied by the U. S. Environmental Protection Agency (EPA). These techniques have been used previously to identify scytonemin and its degradation products (Leavitt et al., 1997).

Algal counts were performed on an inverted microscope at magnifications of 125X and 400X with phase contrast illumination. All counts were done by a modified Utermöhl technique (Nauwerck, 1963). Wet biomass estimates were made from approximations of cell volumes of each species, according to best-fit formulae for different taxa (Vollenweider, 1968).

Invertebrates were hand-picked using a dissecting microscope and identified on dissecting and compound microscopes at up to 400X magnification using standard keys (Clifford, 1991, Pennak, 1989, Thorp and Covich, 1991, Ward and Whipple, 1959). Results are reported as the number of organisms per m^2. Invertebrate and algal community structure were summarized using Simpson's dominance index (D), which emphasizes more abundant species, and Bulla's evenness and diversity indices, which more heavily weight rare species (Bulla, 1994, Washington, 1984).

With the advent of analytical techniques such as redundancy or ordination analyses, complex interactions between community structure and environmental factors can be inferred from what otherwise are cumbersome datasets of descriptive spatial studies, providing the foundation for further experimental probing. Potential community relationships were therefore identified using step-wise forward redundancy analysis (RDA)(ter Braak, 1988), in which environmental variables and algal and invertebrate community structure were examined to infer qualitative relationships. Criteria for inclusion

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or exclusion of a particular environmental variable include the degree of its collinearity with other factors. For example, UV-a has been excluded by the model because it is collinear to both UV-b and PAR. However, UV-b and PAR were not collinear to the degree that dictated exclusion of either. As a result, excluded variables may share relationships to community structure that are demonstrated by those environmental variables to which they are collinear.

The lengths of the arrows indicating environmental variables in the ordination plots correspond to the importance of those variables in explaining the variance in the community data. The proximity of points representing sampling sites or assemblage taxa corresponds to their similarity. Assemblages represented by two points close together are more similar than two points far apart. Likewise, two species that are close together appear under more similar conditions than two species that are further apart on the ordination diagram. Based on these qualitative inferences, further examination of patterns of community and environmental variable interactions were investigated using analysis of variance (ANOVA; SPSS 6.1.1).

Results

Structuring of algal communities

Factors most related to optical environment (DOC fluorescence, PAR flux, [DOC], and UV-b flux) accounted for the majority of variance (0.30 of 0.46 total variance explained) in algal composition and biomass in the shallow littoral zones of the different lakes, based upon step-wise, forward selection redundancy analysis and its ordination (Table 6-3; Figure 6-3). Chironomid density accounted for 3% of variance observed, while pH accounted for 4% of variance. Nitrogen concentrations (NO_3, NO_2, and TDN) accounted for 7% of total variance, and TDP accounted for 2%. All other factors measured either were collinear with one or more of the listed factors, or did not explain more of the data variance, thus not meeting the selection criteria of CANOCO. UV-a was excluded by the model because of its high correlation with both PAR and UV-b, which demonstrated more independence from each other.

PAR exposure at the different sampling sites appeared to have no discernible impact on total algal biomass (Figure 6-4). However, PAR flux correlated to dominance by green algae (Chlorophyta), presented here as the ratio of chlorophyte biomass to the total algal biomass (Figure 6-5). The communities inhabiting the shallowest sampling sites and exposed to the highest PAR fluxes were those most dominated by green algae. Those sites

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with the highest UV-b exposure also had greater chlorophyte dominance than those with low UV-b exposure. The majority of chlorophytes in the lakes were found at sites that had UV-b exposure greater than ~0.5 W/m^2 (Figure 6-6). Of the chlorophytes, filamentous species dominated those sites that were subject to exposure to the highest UV-b flux (Figure 6-7). One exception was acidified L302S, which had high proportions of filamentous green algae in epilithon at all four depths, irrespective of UV-b flux. However, the relationship between filamentous green algae dominance and UV-b flux was positive with either inclusion or exclusion of the L302S sites. Of the chlorophytes, filamentous taxa including *Spondylosium planum*, *Mougeotia* spp., *Zygnema* spp., *Bambusina brebissonii*, *Oedogonium* spp., and *Bulbochaete* spp., accounted for the majority of green algae at sites exposed to high PAR and UV-b fluxes. This also was demonstrated in the ordination plot of taxa and environmental variables, where these taxa were all among those furthest along the plane indicated for PAR and UV-b (Figure 6-3). Complementary to the patterns of chlorophyte dominance, diatom dominance decreased as UV-b exposure increased (Figure 6-8). Other algal classes showed no strong relationships with environmental variables.

The clearest pattern observed was the power function (r^2=0.709) between the ratio of scytonemin concentration to total algal pigment concentration and PAR exposure in shallow-water communities, once attenuation characteristics of the different lake waters were accounted for. Those communities exposed to highest PAR fluxes had the highest degree of scytonemin dominance (Figure 6-9). Similarly, those communities exposed to the highest UV-b fluxes had the greatest scytonemin dominance (Figure 6-10). In the case of UV-b exposure, however, dramatic increases in scytonemin concentrations, from 10 to 40-50%, coincided with small increases of UV-b at very low fluxes (from 0.05 to 0.2 W/m^2) At UV-b fluxes greater than 0.2 W/m^2, scytonemin concentrations increased more slowly.

Structuring of invertebrate communities

The ordination plot of invertebrate communities and environmental variables suggests that algal and optical factors are the most important determinant of invertebrate patterns (Table 6-4; Figure 6-11). According to step-wise, forward selection redundancy analysis, epilithon chl *a* concentration accounted for 23% of variance observed, and diatom biomass accounted for 18% of variance. It is likely that chlorophyll concentrations are somehow indicative of general physiological state or nutritional quality, because they do not correlate well with any particular group of algae. Cyanophyte and chlorophyte biomass accounted for only a total of 4% of variance (Table 6-4). DOC fluorescence ratio and peak

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fluorescence accounted for 18% of variance observed, and PAR and UV-b accounted for 9% of variance, combined. All other factors measured either were collinear with one or more of the listed factors, or did not contribute to the model explaining more of the data variance.

In the invertebrate assemblages, densities of chironomid, oligochaetes, and nematodes were positively correlated with epilithon [chl *a*] (Figure 6-12). Also positively correlated with epilithon [chl *a*] were water mite (Hydrachnidia) densities, *Alluaodomyia* spp. (biting midge), chydorid, cyclopoid copepod, and *Diaphanosoma* spp. densities (Table 6-5).

Among algal-invertebrate relationships, total-diatom biovolume and chironomid densities had a significant positive relationship (Figure 6-13). However, chlorophyte biovolume had a slight negative correlation with *Alluaudomyia* spp. and water mite densities. The highest biting midge and mite densities occurred in those communities with chlorophyte biomass less than 200 $\mu g/m^2$, however trends were not statistically significant (P=0.251 and 0.083, respectively; not shown).

Areal density of chironomids was a negative function of PAR flux (Figure 6-14; P=0.0003), but more strongly negatively with UV-b flux (Figure 6-15; P=0.0017). In addition, densities of nematodes and cyclopoid copepods, ostracods, and oligochaetes were all negative functions of UV-b flux (Table 6-5).

Potential trade-offs between biological and chemical interactions can be illustrated by determining patterns of change of a response variable normalized to another variable. Here, I compared differences in the number of chironomids per unit total algal biomass to infer a potential for change in the relationship between algae and an invertebrate grazer as a function of radiative exposure of the benthic algal associations. There was a negative relationship between chironomids/total algal biomass and PAR flux (Figure 6-16). There was also a negative relationship between chironomids/total algal biomass and UV-b flux (Figure 6-17); as UV-b flux increased from 0 to 2 W/m^2, chironomid densities decreased from 30 to 6 μg^{-1} algal biomass. The number of chironomid larvae per unit diatom biomass, however, was constant over the range of UB-b fluxes, and the relationship between chironomids per unit filamentous green algae was negative (not shown).

Discussion

DOC absorbance of ultraviolet radiation

Ultraviolet radiation spectral intensities (UV-a and UV-b) predicted from DOC concentrations were less than spectroradiometrically measured intensities. In the surveyed lakes, the discrepancy between modeled and observed values increased in lakes with DOC

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concentrations below 4-5 mg/L. The models used involve calculation of flux intensities from [DOC] (Schindler et al., 1996, Scully and Lean, 1994), and do not consider changes in its optical quality, or in other properties of the lake water that can attenuate DOC. The water residence time, catchment size, area of wetlands in a catchment, and productivity of a lake can all contribute to altering the quality of DOC. As a result of interactions between these factors, DOC in low [DOC] lakes of the ELA area tends to be more autochthonous-like in character than in higher [DOC] lakes (Donahue et al., 1998). These lakes thus have lower carbon-specific UV attenuation, and as a result, UV penetration is often greater than predicted from [DOC]-based models. Models that use the peak fluorescence of coloured dissolved organic matter for predicting UV transmittance (Scully and Lean, 1994) would be more accurate than those based on DOC concentration. In the absence of fluorescence data, however, one is limited to using historical [DOC] in reconstructing past UV transmission in lakes.

Algal responses

Taxa and biomass

Results from this spatial survey of multiple sites in and among lakes suggest that radiation exposure (PAR and UVR) is an important factor in structuring epilithon in the shallow littoral zone of boreal lakes. In contrast to the potential importance of PAR and UVR in the structuring of epilithon, nutrient limitation often plays a major role in the development of differences in planktonic algal community structure in many lakes, including those at the ELA (Schindler, 1971a, Schindler et al., 1971, Smith, 1986). Phosphorus availability has also been shown to limit growth in periphyton of shallow experimental streams (Bothwell, 1989) and water temperature was positively correlated to maximum specific growth rates in the same experimental streams (Bothwell, 1992). The depth of the mixed layer of most lakes and optical properties are very closely tied to the amount and colour of DOC (Mazumder and Taylor, 1994, Schindler, 1971b, Schindler et al., 1996, Scully and Lean, 1994). Phytoplankton demonstrates less spatial variability in a given lake than epilithon as a result of relatively homogenous nutrient concentrations and temperatures within the mixed layer, and their movement throughout the mixed layer where exposure to solar radiation is variable. In agreement with my results, light quality and quantity were the most important parameters, plus physical disturbance, determining the distribution of *Cymbella* spp. diatoms in a reservoir (Hoagland and Peterson, 1990). Between-lake differences in epilithon elsewhere may have been in part a result of differences in nutrient concentrations (Sand-Jensen, 1983), however my results do not

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support this. If nutrient availability were driving epilithic algal growth and development in the euphotic zone, epilithon would not exhibit the depth- and flux-dependent patterns that I observed in the ELA lakes. Instead, patterns of solar radiation flux common to all lakes were most strongly associated with patterns in epilithic algal assemblages in the ELA lakes. However, it is impossible to generalize more broadly on the role of inter-lake differences of nutrient concentrations in determining differences in epilithon because all lakes I sampled were ultra- to meso-oligotrophic.

There was no evidence of light limitation (PAR) of total algal biomass in ELA lakes. In contrast, positive correlations between algal biomass (ash-free dry mass) and light exposure have been demonstrated in experimental troughs (DeNicola and McIntire, 1990, Steinman and McIntire, 1986, Triska et al., 1983), although nutrient additions were used by Triska et al. to prevent nutrient limitation. Elsewhere, lower periphyton standing crops were observed in sites exposed to high solar fluxes in a comparison of open- and closed-canopy sites in a 3rd-order stream (Robinson and Rushforth, 1987). In another study at the ELA, half-saturation constants for photosynthetic rates of epilithon were quite low (10.4 - 45.8 μEinsteins/m^2/s), suggesting that epilithon in shallows of ELA lakes is easily light saturated (Turner et al., 1983), alleviating light limitation of photosynthesis or growth.

Although total biomass did not appear to be related to light intensities, differences in proportions of algal taxa in epilithon were closely tied to solar fluxes in the lakes. Diatom taxa were most abundant at sites with low exposure to PAR and UVR, and chlorophytes dominated algal communities at sites with high solar flux. Sites that were exposed to high solar fluxes were at shallower depths in lakes with greater [DOC], and at somewhat deeper depths in the clearer lakes. This is similar to other studies where total benthic algal biomass *in situ* was not necessarily limited by high incident light alone (Allan, 1995). An earlier survey of periphyton communities was performed at the ELA (Stockner and Armstrong, 1971), but samples were taken at each meter of depth, from 0 to 5 m, and integrated so no comparisons of depth trends can be made with my study. However, the higher dominance of diatoms (60-70% of biomass) in integrated samples largely comprised of samples from deeper than 1 m is consistent with my observations. It is possible, however, that Stockner and Armstrong's (1971) observations were biased by a methodological flaw. Their sampling involved collections of rocks, by snorkeling, which were then returned to the lake surface for scraping. It is possible that the normally substantial blue-green component, which is usually loosely associated with the substratum surface, would have been lost during retrieval. The more tightly adhering diatoms would have been deemed to be dominant.

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Photoprotection

Unlike planktonic communities, epilithon are exposed to intensities of solar radiation that change significantly over the short term only as a result of cloud cover and sun angle. Algal community structure and function in epilithon thus may rely more on adaptive or evolutionary changes. These include development of photoprotective mechanisms in response to continual high exposures to solar radiation, such as pigment and mycosporine-like amino acid production (Carreto et al., 1990, Garcia-Pichel and Castenhoz, 1991, Garcia-Pichel et al., 1993, Leavitt et al., 1997, Vincent and Roy, 1993), within-mat vertical migration, or self-screening (Karentz et al., 1994, Losee and Wetzel, 1983, Vincent et al., 1993). The production of UV-absorbing pigments, including scytonemin, appears to be an important adaptive photoprotective mechanism in epilithic algae (Garcia-Pichel and Castenhoz, 1991, Leavitt et al., 1997, Vincent et al., 1993). In the ELA lakes, a strong negative depth gradient existed in the proportion of scytonemin in the total pigment mass present in epilithon. In the majority of lakes, the proportion of scytonemin was ~75-90% of all pigments in epilithon from the 0.1 m sites. Only in L979, the experimentally flooded peat bog in which attenuation of solar radiation is very high, was the proportion of scytonemin low. In all lakes, there was dramatic decrease in the dominance of scytonemin between 0.1 and 1.5 m sites, suggesting that deeper communities need less pigment for protection from high-energy UVR, because of its attenuation by DOC in the lakes.

