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EFFECTS OF ULTRAVIOLET RADIATION ON BENTHIC ASSEMBLAGES OF MONTANE LAKES

by

Suzanne Elizabeth Tank 

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

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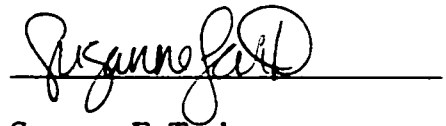

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Effects of Ultraviolet Radiation on Benthic Assemblages of Montane Lakes submitted by Suzanne Elizabeth Tank in partial fulfillment of the requirements for the degree of Master of Science in Environmental Biology and Ecology.

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Date: 07 January, 2002

ABSTRACT

Because of their clarity and altitude, exposure to ultraviolet radiation in mountain lakes can be extremely high. This study was conducted in four montane lakes in Jasper National Park. Its focus was twofold: first, to examine the direct effect of ultraviolet radiation on both benthic invertebrates and epilithon, the rock-dwelling matrix of algae, bacteria, and detritus. Second, to examine the indirect effect of ultraviolet-mediated shifts in epilithic composition on invertebrates. Although ultraviolet radiation decreased epilithic carbon accrual and pigment concentrations, total algal biomass was not affected. Furthermore, although exposure to ultraviolet radiation decreased invertebrate colonization, it increased food quality for invertebrates, through decreased carbon to nutrient ratios and increased fatty acid concentrations. These effects, however, were weak, and not universal across our four study lakes. Our results suggest that although ultraviolet radiation can play an important role in structuring freshwater benthic communities, other factors, such as nutrient availability, may often be of paramount importance.

PREFACE TO THE THESIS

The structure of my thesis is in paper format, and is presented as two manuscripts (Chapters 2 and 3). A general introductory chapter (Chapter 1) is intended to provide a brief background to the field of research, my research objectives, and to outline my experiments. A general conclusion (Chapter 4) is provided as a summary of conclusions drawn from across my experiments and suggestions for future research.

As with almost all scientific endeavors, this work could not have occurred without the collaboration of several individuals. To acknowledge their contribution, I have written my thesis in the plural. Below are cited the manuscripts as they will be submitted for publication in the scientific literature.

Chapter Two:

Tank, SE, Schindler, DW, and Arts, MT. Differential impacts of ultraviolet radiation on epilithic composition and food quality in four diverse montane lakes.

Chapter Three:

Tank, SE and Schindler, DW. The role of ultraviolet radiation in structuring epilithic algal communities in montane lakes of the Rocky Mountains: evidence from pigments and taxonomy.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE: GENERAL INTRODUCTION

The ability of ultraviolet radiation (UVR) to structure aquatic communities has long been recognized (McLeod and McLachlan 1959). Investigation of the ecological importance of UVR, however, has increased dramatically since the discovery of the Antarctic ozone hole (Farman et al. 1985), and later the discovery of a similar hole over the Arctic (Hofmann and Deshler 1991). Although less severe, decreases in stratospheric ozone have also been well documented at temperate latitudes (Kerr and McElroy 1993, Wardle et al. 1997, Madronich et al. 1998), where increased fluxes of UVR are most likely to affect freshwater systems.

Much of the early work on UVR in aquatic systems has been performed in the marine environment. In freshwaters, however, changes in the penetration of UVR through the water column can be expected to continue long after concentrations of stratospheric ozone return to baseline levels. Dissolved organic carbon (DOC) is the main attenuator of UVR in freshwaters (Scully and Lean 1994). Its aromaticity, however, strongly influences its ability to absorb incident radiation. Terrestrially derived (allochthonous) carbon is highly aromatic, and absorbs strongly in the UV portion of the spectra, while internally derived (autochthonous) carbon is much less so (McKnight et al. 2001). At low altitudes, warming-induced drought has been shown to decrease the inflow of allochthonous carbon from the catchment, thus increasing the depth to which meaningful fluxes of UVR penetrate (Schindler et al. 1996). Conversely, in sparsely vegetated mountainous and alpine catchments, containing lakes with high proportions of internally derived carbon, climate warming can be expected to decrease water clarity, through increases in surrounding vegetation (Vinebrooke and Leavitt 1998, Pienitz and Vincent 2000). Acidification, on the other hand, has been shown to modify DOC quality, converting allochthonous carbon to autochthonous-like, and thus greatly increasing the penetration of UVR through the water column (Donahue et al. 1998). This combination of stratospheric ozone depletion, climate warming, and lake acidification has been coined the "three-pronged attack" (Gorham 1996); freshwater organisms may be faced with alterations in UVR exposure that are exceptional in comparison to other ecosystems.

The direct effects of UVR on aquatic organisms

At the cellular level, several biomolecules absorb in the UV portion of the spectra. Exposure to UVR is known to damage both RNA and DNA (Karentz et al. 1991), and proteins (Döhler 1992, but see Buma et al. 1996) at high levels of exposure. Photosynthesis can also be directly inhibited through the inactivation of photosystem II reaction centers, and the enzyme Rubisco (Vincent and Neale 2000).

Such damage at the molecular level may lead to a suite of UVR-induced changes in the physiology of primary producers. UVR has been shown to decrease photosynthesis, both in pelagic (Helbling et al. 1992, Karentz et al. 1994), and benthic (Nadeau et al. 1999, McNamara and Hill 2000, Watkins et al. 2001) algae. Respiration, on the other hand, has been shown to be relatively unaffected by UVR exposure (Vernet 2000, Watkins et al. 2001). Short-term, high-intensity exposure to UVR has also been demonstrated to damage and bleach photosynthetic chlorophylls (Döhler and Buchmann 1995, Döhler and Haas 1995) and carotenoids (Döhler and Haas 1995, Gerber and Häder 1995). However, over the long-term, the response of the cell is usually to increase the concentrations of carotenoids, due to their photoprotective capacity (Buma et al. 1996, Walsch et al. 1997, Underwood et al. 1999, Vernet 2000). Nitrogen uptake rates have also been shown to decrease under UVR exposure (Döhler and Biermann 1987, Döhler and Kugel-Anders 1994), while phosphorus uptake may increase at low, and decrease at higher exposure levels (Hessen et al. 1995). Concomitant decreases in carbon acquisition (McNamara and Hill 2000, Watkins et al. 2001), growth rates (Calkins and Thordardottir 1980, Jokiel and York 1984, Xenopoulos et al. in press), and biomass accrual (Bothwell et al. 1993, Vinebrooke and Leavitt 1996, Francoeur and Lowe 1998, McNamara and Hill 2000) have also been documented.

Inhibition by UVR has been recorded at all trophic levels in aquatic systems. Pelagic (Grad et al. 2001, Leech and Williamson 2001, Williamson et al. 2001) and stream-dwelling benthic (Bothwell et al. 1994, Kiffney et al. 1997a, b, McNamara and Hill 1999, Kelly 2001) invertebrates have shown increased drift and migration, and decreased colonization and survivorship in response to UVR exposure. Benthic invertebrates from lentic systems, conversely, are often shown to be unaffected by UVR

(Francoeur and Lowe 1998, Vinebrooke and Leavitt 1999), presumably because of the greater water depth at which these experiments are conducted. Detrimental effects on fish (Siebeck et al. 1994) and amphibians (Blaustein et al. 1998) have also been demonstrated.

Differential and interactive effects of UVR

Clearly, the potential for aquatic organisms to be negatively impacted by UVR has been well established. Not all organisms, however, are expected to respond similarly to UVR exposure. Algal cells acclimated to similar UVR fluxes have been shown to vary up to 100 fold in their sensitivity (Karentz et al. 1991). Differences in exposure history are also important: studies have found tropical algae to be less susceptible to the effects of UVR than those from the poles, and algae from high-altitudes to be less susceptible than those from lower elevations (Helbling et al. 1992, Xiong et al. 1996). At similar elevations, organisms from clear-water communities have been shown to be less sensitive to UVR exposure than those from darker waters (Kaczmarska et al. 2000, Xenopoulos 2001). Such differences in exposure history likely affect the cell's ability to minimize and repair UVR-induced damage. For example, concentrations of UV-protectant compounds, such as scytonemin and microsporine-like amino acids (MAAs), have been found to increase with altitude (Laurion et al. 2000) and water clarity (Leavitt et al. 1997), and decrease with depth (Donahue 2000).

Variations in environmental conditions may also affect the susceptibility of organisms to UVR exposure. Several recent studies have shown UVR stress to be secondary in algae experiencing severe nutrient stress (Behrenfield et al. 1994, Xenopoulos et al. in press). Studies of the interaction between temperature and UVR damage, conversely, have been mixed, demonstrating both increased rates of UVR-specific damage (Roos and Vincent 1998) and repair (Rae and Vincent 1998, Pakker et al. 2000) with increasing temperature.

The indirect effects of UVR

In order to understand how UVR acts at the level of the ecosystem, however, it is imperative to understand its indirect effects. Because of their multi-dimensionality, such food-web mediated mechanisms can be difficult to study, and have been poorly documented. The results of those studies that do exist are often unexpected, and have been among the most interesting UV work conducted in freshwater systems.

In the water column, UVR has been shown to cleave nutrients from DOC (Francko and Heath 1982, Cottner and Heath 1990, Boavida and Wetzel 1998). The magnitude of this effect can be so drastic that bacteria have actually been shown to increase under low-level UVR exposure (Herndl et al. 1993). UVR may also react with intermediary compounds to form reactive oxygen species (ROS), such as hydrogen peroxide, superoxide, and hydroxyl radicals (Vincent and Neale 2000). These molecules can both directly damage aquatic organisms (Xenopoulos and Bird 1997), and facilitate differential survival between trophic levels, due to differences in ROS susceptibility (Xenopoulos and Bird 1997, Donahue 2000). Such trophic interactions have also been reported in benthic systems, where consumers (chironomids) have been shown to be more susceptible to UVB radiation than algae, thus causing a counterintuitive rise in algal biomass (Bothwell et al. 1994).

UVR has also been predicted to alter the quality of food provided by producers for their consumers. In addition to its ability to decrease nutrient uptake rates (Döhler and Biermann 1987, Döhler and Kugel-Anders 1994, Hessen et al. 1995), UVR has been shown to decrease concentrations of poly-unsaturated fatty acids (Goes et al. 1994) and shift the composition of producer communities towards less edible taxa (by causing a decrease in diatom abundance; Vinebrooke and Leavitt 1996, Xenopoulos et al. 2000). Although exposure to UVR has been suggested to decrease food quality in aquatic systems (Hessen et al. 1997), this question has been particularly poorly studied. To date, only one *ex-situ* study has directly addressed this question, with ambiguous results (McNamara and Hill 2000).

Study rationale and hypotheses

In this study, I endeavored to investigate the importance of UVR in structuring primary producer and primary consumer communities in aquatic systems. To do this, I worked with epilithon, the matrix of algae, bacteria, and detritus dwelling on rocks, and benthic invertebrates. I hypothesized that (1) UVR would be an important factor in structuring the epilithic algal community in these lakes, and effect a shift in algal community composition towards assemblages that are more UV tolerant, (2) that although epilithic accrual may differ between UVR treatments initially in the experiment, shifts in community composition would dampen this effect by the end of the growing season, (3) that exposure to UVR would decrease colonization by benthic invertebrates, and (4) that UVR-mediated shifts in epilithic community structure would decrease the quality of epilithon as food for grazers.

Study design

I conducted my experiments in four oligotrophic lakes of Jasper National Park (Figure 1.1). Leach, Hibernia, Honeymoon, and Saturday Night Lakes lie at the interface between the montane and sub-alpine ecoregions (Holland and Coen 1983). These lakes were chosen both for their range of water clarity levels (1% UVB penetration depths range from 0.34 m to 0.98 m), and their elevation: UVB radiation increases at approximately 20% per thousand meters altitude (Blumthaler et al. 1997). Choosing these study sites allowed me to access relatively clear, high elevation lakes, while still maximizing the ice-free season, and potential experimental length. In each of the four study lakes, UVR exposure at the lake bottom was manipulated by suspending large plastic filters slightly below the water surface. These filters selectively removed the UVA and/or UVB portion of the incident radiation, but did not significantly alter the influx of photosynthetically active radiation. More specific experimental details are provided in Chapters 2 and 3.

The main focus of my thesis was to examine how UVR directly affects the quantity (standing crop), and quality (elemental and biochemical composition) of epilithon, and the importance of these changes for epilithic consumers. Chapter 2

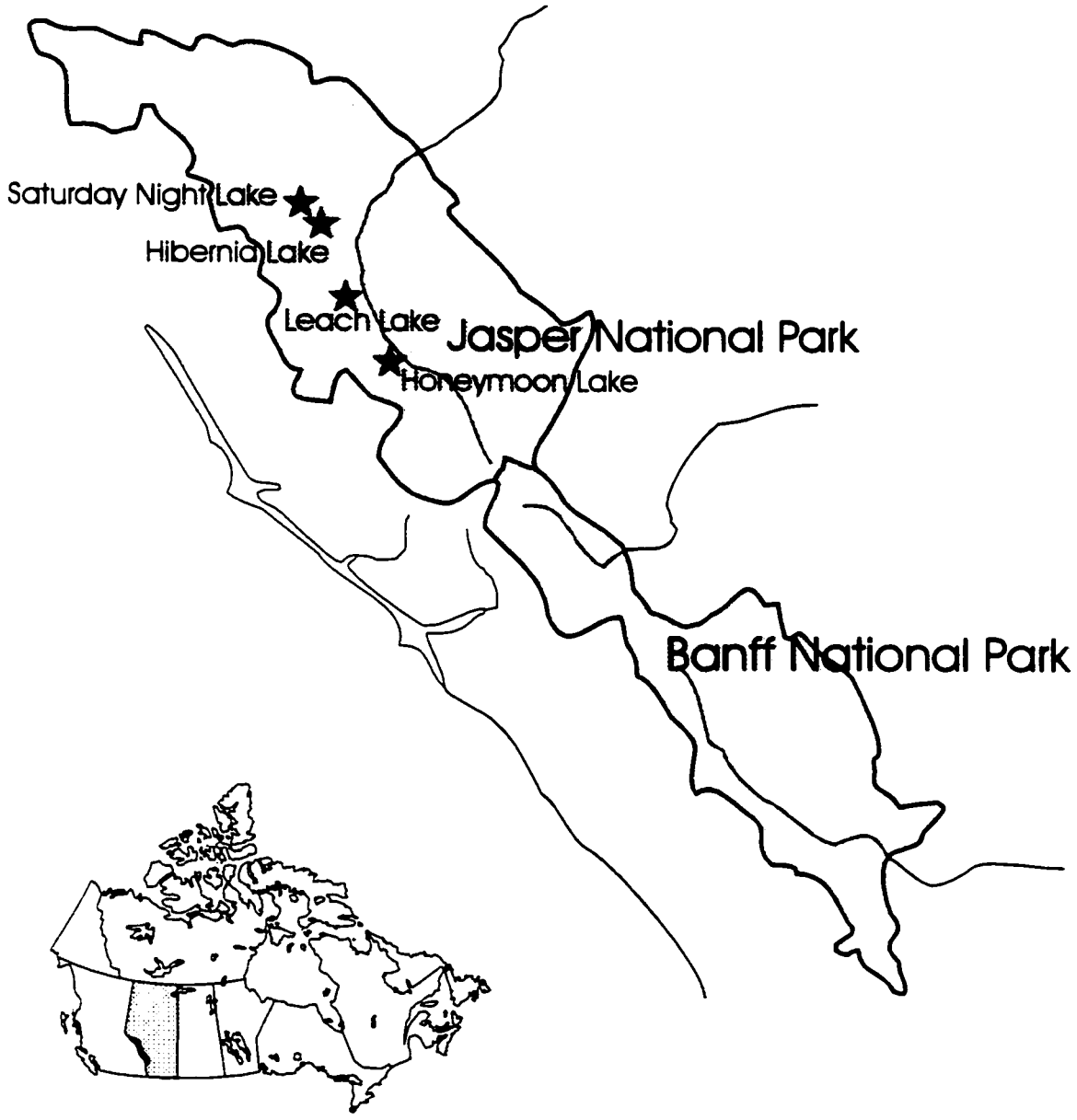

Figure 1.1: Location of Leach, Honeymoon, Hibernia and Saturday Night Lakes in Jasper National Park, Alberta, Canada

addresses these questions, through measuring the direct response of the epilithic community and benthic invertebrates to differential UVR exposure, and the indirect response of invertebrates to differentially irradiated epilithon.

I also believed, however, that is was necessary to understand whether, and how, specific species shifts were occurring in the epilithon in response to UVR exposure. Through the use of ordination techniques I was able to explore the importance of UVR in relation to other factors in structuring the epilithic algal community. In Chapter 3, both taxonomic counts and taxa-specific pigments are used to understand factors important in structuring the algal community of the benthos in my study lakes.

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CHAPTER TWO: DIFFERENTIAL IMPACTS OF ULTRAVIOLET RADIATION ON EPILITHIC COMPOSITION AND FOOD QUALITY IN FOUR DIVERSE MONTANE LAKES

INTRODUCTION

Concurrent with decreases in stratospheric ozone, fluxes of ultraviolet-B (UVB) radiation to the Northern Hemisphere have been documented to be growing (Kerr and McElroy 1993, Wardle et al. 1997). This increase in downwelling UVB is of particular interest in aquatic systems, where acidification, drought, and higher UVR fluxes can increase water clarity by destroying or bleaching dissolved organic carbon (DOC), extending the penetration of all wavelengths of solar radiation through the water column (Schindler and Curtis 1997).

Direct, physiological damage has been well documented as a result of exposure to ultraviolet radiation (UVR), especially in the phytoplankton (Karentz et al. 1994). In epilithic (rock-dwelling) algae, UVR can decrease growth rates, species richness, and biomass in both lakes (Vinebrooke and Leavitt 1996, Santas et al. 1998, McNamara and Hill 2000), and streams (Bothwell et al. 1993, Kelly 2001). However, several field studies have shown no effect of UVR on epilithic algae (e.g., Vinebrooke and Leavitt 1998), or results that are not consistent through the growing season (Francour and Lowe 1998). Furthermore, strong negative physiological responses to UVR by benthic algae may not translate into commensurate decreases in biomass accrual (Bothwell et al. 1994, Watkins et al. 2001).

Benthic invertebrates can also be negatively affected by exposure to UVR. In the laboratory, they have shown increased mortality in response to UVR stress, with smaller organisms being more susceptible to the radiation (McNamara and Hill 1999). The results of field-based investigations, however, have been less clear-cut. Zoobenthos (Chironomidae and *Gammarus*) in one alpine lake did not differ in their abundance in response to UVR (Vinebrooke and Leavitt 1999). Similarly, in a low-altitude study, chironomid density did not differ among radiation treatments, or through time (Francour and Lowe 1998). Conversely, invertebrate abundance decreases with exposure to UVR

both in stream (Kiffney et al. 1997a, Kelly 2001), and flume (Kelly et al. 2001) experiments.

In order to fully understand how UVR might affect aquatic ecosystems, it is imperative to consider its indirect, food-web mediated effects. Although these effects can be great (Bothwell et al. 1994, Kelly et al. 2001) the manner in which UVR-mediated changes in one trophic level might affect another has been poorly documented. In particular, evidence is scant for how UVR might affect the nutritional quality of primary producers as food for their consumers, especially under natural conditions.

There are several mechanisms through which the composition of producer communities may affect consumer growth. For example, stoichiometric investigations have shown that variations in producer elemental ratios, most notably increases in C:P, reduce the growth rates of consumers (e.g., Sterner and Shultz 1998). Although most of this work has been performed in pelagic systems, stoichiometric imbalances are also expected to occur in the benthos (Frost and Elser in press a). Research has also shown that biochemicals can be important for invertebrate growth. Specifically, concentrations of polyunsaturated fatty acids (PUFAs) may limit the growth of organisms of higher trophic levels, which are unable to manufacture these compounds (Müller-Navarra 1995).

There are several mechanisms by which the stoichiometry of UVR-stressed cells might be affected *in situ*. The most obvious of these occurs through changes in nutrient uptake rates. Both ammonia and nitrate have been documented to be incorporated more slowly into laboratory grown, UVR-stressed phytoplankton cells (Döhler and Biermann 1987, Döhler and Kugel-Anders 1994). Phosphorus uptake, on the other hand, has been shown to increase in laboratory phytoplankton under low levels of UVR, but be severely inhibited at higher doses (Hessen et al. 1995). Changes in a cell's growth rate may also alter its elemental composition. Increases in algal growth rates at constant nutrient supply rates have long been known to increase the ratio of carbon to nutrients (Goldman 1986). More recently, the corollary to this has also been observed; slow growing, UVR-stressed phytoplankton have been shown to have lower C:P ratios than non-UVR exposed assemblages (Xenopoulos et al. in press). Finally, UVR-induced changes in grazing pressure might also affect nutrient composition. In phytoplankton, increased herbivory

has been shown to result in decreased carbon to nutrient ratios, brought about by grazer nutrient release, and increases in per capita nutrient supply to a reduced algal pool (Urabe 1995). Decreases in C:P ratios have also been observed with increasing herbivory in the benthos; however, the mechanism for this remains less clear (Frost et al. in press).

Epilithic communities are composed of a complex matrix of algae, bacteria and detritus. In contrast to research done on algae, relatively little work has been performed to suggest how UVR might affect nutrient limitation in bacteria. Research has shown, however, that bacteria can be more sensitive to the effects of UVR than algal cells facing the same level of exposure (Jeffrey et al. 1996, Xenopoulos and Bird 1996), and that bacteria are generally better competitors for nutrients than are algae (Curie and Kalff 1984). Although there is a scarcity of research on the subject, current knowledge suggests that nutrients arrive in the epilithon largely through both algal and bacterial uptake (Hamilton et al. 2001).

UVR-mediated changes in cellular stoichiometry can be expected to further affect cellular biochemistry (Healy and Hendzel 1979, Kilham et al. 1997). Internal stores of carbohydrates have been shown to increase under UVR stress in phytoplankters (Van Donk and Hessen 1995), while protein can increase at low, and decrease at higher, dosage rates (Buma et al. 1996). Short chain storage lipids have been demonstrated to remain unchanged under UVR stress (Goes et al. 1994). Although these patterns are not universal (Hessen et al. 1997), and can be variable between species (Arts and Rai 1997), long chain PUFAs in particular are expected to decrease under UVR stress. UVR is a powerful inducer of cell peroxidation, to which longer chain PUFAs are particularly susceptible. Laboratory studies have shown decreases in two fatty acids considered to be essential for consumer growth: eicosapentanoic (20:5ω3) and docosahexanoic (22:6ω3) acids (Goes et al.1994, Wang and Chai 1994). Over the longer term, chain elongation and desaturation may also be inhibited under UVR stress (Goes et al. 1994).