An inflection point in the relationship between proportion of scytonemin and PAR flux occurs at approximately 1100 - 1400 $\mu E/m^2/s$ exposure at midday. Algae in epilithon of lakes exposed to PAR intensities greater than this may exhibit increased scytonemin concentrations. Sites with the lowest PAR exposure, at 1.5 m of depth, and in the case of highly-coloured L979 at a depth of 0.3 m, had less than 10% of their pigment complements as scytonemin. Exceptions were two of the clearest lakes, L224 and previously acidified L223, in which ~30-40% of total pigment mass was still scytonemin at 1.5 m sites.

As expected, patterns of scytonemin dominance and UV-b flux were similar to those with PAR. Samples from sites exposed to the highest UV-b intensities had the highest % scytonemin, and those at the sites with the lowest UV-b exposure had the lowest % scytonemin. Unlike its relationship with PAR, scytonemin still accounted for the majority of pigment complements under relatively low UV-b exposure, as a percent of surface exposure. Under exposures of ~10% of surface flux of UV-b, approximately half of the total mass of all pigments was still scytonemin. In contrast, sites with 50% PAR exposure had less than 10% of the total mass of algal pigments as scytonemin. Large increases in % scytonemin were seen at much lower relative UV-b exposures than for PAR exposures,

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suggesting that scytonemin production is probably an adaptive physiological response to low-wavelength solar radiation (Garcia-Pichel and Castenhoz, 1991).

Relationships between solar fluxes and filamentous forms of green algae were stronger than with chlorophytes as a whole, suggesting that the filamentous taxa may have some competitive advantage over others in their tolerance of high-intensity PAR and UV-b. Abundance of filamentous green algae was 15x greater in experimental streams exposed to photon flux densities of 450 $\mu E/m^2/s$ than those exposed to fluxes of $50\mu E/m^2/s$, also suggesting a competitive advantage in these taxa under high-light conditions (DeNicola and McIntire, 1990, Steinman and McIntire, 1986). One potential reason for domination of epilithon by filamentous green algae in high-light sites (see also Graham, 1996, and Turner, 1987), may be an increased "photorespiratory capacity" in these taxa. Algae inhabiting shallow waters may have developed photorespiration as a possible photoprotective mechanism to reduce photoinhibition-induced internal production of reactive oxygen species, thus minimizing their potential to damage the photosynthetic apparatus (Kozaki and Takeba, 1996). During experimental acidification of L302S, net photosynthesis of epilithon declined, perhaps as a result of dissolved inorganic carbon limitation as bicarbonate was eliminated (Turner et al., 1994). There was also an increase in dark respiration rates and a decrease in gross photosynthetic rates (Turner et al., 1987, Turner et al., 1995b). Seasonally, net photosynthesis reached its minimum in midsummer - coincident with high water temperatures, low solubility of CO_2, and minimal DIC concentrations (Turner et al., 1995b). Rather than responses to changes in nutrient availability, it is possible that some of the long-term and seasonal metabolic changes that were observed during experimental acidification may have been protective photorespiratory responses to increased UVR fluxes.

Invertebrate responses

Direct and indirect effects of UVR

Studies of invertebrate response to ultraviolet radiation have focused either on drift increases or colonization decreases in natural or experimental streams (Bothwell et al., 1994, DeNicola and Hoagland, 1996, Donahue and Schindler, 1998, Hill et al., 1997, Kiffney et al., 1997a, Kiffney et al., 1997b, Lin-Ki, 1975), or on mesocosm experiments using lentic zooplankton communities (Hessen, 1994, Siebeck et al., 1994, Williamson et al., 1994). Comparative studies and transplantation experiments *in situ* or in mesocosms have also revealed that fish and amphibians are sensitive to UVR (Blaustein et al., 1994, Blaustein et al., 1995, Kiesecker and Blaustein, 1995, Williamson et al., 1996).

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In this study chironomid densities were negatively correlated with both PAR and UVR. Bothwell et al. (1994) found that chironomid grazers were more sensitive to UV radiation than the algae upon which they fed. Algae that were previously identified as sensitive to UVR (Bothwell et al., 1993) were more abundant under UVR exposure in the absence of chironomids, due to their release from grazing pressure. I found that nematodes, oligochaetes, and ostracods densities also decreased with solar exposure, as did densities of other common benthic invertebrates in epilithic algal associations (copepods, water mites, and biting midge larvae).

Many invertebrate taxa and communities exhibit sensitivity or physiological or behavioural adaptations to UVR exposure, including pleurocerid snails (*Elimia* spp.) (Hill et al., 1997, Johnson and Brown, 1997), black fly larvae (Donahue and Schindler, 1998, Kiffney et al., 1997b), hydropsychid caddis-fly larvae (W. F. Donahue, unpublished data), and zooplankton, including *Daphnia* spp. (Hessen, 1994, Siebeck and Böhm, 1994, Siebeck et al., 1994, Zagarese et al., 1994), copepods (Cabrera and Pizarro, 1994, Ringelberg et al., 1984, Williamson et al., 1994), *Leptodora* spp. (P. R. Leavitt, in prep.), and rotifers (Vinebrooke and Leavitt, 1999) and thus my results are not surprising. Densities of predatory species, such as water mites and the biting midge larvae (Hilsenhoff, 1991), may have been lower at high-UVR sites as a result of direct sensitivity to UVR. However, it also may have been a result of lower densities of certain UVR-sensitive prey species, like chironomids. Many invertebrates in epilithon depend upon algae and bacteria for food, including nematodes, oligochaetes, dipteran larvae, ostracods, cladocerans, and copepods. The thick mat of the algae also may shield invertebrates from predation by fish or macrobenthic invertebrate predators.

Invertebrate densities and potential quality of algae as food

In my analyses, diatoms explained much of the variance in invertebrate assemblages. Diatoms may be of higher food quality than other algal groups because they often contain higher concentrations of essential polyunsaturated fatty acids (PUFAs), saturated fatty acids, and total lipids, especially green algae such as *Chlorella* and *Scenedesmus* spp. (Brown et al., 1996, Shifrin and Chisholm, 1981, Volkman et al., 1989). In addition, the relative production of the essential 20:5(n-3) PUFA increases as light intensity decreases (Thompson et al., 1990), suggesting that diatoms growing at a greater depth may be of higher food quality than those from shallow depths exposed to higher radiative fluxes. In experiments, *Daphnia* sp. growth rates decreased with decreases in the relative proportion of essential PUFAs to total fatty acid content in *Cyclotella* sp. (diatom) and *Scenedesmus* sp. (filamentous green alga) (Müller-Navarra, 1995). This, combined with the sensitivity

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of diatoms to UVR, suggests that low-quality, high-chlorophyte assemblages may occur at the shallowest sites of lakes, changing to high-quality, high-diatom assemblages deeper in the littoral zones. The general patterns observed in invertebrate communities may thus respond more to availability and quality of diatom biomass than to changes in other algal taxa.

The lower chironomid densities at higher UV-b fluxes may therefore be either a result of direct sensitivity to UVR and/or a UVR-initiated reduction in food quality. The reduced numbers of chironomid larvae per unit algae at the shallowest sites suggests that chironomids are likely not limited by food, but by UVR. With greater depth or where increased [DOC] decreases UV flux, chironomids may be limited by other factors, such as food. However, diatom-normalized chironomid densities were relatively constant, except in the clearest lakes. Because the proportion of diatoms in total algae increased as UVR decreased, if the chironomid:diatom relationship were constant, the densities of chironomids per μg of total algae would be greater under lower UVR exposures. Similarly, chironomid densities normalized to biomass of filamentous green algae were low at sites where UV-b was highest. If chironomids were feeding primarily on diatoms, a constant chironomid:diatom relationship might indicate that chironomid larvae are food limited at most sites. Diatom-normalized densities of chironomids in the clearest lakes with the greatest UV-b fluxes (>~0.9 W/m^2) increased from approximately 50 μg^{-1} at 0.3 meters to 300 μg^{-1} at 0.1 m (in L224, the clearest lake). This may have been a result of increased refuge from solar radiation for chironomids in algal mats. The biomass of filamentous green algae increased from approximately 50 to 750 μg/m^2 at the same sites. In summary, densities of chironomid grazers that are normally sensitive to UVR may be affected by several factors, including the quality and quantity of food, and shelter from UVR by an overstory of filamentous green algae.

Nematodes and oligochaetes also decreased in density with increasing UV-b fluxes. Because they are detritivores, omnivores, or microbotrophs, nematodes and oligochaetes may be less abundant in the shallow high-UV sites simply because of lack of food (Brinkhurst and Gelder, 1991 , Poinar Jr., 1991). Oligochaetes are also affected by changes in light and by physical disturbance (Brinkhurst and Gelder, 1991). In addition, sediment focussing would result in lower food availability and less shelter for these taxa on shallow bedrock exposed to high solar insolation than in the flocculent detritus that has settled at deeper sites.

Ostracod densities also increased with depth, and were inversely related to solar fluxes. Their distribution has usually been linked to food availability and substratum characteristics. They prefer organic detritus and have lower densities on predominantly

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mineral substrata, to which my sampling was limited. Ostracods also may feed on diatoms (Delorme, 1991), which increased with depth as discussed above. Like other invertebrate taxa, it is difficult to determine the proximal cause of the patterns of ostracod distribution.

My analysis does not consider hydrodynamic energy in shallower waters and its potential to physically act upon algal associations. The greater hydrodynamic energy at shallower depths renders algae less sensitive to low nutrient supplies (Turner et al., 1994). This may also contribute to changes in taxonomic composition and food quality with depth. Boundary layers over algal assemblages are thinner when hydrodynamic energy in greater, accelerating nutrient transfer from water to algae. For example, there is an inverse relationship between hydrodynamic energy and dissolved inorganic carbon supply for algae (R. H. Hesslein and M. A. Turner, unpublished data). As a result of diffusive resistance through boundary layers, inorganic carbon has been found to limit the growth rates of epilithon below the wave zone in ELA lakes (Turner et al., 1994). Filamentous green algae acquire DIC more efficiently than other taxa (Turner et al., 1995a), which may promote the dominance of these taxa in shallower waters. Nutrient availability within the biofilm and in overlying water, and the characteristics of the substrata underlying the biofilms (e.g., determining the potential for accumulation of sedimenting materials) may also affect algal densities and dominance.

Conclusions

A number of features of littoral communities are positively or negatively related to UVR, but in almost every case food quality and quantity, nutrient availability, or hydrodynamics are potential confounding or interacting factors. Further experiments are necessary to determine which of these are the proximal causes, including direct algal and invertebrate responses to UVR and PAR, the potential for interactions between sensitivities to solar radiation and foodweb dynamics, and the role of physical refugia in the mediation of trophic or "solar" cascades (Williamson, 1995).

UVR fluxes are increasing in lakes as a result of combinations of stratospheric ozone depletion, climate change, and acidification (Schindler et al., 1996). Although increases in UV radiation are global, effects of UVR are mediated by its attenuation in any given lake by DOC. In addition to shielding by DOC, protective pigments and behavioural adaptations make the effects of UVR on littoral communities difficult to predict. Communities that have evolved in high-UV waters usually exhibit evidence of physiological resistance or recovery mechanisms and behaviours that enable them to function in a high-UVR environment. It is therefore likely that the depth to which UVR-resistant epilithon occur

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will increase and, as a result, filamentous green algae could become more dominant, reducing diatom biomass. Further, changes in invertebrate communities could result.

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Table 6-1: Water chemistry from 8 lakes sampled at the Experimental Lakes Area (* previously experimentally acidified; ** currently experimentally acidified; *** experimentally flooded peat bog)

Site	NO3	NO2	NH4	TDP	TDN	Suspended particulate					DIC	[DOC]	CHLA	pH
	μM	μM	μM	μM	μM	C	N	P	C:N	N:P	μM	mg/L	μg/L	
223*	3	<1	19	2	255	490	51	3	9.6	17	74	4.20	1.07	6.52
224	1	<1	13	2	170	450	41	2	11.0	20.5	91	3.12	1.10	7.19
239	<1	1	22	1	285	500	38	3	13.2	12.7	153	6.72	1.76	7.43
260	<1	0.5	4.5	2.5	313	500	44	2	11.5	22	158	5.22	1.07	7.35
302S**	<1	1	11	3	260	1110	98	5.6	11.3	19.6	27	3.84	1.96	5.56
373	1	<1	9	4	185	410	60	1	6.8	60	200	3.6	1.22	7.55
468	4.5	<1	9	3	233	550	46	3	12.0	15.3	130	4.92	0.58	7.35
979***	<1	1	15	6	348	695	67	6	10.4	11.2	144	8.64	2.53	6.64

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Table 6-2. DOC quality and UV flux (measured spectroradiometrically and modeled from [DOC]) in 8 lakes sampled at the Experimental Lakes Area. PAR meaurements were also taken spectroradiometrically (*calculated from vertical profiles of PAR flux measured with a Li-Cor light meter). Models used for comparative UV predictions: 1=(Scully and Lean, 1994); 2=(Schindler et al., 1996). DOC peak fluorescence was measured at an excitation wavelength of 354 nm and emission wavelength of 496 nm, and reported as quinine sulfate units (QSU). DOC ratio is the fluorescence emission ratio of 450 and 500 nm emission intensities, with excitation at 370 nm. Water colours were noted as green (gr), brown (br), or orange (or).

Lake	Water colour	Fluorescence		Visible Radiation		1% UV-b depth (m)			1% UV-a depth (m)	
		Peak	DOC ratio	Secchi (m)	1% PAR (m)	Measured	Model 1	Model 2	Measured	Model 1
223	gr-br	5.32	1.49	7.2	11.0	0.92	0.77	0.85	2.23	1.71
224	gr-br	1.73	1.48	7.4	15.4	2.07	1.34	1.29	5.12	2.70
239	br-or	17.17	1.40	4.55	6.3	0.33	0.32	0.32	0.84	0.84
260	gr-br	10.32	1.47	5.25	8.5*	-	0.51	0.58	-	1.23
302S	gr	7.72	1.53	5.15	5.8	0.98	0.91	0.97	2.36	1.97
373	gr	3.54	1.47	7.9	12.1	1.39	1.02	1.07	2.99	2.17
468	gr	6.59	1.45	5.5	10.2	0.74	0.57	0.65	1.67	1.35
979	br	47.96	1.39	1.65	1.6*	-	0.20	0.10	-	0.57

Table 6-3: Percent variance in inter-site, inter-lake epilithon algal species composition and biomass explained by environmental variables (step-wise forward redundancy analysis).

Factor	Variance explained (0.46 total)
DOC fluorescence	.09
PAR	.09
[DOC]	.06
UV-b	.06
pH	.04
chironomid density	.03
NO_3	.03
TDP	.02
TDN	.02
NO_2	.02

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Table 6-4: Percent variance in inter-site, inter-lake epilithic invertebrate species data explained by environmental variables (step-wise forward selection redundancy analysis; Monte Carlo permutations, n=99, P=0.01)

Environmental factor	Variance explained (0.72 total)
Epilithon chl *a*	0.23
Diatom biomass	0.18
DOC ratio	0.09
Fluorescence	0.09
PAR	0.06
UV-b	0.03
Cyanophyte biomass	0.02
Chlorophyte biomass	0.02

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Table 6-5. Densities of invertebrate taxa as a function of chl *a* concentrations in epilithon and UV-b exposure from 4 depths in 6 ELA lakes (July, 1996).

Invertebrate taxon (density; m^{-2})	Relationship with environmental variable	r^2	F	P
	[chl *a*] (mg/m^2)			
Diaphanosoma spp.	$1500(\text{chl } a)^{0.860}$	0.535	18.02	0.0005
Chydorids	2370(chl *a*)+7720	0.248	5.60	0.03
Cyclopoid copepods	$4990(\text{chl } a)^{0.403}$	0.307	7.52	0.01
Alluaodomyia spp.	1935ln(chl *a*)+2470	0.245	5.51	0.03
Total mites	2090ln(chl a)+5170	0.187	3.91	0.06
	UV-b flux (W/m^2)			
Nematodes	-9650(UV-b)+15300	0.347	7.98	0.013
Ostracods	-227ln(UV-b)+169	0.455	12.51	0.003
Cyclopoid copepods	-2530(UV-b)+7350	0.193	3.59	0.078
Oligochaetes (all lakes)	-4460(UV-b)+6820	0.121	2.07	0.171
Oligochaetes (no L302S)	-1908(UV-b)+3560	0.284	5.56	0.034

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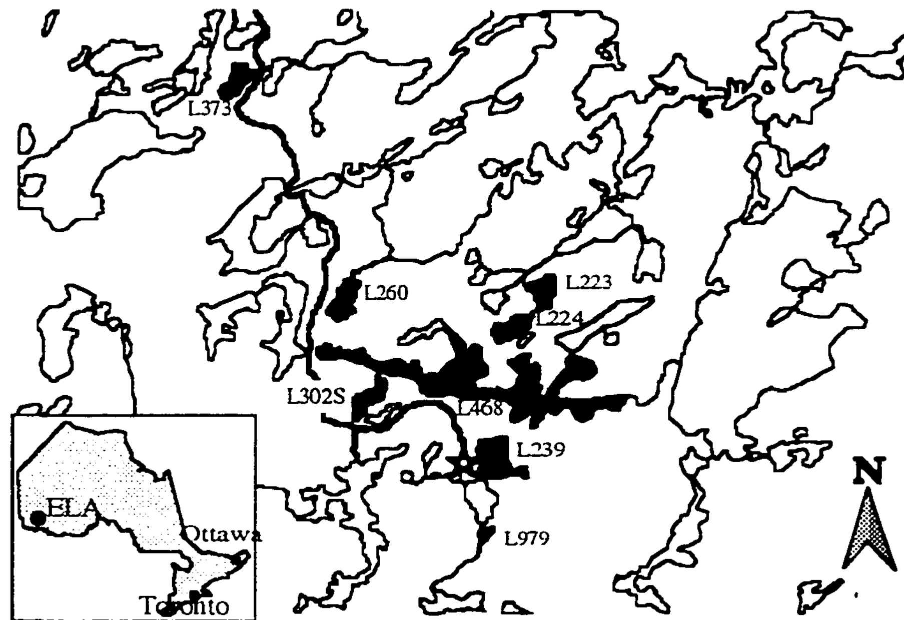

Figure 6-1. Eight lakes (marked in black) surveyed for biological, chemical, and physical parameters in July, 1996, at the Experimental Lakes Area, northwest Ontario, Canada. The star denotes the ELA field station.

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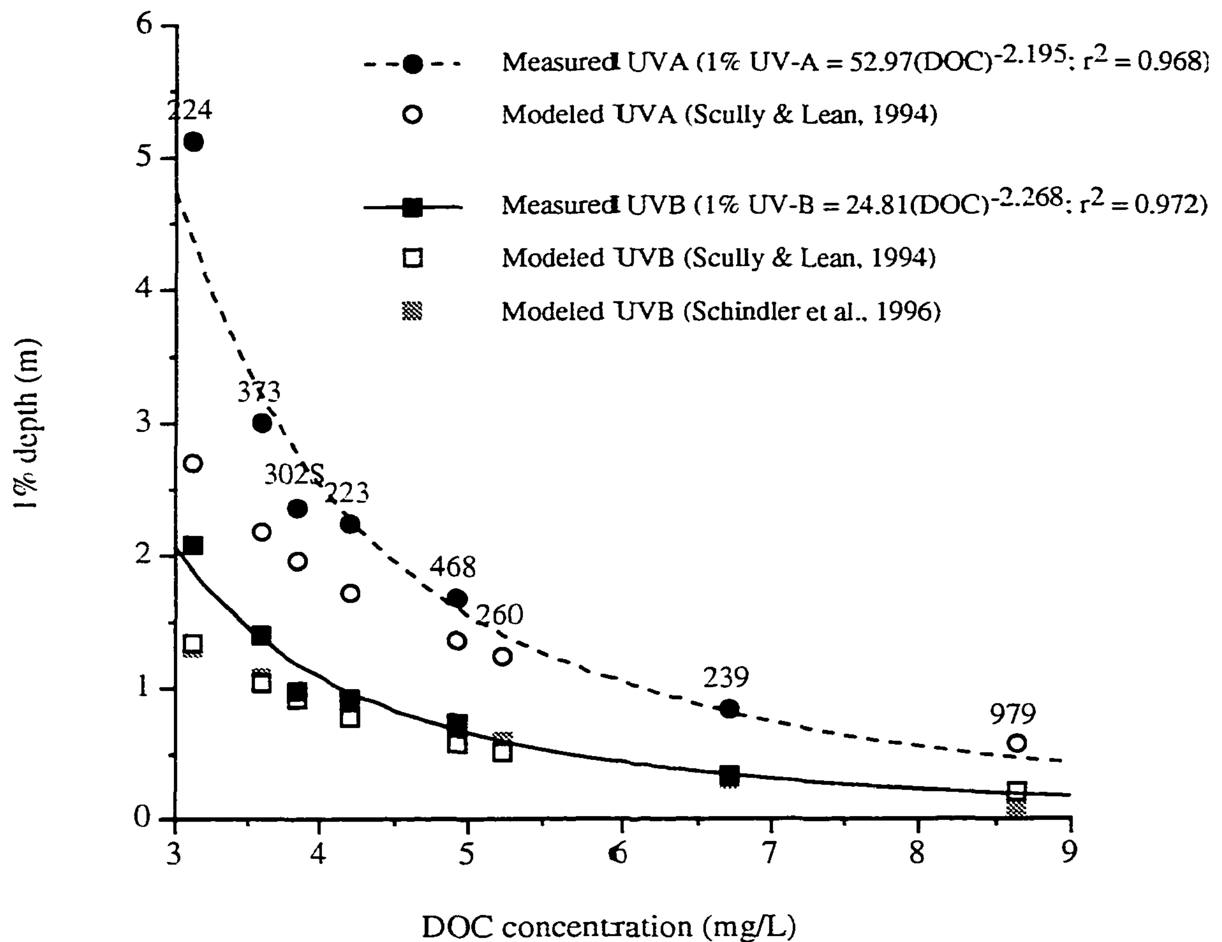

Figure 6-2. DOC concentrations and corresponding 1% depths for UV-a and UV-b in a suite of 8 lakes sampled at the ELA. Modeled values are from Scully and Lean (1994) and Schindler et al. (1996). In situ measurements were performed using a Licor Model LI-1800UM Underwater Spectroradiometer, and mid-July, cloudless solar fluxes were integrated over the ultraviolet spectra. Both UV-b models agree well with each other over the range of DOC concentrations covered by this survey. However, they both increasingly deviated from observed UV-b penetration for lakes with less than 4 mg/L DOC. Similarly, Scully and Lean's UV-a model underestimated UV-a penetration in ELA lakes with less than 5 mg/L DOC.

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Figure 6-3. Ordination plot of redundancy analysis (RDA) of algal species and environmental variables in epilithon of 8 lakes of the Experimental Lakes Area. Epilithon was sampled in triplicate at 0.1, 0.3, 0.7, and 1.5 m in two sites in each lake. The relative length of arrows indicates the relative strength of environmental factors in determining algal assemblages. The relative position of taxa along a line parallel to the direction of any environmental variable's arrow indicates alignment of the taxa along a gradient of that variable. The head of any arrow indicates the direction of a positive increase in that variable.

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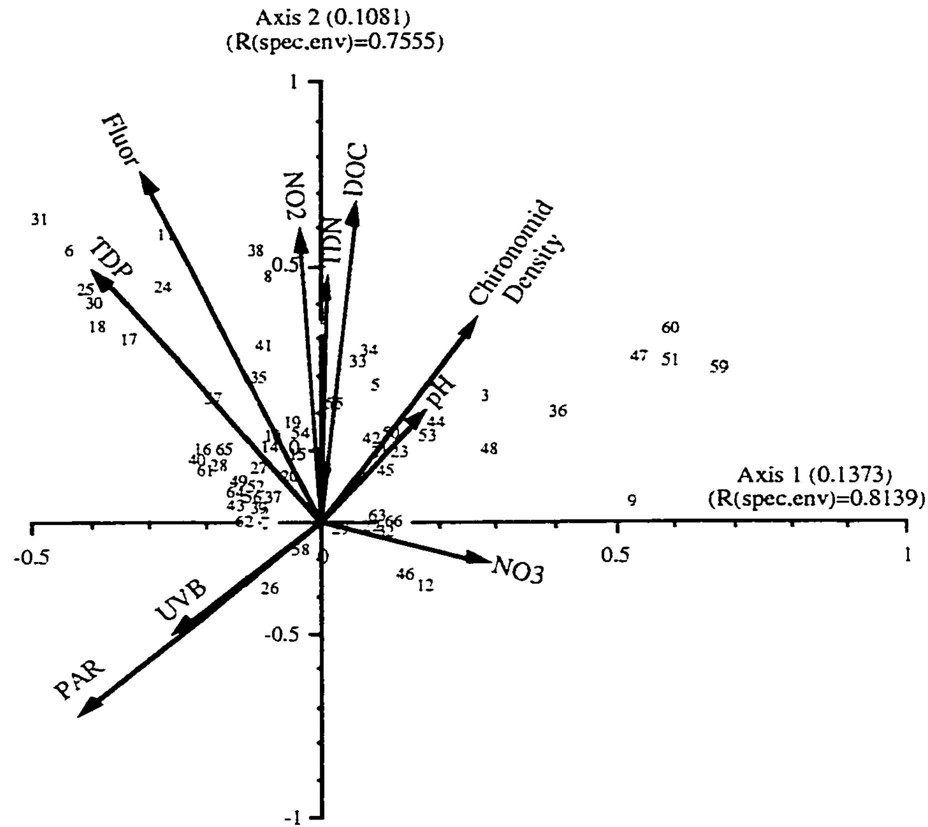

2 Aphanothece sp.
3 Chroococcus limneticus Lemmermann
4 Gomphosphaeria sp.
5 Merismopedia tenuissima Lemmermann
6 Synechococcus sp.
7 Rhabdogloea sp.
8 Anabaena sp.
9 Lyngbya sp.
10 Snowella sp
11 Tolypothrix sp.
12 Rivularia sp.
13 Gloeothece sp.
14 Scytonema sp.
15 Chlamydomonas spp.
16 Pediastrum tetras (Ehrenberg) Ralfs
17 Oocystis borgei Snow
18 Scenedesmus sp.
19 Dictyosphaerium pulchellum Wood
20 Elakatothrix gelatinosa Willen
21 Cosmarium depressum v achondrum (Boldt)
22 Euastrum spp.
23 Micrasterias sp.
24 Staurodesmus paradoxum Meyen
25 Spondylosium planum (Wolle)
26 Mougeotia sp.
27 Mougeotia sp.
28 Mougeotia sp.
29 Zygnema sp.
30 Bambusina brebissonii Kutzing
31 Oedogonium sp.
32 Bulbochaete sp.
33 Dinobryon sertularia Ehrenberg
34 Aulacoseira granulata (Ehrenberg) Simonse
35 Cyclotella stelligera Cleve and Grunow
36 Tabellaria fenestrata (Lyngbye) Kutzing
37 Tabellaria flocculosa (Roth) Kutzing
38 Fragilaria construens (Ehrenberg) Grunow
39 Synedra acus Kutzing
40 Gomphonema sp.
41 Aulacoseira italica v subarctica
42 Pinnularia sp.
43 Nitzschia sp.
44 Achnanthes minutissima Kutzing
45 Cymbella sp.
46 Cyclotella bodanica Eulenst.
47 Epithemia argus Kutzing
48 Eunotia pectinalis (Kutzing) Rabenhorst
49 Gomphonema acuminatum v. coronata
50 Navicula subtilissima Cleve
51 Navicula incerta Grunow
52 Navicula pupula Kutzing
53 Navicula sp.
54 Navicula sp.
55 Neidium sp.
56 Anomoeonies serians (Breb.) Cleve
57 Anomoeonies serians v brachysira Brebisso
58 Pinnularia flexuosa Cleve
59 Pinnularia maior Kutzing
60 Anomoeonies exilis (Kutz.) Cleve
61 Fragilaria pinata Ehrenberg
62 Cymbella gracilis (Rabhorst) Cleve
63 Pinnularia borealis Ehrenberg
64 Neidium sp. a
65 Peridinium inconspicuum Lemmermann
66 Peridinium pusillum (Penard) Lemmermann

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Figure 6-4. Total algal biomass in epilithon at four depths (0.1, 0.3, 0.7, and 1.5 m) in 8 lakes at the Experimental Lakes Area and the flux of photosynthetically active radiation (PAR) to which the epilithic communities were exposed at solar noon on a typical cloudless day in mid-July, 1996 (F=0.031, P=0.861). Points are labeled with the number of the lake.