Coupled with these effects, UVR-mediated shifts in algal taxonomic composition when cells are exposed to UVR might be expected to alter the nutritional value of the epilithic community. UVR-induced changes in morphological characteristics, such as increased cell wall thickness (Van Donk and Hessen 1995) and increased cell size

(Karentz et al. 1991, Bothwell et al. 1993, Van Donk and Hessen 1995) may also decrease epilithic digestibility for consumers.

Most of the studies cited above have been conducted in the laboratory, over the short-term. Although field studies have examined the effect of UVR stress on nutrient ratios (Watkins et al. 2001, Xenopoulos et al. in press), no previous *in-situ* studies are known to have examined the effect of UVR on fatty acid composition, or the degree to which UVR-mediated changes in food quality might affect higher trophic levels. In this study, we experimentally tested how ambient UVA and UVB radiation affect nutrient parameters in the epilithon in a long-term, field setting. We further examined how these changes in nutrient content translate into food quality effects; that is, their effect on epilithic consumers.

METHODS

Study lakes

We conducted our experiments in four oligotrophic lakes of Jasper National Park. Honeymoon, Leach, Hibernia and Saturday Night Lakes were chosen for their ease of access, comparable elevations, and range of water clarity. The lakes lie in the main ranges of the Rocky Mountains, are underlain by calcareous till, and surrounded by brunizolic and luvisolic soils (Holland and Coen 1983). Surrounding vegetation is dominated by lodgepole pine (Holland and Coen 1983). Leach, Hibernia, and Saturday Night Lakes are located in the montane ecoregion, while Honeymoon Lake lies in the lower subalpine (Holland and Coen 1983). Leach and Honeymoon Lakes, which drain to the Athabasca River, are relatively transparent to both ultraviolet and photosynthetically active radiation. Hibernia and Saturday Night Lakes are less transparent, and drain to the Miette River. Common limnological parameters for the four study lakes are given in Table 2.1.

Experimental Design: Direct effects of UVR on epilithon and invertebrates

Three plastic UVR screening treatments (Cadillac Plastics, Edmonton, Canada) were employed to test the effect of UVR on epilithic communities. The first, our "PAR

Table 2.1: Selected limnological parameters for four study lakes in Jasper National Park, Alberta. Measurements were taken every 10 days from early June to early September 2000.

Parameter	Lake			
	Leach	Honeymoon	Hibernia	Saturday Night
Elevation (masl)	1237	1405	1198	1418
Area (ha)	13.1	18.4	9.6	9.7
Z_{mean} (m)	3.1	2.1	3.4	3.3
Z_{max} (m)	11.0	7.0	8.5	8.3
1% UVB depth (m)	0.71-0.95	0.72-0.98	0.34-0.41	0.36-0.46
DOC (mg L^{-1})	8.3-9.7	6.9-8.9	9.0-10.5	8.3-9.3
Suspended chlorophyll (μg L^{-1})	0.4-2.1	0.7-2.4	0.7-2.2	1.0-5.5
TP (μg L^{-1})	7.7-10.2	5.5-7.9	9.1-12.5	6.9-13.3
NH_4^+ (μg L^{-1})	1.7-49.3	5.2-41.8	1.1-23.6	4.7-25.3
$NO_2^- + NO_3^-$ (μg L^{-1})	0.5-8.7	0.6-4.9	0.2-5.0	0.8-11.0
Conductivity (μS cm^{-1})	196-207	173-182	255-258	248-256
pH	7.9-8.5	8.1-8.5	8.1-8.5	8.1-8.5
Alkalinity (mg L^{-1} as HCO_3^-)	126-132	109-115	150-152	147-157
Silica (mg L^{-1})	4.7-5.3	1.5-2.2	5.6-6.3	4.2-5.1

+ UVA + UVB" treatment, allowed penetration of the full solar spectrum (Acrylite OP4, transparent to all radiation > 280 nm). The second, our "PAR+UVA" treatment, blocked UVB radiation (Mylar-D, transparent to radiation > 320 nm). The third, our "PAR" treatment, blocked all UVR (Acrylite OP3, transparent to radiation > 400 nm). The plastic sheets were suspended slightly below the water surface using a frame of ABS plastic piping. Acid washed unglazed ceramic tiles (4.8 cm x 4.8 cm) were placed on the lake bottom below the plastic screens and allowed to colonize for the length of the summer. Tiles were initially placed at a depth of 30 cm, and monitored throughout the summer for changes in depth caused by water column fluctuations. Tiles were not pre-colonized in the study lakes in order that initial community succession might occur under the three optical treatments. The positioning of the screening treatment was such that radiation incident upon the tiles was always filtered, despite daily and seasonal shifts in solar angle.

One replicate of each of the three UVR screening treatments was set up in each of the four study lakes. Experiments were initiated between May 26 and 31, 2000. Samples were collected approximately every 10 days thereafter, by randomly selecting tiles, and scraping off the epilithic community that had accumulated on the tile surface. For particulate analyses (chlorophyll *a*, fatty acids, particulate carbon, nitrogen and phosphorus), four replicates were collected, each replicate consisting of a separate whole or half tile. Replicates were collected in the field on GF/F filters, and either dried at 60°C for 24 hours and frozen (carbon, nitrogen and phosphorus), directly frozen (chlorophyll *a*) or frozen on dry ice (fatty acids) within 2 hours. Fatty acid samples were transferred to a –80°C freezer directly from the dry ice. Filters were pre-combusted (475° C for 2 hours) for carbon, nitrogen, phosphorus and fatty acid collection. Three replicate invertebrate samples were collected and immediately preserved using 4% formalin. Water chemistry samples were also obtained on each sampling day just below the lake surface, near the center of each lake. Samples for the determination of DOC, chlorophyll, TP, NH_4^+, NO_2^-+NO_3^-, conductivity, pH, alkalinity and silica were analyzed using standard methods (Stainton et al. 1978, Prepas and Rigler 1982, APHA 1992, Welschmeyer 1994).

To determine how the effects of ultraviolet radiation might change with water column depth, smaller versions of the above-described set-up were employed in Leach and Hibernia Lakes. Tiles were attached to the bottom of wire baskets, which were covered with UVR screening plastics large enough to block all incident solar radiation. In each lake, one replicate of each of the three radiation treatments was placed at each of two depths: in Leach Lake at approximately 60 and 90 cm, and in Hibernia Lake, at approximately 37.5 and 60 cm. For the purposes of clarity, these experiments will be referred to as "mid" and "deep", while the larger above-mentioned design will be referred to as the "primary" experiment. Sample collection dates and sampling activities for all experiment types are given in Table 2.2.

Experimental Design: Indirect effects of UVR on epilithic food quality

To assess how UVR-induced changes in epilithic composition might affect epilithic grazers, a feeding experiment was performed in Honeymoon Lake. Valvatid snails (*Valvata sincera helicoidea*) were collected, measured, and incubated with epilithon-covered tiles that had been colonized for at least six weeks under one of the three radiation treatments discussed above. At the start of the experiment a sample of snails of similar length was also collected, measured with calipers to obtain the greatest shell diameter, and dried and weighed, to obtain an initial length-weight regression. Incubation trays containing snails and tiles were then covered with one of the three UVR-screening plastics. Five combinations of previous epilithic UVR exposure and ambient UVR shield were employed to allow us to distinguish between effects brought about by UVR incident directly on the snails, and by UVR-induced changes in food quality. These were:

(1) epilithon incubated under PAR, container covered with PAR screen;
(2) epilithon incubated under PAR+UVA, container covered with PAR screen;
(3) epilithon incubated under PAR+UVA+UVB, container covered with PAR screen;
(4) epilithon incubated under PAR+UVA, container covered with PAR+UVA screen;
(5) epilithon incubated under PAR+UVA+UVB, container covered with PAR+UVA+UVB screen.

Table 2.2: Sampling schedule for chlorophyll *a* (chl *a*), carbon, nitrogen, and phosphorus (CNP), polyunsaturated fatty acids (PUFA), and invertebrate taxonomy samples for primary, mid and deep experiments in four study lakes in Jasper National Park, Alberta.

Sampling activity Primary (mid and deep)	Lake			
	Leach	Honeymoon	Hibernia	Saturday Night
Set-up	May 26	May 27	May 29	May 31
Chl *a*	June 9	June 11	June 11	June 12
Chl *a*, CNP, PUFA	June 19	June 20	June 21	June 22
Chl *a*	June 29	June 30	July 1	July 2
Chl *a*, CNP, PUFA, Invertebrate (CNP)	July 9	July 10	July 11	July 12
Chl *a*	July 20	July 20	July 21	July 22
Chl *a*, CNP, PUFA, Invertebrate (CNP)	July 29	July 30	July 31	August 1
Chl *a*	August 8	August 10	August 10	August 11
Chl *a*, CNP, PUFA, Invertebrate (CNP)	August 18	August 19	August 20	August 21
Chl *a*	August 28	August 29	August 30	August 31
Chl *a*, CNP, PUFA	September 7	September 8	September 9	September 10

Each treatment was replicated 5 times, and each replicate consisted of 6 snails incubated in a single container. The incubation continued for 18 days, during which tiles were replaced with new, ungrazed tiles every 3 days. Initial experiments were conducted to ensure that the consumers would not be supply limited at this replacement rate. Tiles were also visually inspected during each replacement to ensure that the epilithic community had not been fully grazed. At the end of the experiment, snails were measured as above, dried and weighed. Initial weights were interpolated by using the initial lengths to solve for weight in the length-weight regression, and a growth rate index was calculated as:

$$\mu = \frac{[\log(b_2) - \log(b_1)]}{time} \quad ,$$

where μ =growth rate, b_1=body weight at the outset of the experiment, and b_2=body weight at the termination of the experiment.

Determination of incident radiation and water clarity

Incident ultraviolet and photosynthetically active (PAR) radiation were measured using a Li-Cor LI1000 data logger equipped with a quantum cosine PAR sensor (Li-Cor Instruments, Lincoln, Nebraska), and broadband UVA and UVB sensors (BW20, Vital Technologies, Toronto, Ontario). The broadband UVB sensor was calibrated against Environment Canada's Brewer spectrophotometer in Edmonton (Alberta, Canada), while the broadband UVA sensor was calibrated against 3 discrete UVA wavelengths (325, 340 and 380 nm) on a Stor-Dat radiometer (Satlantic Inc., Halifax, Canada) placed beside the broadband sensor. Because of equipment failure, the radiation flux values for certain dates were estimated using multivariate regression with daylength, hours of bright sunshine (both provided by Environment Canada), and ozone (obtained from NASA satellite data) as input variables.

On each sampling occasion, water was collected from the center of the lake immediately below the lake surface. Water samples were refrigerated in the dark until analysis (within two weeks), at which time light absorption in the range of 280 to 700 nm was measured through a 2 cm cuvette using a scanning spectrophotometer (Cary WinUV,

Varian Instruments, California). During late summer (August 17-19, 2000) a submersible radiometer was further employed to calculate solar attenuation within the water column (Satlantic Stor-Dat, Satlantic Inc., Halifax, Canada). Estimates of water clarity from these two methods were compared using calculated K_d values from the closest sampling date.

Laboratory analyses

Chlorophyll a: Samples were extracted in the dark at 80°C in 90% ethanol for 5 minutes, and shielded from light upon removal. Extraction continued in darkness at 4°C for 24 hours. Chlorophyll *a* concentration was determined spectrofluorometrically (Shimadzu Model RF-1501, Mandel Scientific, Guelph, Ontario) without acidification, following the method of Welschmeyer (1994).

Carbon, Nitrogen and Phosphorus: To assess particulate carbon, and carbon to nitrogen ratios, epilithic carbon and nitrogen were estimated on individual samples after combustion at 975°C in an elemental analyzer (CEC model 440, Control Equipment Corporation, Lowell, Massachusetts). In order to account for variation between samples, epilithic carbon content was also estimated on samples analyzed for epilithic carbon to phosphorus ratios. Here, carbon was estimated as carbon dioxide after digestion in a closed vessel with potassium persulfate, using gas chromatography (Hewlett Packard 5890 with Chromosorb 102 column; Lampman et al. 2001), followed by phosphorus analysis on the same sample using the molybdate-absorbic acid method (APHA 1992).

Fatty Acids: Two replicate filters for fatty acid analysis were combined, and freeze dried (specimen chamber –20°C, condenser chamber –40°C) prior to extraction. Dry samples were weighed, and extracted three times in chloroform: methanol (2:1 by volume). Each extracted sample was then subject to a salt-rinse, through addition of 0.9% NaCl (weight per volume) at 20% of the volume of the extract. Samples were vortexed and centrifuged, and the salt layer was removed. Rinsed samples were then evaporated to dryness under nitrogen gas, and stored at -80°C until the time of methylation.

To methylate fatty acids to their methyl esters, samples were dissolved in 2 mL hexane, to which 2 mL of BF_3 methanol (10% by weight) was added. Sample tubes were purged with nitrogen, sealed, and incubated at 70°C for 2 hours. Analytical blanks (2 mL of pure hexane and 2 mL of BF_3 methanol) and standards (standard concentrations of fatty acids in 2 mL of hexane, and 2 mL of BF_3 methanol) were also subjected to the methylation process. After the incubation, 1 mL of GC-grade water was added to each tube, and the hexane phase was extracted three times through repeated addition of 1 mL of hexane, which was decanted from the BF_3-water mixture. Methylated samples in hexane were then dried to a volume of 0.2 mL, transferred into clean microvials, and stored at -80°C until the time of analysis.

Methylated samples were analyzed using gas chromatography (Hewlett Packard 5890, Series II), on an HP-5 column (25 m x 0.2 mm, column head pressure = 60 kPa), using a flame ionizing detector (detector temperature = 300°C, injector temperature = 300°C). Injection was splitless, with 1 or 2 μL of sample being injected. Oven temperature was initially set at 50°C, ramped to 180°C at 10°C/minute, ramped to 258°C at 2°C/minute, and finally ramped to 300°C in one minute, where it was held for 15 minutes. Individual fatty acid peaks were identified by comparing retention times with known standards (Supelco 37-component FAME mix), and further verified using GC-MS. Fatty acid concentrations were interpolated from a four point standard curve (Supelco 37-component FAME mix), and were normalized per unit dry weight.

Invertebrate taxonomy: Invertebrate samples were counted, without subsampling, under a dissecting microscope. Identifications were made to class or family. Data are presented as numbers for the most common taxa (Oligochaeta, Nematoda, and Chironomidae), as well as a total count that includes less common taxa.

Statistical analyses

Two-way ANOVAs were used to test for the effects of UVR treatment and time of sampling on the various epilithic characteristics measured (JMP Version 3.2, SAS Institute, 1996). The Tukey-Kramer test was used for post-hoc comparisons where

analyses were significant (SYSTAT Version 8.0, SPSS Inc. 1996). In some cases, where interactions were indicated by the data analysis, simple effects and simple contrasts were calculated to further analyze the data (Keppel 1982). Chlorophyll *a*, particulate carbon, and area-specific fatty acid data were $\log_{10}$ transformed in order that the data met the assumptions of ANOVA. In Leach Lake, dry-weight specific fatty acid data was analyzed as it's inverse, again to meet the assumptions of ANOVA.

Invertebrate count data were first analyzed as a MANOVA before ANOVA tests were employed. Again, in order that the data might meet the assumptions of ANOVA, invertebrate counts were square root (n+1) transformed. When a significant difference was present in the MANOVA analysis, Dunn-Šidák adjusted contrasts were performed to investigate where differences lay (JMP, SAS Institute, 1996). For the feeding experiment, the average of all snails in a replicate was used to calculate a growth rate for that replicate. Growth rates were then analyzed in two one-way ANOVAs. The first analysis compared snails fed differentially irradiated epilithon, but incubated in the absence of UVR (treatments 1, 2, and 3 above), while the second compared snails fed differentially irradiated epilithon, and incubated under the epilithic irradiation regime (treatments 1, 4, and 5 above).

RESULTS

Incident radiation and water clarity

Incident radiation was greatest in early July, with lower values at the beginning and towards the end of the experimental period (Figure 2.1). High cloud cover likely caused the observed decrease in incident radiation during the period directly surrounding the summer solstice, when clear-sky fluxes were expected to be greatest (S. Tank, personal observation). Multiple regressions incorporating daylength and bright sunshine as independent variables explained a significant amount of variation in the measured levels of PAR, UVA, and UVB (Figure 2.1; r^2_{PAR}=0.92, $r^2_{UVA\ (380)}$=0.85, $r^2_{UVA\ (340)}$=0.85, $r^2_{UVA\ (325)}$=0.85, r^2_{UVB}=0.83), and were used to estimate fluxes where data are missing.

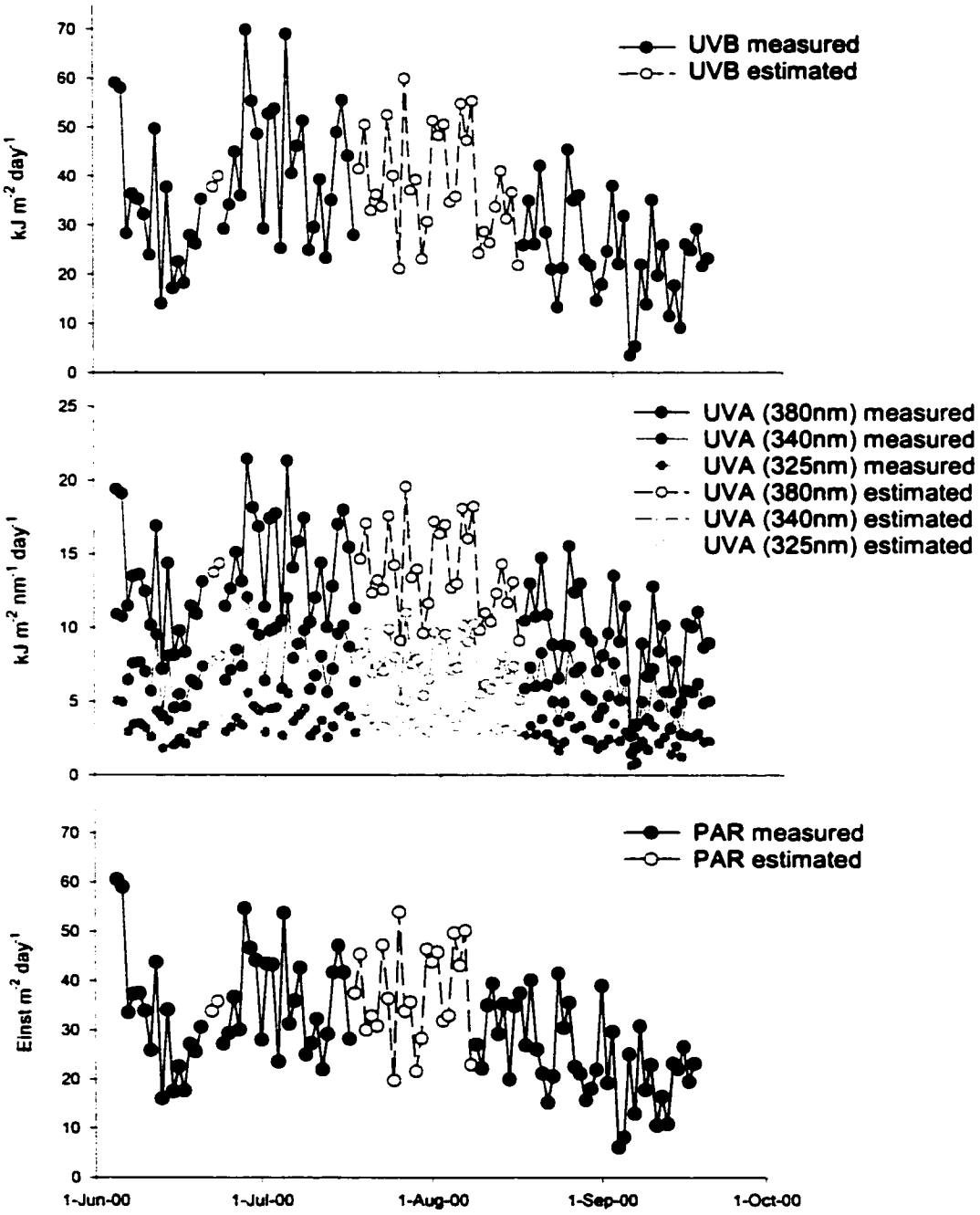

Figure 2.1: Downwelling PAR, UVA and UVB radiation in Jasper, Alberta. Missing data were estimated using the following equations: UVB = -52.600 + 3.220sun + 4.523daylength, $r^2 = 0.83$; $UVA_{(380)}$ = -13.532 + 0.869sun + 1.389daylength, $r^2 = 0.85$; $UVA_{(340)}$ = -7.644 + 0.491sun + 0.782daylength, $r^2 = 0.85$; $UVA_{(325)}$ = -3.566 + 0.229sun + 0.361daylength, r^2=0.85; PAR = -36.444 + 2.833sun + 3.444daylength, $r^2 = 0.92$. Sun = hours of bright sunshine, daylength = hours from dawn to dusk.

Neither breaking the regressions down seasonally, nor incorporating ozone levels into the UVB model, significantly improved model fit.

Spectrophotometric estimates of water clarity showed that Hibernia and Saturday Night Lakes absorb highly in the UVB range, while Leach and Honeymoon Lakes are more transparent (Figure 2.2). In the more coloured lakes, less than 15% of incident UVB penetrated to the tile surface in the primary experiment. This difference in transparency between the lakes decreased with increasing wavelength, to the point where absorption is almost identical in the PAR region, with the exception of Saturday Night Lake. Excluding the mid experiment in Leach Lake, UVB penetration in the mid and deep experiments was negligible (<2%; Figure 2.3). Again, the discrepancy between penetration in the mid and deep experiments decreased as wavelength increased. Penetration of UVR to the Leach Lake mid experiment was similar to that in the Hibernia and Saturday Night Lakes primary experiment, while penetration to the Leach Lake deep experiment was intermediate to that in the Hibernia Lake mid and deep experiments (Figure 2.3).

The *in situ* estimation of water clarity using a submersible radiometer was compared to spectrophotometric measurements from the nearest sampling date. The two estimates agreed well in the UVB and UVA range. Generally, K_d values calculated from spectrophotometric and radiometric measurements differed by less than 20% from each other, and indicate that our spectrophotometric method may have underestimated water clarity.

Estimates of epilithic biomass

Both particulate chlorophyll *a* and carbon were used to infer epilithic biomass. Chlorophyll *a* concentrations increased significantly over time in the primary experiment in all lakes (Table 2.3, Figure 2.4). In Honeymoon and Saturday Night Lakes, the elimination of UVR had no effect on chlorophyll *a* concentrations (Table 2.3). In Leach Lake, removing UVA radiation significantly increased chlorophyll *a* concentrations: the PAR treatment was significantly greater than both the PAR + UVA and PAR + UVA + UVB treatments, which did not differ from each other (Table 2.3, Figure 2.4; Tukey-

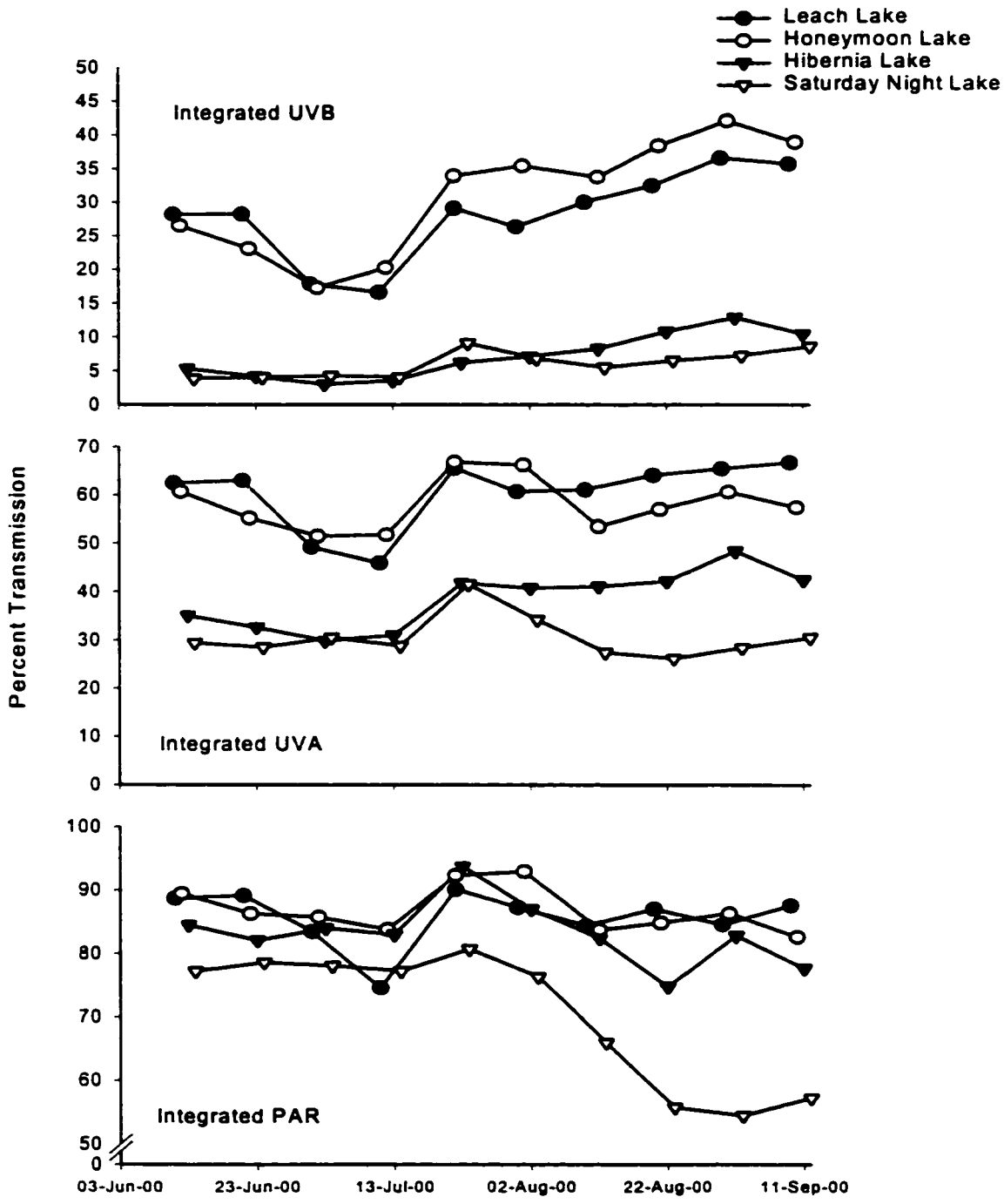

Figure 2.2: Percentage of integrated UVB, UVA, and photosynthetically active radiation at the lake surface that penetrates to the primary experiment tile depth in each of four study lakes in Jasper National Park, Alberta. Penetration was measured spectrophotometrically, and is a measure of fluctuations in tile depth and water clarity on each sampling date. Measurements for UVB radiation are integrated over 290-320 nm, for UVA radiation over 320-400 nm, and for PAR radiation over 400-700 nm.

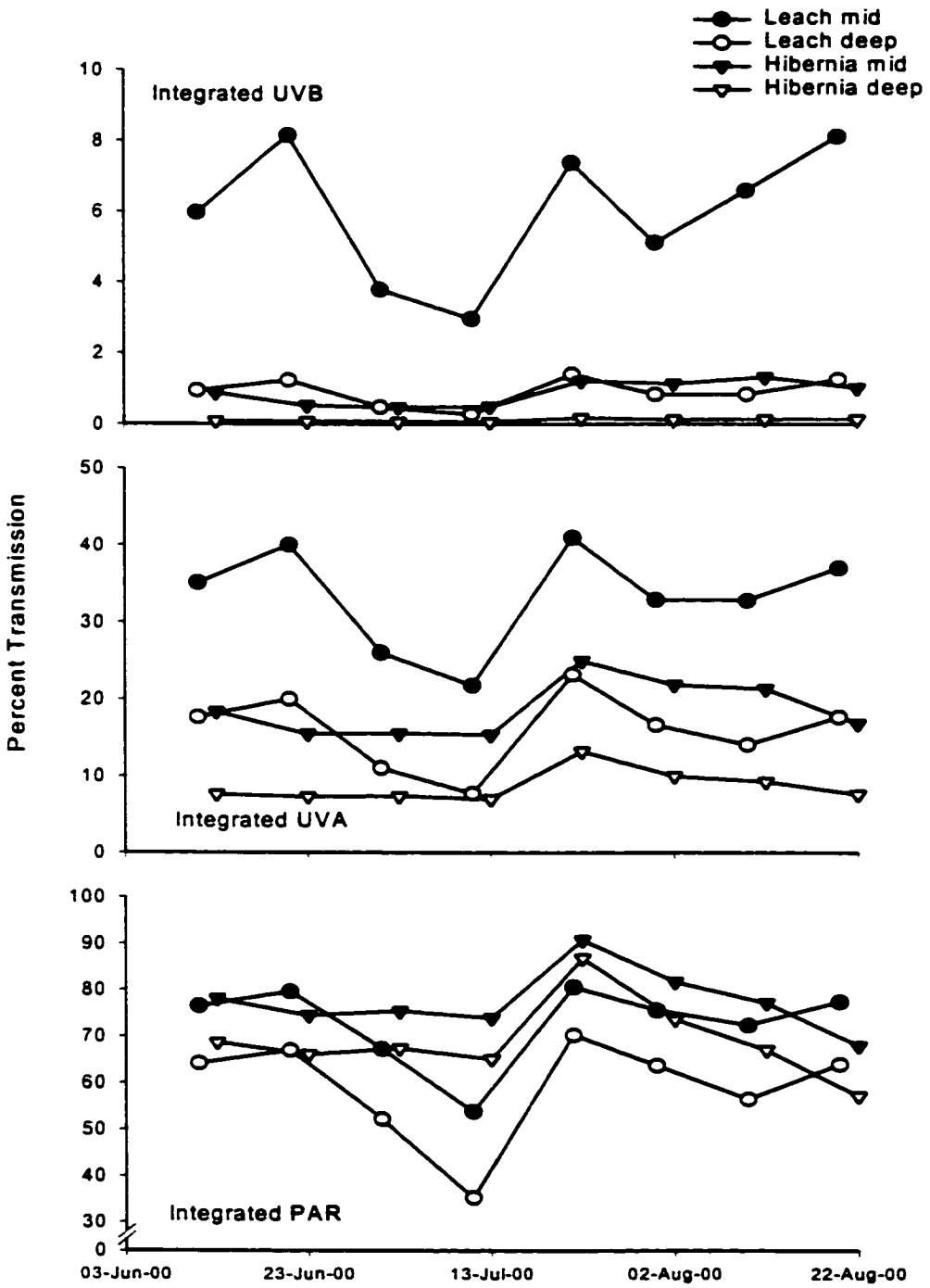

Figure 2.3: Percentage of integrated UVB, UVA, and photosynthetically active radiation at the lake surface that penetrates to the mid and deep experiment tile depths in Leach and Hibernia Lakes in Jasper National Park, Alberta. Penetration was measured spectrophotometrically, and is a measure of fluctuations in tile depth and water clarity on each sampling date. Measurements for UVB radiation are integrated over 290-320 nm, for UVA radiation over 320-400 nm, and for PAR radiation over 400-700 nm.

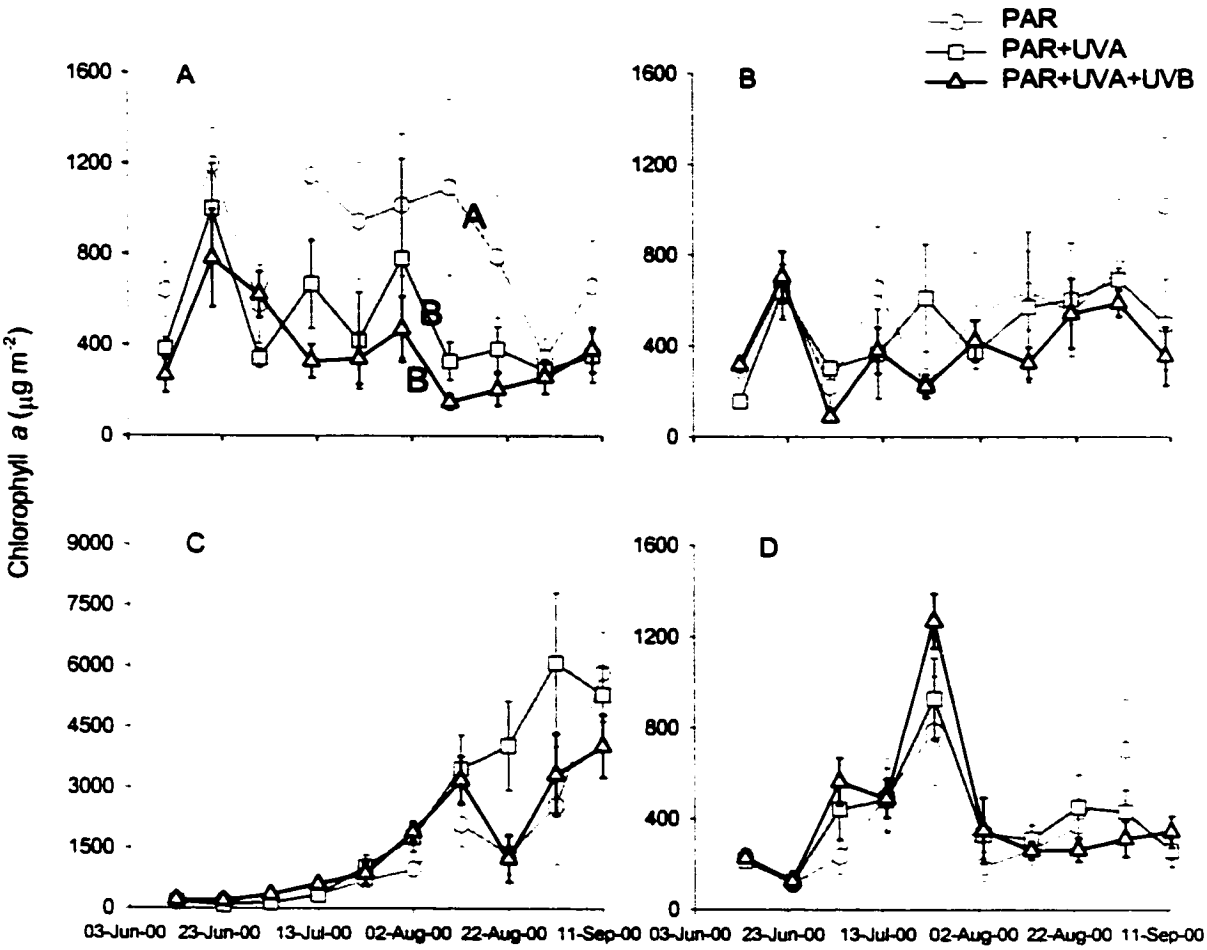

Figure 2.4: Chlorophyll *a* concentrations in epilithon under three different UVR regimes in four study lakes in Jasper National Park, Alberta: (A) Leach Lake, (B) Honeymoon Lake, (C) Hibernia Lake, and (D) Saturday Night Lake. Error bars represent ± standard error for $n = 4$ replicates. Significant differences between treatments (within each panel) are indicated by different letters.

Table 2.3: Two-way ANOVA results for the effects of ultraviolet radiation (UVR) on log-transformed chlorophyll and particulate carbon, and C:P and C:N ratios in epilithon in the primary experiment for study lakes in Jasper National Park, Alberta. Reported are degrees of freedom, F statistics and p-values for $n = 4$ replicates. Significant differences are highlighted in bold.

		Lake							
		Leach		Honeymoon		Hibernia		Saturday Night	
	df	F	p	F	p	F	p	F	p
Chlorophyll									
UVR	2	14.6758	**<.0001**	1.9256	0.1518	3.5562	**0.0327**	1.1688	0.3154
Time	9	2.9497	**0.0041**	5.6947	**<.0001**	58.0962	**<.0001**	10.6498	**<.0001**
UVR x Time	18	0.9415	0.5326	1.4513	0.1281	2.0791	**0.0129**	0.9904	0.4779
Particulate Carbon									
UVR	2	8.5796	**0.0007**	1.8048	0.1765	1.1103	0.3385	0.7881	0.2433
Time	4	13.7690	**<.0001**	9.9285	**<.0001**	25.9523	**<.0001**	13.5685	**0.0014**
UVR x Time	8	2.0722	0.0600	3.4265	**0.0038**	1.3535	0.2435	0.7917	0.6126
Particulate C:N									
UVR	2	3.0767	0.0564	1.2747	0.2896	1.8532	0.1688	1.4660	0.4610
Time	4	11.2141	**<.0001**	3.8358	**0.0093**	18.4328	**<.0001**	5.3437	**<.0001**
UVR x Time	8	0.9412	0.4934	0.8028	0.6034	0.8238	0.5861	0.7917	**0.0413**
Particulate C:P									
UVR	2	3.7222	**0.0321**	1.1542	0.3251	2.8427	0.0693	1.9485	0.1549
Time	4	9.0494	**<.0001**	4.2582	**0.0055**	3.1978	**0.0219**	2.1733	0.0881
UVR x Time	8	0.3378	0.9464	1.5631	0.1653	5.0557	**0.0002**	1.4497	0.2041

Kramer; PAR > PAR + UVA, p=0.002; PAR > PAR + UVA + UVB, p<0.0001; PAR + UVA = PAR + UVA + UVB, p=0.747). In Hibernia Lake, although chlorophyll *a* concentrations did differ significantly between treatments, the effect was not constant over time (Table 2.3, significant interaction term). An analysis of simple effects and contrasts for this lake suggests that differences between treatments were driven by high chlorophyll *a* concentrations under the PAR + UVA treatment on the 8th and 9th sampling dates ($p<0.01$; PAR + UVA > $PAR_{(August\ 20)}$, $F_{1,98}=7.85$; PAR + UVA > PAR + UVA + $UVB_{(August\ 20)}$, $F_{1,98}=14.99$; PAR + UVA > $PAR_{(August\ 30)}$, $F_{1,98}=8.35$).

Trends in the particulate carbon data from the primary experiment resemble those observed for chlorophyll *a* (Table 2.3, Figure 2.5). In Honeymoon and Saturday Night Lakes, differential UVR exposure did not significantly affect particulate carbon concentrations (Table 2.3), despite the large carbon increase under the PAR treatment in Honeymoon Lake on one sampling occasion. In Leach Lake, removal of UVA resulted in significant increases in particulate carbon concentrations (Table 2.3, Figure 2.5; Tukey-Kramer; PAR > PAR + UVA, p=0.005; PAR > PAR + UVA + UVB, p=0.001; PAR + UVA=PAR + UVA + UVB, p=0.860), while in Hibernia Lake no significant treatment effect was observed (Table 2.3).

Particulate carbon results for the mid and deep experiments in Leach Lake suggest that the UVR effect decreased with depth (Figure 2.6). Because of an interaction between UVR and time during the second sampling period the UVR effect was not statistically significant in the mid experiment (Table 2.4, Figure 2.6). However, an analysis of simple effects and contrasts reveals that particulate carbon was significantly higher in the PAR treatment on the third sampling day ($F_{2,27(August\ 18)}=5.299$, $p<0.05$; post hoc PAR>PAR + UVA, $F_{1,27}=8.138$; PAR > PAR + UVA + UVB, $F_{1,27}=7.788$; $p < 0.05$). In the deep experiment removal of UVA resulted in significant increases in particulate carbon concentrations (Table 2.4, Figure 2.6; Tukey-Kramer, PAR > PAR + UVA, p=0.0049; PAR= PAR + UVA + UVB, p=0.0814; PAR + UVA=PAR + UVA + UVB, p=0.4284). However, a comparison of Figures 2.4 and 2.6 clearly demonstrates the magnitude of this effect to be less than that for the primary experiment.

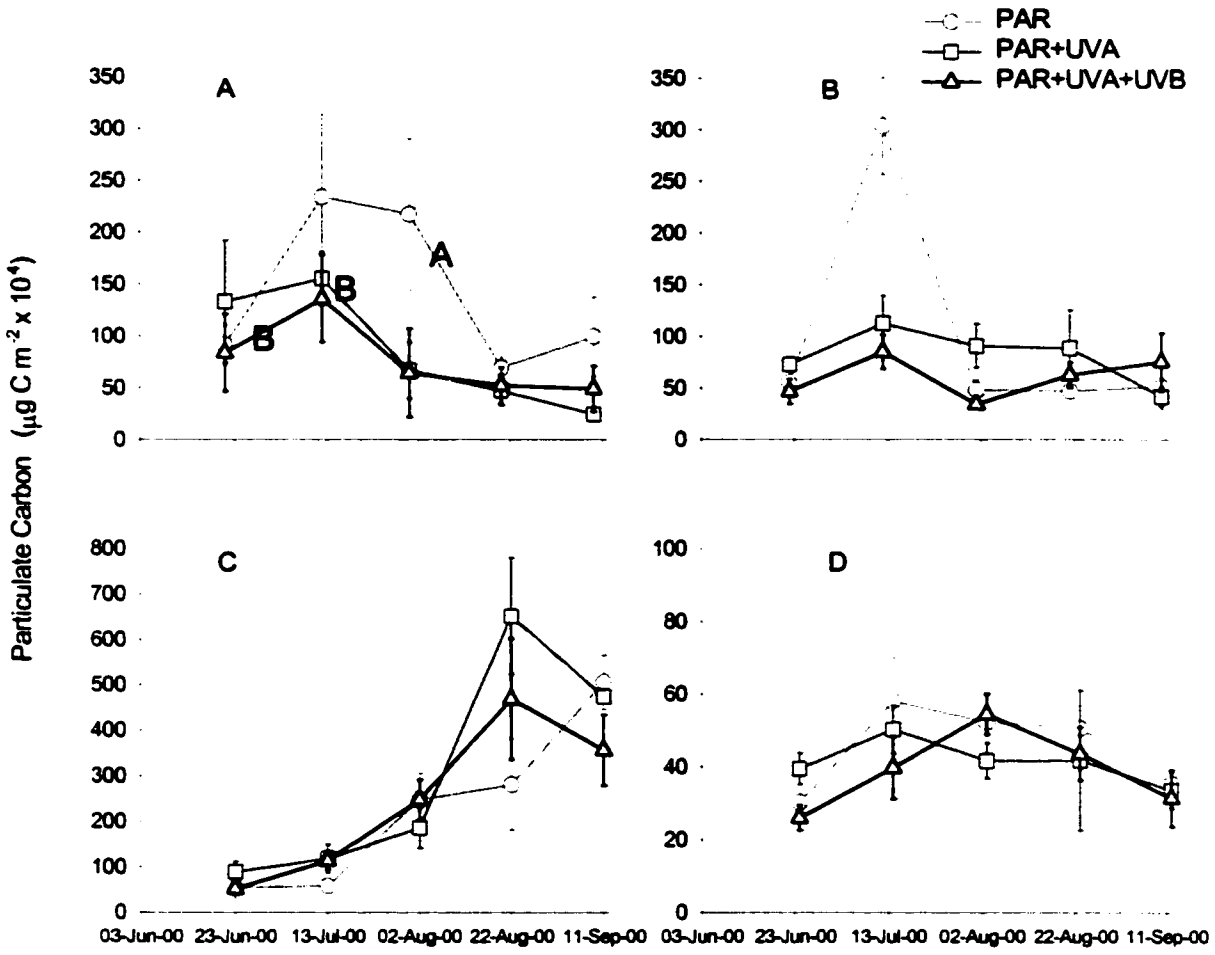

Figure 2.5: Particulate carbon concentrations in epilithon under three different UVR regimes in the primary experiment of four study lakes in Jasper National Park, Alberta: (A) Leach Lake, (B) Honeymoon Lake, (C) Hibernia Lake, and (D) Saturday Night Lake. Error bars represent ± standard error for $n = 4$ replicates. Significant differences between treatments (within each panel) are indicated by different letters.