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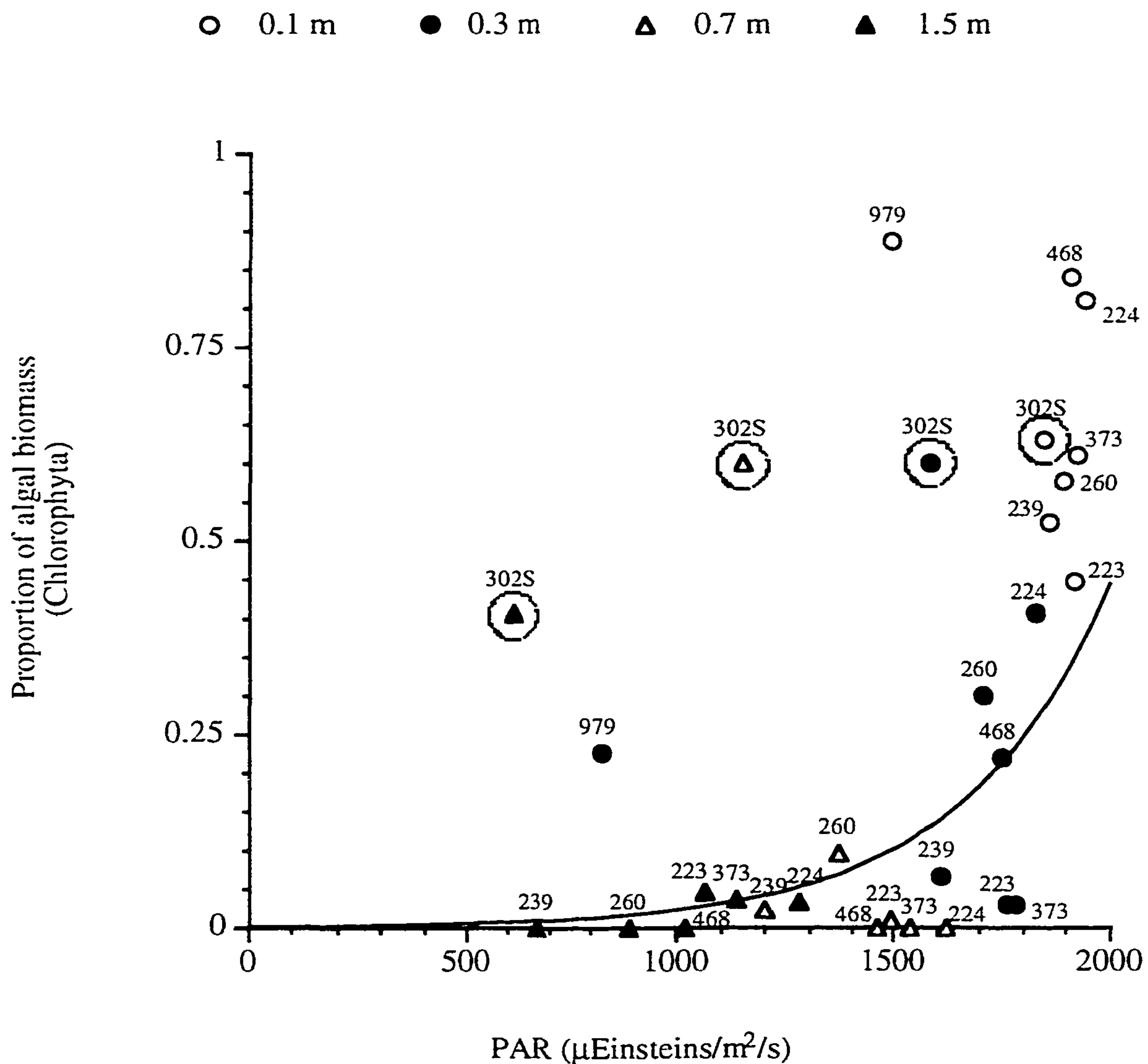

Figure 6-5. Dominance of green algae (Chlorophyta), as a proportion of the total algal biomass, in algal assemblages subject to high PAR exposure in epilithon from 4 depths of 8 ELA lakes. Sites exposed to high PAR fluxes were dominated by chlorophytes (F=7.083; P=0.013). Experimentally acidified L302S (circled), although allowed to recover to pH 5.6, had assemblages with higher proportions of chlorophytes than at sites with similar PAR exposure in unmanipulated lakes.

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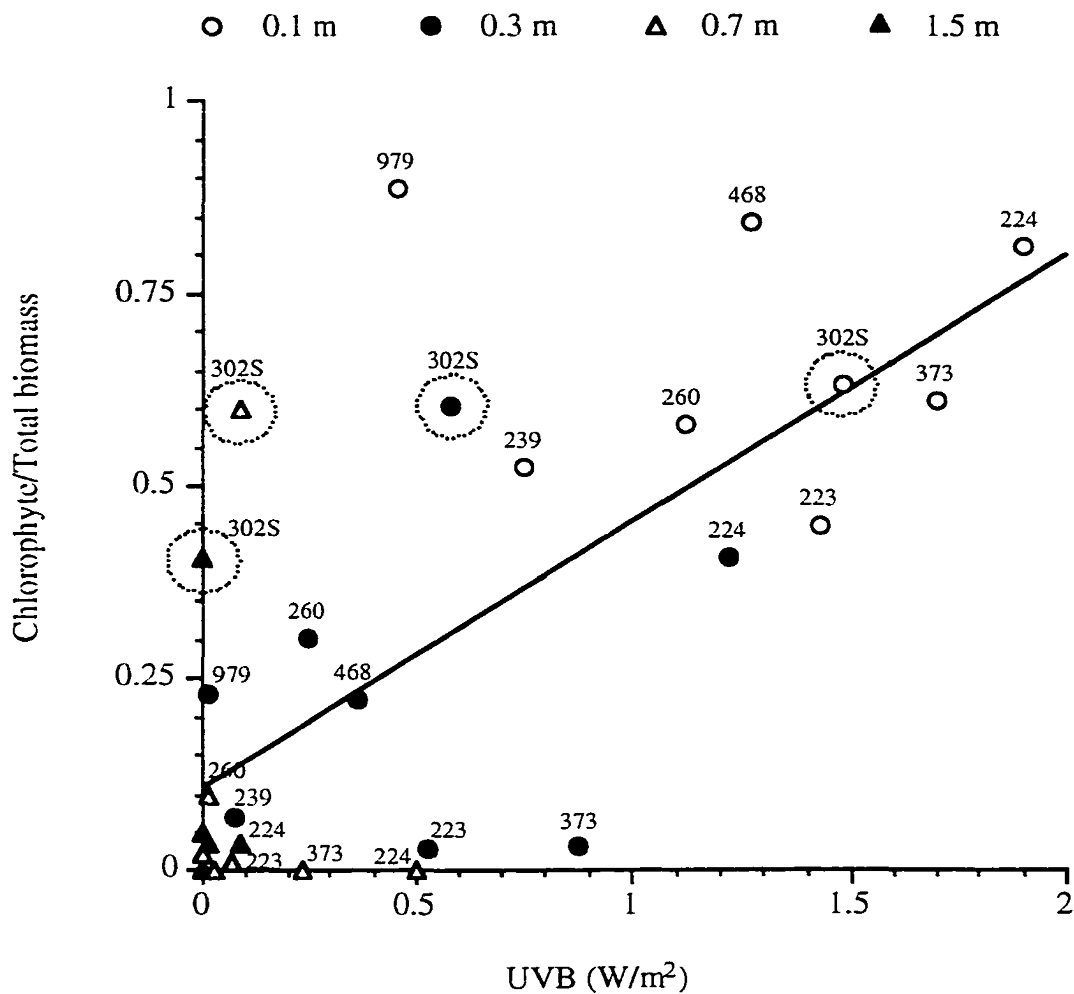

Figure 6-6. Chlorophyte dominance in epilithon subject to high in situ solar UV-b fluxes (% chlorophytes = 0.345(UVB)+0.110; r^2=0.47; F=24.6, P<0.001). Many sites with very low UV-b insolation had low proportions of green algae, although experimentally acidified L302S (circled) had relatively high chlorophyte dominance despite comparable UV-b exposure.

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Figure 6-7. Filamentous green algae dominance in epilithon exposed to a gradient of solar UV-b fluxes in 8 ELA lakes. Depths are 0.1, 0.3, 0.7, and 1.5 m, and fluxes are typical of a cloudless day in mid-July, 1996 (proportion filamentous greens = 0.351(UV-b) + 0.100; r^2=0.48; F=26.1, P<0.001). The circled data points represent algal assemblages in experimentally acidified L302S, at pH 5.6 at the time of sampling.

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Figure 6-8. Patterns of diatom dominance in epilithon from sites exposed to different incident solar UV-b fluxes in 8 ELA lakes (diatoms = $0.3307e^{(-1.022UV\text{-}b)}$; $r^2=0.300$; $F=9.41$, $P=0.0056$).

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Figure 6-9. Dominance of the total pigment complement in epilithon by scytonemin, a photoprotective pigment. The epilithon from sites with the greatest PAR exposure in 8 ELA lakes contained scytonemin comprising 80-90 % of the total pigment mass (Proportion scytonemin=$0.0064e^{0.00258PAR}$; $r^2=0.709$; $F=68.27$, $P<0.0001$).

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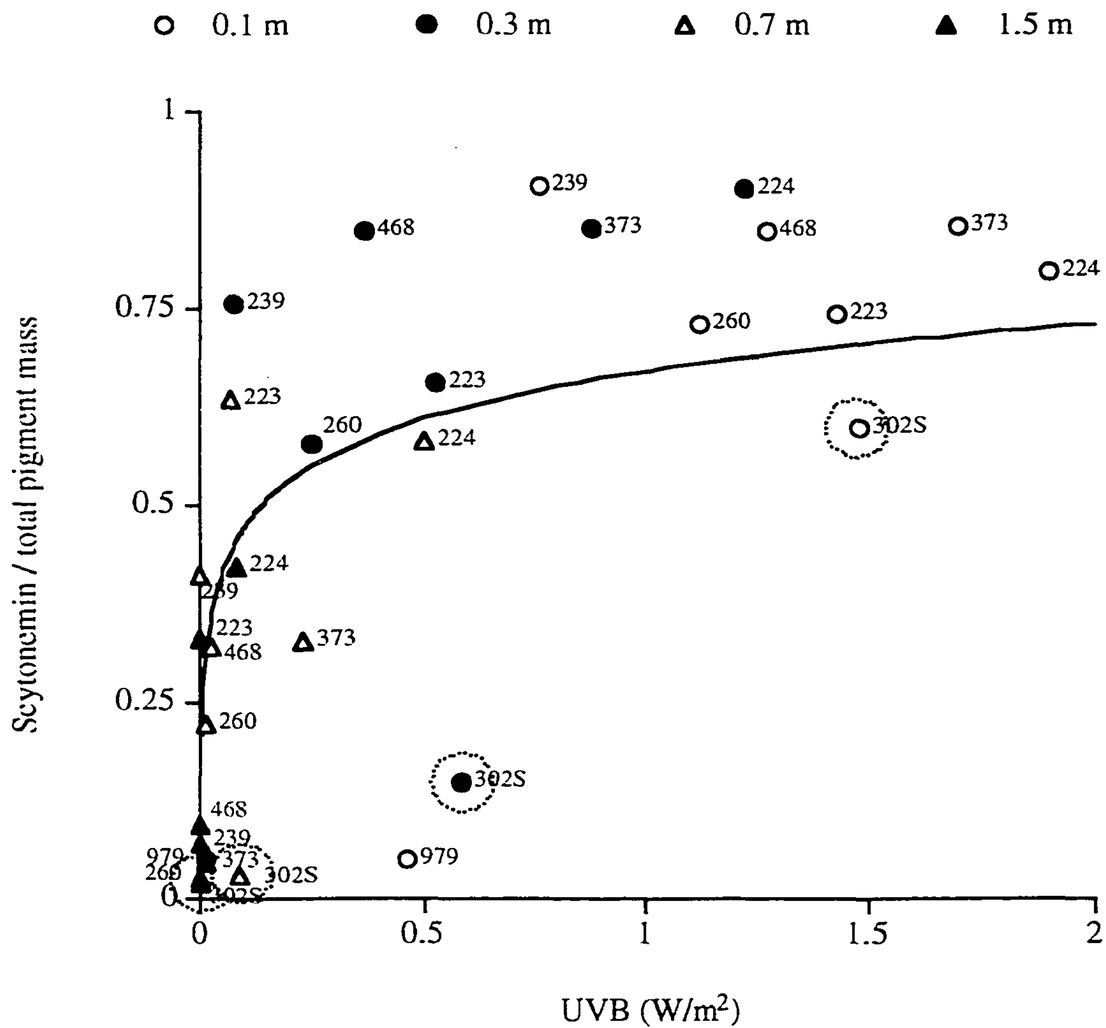

Figure 6-10. Portion of scytonemin in the total pigment complement in epilithon from sites exposed to different solar UV-b fluxes in 8 ELA lakes (scytonemin = 0.200log(UV-b) + 0.672; F=13.16, P=0.0015).