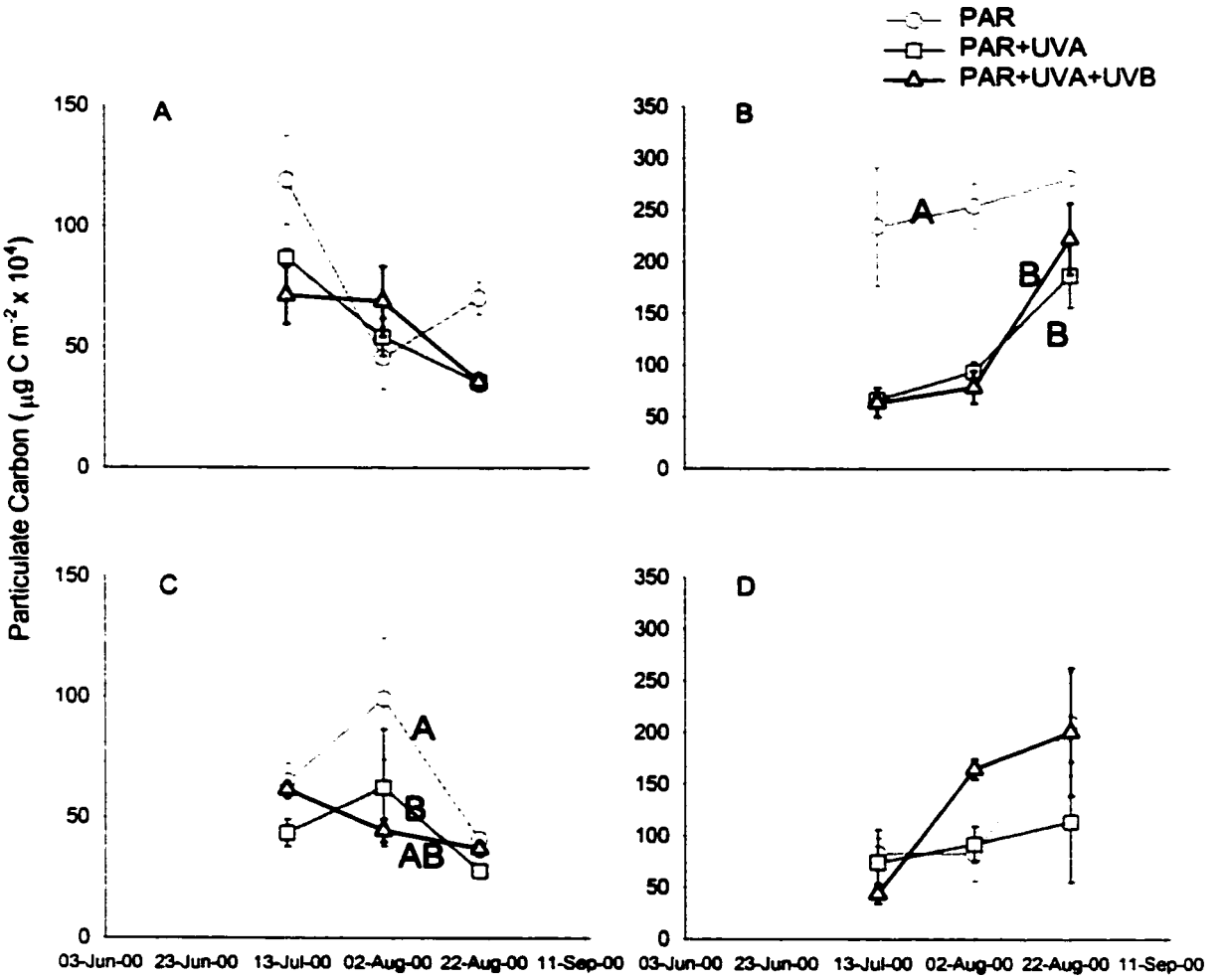

Figure 2.6: Particulate carbon concentrations in epilithon under three different UVR regimes for the mid and deep experiments of Leach and Honeymoon Lakes in Jasper National Park, Alberta: (A) Leach Lake, mid (B) Hibernia Lake, mid (C) Leach Lake, deep, and (D) Hibernia Lake, deep. Error bars represent ± standard error for $n = 4$ replicates. Significant differences between treatments (within each panel) are indicated by different letters.

Table 2.4: Two-way ANOVA results for the effects of ultraviolet radiation (UVR) on log-transformed particulate carbon, and C:P and C:N ratios in epilithon in the mid and deep experiments in Leach and Hibernia Lakes. Reported are degrees of freedom, F statistics and p-values for $n = 4$ replicates. Significant differences are highlighted in bold.

		Lake							
		Leach Mid		Leach Deep		Hibernia Mid		Hibernia Deep	
	df	F	p	F	p	F	p	F	p
Particulate Carbon									
UVR	2	2.0719	0.1455	6.1729	**0.0064**	24.5281	**<.0001**	1.0421	0.3695
Time	2	13.8516	**<.0001**	9.9511	**0.0006**	17.6806	**<.0001**	6.7181	**0.0046**
UVR x Time	4	3.6072	**0.0176**	1.6924	0.1820	2.7486	**0.0496**	2.2393	0.0935
Particulate C:N									
UVR	2	0.3963	0.6767	6.0495	**0.0070**	0.9139	0.4134	0.5432	0.5876
Time	2	0.7200	0.4959	20.4928	**<.0001**	32.5259	**<.0001**	6.3328	**0.0060**
UVR x Time	4	1.1924	0.3367	3.1172	**0.0320**	0.4726	0.7554	1.0329	0.4098
Particulate C:P									
UVR	2	1.3578	0.2749	1.0745	0.3562			0.4099	0.6681
Time	2	15.3610	**<.0001**	1.5276	0.2359	see text		3.6356	**0.0411**
UVR x Time	4	1.9006	0.1404	2.3642	0.0792			2.1396	0.1056

In Hibernia Lake, particulate carbon concentrations were significantly higher under the PAR treatment than under the UVR exposed treatments in the mid experiment (Table 2.4, Figure 2.6; Tukey-Kramer; PAR > PAR + UVA, $p<0.0001$; PAR > PAR + UVA + UVB, $p<0.0001$; PAR + UVA=PAR + UVA + UVB, $p=0.9123$). Although the interaction term was significant for this analysis it is marginal in comparison with the treatment effect, and will not be discussed further. There was no significant difference between radiation treatments in the deep experiment (Table 2.4, Figure 2.6).

Epilithic stoichiometry

Manipulation of the ultraviolet environment had no effect on C:N ratios in either Honeymoon or Saturday Night Lakes (Table 2.3, Figure 2.7). In the Leach Lake primary experiment, average C:N ratios in the PAR treatment were 5.1% higher than in the PAR + UVA treatment, and 4.7% higher than in the PAR + UVA + UVB treatment. This effect was marginally non-significant ($p=0.0564$; Table 2.3, Figure 2.7). UVR manipulation did not significantly affect C:N ratios in the mid experiment of Leach Lake. In the deep experiment, C:N ratios were significantly decreased in the PAR treatment (Table 2.4, Figure 2.8; Tukey-Kramer, PAR < PAR + UVA, $p=0.0082$; PAR < PAR + UVA + UVB, $p=0.0294$; PAR + UVA=PAR + UVA + UVB, $p=0.8422$). There was no effect of light manipulation on epilithic C:N in Hibernia Lake (Tables 2.3 and 2.4, Figures 2.7 and 2.8). In the Leach deep, and Hibernia mid and deep experiments, C:N ratios decreased significantly with time (Table 2.4, Figure 2.8).

Epilithic C:P ratios did not differ significantly between radiation treatments in either Honeymoon or Saturday Night Lakes (Table 2.3, Figure 2.9). In the Leach Lake primary experiment, the removal of both UVA and UVB radiation significantly decreased C:P ratios (Table 2.3, Figure 2.9; Tukey-Kramer, PAR >PAR + UVA + UVB, $p=0.0290$; PAR=PAR + UVA, $p=0.1655$, PAR + UVA=PAR + UVA + UVB, $p=0.6827$). No significant difference existed between radiation treatments in the mid or deep experiments of Leach Lake (Table 2.4, Figure 2.10). In Hibernia Lake, epilithic C:P ratios did not differ significantly between radiation treatments in the primary experiment

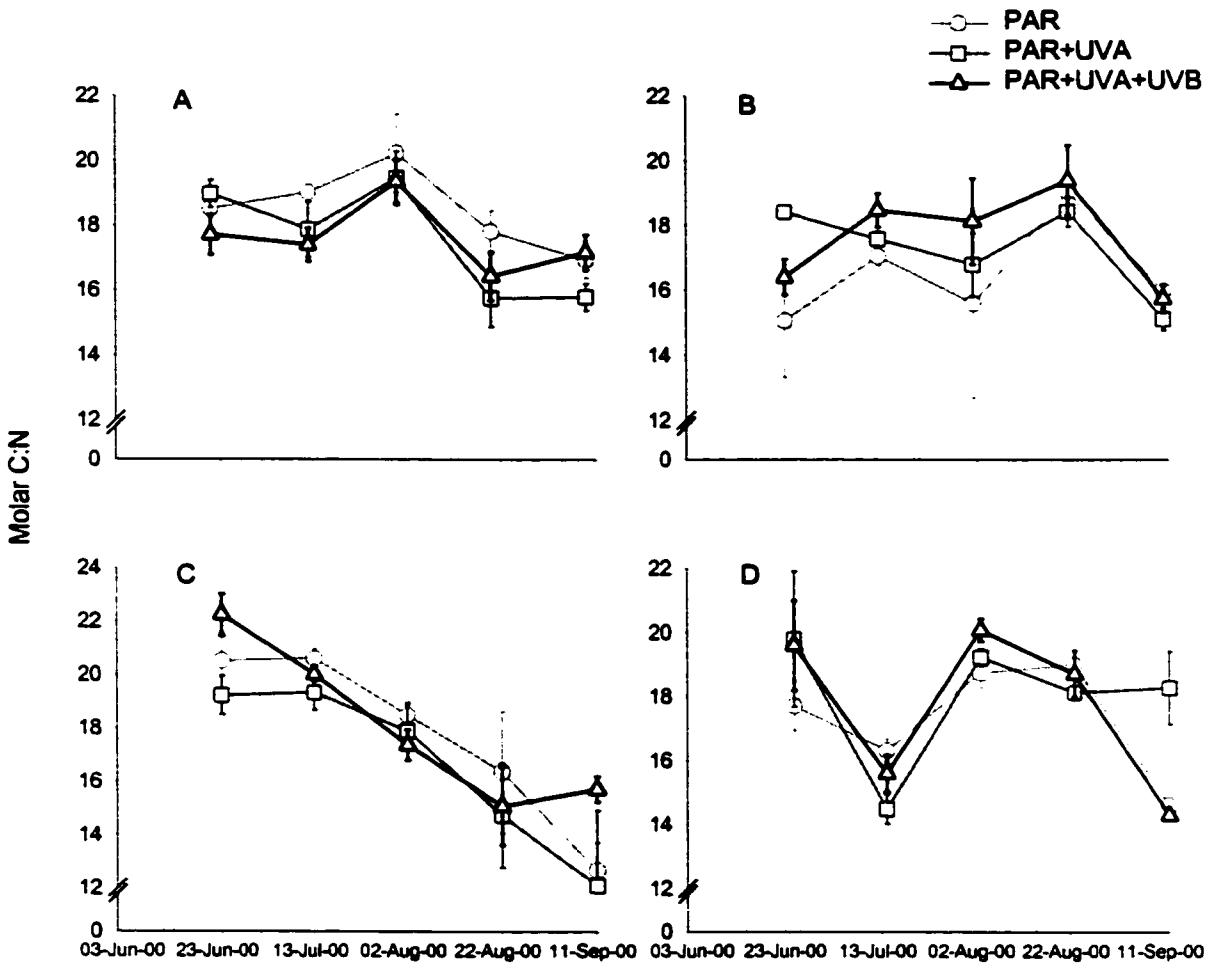

Figure 2.7: Molar C:N ratios in epilithon under three different UVR regimes in the primary experiment of four study lakes in Jasper National Park, Alberta: (A) Leach Lake, (B) Honeymoon Lake, (C) Hibernia Lake, and (D) Saturday Night Lake. Error bars represent ± standard error for $n = 4$ replicates.

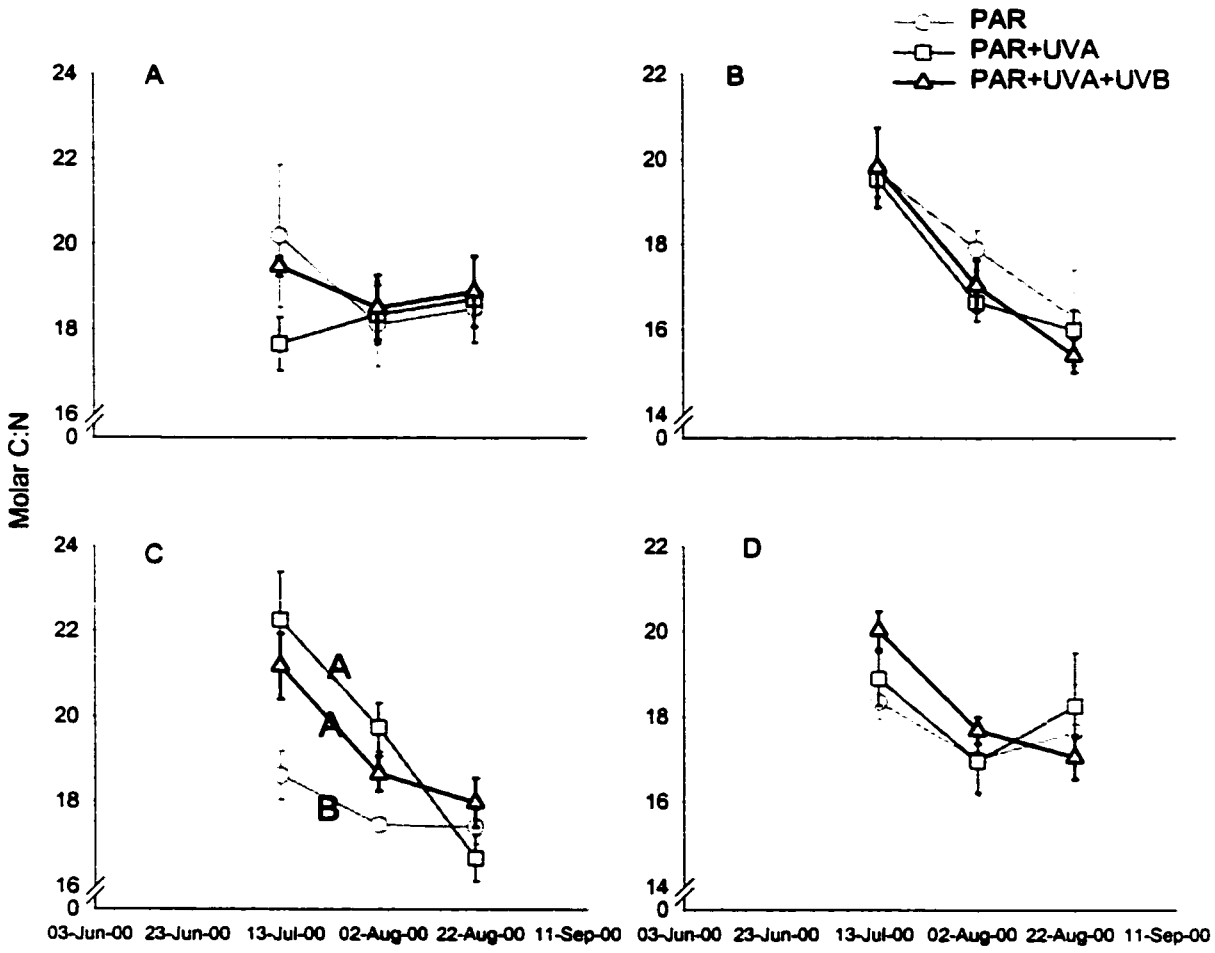

Figure 2.8: Molar C:N ratios in epilithon under three different UVR regimes in the mid and deep experiments of Leach and Honeymoon Lakes of Jasper National Park, Alberta: (A) Leach Lake, mid (B) Hibernia Lake, mid (C) Leach Lake, deep, and (D) Hibernia Lake, deep. Error bars represent ± standard error for $n = 4$ replicates. Significant differences between treatments (within each panel) are indicated by different letters.

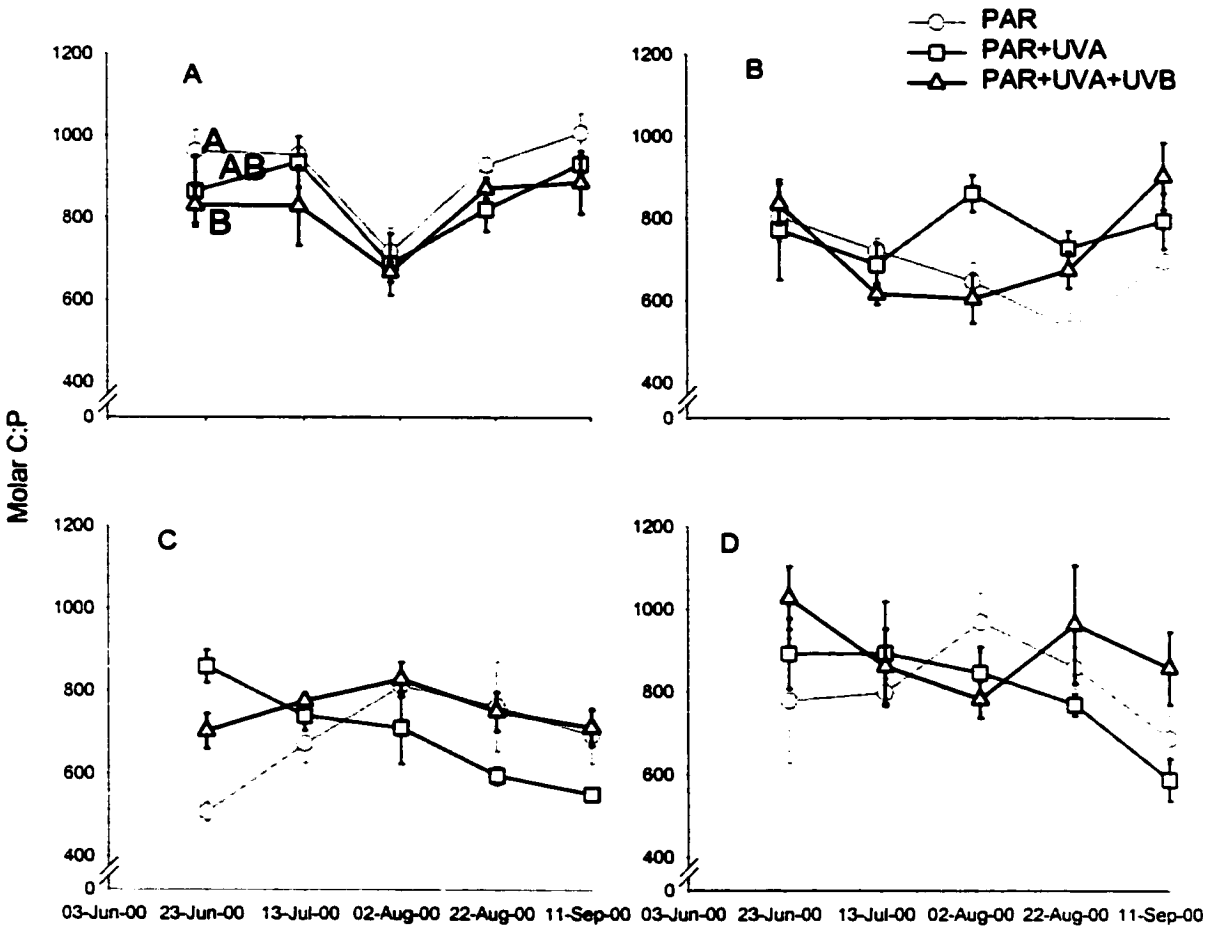

Figure 2.9: Molar C:P ratios in epilithon under three different UVR regimes in the primary experiment of four study lakes in Jasper National Park, Alberta: (A) Leach Lake, (B) Honeymoon Lake, (C) Hibernia Lake, and (D) Saturday Night Lake. Error bars represent ± standard error for $n = 4$ replicates. Significant differences between treatments (within each panel) are indicated by different letters.

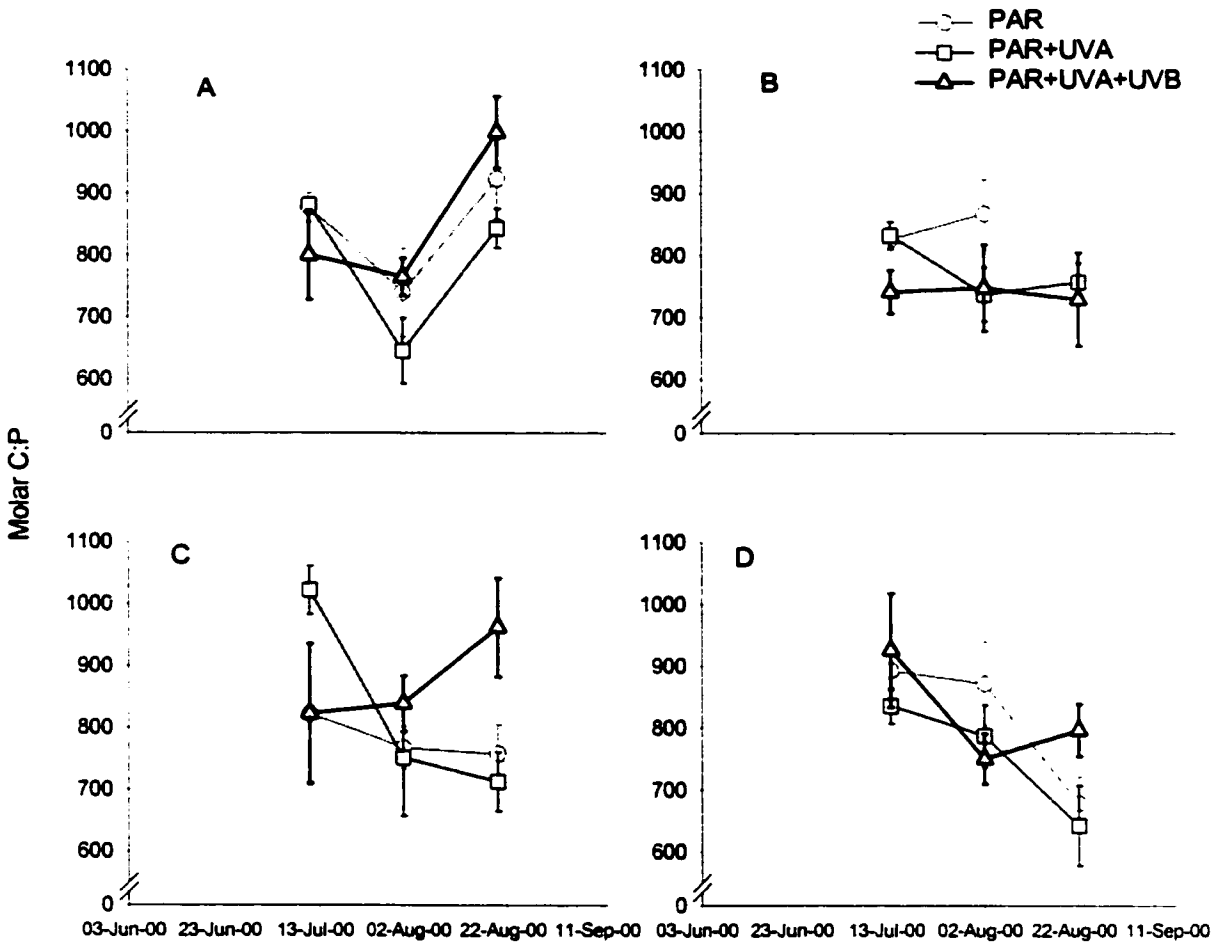

Figure 2.10: Molar C:P ratios in epilithon under three different UVR regimes in the mid and deep experiments of Leach and Honeymoon Lakes of Jasper National Park, Alberta: (A) Leach Lake, mid (B) Hibernia Lake, mid (C) Leach Lake, deep, and (D) Hibernia Lake, deep. Error bars represent ± standard error for $n = 4$ replicates.