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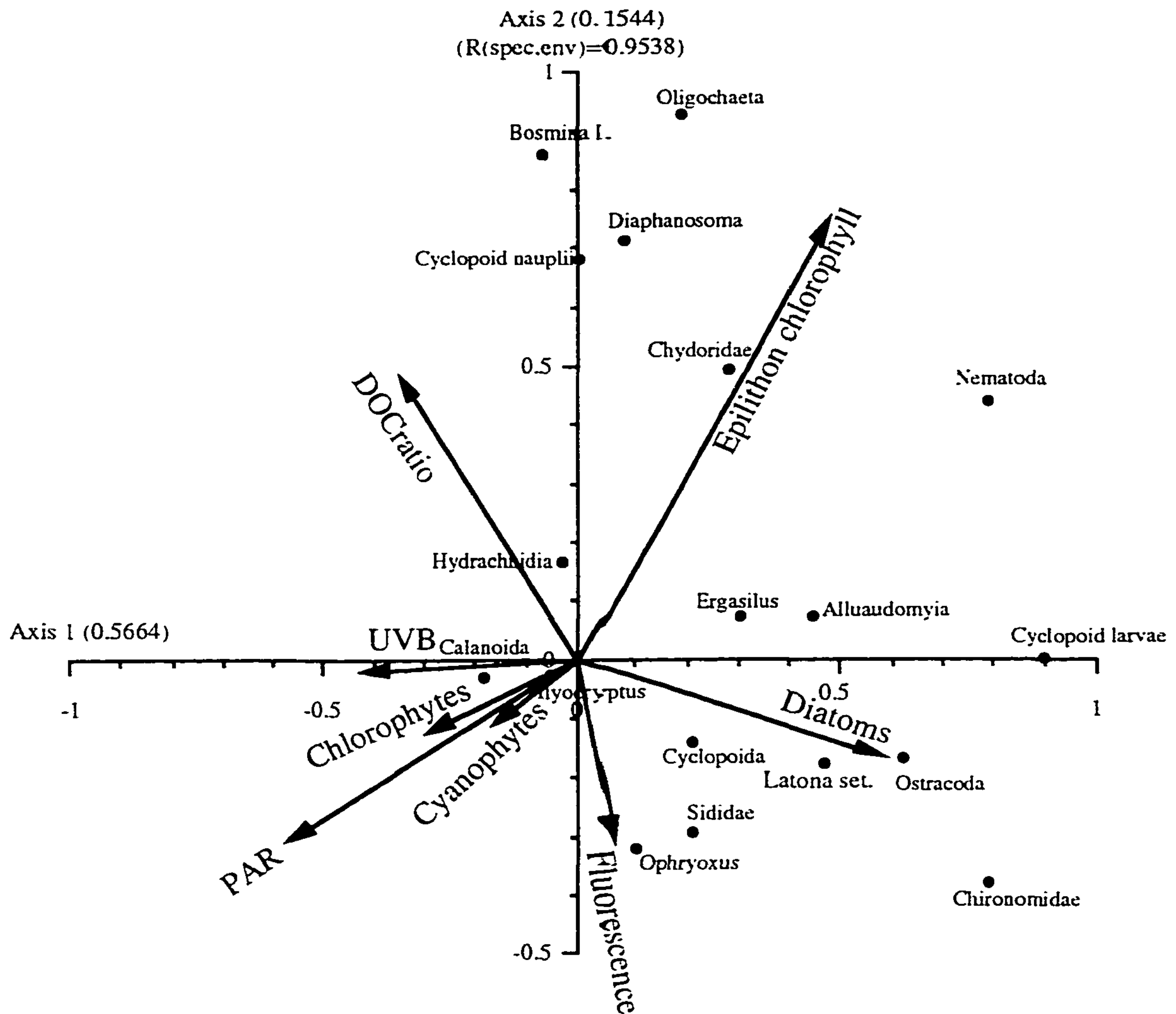

Figure 6-11. Ordination plot of the redundancy analysis (RDA) of epilithon invertebrate assemblages and environmental variables in 8 ELA lakes. Epilithon was sampled on south-facing shores at 0.1, 0.3, 0.7, and 1.5 m in each lake.

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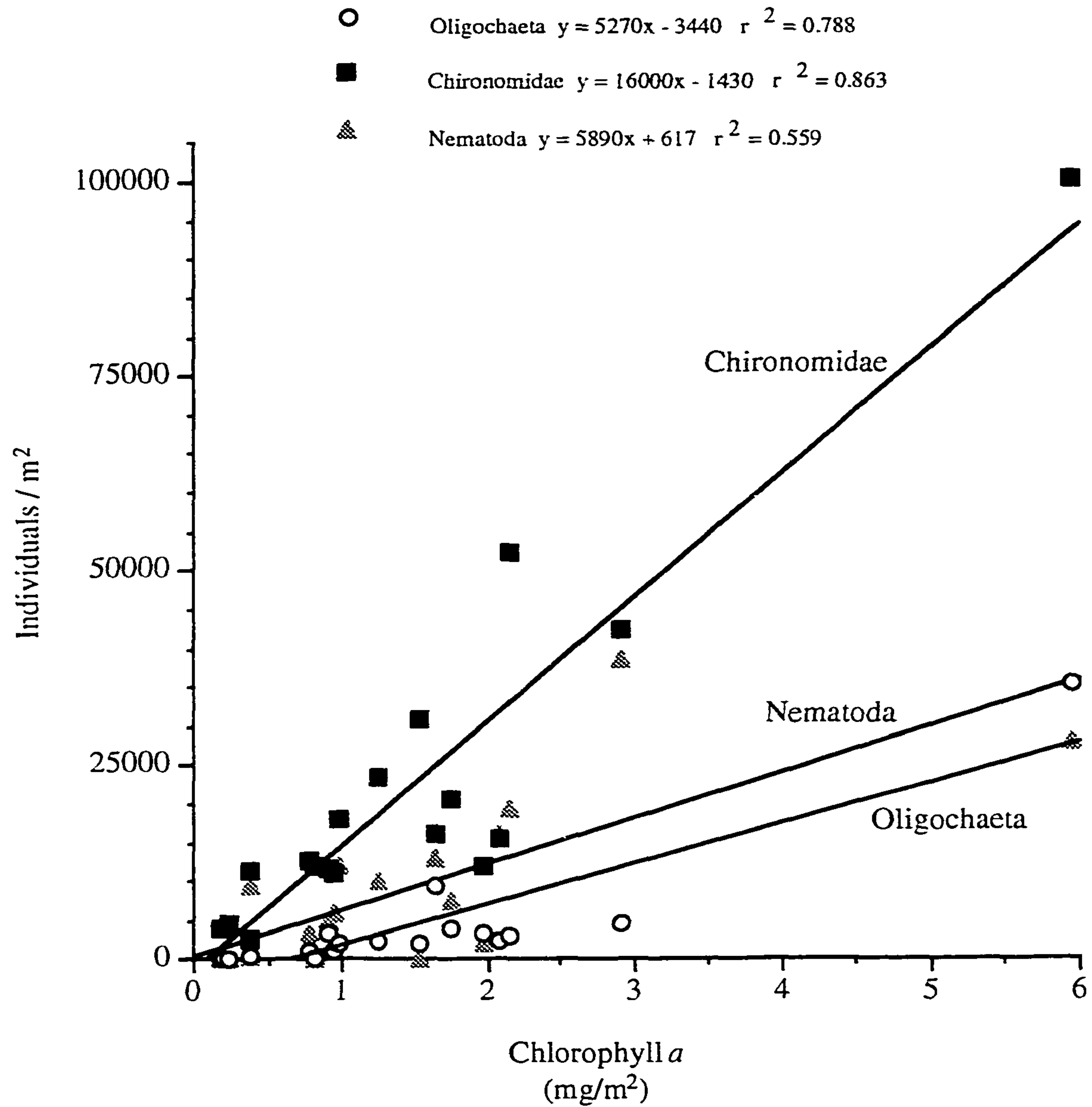

Figure 6-12. Trends of invertebrate densities along a chl a *gradient in epilithon from 0.1, 0.3, 0.7, and 1.5 m in 8 ELA lakes: chironomid larvae (F= 107, P<0.0001); oligochaetes (F=63.2, P<0.0001); and nematodes (F=21.5, P=0.002).*

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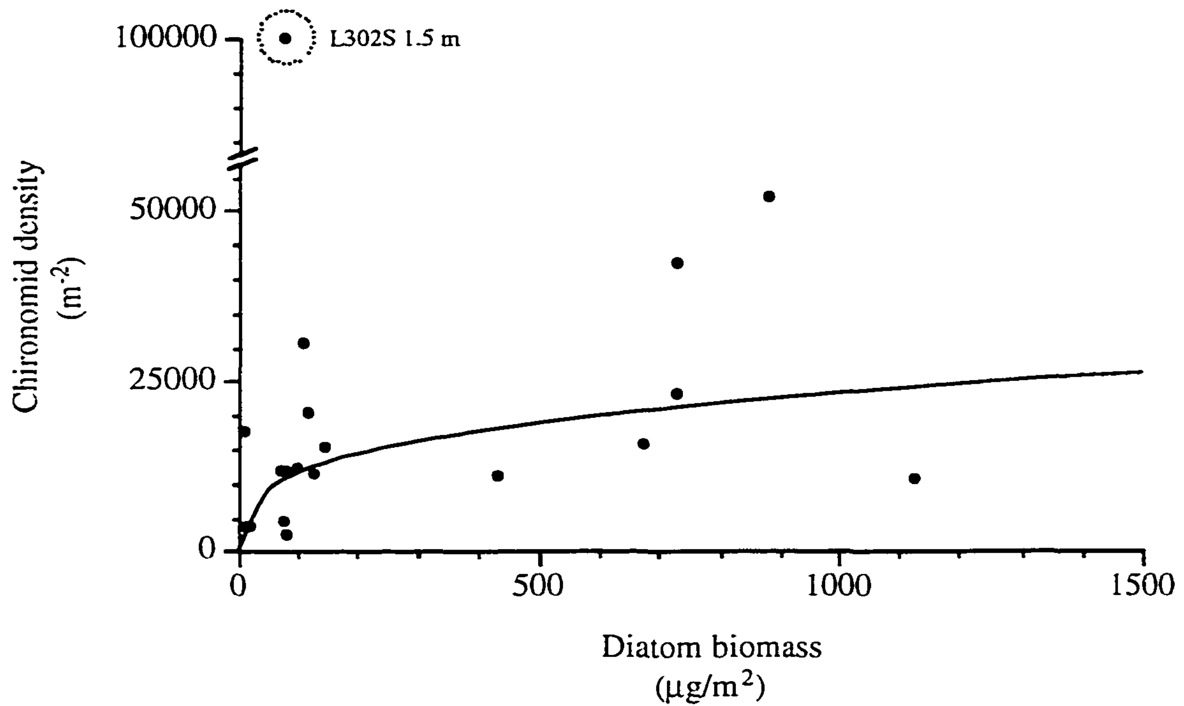

Figure 6-13. Chironomid densities versus diatom biomass in littoral epilithon of 8 ELA lakes. Experimentally acidified L302S had a much higher chironomid density than any other lake, perhaps as a result of release from predation pressure due to the extirpation of minnow populations. For lakes excluding L302S, chironomid density = 2878(diatom biomass)$^{0.303}$ (r^2=0.296, F=6.73, P=0.020).

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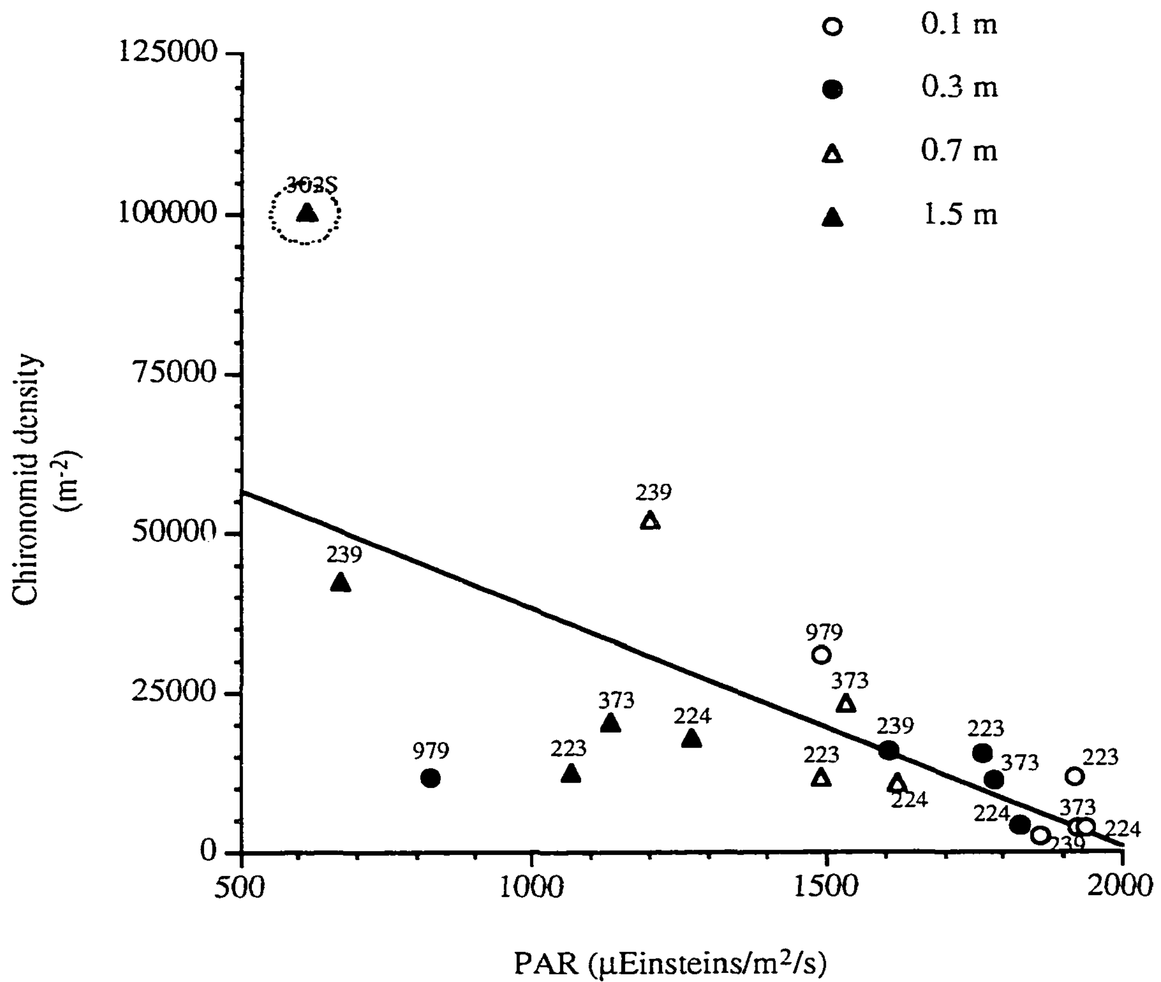

Figure 6-14. Trend in chironomid larvae densities in epilithon along a gradient of exposures of solar PAR in 6 ELA lakes. Those sites exposed to the greatest noon PAR flux on a typical July day had the lowest densities of chironomids (chironomid density = 140200($e^{-0.00158PAR}$); r^2=0.543; F=20.21, P=0.0003).