(Table 2.4, Figure 2.10). In the mid experiment, a missing PAR sample on the third sampling day meant that a complete two-way ANOVA was not possible. Analyzing the PAR + UVA and PAR + UVA + UVB treatments over the three sampling days revealed no significant effects (p_{UVR}=0.3868, p_{time}=0.6088, $p_{interaction}$ =0.6001). Including the PAR treatment, but excluding the third sampling day also reveals no significant effects (p_{UVR}=0.0956, p_{time}=0.6678, $p_{interaction}$ =0.3050). The effect of UVR on the Hibernia deep experiment was not significant (Table 2.4, Figure 2.10).

Poly-unsaturated fatty acids

Because concentrations of docosahexanoic acid (22:6ω3) were below detection limits in many of our samples, these data are not discussed. In Honeymoon Lake, exposure to UVR did not affect dry weight-normalized concentrations of eicosapentanoic acid (EPA, 20:5ω3; Table 2.5, Figure 2.11). When concentrations were normalized per unit area, a significant interaction effect occurred. Further analysis suggests that on the last sampling day, area-specific concentrations of EPA were highest in the PAR treatment (Table 2.5, Figure 2.11; PAR > PAR + UVA, F=16.80, p=0.0015). In Leach Lake, exposure to UVA radiation significantly increased dry-weight normalized concentrations of EPA (Table 2.5, Figure 2.11; Tukey-Kramer, PAR<PAR + UVA + UVB, p=0.0019; PAR<PAR + UVA, p=0.0138, PAR + UVA=PAR + UVA + UVB, p=0.3525). Concentrations per unit area, however, were not significantly affected by UVR exposure (Table 2.5, Figure 2.11).

Invertebrate colonization

In Leach and Hibernia Lakes, invertebrate colonization differed significantly between radiation treatments (two-way MANOVA; Table 2.6, Figure 2.12). In both lakes, this trend was largely driven by chironomid and oligochaete densities. In Leach Lake, colonization in the PAR treatment was significantly greater than in the PAR + UVA + UVB treatment, while other comparisons did not differ (MANOVA contrasts; Table 2.6). In Hibernia Lake, invertebrate colonization in the PAR + UVA treatment was significantly greater than the PAR treatment, with no significant difference occurring

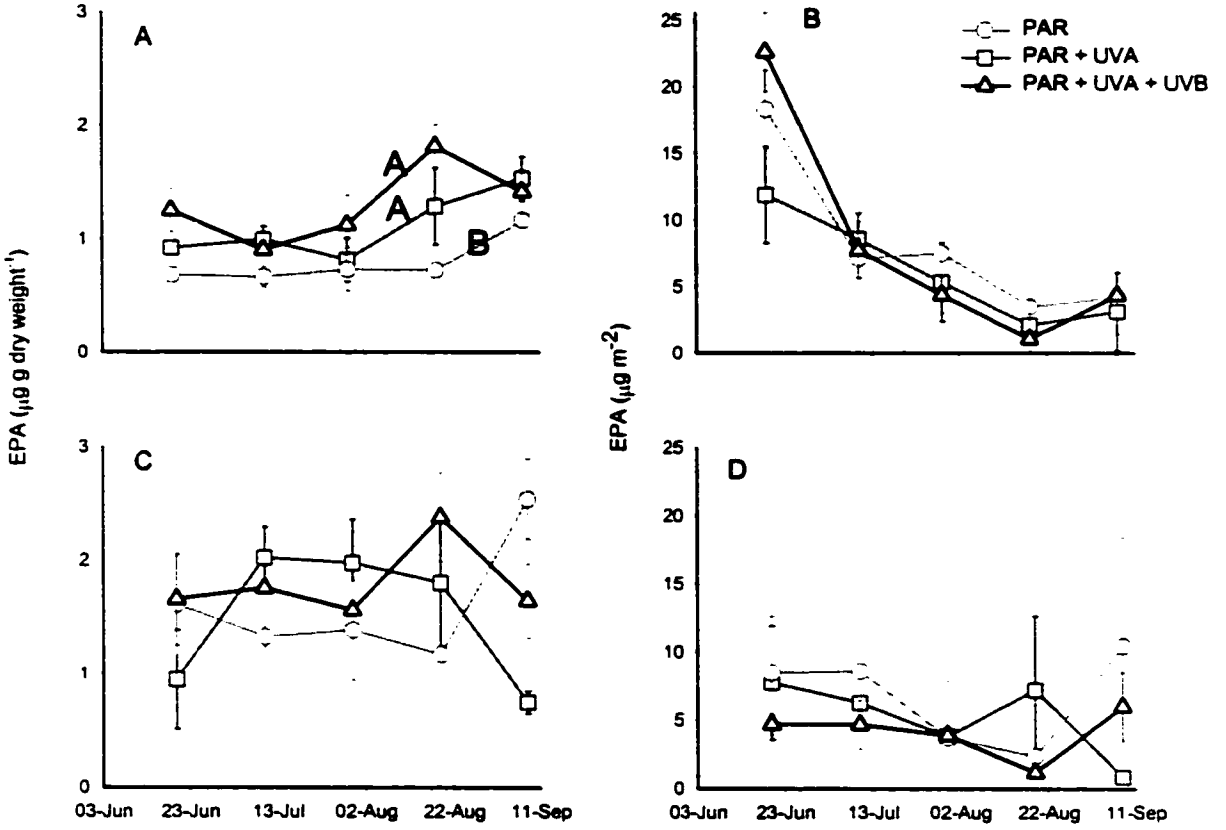

Figure 2.11: Concentrations of eicosapentanoic acid (EPA, 20:5ω3) in epilithon under 3 different UVR regimes normalized per mg dry weight for (A) Leach and (C) Honeymoon Lakes, and per m^2 for (B) Leach and (D) Honeymoon Lakes. Error bars represent ± standard error for $n = 2$ replicates. Significant differences between treatments (within each panel) are indicated by different letters.

Table 2.5: Two-way ANOVA results for the effects of ultraviolet radiation (UVR) on concentrations of eicosapentanoic acid (EPA, 20:5ω3) in epilithon, normalized per unit dry weight, and per m^2 . Reported are degrees of freedom, *F* statistics and p-values for $n = 2$ replicates. Significant differences are highlighted in bold.

		Lake			
		Leach		Honeymoon	
	df	*F*	p	*F*	p
EPA ($\mu g\ g^{-1}$)					
UVR	2	13.7372	**0.0018**	0.6021	0.5634
Time	4	5.2063	**0.0189**	0.6646	0.6286
UVR x Time	8	0.8248	0.6017	4.2583	**0.0124**
EPA ($\mu g\ m^{-2}$)					
UVR	2	1.9357	0.1199	0.9560	0.4119
Time	4	25.1224	**<.0001**	2.2445	0.1250
UVR x Time	8	1.1856	0.3995	2.2113	0.1041

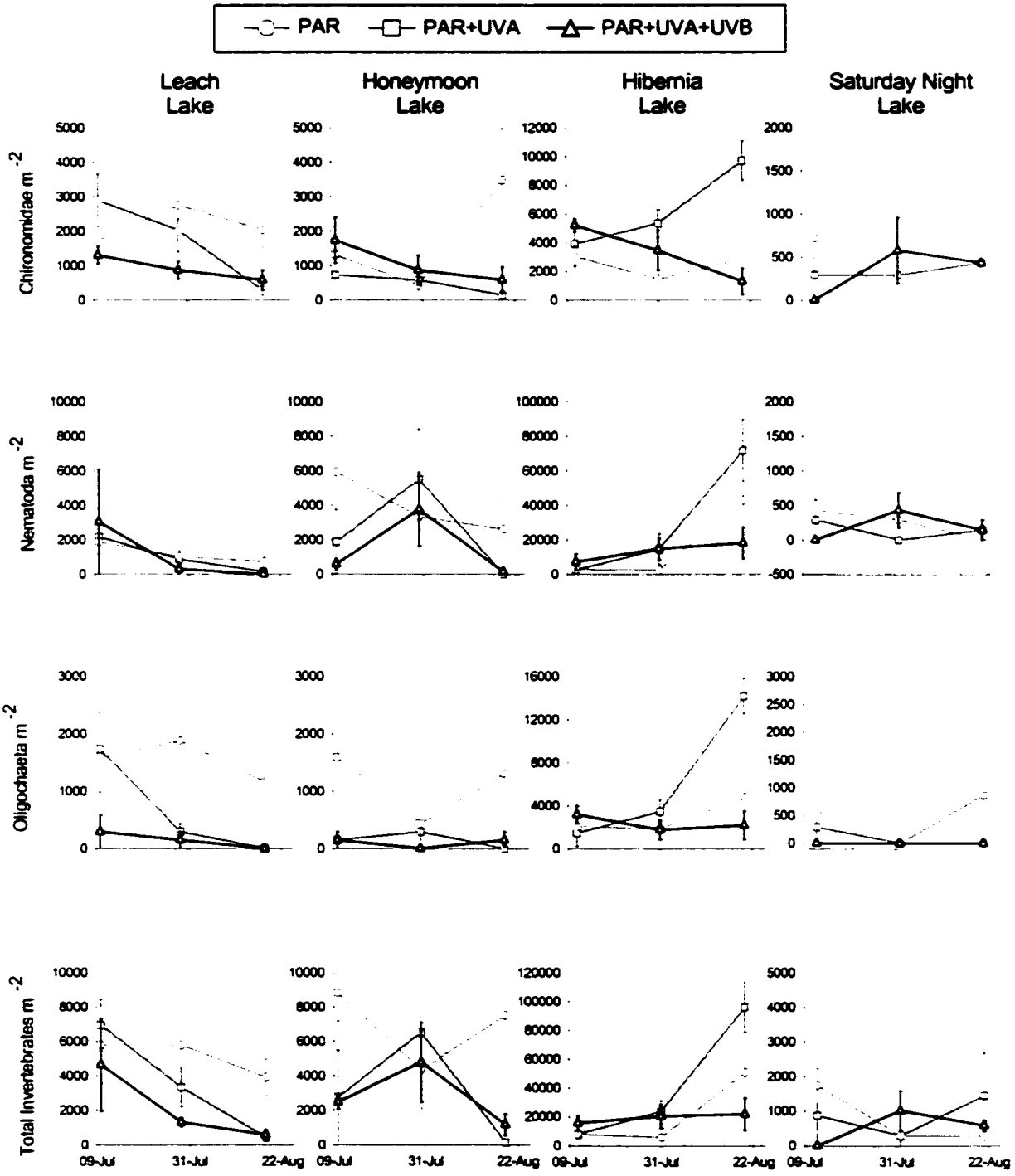

Figure 2.12: Mean abundance (mean ± 1 standard error, $n = 3$) of Chironomidae, Nematoda, Oligachaeta, and total invertebrates under 3 different UVR regimes, in four study lakes in Jasper National Park, Alberta.

Table 2.6: Multivariate and two-way ANOVA results for the effects of ultraviolet radiation (UVR) on square root (n+1) transformed invertebrate counts in the primary experiment in four study lakes in Jasper National Park, Alberta. Reported are degrees of freedom, F statistics and p-values for $n = 4$ replicates. The Hotelling-Lawley F statistic is used in the MANOVA test. Significance levels for the ANOVA and MANOVA contrasts have been adjusted to 0.0170 to reflect multiple comparisons. Significant differences are highlighted in bold.

		Lake							
		Leach		Honeymoon		Hibernia		Saturday Night	
	df	F	p	F	p	F	p	F	p
Chironomidae									
UVR	2	5.9811	**0.0131**	2.5146	0.1089	9.4286	**0.0016**	0.0302	0.9703
Time	2	5.8572	**0.0018**	0.6350	0.5414	0.5208	0.6027	0.2499	0.7815
UVR x Time	4	3.1972	0.2243	2.2220	0.1973	5.7381	**0.0037**	2.1689	0.1139
Oligochaeta									
UVR	2	6.6752	**0.0068**	4.2912	0.0299	5.0468	0.0182	1.3623	0.2812
Time	2	2.6672	0.0967	0.3398	0.7164	10.6467	**0.0009**	1.4032	0.2714
UVR x Time	4	0.9901	0.4380	0.5277	0.7168	6.8875	**0.0015**	1.4663	0.2537
Nematoda									
UVR	2	0.5735	0.5725	2.4074	0.1184	2.1600	0.1443	0.1433	0.8675
Time	2	3.2275	0.0634	3.3413	0.0583	23.4553	**<.0001**	0.4417	0.6497
UVR x Time	4	0.1445	0.9631	1.4062	0.2719	3.7202	0.0224	1.1864	0.3503
Total Invertebrates									
UVR	2	5.5682	**0.0131**	4.5351	0.0254	4.5309	0.0255	0.1755	0.8404
Time	2	9.1733	**0.0018**	1.5939	0.2305	23.0816	**<.0001**	0.1538	0.8586
UVR x Time	4	1.5732	0.2243	1.8808	0.1576	5.8858	**0.0033**	2.3214	0.0961
MANOVA									
UVR	2	2.7555	**0.0297**	2.1426	0.0774	3.2279	**0.0144**	0.8780	0.5227
Time	2	2.8993	**0.0238**	1.3057	0.2849	8.8649	**<.0001**	0.7963	0.5803
UVR x Time	4	1.0426	0.4239	1.1014	0.3830	2.9604	**0.0042**	1.4517	0.1797
MANOVA Contrasts									
PAR vs. PAR+UVA		=	0.2023	NSD		>	**0.0103**	NSD	
PAR vs. PAR+UVA+UVB		>	**0.0072**			=	0.5645		
PAR+UVA vs. PAR+UVA+UVB		=	0.2997			=	0.0197		

between the other treatments (MANOVA contrasts; Table 2.6). In Leach Lake, invertebrate numbers decreased significantly with time, while in Hibernia Lake they increased (Table 2.6, Figure 2.12). The significant interaction term in the Hibernia Lake analysis appears to be mediated by the large divergence of treatments on the last sampling day.

In Honeymoon Lake, the effect of UVR on invertebrate density was marginally non-significant (Table 2.6), as assessed both by the MANOVA results, and ANOVAs performed on individual taxa, where p-values for oligochaete and total counts fell just above the Dunn-Šidák adjusted value of 0.0170. Visual inspection of the data reveals that these marginal differences occurred largely as a result of increases in abundance in the PAR treatment (Figure 2.12). No significant difference existed between treatments in Saturday Night Lake.

Feeding experiment

Snails fed algae irradiated with the full solar spectrum had, on average, growth rates 1.55 times greater than those fed non-UVR irradiated food (Figure 2.13). Conversely, when both snails and their food source were exposed to the full solar spectrum, their growth rate was 2.0 times less than those exposed to PAR alone (Figure 2.13). Despite this fact, the growth rates of incubated snails did not differ significantly among UVR treatments. Both comparisons among snails which were fed differentially-irradiated epilithon in the absence of UVR ($F_{2,10}$=1.8836, p=0.2022), and in the presence of the UVR treatment that their food source had been subjected to ($F_{2,10}$=0.9756, p=0.4229) were non-significant (Figure 2.13).

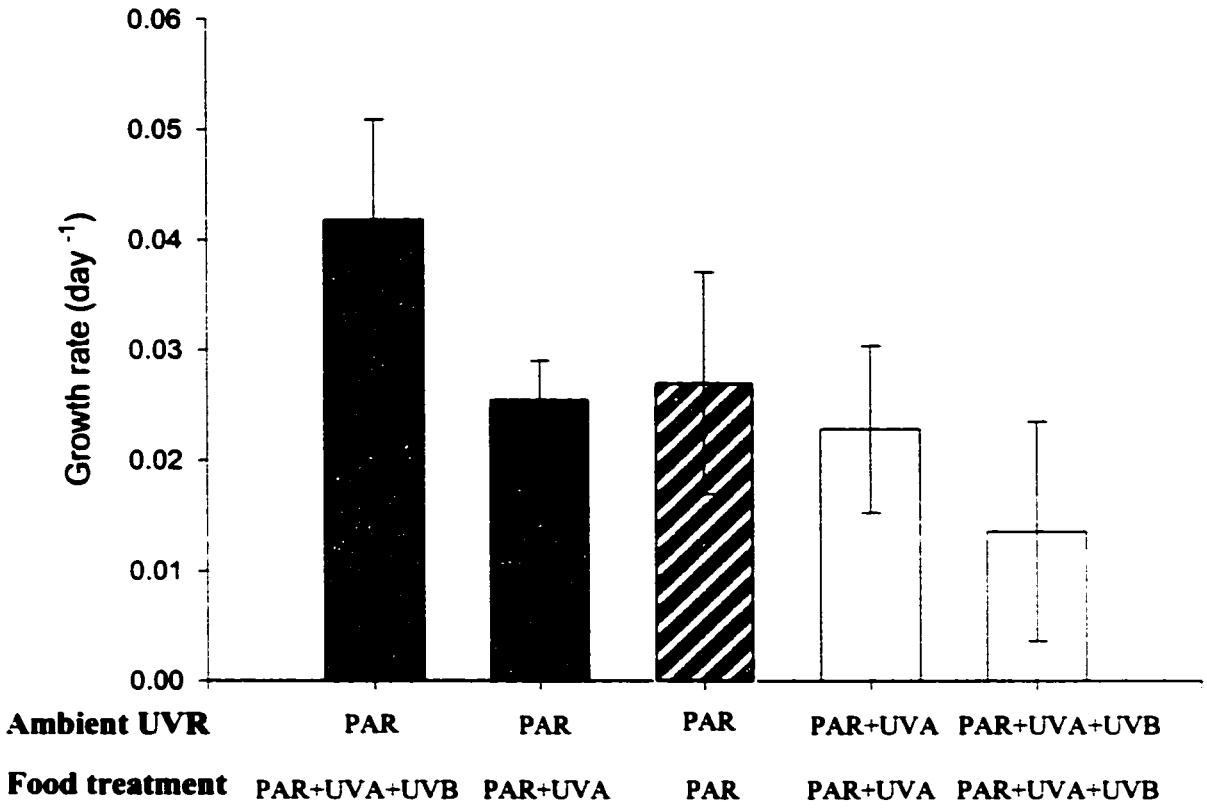

Figure 2.13: Mean growth rates for valvatid snails under differing food and UVR treatments. Gray and cross-hatched bars indicate trials where food exposed to differing UVR treatments was provided to snails in absence of UVR. White and cross-hatched bars indicate trials where both food quality and ambient UVR was modified. Each treatment mean is an average of 5 replicates (mean ± standard error); each replicate is an average of 6 organisms.

DISCUSSION

Water quality and clarity

Both dissolved organic carbon (DOC) and suspended chlorophyll *a* are known to regulate the transmission of solar radiation through the water column (Xenopoulos and Schindler 2001). Although the concentration of chlorophyll *a* has little impact on the attenuation of radiation in the UV range in oligotrophic freshwaters (Scully and Lean 1994), it can be important for controlling the transmission of photosynthetically active radiation (Tilzer et al. 1995). This relationship between suspended chlorophyll *a* concentrations and PAR transmission is seen in Saturday Night Lake. Here, an increase in chlorophyll *a* concentrations, from 1-2 μg/L in early summer to 5-6 μg/L later in the season, was accompanied by a large late-summer decrease in the transmission of PAR through the water column (Figure 2.2).

DOC has been shown to be the major attenuator of ultraviolet radiation in freshwater systems (Scully and Lean 1994). Several models that successfully predict UVR attenuation in freshwaters are based solely on DOC concentrations (Scully and Lean 1994, Morris et al. 1995). In our study lakes, however, DOC concentrations were extremely high in comparison to UVR penetration depths. For example, Scully and Lean's (1994) formula predicts 1% UVB penetration depths in Leach, Honeymoon, Hibernia and Saturday Night Lakes to be 0.22, 0.31, 0.19, and 0.22 m, respectively. This compares to measured 1% depths of 0.95 and 0.98 m in our two clearwater lakes (Leach and Honeymoon), and 0.41 and 0.46 m in our more coloured lakes (Hibernia and Saturday Night; Table 2.1).

Recent investigations have revealed that the quality, in addition to the quantity, of DOC strongly influences its ability to absorb incident radiation. Specifically, terrestrially derived carbon is highly aromatic, and absorbs strongly in the UV portion of the spectrum, while internally derived carbon is much less so (McKnight et al. 2001). In lakes of the Canadian Rocky Mountains, high concentrations of internally derived relative to terrestrially derived DOC are common (Donahue et al. 1998), as would be expected in lakes with mountainous catchments. These results further reinforce the findings of other studies that suggest that DOC quality varies regionally (Curtis and

Adams 1995, Morris et al. 1995, Williamson et al. 1996, Arts et al. 2000), and indicate that estimates of UVR penetration using only DOC measurements should be undertaken with caution.

The effect of UVR on epilithic carbon and chlorophyll a

We found varying effects of UVR exposure on the epilithic standing crop of our study lakes, as assessed by chlorophyll *a* and particulate carbon concentrations. Of our two clearwater lakes, epilithon in Leach Lake decreased strongly with UVR exposure. The magnitude of this effect decreased with depth. Honeymoon Lake, conversely, appeared to be largely unaffected by UVR. The effect of UVR exposure on epilithic carbon and chlorophyll *a* was non-significant in our more colored lakes: although estimates differed between radiation treatments in Hibernia Lake, the effect was erratic when compared between replicates at differing depths. These disparate responses between lakes occurred despite the fact that fluxes of UVR to the primary experiment in Leach and Honeymoon Lakes were similar, as were fluxes to the primary experiments of Hibernia and Saturday Night Lakes and the Leach Lake mid and deep experiments.

Decreased epilithon in the presence of UVR has been previously observed as assessed by chlorophyll *a* (Bothwell et al. 1994, Vinebrooke and Leavitt 1996, Francoeur and Lowe 1998), particulate carbon (Kelly 2001), and taxonomic biomass counts (Bothwell et al. 1993, Vinebrooke and Leavitt 1999). In our study, several mechanisms may have been responsible for the variability of our observations between lakes. Our results in Leach Lake may represent a UVR-specific biomass response that did not occur in our other study lakes. The susceptibility of organisms to UVR exposure is known to vary both among taxa (Jokiel and York 1984, Karentz et al. 1991) and environments (Helbling et al. 1992, Xiong et al. 1996). The taxonomic composition of the experimental communities in Leach Lake was greater than 90 percent diatoms (Chapter 3), which have often been suggested to be most susceptible to the effects of UVR in freshwaters (Vinebrooke and Leavitt 1996, Xenopoulos et al. 2000). This was a proportion greater than for any of our other study lakes, and contrasted most sharply with the community in Honeymoon Lake, whose composition was up to 50 percent

chorophytes and Cyanobacteria (Chapter 3). Environmental conditions also differed between our lakes (Table 2.1), which may have affected the response of the epilithic community to UVR exposure, as has been shown for variations in nutrient availability, and temperature (Behrenfield et al. 1994, Rae and Vincent 1998, Roos and Vincent 1998, Xenopoulos et al. in press).

Conversely, it may be that physiological shifts occurred in response to UVR exposure in the Leach Lake epilithic community. Chlorophyll *a* concentrations have been shown to decrease under high light and UVR exposures (Falkowski and LaRoche 1991, Döhler and Buchmann 1995, Döhler and Haas 1995), while UVR-stressed cells of the benthos have been shown to fix less carbon (McNamara and Hill 2000, Watkins et al. 2001). Clearly, decreases in epilithic standing crop (as assessed by particulate carbon concentrations) occurred under UVR exposure in Leach Lake. However, potential UVR-induced decreases in cell-specific chlorophyll *a* concentrations and carbon fixation may preclude the conclusion that this decrease in standing crop was driven by a biomass response. Finally, the increased numbers of invertebrate grazers that occurred in the absence of UVR exposure in some of our lakes may have decreased the epilithon to a point where differences between treatments were no longer discernable.

The effect of UVR on epilithic stoichiometry

The ratios of C:N and C:P in our epilithic communities were extremely high (C:N between 12 and 22, and C:P between 500 and 1100), and indicative of nutrient limitation. In the benthos, maximal growth has been found in epilithic communities which exhibit C:N ratios of roughly 7.5, and C:P ratios of 130 (Hillebrand and Sommer 1999). Such low nutrient concentrations are also likely to limit the growth of epilithic consumers. In pelagic systems, previous studies have shown that *Daphnia magna* fed on algae with C:P ratios greater than 300 were nutrient limited, and exhibited reduced growth rates (Sterner and Hessen 1994). More recently, similar results have been observed in the benthos, where reductions in epilithic C:P ratios from approximately 600 to between 80 and 330 resulted in significant increases in grazer growth (Frost and Elser in press b). Nitrogen limited growth has been shown to occur in benthic consumers when epilithic C:N ratios

exceed 12-16 (Söderström 1988, Dorgelo and Leonards 2001). At the high C: nutrient concentrations observed in our study, any decrease in epilithic carbon to nutrient ratio of the food source is likely to increase the growth rates of epilithic consumers.

In three of our four study lakes, UVR exposure did not affect epilithic carbon to nutrient ratios. In our fourth lake, Leach, both C:N and C:P ratios decreased under UVR exposure in the primary experiment, although this effect was marginally non-significant for the nitrogen result. Because both ammonium and nitrate uptake rates have been shown to decrease under UVR exposure in the laboratory (Döhler and Biermann 1987, Döhler and Kugel-Anders 1994), the assumption has been that UVR should decrease food quality by increasing carbon to nutrient ratios (Hessen et al. 1997). Recently, however, *in-situ* studies have shown lowered epilithic and sestonic C:P ratios under UVR exposure (Watkins et al. 2001, Xenopoulos et al. in press). These studies suggested that lower carbon concentrations in UVR exposed cells, presumably through decreased carbon acquisition, led to decreases in C:P ratios. Our study suggests that this effect may occur for both C:N and C:P ratios in the shallow-water benthos of montane lakes, although the lower magnitude of the nitrogen effect may reflect strong UVR-induced decreases in N-uptake counterbalancing decreases in carbon accumulation.

In contrast to the results of the primary experiment, C:N ratios increased in our UVR-transparent treatments in the deep incubation. These divergent responses of C:N ratios as the depth of the experimental community increased may have occurred because rates of nitrogen uptake and carbon accumulation did not change uniformly in response to variations in UVR exposure. Such differences in the relative sensitivity to UVR of other physiological processes have been shown elsewhere (e.g., Buma et al. 1996). The UVR-induced decreases in nitrogen uptake discussed above occur at, and below, exposures found in our deepest incubation (Döhler and Biermann 1987). Thus, although carbon concentrations decreased under UVR exposure at this depth, the level of reduction may not have been large enough to overcome decreased nitrogen uptake rates, as likely occurred in the shallower incubation. Increased grazer abundances in the UVR-shielded deep incubations could further account for our observations in the deep experiment. Invertebrates have been found to be more sensitive to the effects of UVR than algal

communities (Bothwell et al. 1994), suggesting that their colonization could have been higher in UVR-screened than UVR-transparent treatments at this depth. Increases in grazer densities may, in turn, have decreased carbon to nutrient ratios, as has been shown in pelagic (Urabe 1995) and benthic systems (Frost et al. in press).

In Hibernia Lake, although there was no effect of UVR on epilithic nutrient ratios, C:N ratios decreased considerably over time in both the primary and deep incubations. This is contrary to what would usually be expected in the benthos, where carbon generally accumulates as detritus, and carbon to nutrient ratios increase as colonization progresses. Our observation likely occurs because of the unusually high abundance of diatoms capable of forming endosymbiotic relationships with Cyanobacteria in these experimental communities. In the primary experiment, *Epithemia argus*, *Rhopalodia* sp., and *Denticula elegans* composed as much as 75% of the algal biomass in this lake (Chapter 3). Relative ratios of N:P further suggest that the epilithon of the experimental communities in this lake may have been co-limited by nitrogen and phosphorus towards the beginning of the experimental period (Hillebrand and Sommer 1999).

The effect of UVR on poly-unsaturated fatty acids

Despite some debate on the subject (von Elert and Wolffrom 2001), the content of long chain PUFAs in producer organisms has been proposed to be critical for controlling the growth and fecundity of consumer organisms (e.g., Müller-Navarra 1995). Several studies have found decreased cell-specific concentrations of PUFAs in laboratory grown phytoplankters under UVR exposure (Goes et al. 1994, Wang and Chai 1994), a result suggested to lead to decreased food quality under UVR exposure (Hessen et al. 1997).

Our, results, however, suggest that such decreases in PUFA concentrations may not occur in the benthos. Exposure to UVR did not significantly affect total areal concentrations of EPA. From our experimental design, it is impossible to infer how *in situ* exposure to UVR affected cellular-specific EPA concentrations. However, we show that increased epilithic accumulation in the Leach Lake PAR treatment led to significantly greater dry-weight specific concentrations of EPA under UVR exposure. Such results, in concert with our stoichimetric findings, suggest that the absolute effect of

UVR exposure on nutrient uptake and biomolecule synthesis may be less important than its effect on carbon acquisition (see also Watkins et al. 2001, Xenopoulos et al. in press). This may be especially true in benthic systems, where large decreases in detrital accumulation under UVR exposure may be able to override any UVR-specific decreases in nutrient and biochemical concentrations.

Direct and indirect effects of UVR on primary consumers

Decreased benthic invertebrate colonization as a result of UVR exposure has been shown in several stream and flume experiments (Bothwell et al. 1994, Kiffney et al. 1997a, b, Donahue and Schindler 1998, Kelly 2001). In lentic systems, however, the effect of UVR exposure has been poorly studied. The results that do exist suggest no overall effect of UVR exposure on the zoobenthos (Francoeur and Lowe 1998, Vinebrooke and Leavitt 1999), presumably because of the increased water depth at which most lentic studies are conducted.

In our lakes, however, UVR exposure significantly decreased invertebrate colonization. Combined exposure to both UVA and UVB radiation decreased invertebrate abundance in Leach Lake, while a moderately non-significant decrease in colonization with UVR exposure occurred in Honeymoon Lake. In Hibernia Lake, invertebrate colonization was greatest under the PAR+UVA treatment, suggestive of another, unknown, factor controlling invertebrate distribution. Overall, decreases in colonization were caused largely by decreases in the Chironomidae and Oligochaeta, while Nematoda densities did not change significantly between treatments. Such differential sensitivities have been shown in several other studies (Kiffney et al. 1997a, b). In our lakes, nematodes may not have reacted significantly to UVR exposure because they were able to burrow within the thick chironomid cases on our experimental tiles.

The decreased colonization rates observed under UVR exposure could have occurred both as a result of UVR avoidance, and direct damage. Because of their optical sensors, invertebrates are able to detect UVR, especially in the UVA range (Tovée 1995). Migration to avoid exposure to UVR has been shown for zooplankters (Leech and Williamson 2001), and might also be expected to occur in the benthos (Kiffney 1997b).

Significant, direct damage to invertebrates has been shown in the presence of UVR (McNamara and Hill 1999). In our study, the effect of direct UVR exposure on snail growth rates was non-significant. We did, however, observe a general decrease in growth rates with increasing UVR exposure. Several reasons may exist for our lack of a significant finding. First, snails have been shown to be more resilient to the effects of UVR than other invertebrates (McNamara and Hill 1999), possibly because their shell offers a protective shield that other invertebrate species lack. Second, food quality, as discussed below, may have increased under UVR stress. This result, acting in a direction opposite to that of the direct effects of UVR, may have dampened the significance of our observations. Finally, it is possible that we have committed a type 2 error in our analysis because our level of replication was not large enough to detect significance in our results. A post-hoc power analysis suggests that with 36 replicates spread over our 3 treatments (i.e., 12, rather than 5 replicates per treatment), a significant response may have been observed.

Although several studies suggest that food quality should be affected in UVR-exposed communities (Watkins et al. 2001, Xenopoulos et al. in press), no study has tested the effects of UVR-mediated shifts in food quality on consumer organisms. Ultraviolet radiation has been proposed to reduce the food quality of aquatic producer communities (Hessen et al. 1997). In our study, however, food quality improved (as assessed by carbon to nutrient ratios and EPA concentrations) with exposure to UVA radiation. This effect occurred in only one of our four study lakes. Our feeding experiment, conducted in Honeymoon Lake, was carried out with epilithon that did not show significant UVR-induced changes in our measured food quality parameters. The effect of food quality on snail growth was non-significant. We did, however, see a trend towards increasing growth rates in UVR exposed food, which power analysis again suggests may have been significant with greater replication (here, a total of 24 replicates, or 8 per treatment).

In our study, organisms were not food limited. Thus, a mechanism other than stoichiometric ratios, EPA content, or food availability, must explain the observed tendency towards increasing growth on UVR-irradiated food. Changes in food quality

other than those measured in this study may account for our observed result. For example, exposure to UVR, at low intensities, has been shown to increase proteins (Buma et al. 1996). Decreases in grazer density in the presence of UVR may also mean that irradiated communities are less recalcitrant, and contain nutrients and biomolecules in a form more available for consumers, having been less subjected to modification by grazing pressure.

Clearly, ultraviolet radiation can affect structure and function in the benthos. In this study, where benthic communities in four lakes were simultaneously examined for their response to variations in UVR exposure, we found that this response was not constant across lakes, and was often weak. While we show that UVR exposure can decrease epilithic standing crop, increase food quality (as assessed by stoichiometric ratios and EPA concentrations), and decrease grazing pressure, we also show that this effect can both decrease and shift rapidly with depth. Our study further demonstrates that UVR may increase the quality of primary producers as food for their consumer organisms. The mechanism for this effect, however, and for variations in UVR response between divergent aquatic systems, remains unclear.

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CHAPTER THREE: THE ROLE OF ULTRAVIOLET RADIATION IN STRUCTURING EPILITHIC ALGAL COMMUNITIES IN MONTANE LAKES OF THE ROCKY MOUNTAINS: EVIDENCE FROM PIGMENTS AND TAXONOMY

INTRODUCTION

Algal growth depends on the presence of visible light for photosynthetic function, limiting primary production to the near-surface region of aquatic systems. This same requirement for the visible wavelengths, however, engenders exposure to ultraviolet radiation (UVR). High radiation levels, both in the photosynthetically active region (Neale 1987) and the ultraviolet region (Vincent and Neale 2000), are known to be potentially damaging. Primary producers experiencing UVR stress exhibit decreased carbon uptake (see, for example, Worrest et al. 1981a, b, Helbling et al. 1992), and altered nutrient metabolism (Döhler 1985, Döhler and Buchmann 1995), amongst other processes.

Whether or not UVR inhibits photosynthesizing organisms depends upon the balance between damage and repair (Vincent and Roy 1993). This balance is neither constant across organisms nor environments. Alterations in light quality, and the interplay between different wavelengths, can fundamentally alter the UVR response of photosynthesizing organisms (Cullen et al. 1992). For example, UVA radiation has been shown to trigger both cellular repair mechanisms (Quesada et al. 1995), and the production of UVR-absorbing pigments in some algal species (Carreto et al. 1990, Garcia-Pichel and Castenholtz 1991, Leavitt et al. 1997). Variations in ambient temperature may also regulate the response to UVR. Research has shown both decreases in the rate of repair processes at cooler temperatures (Roos and Vincent 1998, Pakker et al. 2000), and increases in the rate of damage at warmer temperatures (Rae and Vincent 1998) in UVR-exposed algae. In addition, the nutrient status of the cell can also influence UVR susceptibility. In some studies, extremely nutrient-limited algae have shown little response to fluctuations in UVR exposure (Behrenfield et al. 1994, Xenopoulos et al. in press). In severely nutrient-stressed individuals, nutrient availability, and not UVR, may regulate growth and production.

In addition to environmentally imposed variations in UVR susceptibility, different taxa are also known to differ widely in their response to UVR exposure (Calkins and Thordardottir 1980, Jokiel and York 1984, Karentz et al. 1991). Because of these differences in susceptibility, changes in community structure may occur as a result of UVR stress (Worrest et al. 1981b). Despite these predictions, *in situ* studies of how UVR affects community structure have been few. Several freshwater studies suggest that diatoms should be most sensitive, and Cyanobacteria most resilient, to the effects of UVR (Vinebrooke and Leavitt 1996, Wängberg et al. 1996, Laurion and Vincent 1998, Xenopoulos et al. 2000). This is suggested to occur because of the production of UVR-protective pigments and strong capacity for repair in the Cyanobacteria (Quesada and Vincent 1997). These results, however, have been far from universal (see, for example Vernet 2000). Such UVR-mediated alterations in food quality due to taxonomic shifts, and decreases in food quantity could fundamentally alter energy flow through aquatic systems.

Before the onset of taxonomic shifts, however, it is likely that organisms will undergo physiological changes as a result of UVR stress. Although they are often used as biomass indicators (Millie et al. 1993), changes in pigment concentrations within the cell have been well documented in both high light (Paerl et al. 1983, Falkowski and LaRoche 1991), and high UVR environments. UVR-stress has been shown to bleach photosynthetic pigments (Döhler and Buchmann 1995, Döhler and Haas 1995, Gerber and Häder 1995). However, over the long term, several authors have observed increasing carotenoid concentrations, or increases in carotenoids relative to chlorophylls (Buma et al. 1996, Goes et al. 1994), that may confer photoprotective ability upon the cell. Changes in growth rates, nutrient availability, and grazing pressure may further affect pigment concentrations within the community (Chalup and Laws 1990, Poister et al. 1999).

In this study, we experimentally investigated changes in the community structure of epilithic (rock dwelling) algae brought about by UVA and UVB radiation, both through the analysis of photosynthetic pigments, and taxonomic counts. This study was conducted simultaneously in four montane lakes of varying water transparency, in order

that differences in response caused by previous and current UVR exposure might be examined. We further investigated whether our two methods of assessment were congruent; that is, whether the analysis of benthic algal composition by taxonomic counts and pigment signatures gives equivalent results. Our analyses place UVR in the context of other stressors, and assess its relative role in structuring epilithic algal communities.

METHODS

Study lakes

Our experiments were conducted in four oligotrophic lakes of Jasper National Park. Honeymoon, Leach, Hibernia, and Saturday Night Lakes lie in the main ranges of the Canadian Rocky Mountains, and are underlain by calcareous till (Holland and Coen 1983). The lakes are of similar elevations (1198 – 1418 m above sea level); however, because of microclimatological differences, Leach, Hibernia, and Saturday Night Lakes are located in the montane ecoregion, while Honeymoon Lake lies in the lower subalpine. The lakes were chosen to span a gradient of water clarity; Leach and Honeymoon Lakes are relatively clear, and have high UVR penetration (summer 1% UVB penetration = 0.71-0.98 m), while Hibernia and Saturday Night Lakes are much less transparent (summer 1% UVB penetration = 0.34-0.46 m).

Experimental Design

We used three optical screening treatments (Cadillac Plastics, Edmonton, Alberta, Canada) to test the effects of UVR on epilithic communities. The first, our "PAR + UVA + UVB" treatment, allowed penetration of the full solar spectrum (Acrylite OP4, transparent to all radiation > 280 nm). The second, our "PAR+UVA" treatment, blocked UVB radiation (Mylar-D, transparent to radiation > 320 nm). The third, our "PAR" treatment, blocked all UVR (Acrylite OP3, transparent to radiation > 400 nm). The plastic sheets were suspended slightly below the water surface using a frame of ABS plastic piping. Artificial substrata (acid washed, unglazed ceramic tiles, 23 cm^2) were placed on the lake bottom below the plastic screens. The positioning of the screening

treatments relative to the tiles ensured constant filtration of incoming radiation, despite daily and seasonal solar angle shifts. Tiles were initially placed at a depth of 30 cm, and were monitored throughout the summer for changes in depth.

One replicate of each of the three UVR screening treatments was set up in each of the four study lakes. Experiments were set up between May 26 and 31, 2000, and samples collected on days 40, 60, and 80 thereafter. Samples were collected by randomly selecting tiles, and scraping off the epilithic community that had accumulated on the tile surface. For photosynthetic pigments, four replicate samples consisting of one tile each were collected in the field on GF/F filters, and frozen. For algal taxonomy, four replicate tile samples were combined to form a single sample, and immediately preserved using Lugol's solution. On each sampling occasion, a water sample was collected from the surface over the deepest point of the lake, and analysed for general water chemistry using standard methods (Stainton et al. 1977, Prepas and Rigler 1982, APHA 1992). In order to account for differences in invertebrate abundance when considering algal taxonomic shifts, samples of invertebrates were collected from three replicate tiles and preserved in 4% formalin until the time of counting.

Determination of water clarity

Every 10 days after experimental setup, water was collected from the center of the lake immediately below the surface. Water samples were kept in the dark and refrigerated until analyses (within two weeks), at which time light absorption in the range of 280 to 700 nm was measured through a 2 cm cuvette using a scanning spectrophotometer (Cary WinUV, Varian Instruments, California).

Laboratory analyses

Epilithic pigments: Epilithic pigments were determined using reverse-phase high performance liquid chromatography (HPLC; Leavitt and Carpenter 1990). Taxonomic pigments representative of the major algal groups present in our study communities are given in Table 3.1. Two replicate filters containing epilithon were combined and freeze dried overnight (Hansson 1988), directly immersed in 10 mL of extraction solvent (85%

Table 3.1: Algal chlorophyll and carotenoid pigments and their representative benthic taxa, as modified from Leavitt (1993).

Pigment	Representative taxa
Chlorophyll *a*	all algae
Chlorophyll *b*	chlorophytes
Chlorophyll *c*	diatoms, chrysophytes
β-carotene	all algae
Canthaxanthin	Cyanobacteria
Diatoxanthin	diatoms
Echinenone	Cyanobacteria
Fucoxanthin	Diatoms, chrysophytes
Violaxanthin	chlorophytes

acetone, 15% methanol by volume), and allowed to extract for 24 hours at 4 °C. Samples were sonicated at the onset of the extraction process to increase extraction efficiency (Wright et al. 1997). Extracted samples were filtered through a 0.2 µm membrane filter, evaporated to dryness under nitrogen gas, and frozen under nitrogen gas until the time of analysis. Dried extracts were dissolved in a known volume of injection solvent (70% acetone, 25% ion-pairing reagent, 5% methanol by volume) containing 3.2 mg/L of the internal standard Sudan II. Pigments were separated on a HPLC Model 1100 equipped with inline diode array detector and fluorescence detector and a 10 cm Varian Microsorb C18 column with 100-angstrom beads.

Algal taxonomic composition: Preserved algal samples were sonicated for two 15-s intervals at 20 kHz (Sonifer Cell Disruptor, Model W140, Heat Systems, Ultrasonic Inc.), and a 2 mL aliquot removed, stained with Fast Green FCF (Fisher Scientific), and allowed to settle overnight. Algal cells were identified to lowest taxonomic unit on an inverted microscope at magnifications of 125 and 400x, using phase contrast illumination and a modified Utermöhl technique (Nauwerck 1963). Cells in random fields were enumerated until 100 viable cells of the dominant taxon had been observed, at which time the final field was completely counted. Viable cells were distinguished by the presence of cellular structures stained with FCF (Owen et al. 1978). Approximation of cell volumes of each species were made according to best-fit formulae for different taxa (Rott 1981), and converted to wet biomass estimates (Nauwerck 1963).