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Figure 6-15. Chironomid larvae densities in epilithon at four depths in 6 ELA lakes along a gradient of solar UV-b exposure. Chironomid densities were greatest at those sites exposed to the lowest noon UV-b flux on a typical July day (chironomid density = $20500e^{-0.912(UV\text{-}b)}$; $r^2=0.518$; $F=0.518$, $P=0.0017$).

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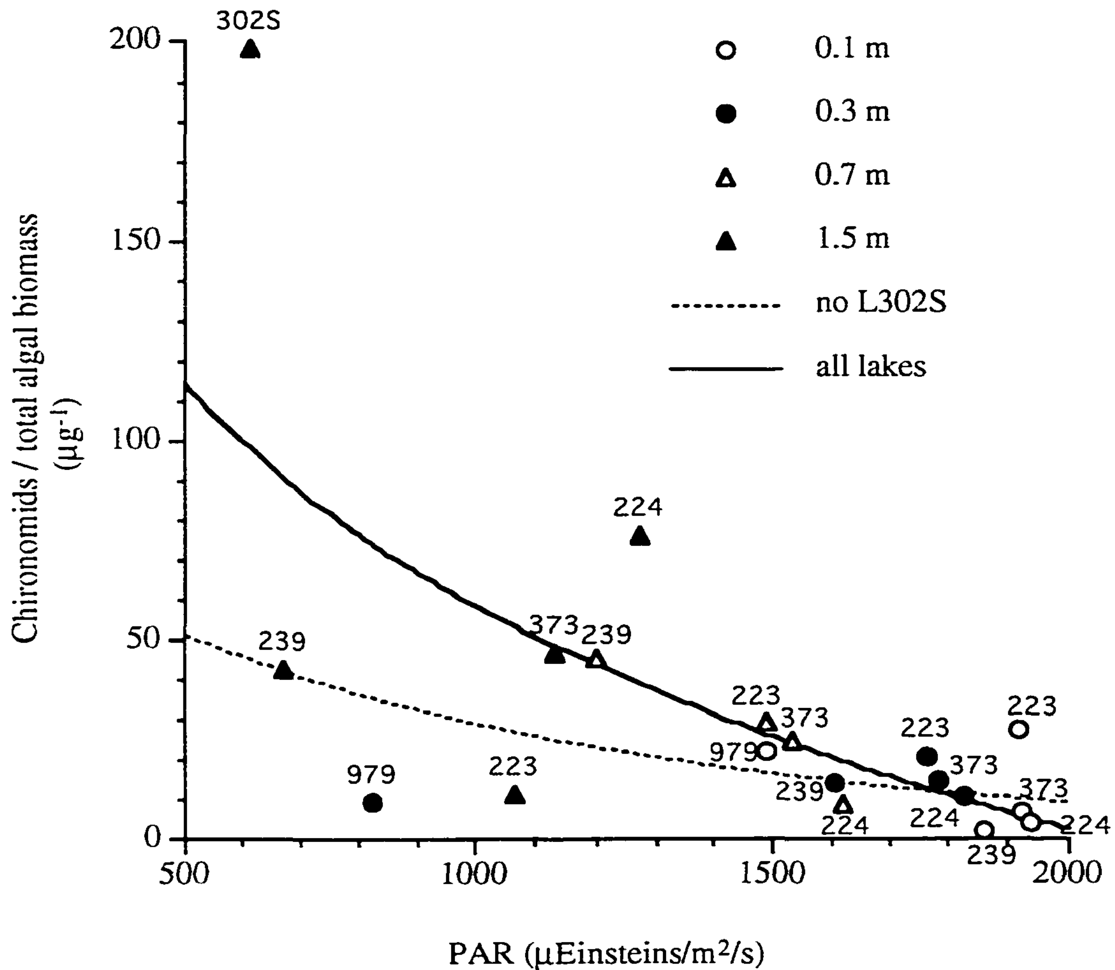

Figure 6-16. Chironomid larvae per unit algal biomass in epilithon from four depths exposed to different solar PAR fluxes in littoral zones of 6 ELA lakes (with L302S: chironomids/µg algae = -80.4ln(PAR) + 614, r^2=0.420, F=12.29, P=0.0027; without L302S: chironomids/µg algae = 90.237$e^{-0.00113PAR}$, r^2=0.246, F=5.21, P=0.037). At sites exposed to high fluxes of PAR, there were fewer chironomid larvae per unit algae.

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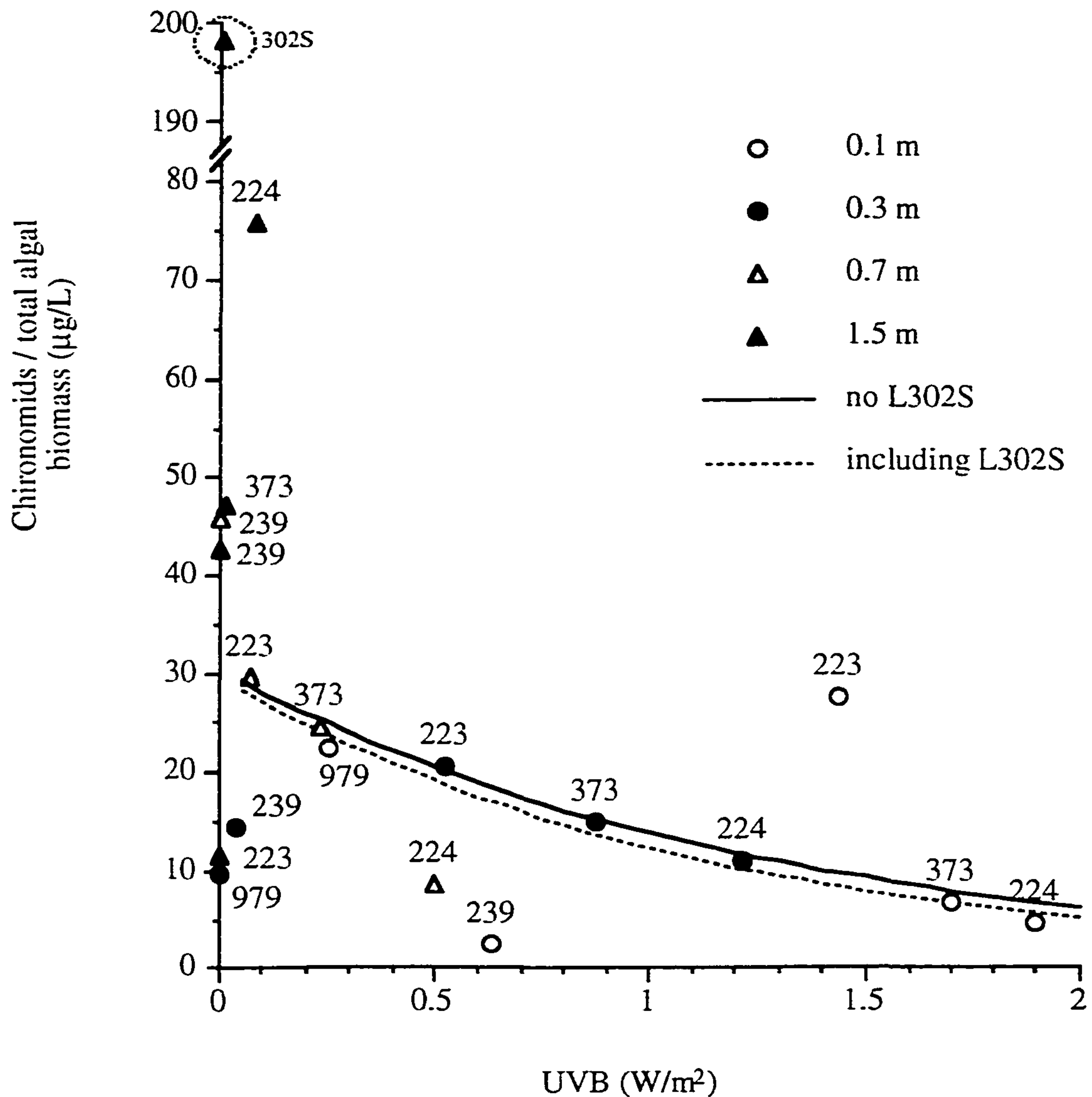

Figure 6-17. Differences in densities of chironomid larvae per total algal biomass (μg^{-1}) in epilithon exposed to a gradient of natural UV-b flux at different depths in 6 ELA lakes (no L302S: chironomids/algal biomass = $e^{(3.31-0.812/UVB)}$, r^2=0.333, F=6.98, P=0.019; all lakes: chironomids/algal biomass = $e^{(3.51-0.971/UVB)}$, r^2=0.289, F=7.92, P=0.013). There were fewer chironomid larvae per mass algal biomass at sites that were exposed to higher fluxes of UV-b than at those sites exposed to low fluxes.

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7. General Discussion

My research program included various studies that highlight the role of ultraviolet radiation (UVR) in chemical, physical, and biological processes in boreal lakes at the Experimental Lakes Area. Before I was able to proceed with experiments on effects of H_2O_2 on DOC and planktonic communities, I first had to overcome problems with a hydrogen peroxide determination technique. Using scanning spectrofluorometry, I described problems of interference in fluorometric determinations of nanomolar concentrations of H_2O_2, as a result of a combination of photobleaching and complex emission response of the fluorophore scopoletin, as well as interactions with horseradish peroxidase (Chapter 3). By eliminating these modes of interference, a very problematic technique used in aquatic photochemistry, groundwater studies, and physiology is now relatively easy and dependable without having sacrificed previously reported levels of sensitivity.

The transmittance of UVR can change in lakes as a result of changes in the quantity and quality of DOC (Schindler et al., 1996, Yan et al., 1996). Although I found no decrease in concentrations of DOC in my 5-week mesocosm experiments, large increases in transmittance of UVR occurred as a result of photobleaching of DOC by UVR (Chapter 2). Further experimentation suggested that nanomolar concentrations of hydrogen peroxide, a reactive photochemical byproduct of the absorbance of UVR by DOC, contribute to the bleaching of DOC, independent of direct UVR-induced photobleaching (Chapter 4). At the same time that photobleaching and chemical bleaching were occurring, coloured, highly aromatic allochthonous DOC originating from the degradation of terrestrial organic matter was replaced with clearer, less aromatic autochthonous DOC originating from planktonic metabolism.

Using spectrofluorometry, I described an acidification-induced change in the nature of DOC (Chapter 5). This was likely due to increased chemical oxidation or precipitation of UV-absorbent aromatic portions of terrestrially-derived DOC molecules, leaving behind more UV-transparent dissolved aliphatic chains. My interpretation provides a mechanism by which acidified lakes undergo dramatic decreases in DOC concentration and a corresponding 2-3-fold increase in mass-specific UV-transmittance. These qualitative DOC changes may have important implications for other physical, biological, and chemical processes within acidified lakes, which are common to many parts of the industrialized world. Decreased DOC inputs and increased residence times in reference lakes as a result of regional climate warming during the 1970s and 1980s resulted in increased photobleaching of allochthonous DOC and increased proportions of autochthonous DOC

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(Chapter 5). Thus, changes in DOC chemical structure can lead to dramatic increases in transmittance of UVR as a result of both direct and indirect chemical, photochemical, and biological activity.

My identification of numerous DOC bleaching mechanisms suggests that conventional UV-flux models based on concentration of DOC and not might be improved by incorporating DOC quality . The variability of absorbance qualities of DOC (Chapters 2, 4, 5, 6) provides a mechanism for explaining the nature of the negative power functions that have been reported between transmittance of UVR and DOC concentration (Schindler et al., 1996, Scully and Lean, 1994). Lakes with low concentrations of DOC used in the construction of these models were from arctic or alpine regions, and had DOC pools that were dominated by relatively colourless autochthonous or photobleached allochthonous DOC. Lakes with moderate to high DOC concentrations that were used in these models, common in boreal regions, were dominated by coloured allochthonous DOC. My work suggests that these models do not account for seasonal changes in UV penetration lakes as a result of altered DOC quality. For example, combined bleaching and greater proportions of autochthonous DOC in boreal lakes can result in much higher penetration of UV-b (+90%) than predicted from DOC concentrations alone (Chapter 6).

Because DOC preferentially absorbs lower wavelengths of solar radiation, my work suggests that decreases in both DOC concentrations and mass-specific UV-absorbance will increase the underwater ratio of radiative fluxes of UV-b to UV-a, in addition to that caused by increases in incident UV-b as a result of stratospheric ozone depletion (Crutzen, 1992, Kerr and McElroy, 1993).

In addition to causing changes in the quality of DOC, my work has shown that UVR has extensive effects on biota of lakes at the ELA. Experimental manipulation of UVR exposure in shallow mesocosms led to reduced algal biomass, largely as a result of strong sensitivities of a few species of diatom (*Tabellaria fenestrata*) and cyanobacteria (*Coelosphaerium* spp. and *Radiocystis geminata* Skuja) (Chapter 2). In addition, *Staurastrum* spp. (Chlorophyta) appeared to be very sensitive. This result suggests that UVR may play an important role in algal succession. The lack of effect of zooplankton in these experiments suggests that UVR effects on many organisms may depend on their ability to migrate or circulate deep enough to escape high-UVR exposure.

My work also suggests that there are indirect responses of organisms to UVR via physical, chemical, and biological linkages that lead to density and assemblage changes in planktonic communities. The increases in algal biomass that I observed in H_2O_2-treated mesocosms suggest that low concentrations of photochemically produced H_2O_2 can affect plankton by stimulating primary production, reducing bacterial resource competition, or

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perhaps altering nutrient speciation (Chapter 4). The algal blooms observed in the H_2O_2-treated mesocosms were consistent with increases in algal carbon fixation observed in low-volume, short-term bottle incubations (Xenopoulos and Bird, 1997). My experiments serve as a spatial and temporal extrapolation of Xenopolous and Bird's observations, and suggest that these processes are occurring in lakes.

As a result of the various ways by which organisms respond to UVR, assemblages often reflect some adaptation to UVR when exposed to high UVR fluxes (Bothwell et al., 1994, Bothwell et al., 1993, Vinebrooke and Leavitt, 1996). My work has shown that UVR is also an important environmental variable in the structuring of benthic algal communities in boreal lakes (Chapter 6). In addition, it has confirmed that many freshwater algal taxa are sensitive to UVR, especially diatoms. Contrary to these general sensitivities, filamentous chlorophytes such as *Oedogonium* spp., *Mougeotia* spp., *Spondylosium planum*, *Zygnema* spp., *Bulbochaete* spp., and *Bambusina brebissonii* dominated epilithon in high-light littoral areas and thus appear to be less sensitive to UVR.

Increased respiration, such as observed in benthic algal associations in acidified lakes (Turner et al., 1995), also may be a protective response to increased UVR exposure. The algae may divert excess radiative energy, that otherwise would result in intracellular production of damaging reactive oxygen species, into the production of phosphoglycolate (Kozaki and Takeba, 1996). The phosphoglycolate then is metabolized via photorespiration to produce CO_2 and phosphoglycerate, thereby protecting themselves from indirect damage by UVR (Kozaki and Takeba, 1996). The dominance of filamentous green algae in sites exposed to high fluxes of solar ultraviolet and visible radiation may be a result of their high photorespiratory capacity, and thus photoprotective capacity. The increase in the proportion of scytonemin in total pigments in the shallowest waters suggests that filamentous green algae also may produce scytonemin as a protective response to high fluxes of low-wavelength solar radiation (Chapter 6). Likewise, it is possible that diatoms, which dominated more sheltered communities, are less able to produce sufficient quantities of photoprotective pigments than filamentous green algae.

Epilithic invertebrate communities in lakes were more sensitive to changes in the algal communities that they inhabited than to UVR. Not only do many invertebrates feed on primary producers, but many benthic invertebrates seek refuge from UVR in the algal mats. Chironomid larvae, nematodes, cyclopoid copepods, and ostracods were less dense at the sites exposed to the greatest UVR fluxes (Chapter 6). There was also evidence that high solar fluxes reduced the algae-specific density of chironomid larvae. Coincident with this decrease, however, was a shift in algal assemblages from diatom-dominated at sites with low UVR intensities to filamentous-green-dominated under high UVR intensities. As a

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result of this taxonomic shift in algae, there may also have been a change in the quality of food for grazing chironomid larvae between the low and high UVR sites. Although many of the invertebrates may have been sensitive to UVR, they may have been able to seek refuge from excessive UVR exposure in the thick algal mats themselves. In this case, a switch in the limiting variable may occur, from the danger of systemic cellular damage as a result of exposure to high solar UVR fluxes to quality and quantity of food.

Previous studies have predicted that UVR fluxes within lakes increased by 900%, as a result of acidification and climate-induced reductions in stream inputs (Schindler et al., 1996). My work suggests that these factors, in concert with increased in-lake bleaching, oxidation, or precipitation of coloured dissolved organic matter, may result in aquatic UVR flux increases of up to 2000-3000%. The relatively short periods over which these processes have been occurring is alarming, and their effects on carefully studied freshwater ecosystems have been extreme. If climatic warming, ozone depletion, and acidification continue according to predictions, lakes may reflect more and more the chemical, physical, and biological impacts of large and rapid increases in UVR exposure. Fundamentally, and in the long run, the problem that is posed by the global nature of current anthropogenic environmental assaults is a problem of the ability of the human race to govern itself responsibly.

Possible areas of future research

My mesocosm experiments in L239 (Chapters 2 and 4) and my description of the acidification-induced alteration of DOC (Chapter 5) provide a starting point for the testing of effects of *in situ* UV-DOC interactions on dissolved organic compounds. Changes in optical and chemical qualities of DOC as a result of dilute H_2O_2 indicate that photochemically produced oxygen radicals are still effective oxidizing agents, even when present in nanomolar amounts. It is possible that other compounds, including organic toxicants, may undergo chemical alteration as a result of oxidation by oxygen radicals produced by Fenton reactions, including H_2O_2, superoxide, and hydroxyl radicals (Zepp et al., 1992), as has been shown in the atmosphere (Friesen et al., 1999). Similarly, acid-induced reductions in aromaticity of DOC (Chapter 5) suggest that anthropogenic acidification has the potential to alter the biogeochemistry of organic pollutants, including chemical alteration and changes in bioavailability and removal processes.

There is a need for further investigations of the effects of dilute photochemicals on *in situ* plankton. The stimulation of phytoplankton that occurred in UV-shielded enclosures in the presence of nanomolar H_2O_2 suggests there is a tradeoff between photochemical stimulation and UV-induced suppression of natural phytoplankton communities. Studies

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of the differential effects of these two environmental variables on microbial and phytoplankton production also need to be pursued. Where mixing moves H_2O_2 profiles deeper and into more UVR-shielded waters (Cooper et al., 1994, Scully et al., 1995), consequences for planktonic communities may be different than in surface waters.

Fluxes of solar radiation dominated the structuring of epilithon in ELA lakes (Chapter 6). Comparisons of boreal benthic communities and patterns of change with those in arctic lakes and those over an elevational gradient, including montane, subalpine, and alpine lakes, might allow one to tease apart the importance and efficacy of various mechanisms that organisms use to protect themselves from excess UVR exposure. It has been shown that short-term invertebrate behavioural responses can be induced when they are exposed to solar UVR (Donahue and Schindler, 1998, Kiffney et al., 1997). Experiments in these systems covering an elevational gradient would allow one to determine whether mechanisms of protection from UVR that involve physiological change, like the production of scytonemin or the production of the caroteno-protein astacene (Vinebrooke and Leavitt, 1999), are environmentally or genetically determined.

From these studies, better predictions could be made of the ability of the systems to withstand or recover from effects of large-scale environmental changes on aquatic systems, including anthropogenic acidification, climate change, and stratospheric ozone depletion.

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APPENDIX A. Algal biovolume, density, and biomass on day 35 in 0.75 m UV-exposed and UV-shielded enclosures, and results of t-tests.

	Biovolume (μm ^ 3)			Density (x10 ^ 6 cells/m ^ 3)			Biomass (mg/m ^ 3)		
Taxon	UV	No UV	P	UV	No UV	P	UV	No UV	P
Anabaena spp.1	320454	44	0.37	46	33	0.80	32	0	0.38
Anabaena spp.2	238	119	0.52	2	85	0.26	1	0	0.40
Anabaena spp.3		65		0	1	0.37		0	0.37
Anabaena spp.4		268		0	1	0.37		0	0.37
Coelosphaerium minutissimum Lemmermann	1206	813	0.37	21	111	0.10	22	71	0.05
Coelosphaerium Kuetzingianum Naegeli		2298		0	74	0.01		171	0.20
Dactylococcopsis linearis Geitler	36	26	0.12	0	18	0.37		0	0.37
Gleothece linearis	29	29	1.00	0	0				
Gomphosphaerium lacustris var. *compacta*	26180	26180		0	2	0.36		62	0.36
Merismopedia punctata Meyen	2	2	1.00	582	20	0.36	1	0	0.36
Microcystis aeruginosa	3	120		2	49	0.03	0	5	0.14
Radiocystis geminata Skuja	1133	957	0.79	18	129	0.04	23	133	0.12
Rhabdoderme lineare	9	9		5	5	0.94	0	0	0.94
Rhabdoderme sigmoidea	5	5		134	136	0.95	1	1	0.95
Ankistrodesmus spp.	17	17	1.00	2	3	0.89	0	0	0.89
Arthrodesmus incus (Brebisson) Haslow	972	972		1	0	0.64	1	0	0.64
Chlamydomonas	28	28	1.00	0	0				
Chlorella vulgaris	4	12	0.00	2922	2261	0.37	12	26	0.01
Chlorella spp	2	3	0.37	243	277	0.63	0	1	0.13
Closterium spp. 1	327	327		0	0				
Closterium spp. 2	5764	5764	1.00	0	0				
Closterium spp. 3	982	982		0	0				
Cosmarium commissurale		997		0	5	0.37		5	0.37
Cosmarium depressum	246	766	0.12	0	19	0.11		19	0.11
Cosmarium spp.	10942	5648	0.15	0	24	0.36	2	19	0.33
Crucigenia rectangularis		65		0	55	0.37		4	0.37
Crucigenia tetrapedium	41	41		28	22	0.77	1	1	0.77
Dictyosphaerium simplex	6	99	0.37	0	106	0.12		17	0.36
Elakatothrix gelatinosa Willen	209	80	0.12	0	20	0.31		1	0.13
Gleococcus Schroeteri (Chodat) Lemmerman	21	29	0.33	3395	3767	0.69	77	106	0.49
Gleocystis planktonica	65	65		0	0				
Lyngbya spp. 1	497	397	0.61	7	7		3	3	0.61
Lyngbya spp. 2	86	463	0.32	7	7		1	3	0.32
Filamentous green		88357		0	0	0.37		6	0.37
Monoraphidium contortum	84	84		0	0				
Monoraphidium minutum	115	115		53	42	0.76	6	5	0.76
Monoraphidium setiforme	142	142		0	0				
Mougeotia spp.	1178	2000	0.57	9	1	0.13	10	1	0.15
Mougeotia spp.	283	679	0.37	0	1	0.37		1	0.37
Nephrocytium lunatum	170	170		14	23	0.72	2	4	0.72
Oocystis borgei	92	92		0	0				
O. lacustris	1206	1206		46	65	0.65	56	78	0.65
O. submarina	95	95	1.00	35	102	0.10	3	10	0.10
Pediastrum duplex	34	34		0	1	0.37		0	0.37

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	Biovolume (μm ^ 3)			Density (x10 ^ 6 cells/m ^ 3)			Biomass (mg/m ^ 3)		
Taxon	UV	No UV	P	UV	No UV	P	UV	No UV	P
Phacomyxa	170	81	0.12	0	72	0.15		3	0.15
Planktonema lauterbornii	13642	13642		0	0				
Planktosphaerium gelatinosa	611	524	0.37	37	7	0.19	19	4	0.19
Quadrigula closterioides (Bohlin) Printz	59	59		0	53	0.11	0	3	0.11
Scenedesmus spp.	18	28	0.52	28	35	0.85	1	1	0.85
Scenedesmus spp.	47	47		0	0	0.37	0		0.37
Scourfieldia cordiformis	34	34		0	0				
Selenastrum minutum	50	50		166	74	0.51	8	4	0.51
Sphaerozosma granulatum	188	188		0	0	0.37	0		0.37
Spondylosium planum		94		0	42	0.37		4	0.37
Staurastrum arachne	171	242	0.37	0	0	0.37		0	0.37
Staurastrum curvatum W. West		3847		0	0	0.12		1	0.13
Staurastrum dejectum		2671		0	0	0.37		1	0.37
Staurastrum gladiosum	11159	11159		0	0				
Staurastrum jaculiferum	1368	1368	1.00	0	0	0.37		0	0.37
Staurastrum leptodermum	66	1070	0.37	0	0	0.37		1	0.37
Staurastrum paradoxum var. *parvum*	2671	1004	0.12	0	0	0.16		0	0.30
Staurastrum pentacerum (Wolle) G. M. Smith	20115	9574	0.02	0	0	0.37	1	2	0.78
Tetraedron minimum		250		0	3	0.27		1	0.37
Tetraspora gelatinosa		14		0	16	0.12		0	0.12
Zygnema spp.	98	98	0.35	63	0	0.37	6		0.37
Large chlorophyte	10996	10996		0	0	0.07	4		0.07
Trachelomonas armata	2463	2463		5	9	0.04	13	21	0.04
Chrysochromulina frigida Holmgren	628	628		44	67	0.25	28	42	0.25
Chrysochromulina parva Lackey	151	151		439	499	0.77	66	75	0.77
Chrysosphaerella longispina	34	34		0	0				
Dinobryon bavaricum	188	119	0.12	0	90	0.36		8	0.36
Uroglena volvox	132	132		0	0				
Achnanthes minutissima	26	26		37	14	0.25	1	0	0.25
Achnanthes spp.	26	26		1	5	0.46	0	0	0.46
Asterionella formosa	11	11		18	18	0.97	0	0	0.97
Cyclotella stelligera Cleve and Grunow	1069	1069		13	26	0.23	14	28	0.23
Cymbella spp.	2648	2648		0	0				
Eunotia spp.	331	331		0	0				
Fragilaria construens (Ehrenberg) Grunow	294	294		1	3	0.61	0	1	0.61
pennate diatom	329	329		7	5	0.56	2	2	0.56
Synedra acus Kuetzing	490	490		7	7	0.90	3	3	0.90
Tabellaria fenestrata (Lyngbya) Kuetzing	1086	711	0.12	9	123	0.11	10	69	0.04
Tabellaria flocculosa (Rothest) Kuetzing	1762	1762		3	11	0.20	5	19	0.20
Cryptomonas erosa Ehrenberg	652	591	0.52	16	58	0.36	10	39	0.39
Cryptomonas rostatiformis	5081	4926	0.52	2	6	0.46	12	28	0.47
Ketablepharis ovalis Skujai	90	90							

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APPENDIX B. Zooplankton densities (/L) in 0.75 m UV-shielded and UV-exposed enclosures and P-values from t-tests.

Invertebrate taxon		No UV 0.9	UV 0.9	P
Acroperus cf.harpae		0.08	1.07	0.01
Chydorus brevilabris		5.80	4.70	0.77
Bosmina longirostris		5.36	1.77	0.46
Sida crystallina		0.00	1.05	0.36
Diaphanosoma birgei		4.25	5.95	0.37
Holopedium gibberum		0.92	0.07	0.13
Polyphemus pediculus		0.85	0.44	0.32
Diacyclops bicuspidatus thomasi	(females)	0.78	0.43	0.41
	(males)	0.21	0.36	0.58
	(c3-5)	1.21	1.08	0.64
Tropocyclops prasinus mexicanus	(females)	0.86	0.72	0.80
	(males)	0.65	0.44	0.63
	(c3-5)	0.28	0.49	0.48
Cyclopoida	(c1-2)	0.00	0.08	0.42
Diaptomus minutus	(females)	0.87	0.57	0.59
	(males)	0.43	1.35	0.06
	(c3-5)	1.36	1.30	0.96
Epischura lacustris	(females)	0.00	0.00	
	(males)	0.00	0.15	0.00
	(c3-5)	0.15	0.00	0.42
Calanoida	(c1-2)	0.08	0.07	0.97
copepod nauplii		0.36	0.98	0.40
Hydrachnidia		0.22	0.21	0.97
Oligochaeta		0.29	0.00	0.42
Chironomidae		0.00	0.15	0.42
Ephemeroptera		0.00	0.07	0.42

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APPENDIX C.