Statistical analyses

Richness and diversity: Algal community richness was calculated as the total number of species present in each sample, under each screening treatment. Shannon-Wiener and Simpson's diversity indices were calculated using common formulae (Krebs 1989); the calculation of the Shannon-Wiener index was performed using logs to the base of 2, while the Simpson's index is represented as (1-S). Two-way ANOVAs were used to test for the effects of UVR treatment and time of sampling on untransformed algal richness and diversity indices (JMP Version 3.2, SAS Institute, 1996). Tukey-Kramer pairwise comparisons were used for post-hoc comparisons where analyses were significant

(SYSTAT Version 8.0, SPSS Inc. 1996). Significance was at the 0.05 level.

Direct gradient analysis: The relationship between algal community composition and environmental factors was investigated using ordination analysis (CANOCO version 4; ter Braak and Šmilauer 1998). Detrended Correspondence Analysis (DCA) was first performed to determine the maximum variance present within the taxonomic biomass dataset. This method allows for determination of whether linear (e.g., Redundancy Analysis, RDA) or unimodal models (e.g., Canonical Correspondence Analysis, CCA) best suit the dataset (ter Braak and Šmilauer 1998).

Values for algal biomass and environmental variables were $\log_{10}$ transformed to downweight the importance of large celled taxa, which might otherwise dominate the analysis, and to approximate normality (ter Braak and Šmilauer 1998, Zar 1999). Throughout our analyses, Monte-Carlo permutations were restricted by specifying repeated samples from one experimental replicate as members of a time series (ter Braak and Šmilauer 1998). The environmental dataset was screened for redundant variables in order to eliminate those variables that did not exert a significant influence on algal distributions (Hall and Smol 1992). First, significantly related environmental variables were identified by way of a Pearson product-moment correlation matrix (Dunn-Šidák adjusted $p < 0.05$). Second, a preliminary RDA was run with forward selection to rank the variables in order of percent taxonomic variance explained by each. Finally, using this ranking, each significantly correlated pair of variables was run through a partially constrained RDA, where the higher-ranked correlated environmental variable was input as the sole variable, and the lower-ranked correlated environmental variable was input as the sole co-variable. The independence of the co-variable was determined using Monte-Carlo permutations for significance of the first canonical axis (199 permutations; ter Braak 1988). Those variables that did not exert significant influence were removed. Following this procedure, a second RDA with forward selection was run and inspected for environmental variables with high variance inflation factors, outlier variables, and those variables that did not significantly improve the fit of the model. Non-significant variables, and those that skewed the model fit, were further removed (ter Braak and Šmilauer 1998).

The significance of the final RDA model was determined by testing the eigenvalues of each of the four canonical axes (Monte-Carlo test with 199 permutations). In RDA, the lengths of the arrows representing environmental variables correspond to their importance in explaining the observed variance in the species data. The proximity of the arrows to one another represents their similarity: environmental variables whose arrows are placed close together are highly positively correlated, while arrows at a 90 degree angle to one another are not related, and arrows facing in opposite directions are highly inversely related. Likewise, species and site points placed close together in the ordination diagram are more similar than points far apart, and two species points found in close proximity to one another will be found in the environment under similar conditions. The relationship between a species and a given environmental variable can be obtained by projecting a line from the species point to the arrow representing the environmental variable, at an angle perpendicular to the environmental variable. The further along the arrow in the positive direction that this projected line falls, the more positively correlated with the environmental variable is the species (ter Braak 1994).

Analysis of pigment composition: The effects of UVR and time of sampling on log-transformed concentrations of both chlorophyll and carotenoid pigments were analyzed by way of two-way MANOVA (JMP Version 3.2, SAS Institute, 1996). Two-way MANOVA was also used to analyze treatment differences in chlorophyll *a* to carotenoid ratios. These ratios are graphically represented as carotenoid: chlorophyll *a*, for consistency with other studies, but were analyzed as this ratio's inverse to achieve normality. Further investigation of individual chlorophyll and carotenoid concentrations, and pigment ratios under the UVR screening treatments was done by way of two-way ANOVAs. When a significant result was present in the MANOVA analysis, Dunn-Šidák adjusted contrasts were performed to investigate where differences lay (JMP Version 3.2, SAS Institute, 1996). When investigations of specific differences in two-way ANOVA results were performed, the Tukey-Kramer test was used (SYSTAT Version 8.0, SPSS Inc. 1996).

RESULTS

Incident radiation and water clarity and chemistry

The number of daily total hours of bright sunshine (i.e., near cloud-free conditions) was generally low through the summer, most noticeably around the time of summer solstice (Figure 3.1). Measurements of actual UVB, UVA, and PAR fluxes are given in Chapter 2. Spectrophotometric estimates of water clarity showed that Hibernia and Saturday Night Lakes absorbed strongly in the UVB range, while Leach and Honeymoon Lakes were more transparent (Chapter 2, Figure 2.2). In the more coloured lakes, less than 10% of incident UVB reached the tile surface during the experimental period. The difference in transparency between the lakes decreases with increasing wavelength, to the point where absorption is almost identical in the PAR region, with the exception of Saturday Night Lake (Chapter 2).

The two transparent lakes, Leach and Honeymoon, had lower concentrations of TDP, alkalinity, and conductivity, and higher concentrations of NH_4^+ (Figure 3.2). Concentrations of Si were lowest in Honeymoon Lake, and similar in the other three lakes. DOC concentrations were higher than expected in Leach and Honeymoon Lakes, given their clarity (Chapter 2), and NO_2^-+ NO_3^- concentrations showed no clear trends between lakes. Although nitrogen concentrations decreased as the summer progressed, this trend does not appear in the phosphorus data (Figure 3.2).

Algal taxonomy: species richness and diversity

Epilithic species richness varied between 12 and 26 species in all treatments, for all dates, and did not show a trend with time (Table 3.2, Figure 3.3). A combined analysis for all four of the study lakes showed no significant UVR effect (Table 3.2). Although visual inspection of the data suggests that removing the influence of UVR in the two clear study lakes (Leach and Honeymoon) may increase richness values, this trend was also non-significant (p_{UVR}=0.0958, p_{TIME}=0.9051, $p_{interaction}$=0.8642).

Trends for the Shannon-Wiener diversity index were similar to those for species richness. Calculated diversity values varied from 1.5 to 3.5, and, except in Hibernia Lake, decreased with time (Table 3.2, Figure 3.3). Removing both UVA and UVB

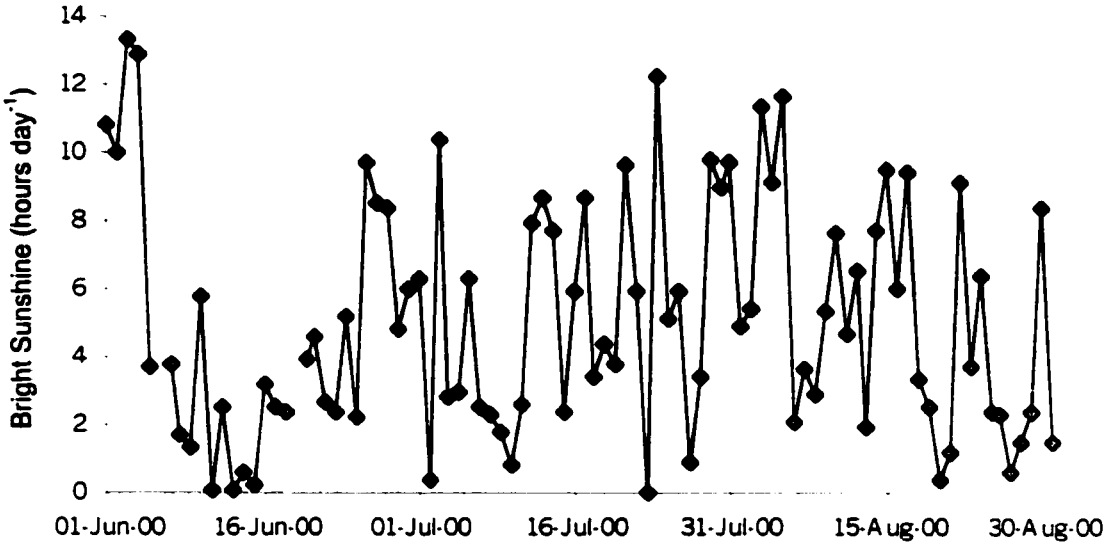


Figure 3.1: Daily total hours of bright sunshine during summer, 2000 in Jasper, Alberta, Canada.

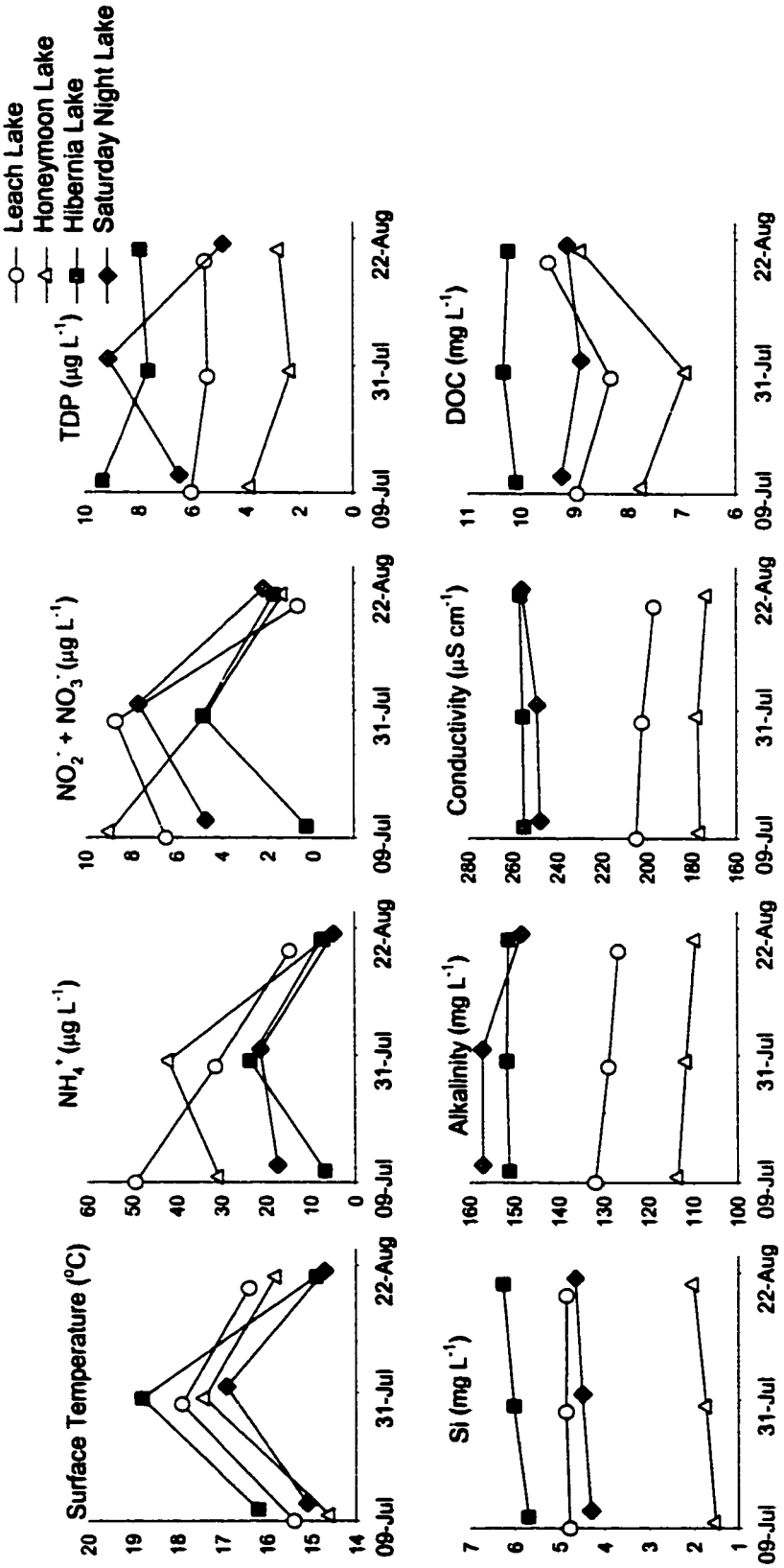


Figure 3.2: Limnological parameters for four study lakes in Jasper National Park, Alberta. Measurements were taken every 10 days from early June to late August 2000. Alkalinity is measured as HCO_3^-.

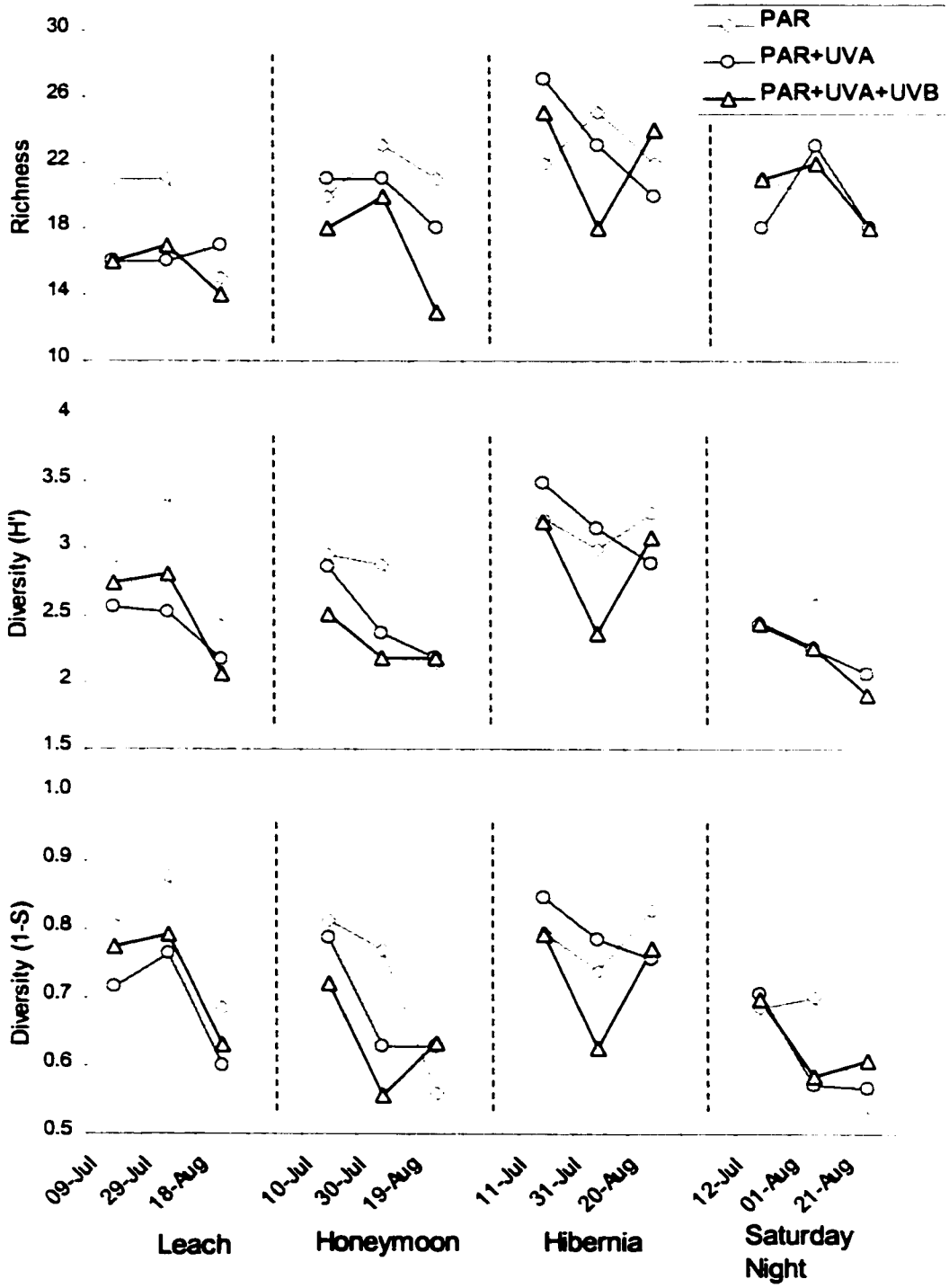


Figure 3.3: Richness, Shannon-Wiener (H'), and Simpson's (1-S) diversity indices under three different UVR regimes in four study lakes in Jasper National Park, Alberta.

Table 3.2: Two-way ANOVA results for the effects of ultraviolet radiation (UVR) on richness and Shannon-Wiener and Simpson's diversity indices in epilithon of four study lakes in Jasper, Alberta. Reported are degrees of freedom, *F* statistics and p-values. Significant differences are highlighted in bold.

		Richness		Shannon-Wiener Diversity (H')		Simpson's Diversity (1-S)	
	df	*F*	p	*F*	p	*F*	p
UVR	2	1.4936	0.2425	6.0384	**0.0068**	2.4850	0.1022
Time	2	0.3567	0.7032	6.7001	**0.0043**	5.5296	**0.0097**
UVR x Time	4	0.1178	0.9750	1.7701	0.1152	1.7703	0.1640

radiation significantly increased species diversity as assessed by this metric (Tukey-Kramer; PAR > PAR + UVA + UVB, p=0.0056; PAR = PAR + UVA, p=0.0790; PAR + UVA = PAR + UVA + UVB, p=0.4897). Although trends for the Simpson's diversity index were again similar to those for Shannon-Wiener (Figure 3.3), these results are non-significant (Table 3.2).

Algal taxonomy: biomass and community composition

In all lakes, the diatoms were clearly the largest contributor to algal biomass (Figure 3.4), with the Cyanobacteria and chlorophytes composing a smaller portion of the total. The relative contribution of non-diatom taxa was much higher in Honeymoon Lake than in the other three study lakes. In all lakes except Hibernia, overall biomass decreased with time, a trend driven by decreases in diatom biomass on the last study date (Figure 3.4). The dominant algal species in our experimental communities are presented as a percentage of total biomass in Table 3.3.

Exposure to UVA and UVB did not change the biomass or percent composition of epilithon in the four study lakes (Figure 3.4). However, several broad taxonomic shifts occurred. In Honeymoon Lake on the second study date, the chlorophytes *Mougeotia* sp. and *Bulbochaete* sp. increased under the PAR treatment, while the diatoms *Cymbella* sp. and *Navicula rosa* increased in the PAR + UVA + UVB treatment. This increased diatom biomass is largely responsible for the observed increase in total biomass (Figure 3.4). In Honeymoon Lake on the last study date, the observed increase in Cyanobacteria biomass under the PAR + UVA + UVB treatment is driven by increases in *Scytonema* sp. In Hibernia Lake, increased chlorophyte volume on the last study date under the PAR + UVA + UVB treatment is caused by a proliferation of a large celled *Bulbochaete* sp. No trend of increasing cell size with increasing UVR was observed (data not shown).

Multiple environmental factors and algal community structure: ordination analysis

Preliminary investigation of species data by detrended correspondence analysis (DCA) revealed that the maximum gradient length represented by the canonical axes was less than 3 units of standard deviation, verifying that a linear (RDA), rather than unimodal, response model would best fit our data (maximum length =2.8; ter Braak and

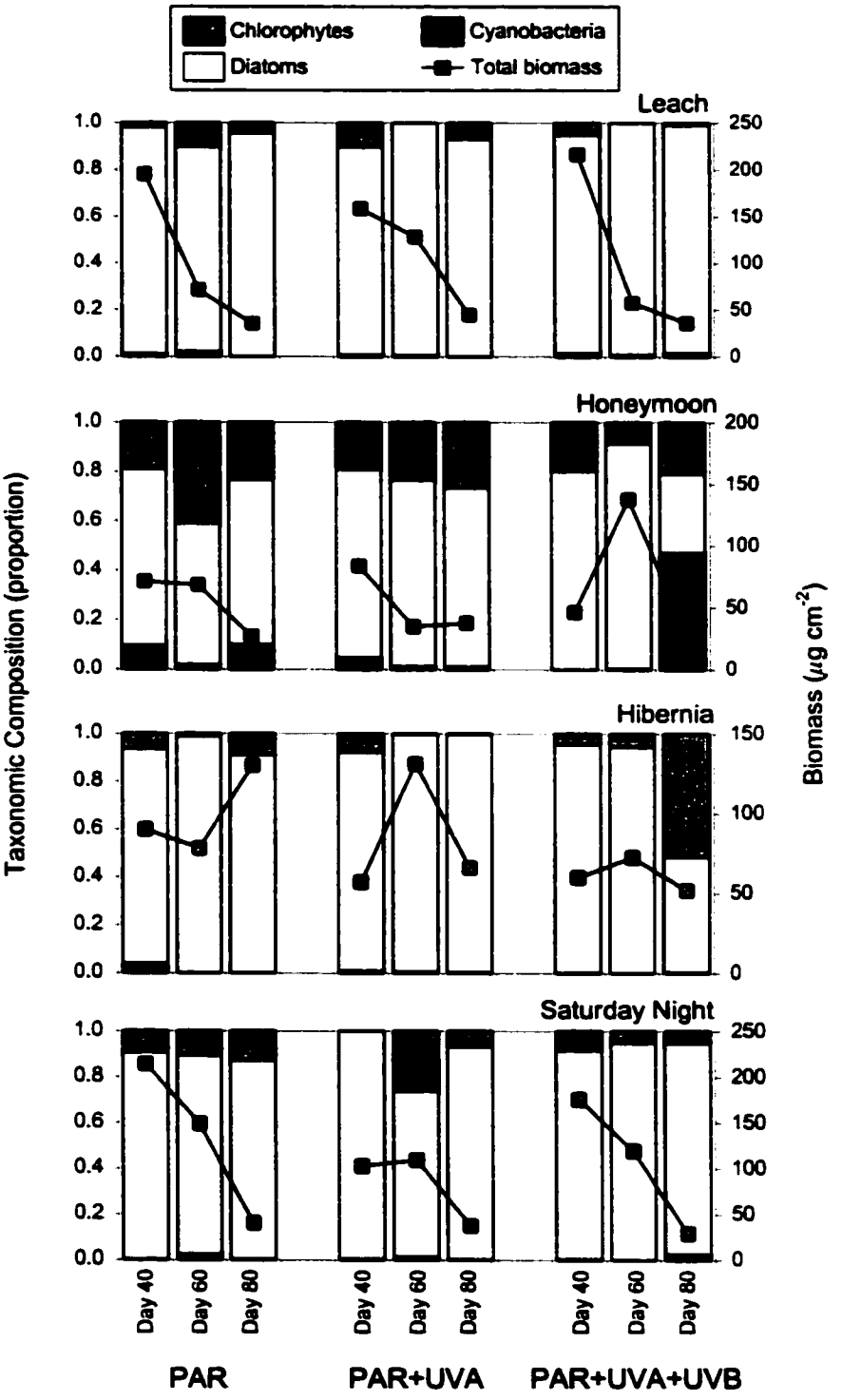


Figure 3.4: Algal taxonomic composition and biomass under three different UVR regimes in four study lakes in Jasper National Park, Alberta.