C-1. Algal taxa and biomass in epilithon at 0.1, 0.3, 0.7, and 1.5 m in Lakes 223 and 224.

	L223				L224			
Species	0.1	0.3	0.7	1.5	0.1	0.3	0.7	1.5
Aphanothece spp.							10.3	
Chroococcus limneticus Lemmermann	2.3	5.6	24.0	11.0	5.0	1.9	1.2	7.2
Gomphosphaeria spp.				55.1				
Merismopedia tenuissima Lemmermann				591.1				
Synechococcus spp.								
Rhabdogloea spp.								
Anabaena spp.	15.6							7.0
Lyngbya spp.	150.3	283.7	240.1	278.8	77.7	149.4	128.6	204.8
Snowella spp.								
Tolypothrix spp.								
Rivularia spp.					70.4	18.7		
Gloeothece spp.	2.8	3.9	2.5	1.4				0.7
Scytonema spp.		292.2						
Chlamydomonas spp.	1.6							
Pediastrum tetras (Ehrenberg) Ralfs								
Oocystis borgei Snow								6.5
Scenedesmus spp.								1.9
Dictyosphaerium pulchellum Wood			4.6					
Elakatothrix gelatinosa Willen								
Cosmarium depressum v achondrum								
Euastrum spp.								
Micrasterias spp.								
Staurodesmus paradoxum Meyen				24.4				
Spondylosium planum (Wolle)								
Mougeotia spp.	77.9				706.6	108.2		
Mougeotia spp.	116.9			29.2				
Mougeotia spp.								
Zygnema spp.								
Bambusina brebissonii Kutzing								
Oedogonium spp.		22.7			12.6	59.8		
Bulbochaete spp.								
Dinobryon sertularia Ehrenberg								
Aulacoseira granulata (Ehrenberg) Simonse								
Cyclotella stelligera Cleve and Grunow				13.5	3.9			8.7
Tabellaria fenestrata (Lyngbye) Kutzing					2.6			
Tabellaria flocculsa (Roth) Kutzing	10.6							
Fragilaria construens (Ehrenberg) Grunow			13.0	60.9				
Synedra acus Kutzing								
Gomphonema spp.								
Aulacoseira italica v subarctica			43.8					
Pinnularia spp.								
Nitzschia spp.								
Achnanthes minutissima Kutzing	16.0	46.9	4.0	14.1	7.0	5.5	5.6	
Cymbella spp.	23.2		4.1			9.7		
Cyclotella bodanica Eulenst.								
Epithemia argus Kutzing								

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Species	L223				L224			
	0.1	0.3	0.7	1.5	0.1	0.3	0.7	1.5
Eunotia pectinalis (Kutzing) Rabenhorst								
Gomphonema acuminatum v. coronata (Ehrenberg)		32.5						
Navicula subtilissima Cleve								
Navicula incerta Grunow		12.2	1.1					
Navicula pupula Kutzing								
Navicula spp.		48.7	47.4			59.2	7.8	
Navicula spp.			13.5					
Neidium spp.		2.4		2.8				
Anomoeonies serians (Breb.) Cleve								
Anomoeonies serians v brachysira Brebisson				5.4				
Pinnularia flexuosa Cleve							938.1	
Pinnularia maior Kutzing								
Anomoeonies exilis (Kutz.) Cleve								
Fragilaria pinata Ehrenberg								
Cymbella gracilis (Rabhorst) Cleve	21.0						175.3	
Pinnularia borealis Ehrenberg								
Neidium spp. a								
Peridinium inconspicuum Lemmermann								
Peridinium pusillum (Penard) Lemmermann								

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C-2. Algal taxa and biomass in epilithon at 0.1, 0.3, 0.7, and 1.5 m in Lakes 239 and 260.

Species	L239 0.1	L239 0.3	L239 0.7	L239 1.5	L260 0.1	L260 0.3	L260 0.7	L260 1.5
Aphanothece spp.								
Chroococcus limneticus Lemmermann	15.2	17.5	13.4	16.1	4.2	4.5	12.9	16.0
Gomphosphaeria spp.								
Merismopedia tenuissima Lemmermann								
Synechococcus spp.	2.8							
Rhabdogloea spp.	1.9							
Anabaena spp.			2.7	4.1			18.8	2.3
Lyngbya spp.	228.6	289.1	195.3	245.5	165.7	150.4	144.4	23.8
Snowella spp.							15.1	
Tolypothrix spp.								
Rivularia spp.	182.8	63.6	21.7		97.4			52.8
Gloeothece spp.								
Scytonema spp.							67.0	
Chlamydomonas spp.								
Pediastrum tetras (Ehrenberg) Ralfs								
Oocystis borgei Snow								
Scenedesmus spp.						1.1		
Dictyosphaerium pulchellum Wood								
Elakatothrix gelatinosa Willen					0.6			
Cosmarium depressum v achondrum		54.1						
Euastrum spp.								
Micrasterias spp.		12.0						
Staurodesmus paradoxum Meyen								
Spondylosium planum (Wolle)								
Mougeotia spp.	323.0				20.3	190.5		
Mougeotia spp.					427.9			
Mougeotia spp.								
Zygnema spp.			20.2					
Bambusina brebissonii Kutzing								
Oedogonium spp.	146.1	11.3	7.0		13.0	8.1	40.6	
Bulbochaete spp.	93.4							
Dinobryon sertularia Ehrenberg								
Aulacoseira granulata (Ehrenberg) Simonse								
Cyclotella stelligera Cleve and Grunow								
Tabellaria fenestrata (Lyngbye) Kutzing			53.4	9.6	10.8			
Tabellaria flocculsa (Roth) Kutzing	9.7	42.4			5.5			
Fragilaria construens (Ehrenberg) Grunow			40.6					
Synedra acus Kutzing								
Gomphonema spp.						67.7		
Aulacoseira italica v subarctica								
Pinnularia spp.		1.1						
Nitzschia spp.		52.0				108.2		
Achnanthes minutissima Kutzing	19.7	43.7	44.6	16.4	8.3	15.5	14.4	
Cymbella spp.		321.5			1.5	52.0	78.6	
Cyclotella bodanica Eulenst.								
Epithemia argus Kutzing				32.5	5.1		4.9	23.4

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Species	L239				L260			
	0.1	0.3	0.7	1.5	0.1	0.3	0.7	1.5
Eunotia pectinalis (Kutzing) Rabenhorst								
Gomphonema acuminatum v. coronata (Ehrenberg)						19.5		
Navicula subtilissima Cleve		191.7						20.8
Navicula incerta Grunow	23.4	18.8	23.8	32.8				2.0
Navicula pupula Kutzing								
Navicula spp.			77.1	24.4	15.2	10.4	16.2	
Navicula spp.								
Neidium spp.								
Anomoeonies serians (Breb.) Cleve								
Anomoeonies serians v brachysira Brebisson								
Pinnularia flexuosa Cleve	27.1							
Pinnularia maior Kutzing			631.4	613.4				
Anomoeonies exilis (Kutz.) Cleve				2.0				
Fragilaria pinata Ehrenberg						0.4		
Cymbella gracilis (Rabhorst) Cleve			9.1		22.6	33.8		28.4
Pinnularia borealis Ehrenberg								
Neidium spp. a								
Peridinium inconspicuum Lemmermann								
Peridinium pusillum (Penard) Lemmermann								

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C- 3. Algal taxa and biomass in epilithon at 0.1, 0.3, 0.7, and 1.5 m in Lakes 373 and 468.

	L373				L468			
Species	0.1	0.3	0.7	1.5	0.1	0.3	0.7	1.5
Aphanothece spp.								
Chroococcus limneticus Lemmermann	12.1	6.9	27.2	77.5	15.6	85.2	10.6	91.9
Gomphosphaeria spp.				1.9				
Merismopedia tenuissima Lemmermann	0.2							
Synechococcus spp.								
Rhabdogloea spp.								
Anabaena spp.		2.7		5.8		3.2	20.7	8.7
Lyngbya spp.	83.8	268.3	195.7	189.9	80.2	141.0	262.7	371.6
Snowella spp.								
Tolypothrix spp.								
Rivularia spp.	114.9	26.0		28.9	84.4	147.2	277.1	
Gloeothece spp.		1.1	1.3	4.0	1.8			
Scytonema spp.								
Chlamydomonas spp.								
Pediastrum tetras (Ehrenberg) Ralfs								
Oocystis borgei Snow						7.2		
Scenedesmus spp.								1.9
Dictyosphaerium pulchellum Wood								
Elakatothrix gelatinosa Willen								
Cosmarium depressum v achondrum								
Euastrum spp.						33.8		
Micrasterias spp.								
Staurodesmus paradoxum Meyen								
Spondylosium planum (Wolle)								
Mougeotia spp.	199.8	25.4			649.4	248.0		
Mougeotia spp.					81.2			
Mougeotia spp.								
Zygnema spp.	124.9				87.7			
Bambusina brebissonii Kutzing								
Oedogonium spp.	11.2			16.2		27.1		
Bulbochaete spp.	22.9				568.3			
Dinobryon sertularia Ehrenberg								
Aulacoseira granulata (Ehrenberg) Simonse								
Cyclotella stelligera Cleve and Grunow								
Tabellaria fenestrata (Lyngbye) Kutzing			8.7		5.7		4.7	7.5
Tabellaria flocculsa (Roth) Kutzing		183.9						
Fragilaria construens (Ehrenberg) Grunow								
Synedra acus Kutzing	3.5		0.8					
Gomphonema spp.								
Aulacoseira italica v subarctica			48.7	97.4				
Pinnularia spp.								
Nitzschia spp.		54.1				25.3		
Achnanthes minutissima Kutzing	13.7	28.2	11.9	1.8	10.8	4.7	1.6	9.4
Cymbella spp.				16.6			135.3	
Cyclotella bodanica Eulenst.		50.4			46.8	38.1	46.2	
Epithemia argus Kutzing							22.1	

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	L373				L468			
Species	0.1	0.3	0.7	1.5	0.1	0.3	0.7	1.5
Eunotia pectinalis (Kutzing) Rabenhorst								26.8
Gomphonema acuminatum v. coronata (Ehrenberg)						17.3		
Navicula subtilissima Cleve			15.6					
Navicula incerta Grunow								
Navicula pupula Kutzing		17.8						
Navicula spp.			11.4					
Navicula spp.								
Neidium spp.								
Anomoeonies serians (Breb.) Cleve	0.9							
Anomoeonies serians v brachysira Brebisson								
Pinnularia flexuosa Cleve			631.4			631.4		
Pinnularia maior Kutzing								902.0
Anomoeonies exilis (Kutz.) Cleve								
Fragilaria pinata Ehrenberg								
Cymbella gracilis (Rabhorst) Cleve		39.8						
Pinnularia borealis Ehrenberg						11.3		
Neidium spp. a		57.7						
Peridinium inconspicuum Lemmermann								
Peridinium pusillum (Penard) Lemmermann					14.6			

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C- 4. Algal taxa and biomass in epilithon at 0.1, 0.3, 0.7, and 1.5 m in Lakes 979 and 302S.

	L979		L302S			
Species	0.1	0.3	0.1	0.3	0.7	1.5
Aphanothece spp.						
Chroococcus limneticus Lemmermann	17.3	5.6	2.1	5.5	79.5	115.4
Gomphosphaeria spp.		18.1				
Merismopedia tenuissima Lemmermann						
Synechococcus spp.	6.0	2.7				
Rhabdogloea spp.						
Anabaena spp.		48.5				
Lyngbya spp.	25.1	63.3	39.3	90.0	58.9	97.0
Snowella spp.						
Tolypothrix spp.		746.8				
Rivularia spp.						
Gloeothece spp.						
Scytonema spp.						
Chlamydomonas spp.						
Pediastrum tetras (Ehrenberg) Ralfs				0.2		
Oocystis borgei Snow	21.7					
Scenedesmus spp.	8.7			3.9		
Dictyosphaerium pulchellum Wood						
Elakatothrix gelatinosa Willen						
Cosmarium depressum v achondrum						
Euastrum spp.						
Micrasterias spp.						
Staurodesmus paradoxum Meyen	34.6					
Spondylosium planum (Wolle)	5.2					
Mougeotia spp.	97.4	9.7	11.2	18.3	233.8	
Mougeotia spp.	54.1		2.9	58.4		
Mougeotia spp.			41.6			
Zygnema spp.						
Bambusina brebissonii Kutzing	701.4					
Oedogonium spp.	295.5	277.6	26.2	187.2	43.8	205.8
Bulbochaete spp.			46.8			
Dinobryon sertularia Ehrenberg						12.2
Aulacoseira granulata (Ehrenberg) Simonse						4.3
Cyclotella stelligera Cleve and Grunow	6.9					
Tabellaria fenestrata (Lyngbye) Kutzing			12.8			
Tabellaria flocculsa (Roth) Kutzing			8.2	13.3		
Fragilaria construens (Ehrenberg) Grunow	86.6				19.7	58.6
Synedra acus Kutzing						
Gomphonema spp.	5.3					
Aulacoseira italica v subarctica		58.5				
Pinnularia spp.						
Nitzschia spp.						
Achnanthes minutissima Kutzing	7.2	14.1			1.6	
Cymbella spp.		2.9	4.2			
Cyclotella bodanica Eulenst.						
Epithemia argus Kutzing						

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	L979		L302S			
Species	0.1	0.3	0.1	0.3	0.7	1.5
Eunotia pectinalis (Kutzing) Rabenhorst						
Gomphonema acuminatum v. coronata (Ehrenberg)						
Navicula subtilissima Cleve						
Navicula incerta Grunow						
Navicula pupula Kutzing						
Navicula spp.		3.3				
Navicula spp.						
Neidium spp.						
Anomoeonies serians (Breb.) Cleve						
Anomoeonies serians v brachysira Brebisson			8.3	22.3	25.3	11.7
Pinnularia flexuosa Cleve						
Pinnularia maior Kutzing						
Anomoeonies exilis (Kutz.) Cleve						
Fragilaria pinata Ehrenberg						
Cymbella gracilis (Rabhorst) Cleve						
Pinnularia borealis Ehrenberg						
Neidium spp. a						
Peridinium inconspicuum Lemmermann				45.1		
Peridinium pusillum (Penard) Lemmermann						

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