Table 3.3: Dominant algal taxa under three different UVR regimes in four study lakes in Jasper National Park, Alberta, given as a percentage of total biomass on each date, under each screening treatment.

	Day 40			Day 60			Day 80		
	PAR	PAR+ UVA	PAR+ UVA+ UVB	PAR	PAR+ UVA	PAR+ UVA+ UVB	PAR	PAR+ UVA	PAR+ UVA+ UVB
Leach Lake									
Rhopalodia sp. O. Muller	25.91	0.00	26.05	30.53	41.54	44.89	20.83	28.97	23.44
Cyclotella meneghiniana Kützing	24.38	14.30	26.54	17.69	18.04	22.01	16.61	9.38	15.60
Cymbella sp.	13.07	58.81	0.11	0.00	0.00	0.00	0.28	0.00	29.70
Epithemia argus Kützing	7.07	0.00	0.00	0.00	13.35	0.00	31.75	22.08	0.00
Achnanthes minutissima Kützing	4.98	7.62	3.44	4.03	4.47	0.00	10.86	14.04	8.51
Gomphonema olivaceum (Lyngbye) Kützing	1.38	2.14	1.68	6.93	3.98	3.75	6.38	3.38	3.15
Bulbochaete sp.	0.00	10.01	3.42	10.02	0.00	0.00	0.00	3.87	0.00
Cymbella microcephala Grunow	2.39	1.34	1.60	3.79	2.43	6.26	0.60	5.33	1.26
Denticula elegans Kützing	0.00	0.00	13.59	0.00	10.51	0.00	0.00	0.00	0.00
Tabellaria fenestrata (Lyngbye) Kützing	0.00	2.78	0.00	11.71	0.00	3.80	0.00	0.92	0.61
Cymbella gracilis (Rabhorst) Cleve	2.01	2.82	1.25	4.06	0.47	3.07	0.00	3.13	0.00
Cymbella pusilla Grunow	0.00	0.00	15.93	0.00	0.00	0.00	0.00	0.00	0.00
Navicula subtilissima Cleve	1.71	0.27	2.04	2.43	1.92	3.94	1.42	0.25	0.17
Mougeotia sp.	1.69	0.00	1.63	0.00	0.00	0.00	3.94	2.90	0.95
Gyrosigma sp.	9.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chroococcus limneticus Lemmermann	1.02	0.83	1.28	1.96	0.61	0.82	0.00	0.00	1.48
Honeymoon Lake									
Cymbella sp.	0.00	27.02	27.46	0.00	8.66	45.45	2.92	16.06	3.32
Achnanthes minutissima Kützing	10.68	6.92	10.28	9.01	24.51	7.43	11.28	19.97	10.90
Mougeotia sp.	13.28	4.74	10.80	23.35	16.02	5.52	11.96	0.00	9.77
Bulbochaete sp.	5.80	13.23	5.12	17.51	6.95	2.88	2.32	23.55	11.01
Gomphonema olivaceum (Lyngbye) Kützing	13.28	0.00	3.03	10.20	15.81	1.53	7.37	7.38	6.83
Cyclotella meneghiniana Kützing	22.02	11.30	19.75	1.57	2.33	0.33	0.99	1.54	5.12
Navicula bacillum Ehrenberg	7.37	3.31	4.75	13.86	0.00	18.88	1.44	11.43	0.00
Scytonema sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	45.77
Cymbella gracilis (Rabhorst) Cleve	3.61	1.09	5.77	3.65	5.78	1.73	3.61	4.37	0.00
Tabellaria fenestrata (Lyngbye) Kützing	2.86	10.45	0.85	3.20	2.62	3.20	1.52	0.00	0.00
Coccones sp.	0.00	1.24	0.00	1.88	7.30	3.20	3.64	4.85	1.96
Hibernia Lake									
Rhopalodia sp. O. Muller	51.30	66.69	36.94	70.95	59.89	48.00	34.16	56.17	15.99
Denticula elegans Kützing	9.10	7.23	6.87	1.83	6.05	11.73	11.55	9.55	4.35
Epithemia argus Kützing	7.65	0.00	0.00	1.03	11.62	19.82	6.60	9.98	6.16
Bulbochaete sp.	0.00	5.62	2.44	0.00	0.00	0.00	0.00	0.00	51.12
Coccones sp.	3.57	0.86	3.19	1.50	4.67	4.23	10.67	10.07	4.77
Achnanthes minutissima Kützing	3.77	3.12	3.47	3.07	3.67	4.37	2.91	2.25	3.38
Navicula bacillum Ehrenberg	1.52	1.12	10.46	1.99	3.63	0.84	6.50	1.00	0.87
Cymbella sp.	2.23	0.12	8.81	2.95	3.27	0.00	0.00	4.89	4.60
Cyclotella bodanica Eulenst.	0.00	0.00	2.70	8.06	1.91	0.74	5.38	0.00	0.00
Navicula sp.	1.97	3.12	8.36	0.00	0.00	0.00	0.34	2.14	1.18
Mougeotia sp.	5.90	1.74	1.57	0.81	0.00	0.53	6.38	0.00	0.00
Brachysira brebissonii	0.29	0.11	0.37	2.07	0.61	3.14	2.34	0.32	1.70
Gomphonema olivaceum (Lyngbye) Kützing	3.08	0.70	1.75	0.53	0.19	0.00	1.30	0.36	0.81
Tabellaria fenestrata (Lyngbye) Kützing	1.64	0.00	0.00	0.64	1.67	0.00	3.12	0.00	0.00
Saturday Night Lake									
Rhopalodia sp. O. Muller	0.00	17.96	16.93	16.14	30.70	34.53	0.00	31.18	9.18
Achnanthes minutissima Kützing	23.86	15.90	11.83	14.27	9.67	13.65	10.33	13.48	11.49
Cymbella sp.	12.90	8.48	21.02	13.28	10.66	7.25	20.36	20.51	9.66
Cyclotella meneghiniana Kützing	19.03	2.32	2.68	13.20	0.62	1.15	11.78	4.05	0.10
Gomphonema olivaceum (Lyngbye) Kützing	10.64	6.37	6.38	5.49	6.87	3.48	6.51	5.46	2.14
Denticula elegans Kützing	7.16	0.00	13.46	2.52	6.60	8.38	0.00	4.22	0.00
Tabellaria fenestrata (Lyngbye) Kützing	0.86	1.41	3.89	1.11	0.00	0.00	2.41	2.62	25.35
Bulbochaete sp.	0.00	0.49	0.00	2.80	3.34	5.30	8.36	4.68	4.04
Epithemia argus Kützing	0.00	0.00	0.00	0.36	0.00	5.23	21.63	0.00	0.00
Mougeotia sp.	0.00	0.00	5.36	5.80	7.43	7.35	0.00	0.00	0.97
Cyclotella stelligera Cleve and Grunow	8.29	1.95	1.64	4.57	2.71	0.00	3.14	3.10	0.00
Closterium kuetzingii Brebisson	0.00	23.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gomphonema constrictum Ehrenberg	0.54	1.11	0.00	0.82	7.40	0.00	1.00	0.85	0.00
Neidium iridis (Ehrenberg) Cleve	0.00	0.00	0.00	8.25	0.00	0.00	0.00	0.00	0.00

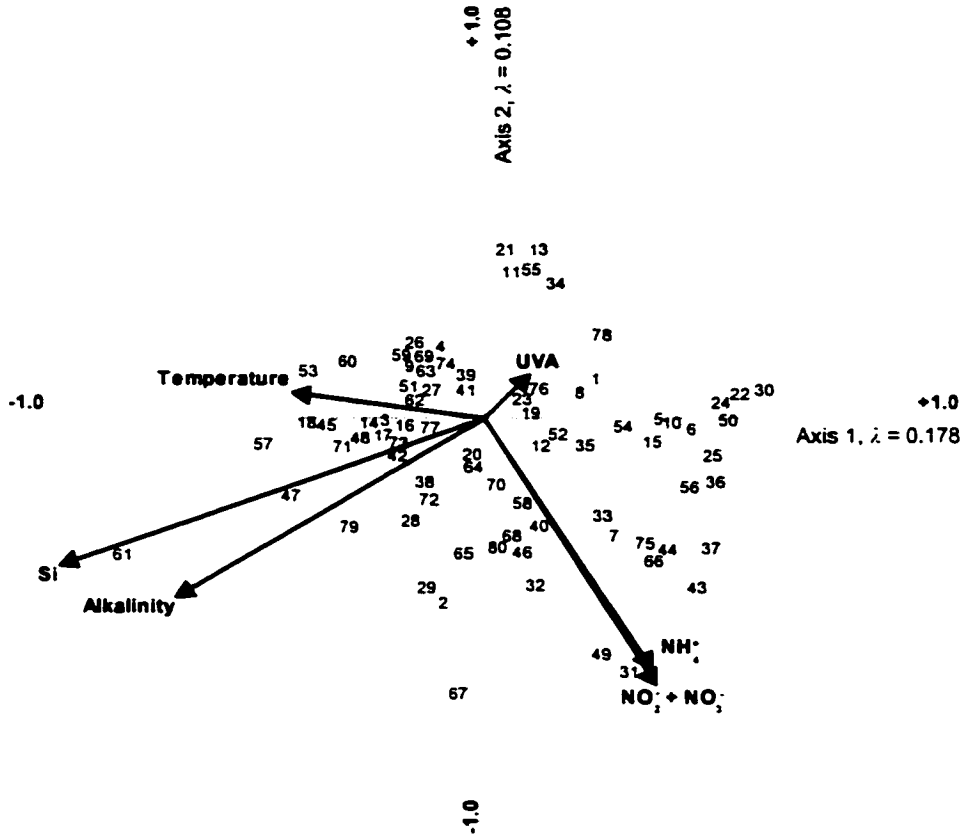
Prentice 1988). A Pearson product-moment correlation matrix was used to identify environmental variables that were significantly correlated with one another (Table 3.4). Preliminary forward selection of all environmental variables resulted in a ranking order of Si, $NO_2^-+NO_3^-$, alkalinity, conductivity, PAR, UVA, chironomid density, temperature, NH_4^+, TDP, DOC, and UVB.

Initial screening eliminated redundant variables that did not contribute significantly to the ordination. Preliminary, partially constrained RDAs suggested that of the significantly correlated variables (Table 3.4), only conductivity was redundant when compared solely to the higher ranked variable alkalinity. Forward selection in the absence of the variable conductivity further removed PAR, TDP, UVB, and DOC as variables that did not significantly improve the fit of the model ($p = 0.45$, 0.53, 0.62, and 0.88 respectively, Monte-Carlo test with 199 permutations). Chironomid density was finally removed because it exerted a significant skewing effect on several of our sites, and was not a significant descriptor of algal distribution when these sites were removed.

The first four axes of RDA with the remaining, significant environmental variables explained 38.5% of the species variance found in these study lakes (Table 3.5). Silica and alkalinity were most strongly related to the first canonical axis, which was significant ($p=0.010$, Monte-Carlo test with 199 permutations), and explained 17.8 percent of the variance in the species assemblage data (Table 3.5, Figure 3.5). The second canonical axis was also significant ($p=0.005$, Monte-Carlo test with 199 permutations), and explained a further 10.8 percent of the species variance. This axis largely explained an available N gradient, with NH_4^+ and $NO_2^-+NO_3^-$ being its largest contributors (Table 3.5, Figure 3.5). The first and second canonical axes tend to contrast diatoms with Cyanobacteria and chlorophytes. Not surprisingly, chlorophytes and Cyanobacteria are inversely related with the silica vector, a relationship that is borne out when further investigated with univariate regression (Figure 3.6). Diatoms clustered in a strong negative relationship with the second axis. Several of the diatom species that were poorly related with the nitrogen vectors (*Epithemia argus*, *Rhopalodia* sp., and *Denticula elegans*) have the ability to form endosymbiotic relationships with Cyanobacteria (Graham and Wilcox 2000). Further investigation of the relationship between total algal

Table 3.4: Matrix of Pearson product-moment correlations for log-transformed environmental variables in four lakes in Jasper National Park, Alberta. Bolded values are significant at Dunn-Šidák adjusted p-values (p=0.05).

	PAR	UVA	UVB	Chironomid Density	Surface Temperature	NH_4^+	$NO_2^- + NO_3^-$	TDP	DOC	Si	Alkalinity
UVA	0.0760										
UVB	0.0870	0.4976									
Chironomid Density	0.2210	0.0153	-0.0311								
Surface Temperature	**0.6511**	0.0396	0.0286	0.1089							
NH_4^+	0.5034	0.0537	0.0650	-0.0187	0.3871						
$NO_2 + NO_3$	0.0876	0.0137	0.0177	-0.1937	0.1833	**0.7706**					
TDP	-0.1025	-0.682	-0.1454	0.0459	0.0690	-0.1772	-0.1010				
DOC	-0.1231	-0.0585	-0.1133	0.1823	-0.0367	**-0.5578**	-0.5198	**0.7704**			
Si	-0.0806	-0.0542	-0.1130	0.1480	0.1722	-0.2636	-0.2595	**0.8640**	**0.8237**		
Alkalinity	-0.3648	-0.0936	-0.1737	-0.1285	-0.0002	-0.3044	-0.0865	**0.8842**	**0.7124**	**0.8265**	
Conductivity	-0.4297	-0.0953	-0.1739	-0.0511	-0.0092	-0.4153	-0.1790	**0.8353**	**0.7357**	**0.8170**	**0.9795**



Cyanobacteria
1 *Aphanothece* sp.
2 *Chroococcus limneticus* Lemmermann
3 *Merismopedia glauca* (Ehrenberg) Kützing
4 *Anabaena* sp.
5 *Phormidium tenue* Anagnostidis and Komarek
6 *Lyngbya* sp.
7 *Cylindrospermum* sp.
8 *Anabaenopsis* sp.
9 Heterocysts
10 *Pseudoanabaena* sp.
11 *Rivularia* sp.
12 *Gloeothece* sp.
13 *Scytonema* sp.

Chlorophytes
14 *Pediastrum duplex* Meyen
15 *Pediastrum tetras* (Ehrenberg) Ralfs
16 *Oocystis borgei* Snow
17 *Scenedesmus quadricauda* (Turp.) Brebisson
18 *Scenedesmus denticulatus* Lagerheim
19 *Scenedesmus* sp.
20 *Closterium kuetzingii* Brebisson
21 *Cosmarium depressum* v achondrum (Boldt)
22 *Euastrum denticulatum* Gay
23 *Euastrum* spp.
24 *Mougeotia* sp.
25 *Bulbochaete* sp.

Chrysophytes
26 *Kephyrion spirale* (Lackey) Conrad
27 *Dinobryon sertularia* Ehrenberg

Diatoms
28 *Fragilaria construens* v binodis Grunow
29 *Gomphonema constrictum* Ehrenberg
30 *Gomphonema constrictum* v capitata
31 *Cyclotella meneghiniana* Kützing
32 *Cyclotella stelligera* Cleve and Grunow
33 *Tabellaria fenestrata* (Lyngbye) Kützing
34 *Fragilaria crotonensis* Kitton
35 *Fragilaria construens* (Ehrenberg) Grunow
36 *Synedra acus* Kützing
37 *Synedra ulna* (Nitzsch) Ehrenberg
38 *Gomphonema* sp.
39 *Pinnularia* sp.
40 *Gyrosigma* sp.
41 *Frustulia rhomboides* (Ehrenberg) de Toni
42 *Achnanthes linearis* W. Smith
43 *Achnanthes minutissima* Kützing
44 *Cymbella* sp.
45 *Cyclotella bodanica* Eulenst.
46 *Eucocconeis* sp.
47 *Epithemia argus* Kützing
48 *Frustulia vulgaris* Thwaites
49 *Navicula subtilissima* Cleve
50 *Sellaphora pupula* (Kützing) Mereschkow
51 *Navicula* sp.
52 *Nitzschia fonticola* Grunow
53 *Nitzschia linearis* W. Smith
54 *Neidium* sp.
55 *Stauroneis anceps* Ehrenberg
56 *Eunotia* sp.
57 *Brachysira brebissonii* (Ross in Hartley)
58 *Neidium iridis* (Ehrenberg) Cleve
59 *Pinnularia subcapitata* Gregory
60 *Cocconeis* sp.
61 *Rhopalodia* sp. O. Muller
62 *Brachysira exilis* (Kütz.) Cleve
63 *Brachysira follis* (Ehrenberg) R.Ross
64 *Cymbella amphicephala* Naegeli
65 *Fragilaria pinnata* Ehrenberg
66 *Cymbella gracilis* (Rabhorst) Cleve
67 *Cymbella microcephala* Grunow
68 *Cymbella pusilla* Grunow
69 *Surirella linearis* v constricta (Ehrenberg)
70 *Pinnularia borealis* Ehrenberg
71 *Amphora ovalis* Kützing
72 *Nitzschia filiformis* (W. Smith) Hustedt
73 *Diatoma vulgare* Bory
74 *Pinnularia gibba* Ehrenberg
75 *Gomphonema olivaceum* (Lyngbye) Kützing
76 *Diatoma* sp.
77 *Neidium gracile* Hustedt
78 *Navicula bacillum* Ehrenberg
79 *Denticula elegans* Kützing

Dinoflagellate
80 *Peridinium pusillum* (Penard) Lemmermann

Figure 3.5: Correlation biplot of algal species and environmental variables in four lakes in Jasper National Park, Alberta.

Table 3.5: Eigenvalues, and their associated significance levels, and cumulative percent variance of the species data explained by the four ordination axes. Each of the 6 environmental variables is listed with its r-value correlation to each axis. P-values are calculated by Monte-Carlo methods, with 199 permutations.

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.1782	0.1080	0.0644	0.0342
p	0.0100	0.0050	0.0250	0.2100
Cumulative explanation (%)	17.8	28.6	35.1	38.5
Correlation of environmental variables with axes (r)				
UVA	0.0839	0.0834	0.0189	-0.7198
Temperature	-0.3620	0.0494	0.0135	0.1982
NH_4^-	0.3096	-0.4903	0.2703	0.1239
$NO_2^- + NO_3^-$	0.3177	-0.5231	0.0726	0.0730
Si	-0.7929	-0.2905	-0.0728	-0.1131
Alkalinity	-0.5798	-0.3530	-0.4833	-0.0917

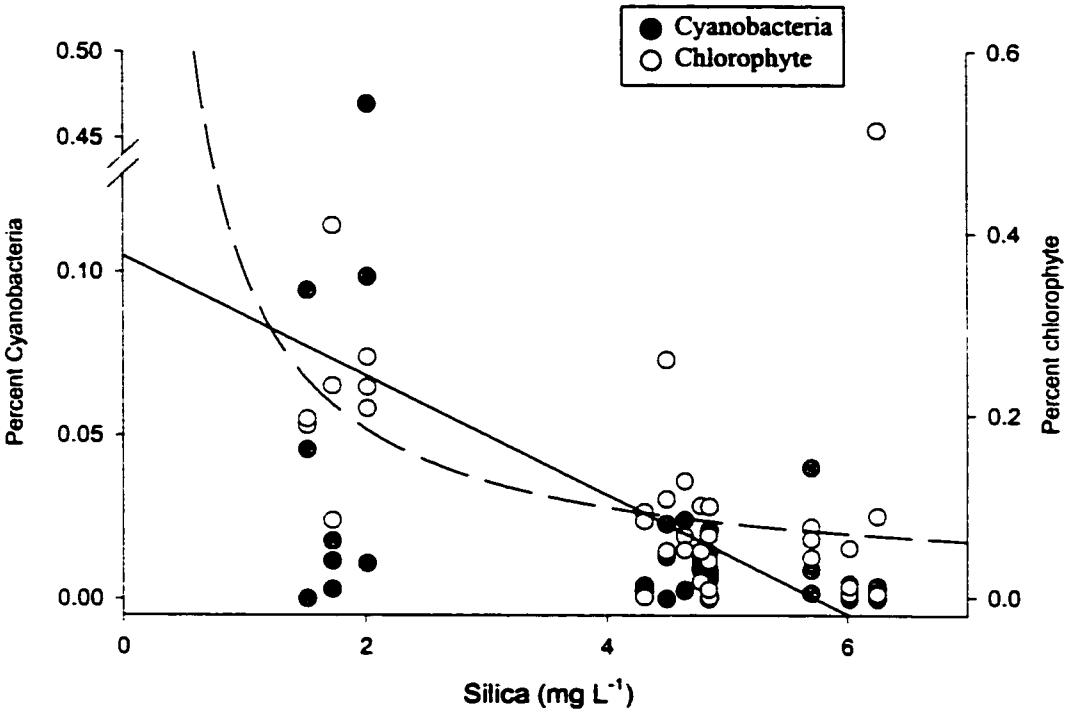


Figure 3.6: Percent Cyanobacteria and chlorophyte biomass decreases with increasing silica concentration. Percent Cyanobacteria = 0.1049 – 0.0183(Si), solid line, r^2=0.134, p=0.0283. Percent chlorophyte = 0.0119 + (0.34561/Si), r^2=0.2557, p=0.0017.

biomass and nutrient concentrations reveals a strong correlation with NH_4^+ (Figure 3.7). Furthermore, despite its removal from our model, total algal biomass is also positively correlated with TDP (Figure 3.7).

The third axis, also significant (Table 3.5), is again dominated by measures of nutrient status. UVA is strongly correlated only to axis four, which does not significantly explain additional variance in the species assemblages. Although variations in nutrient concentration are clearly the largest contributor to species variation in these lakes, an investigation of the fourth ordination axis reveals some patterns in the species data with respect to UVR (Figure 3.8). None of the taxonomic groups showed an overall negative correlation with the UVA vector. However it was specific chlorophyte (*Mougeotia* sp.), and diatom (*Gomphonema constrictum* and *Synedra acus*) species which were found to be most strongly negatively related to UVA radiation (Figures 3.8 and 3.9). Few species had a strong positive relationship with UVA. Those that did correlate positively with the UVA axis (e.g., *Navicula* sp., *Nitzchia linearis, and Fragilaria crotonensis*) showed constant biomass across all radiation levels, rather than increases in the presence of UVA.

Algal pigment composition

In Leach, Honeymoon, and Saturday Night Lakes, there was an overall trend for algal carotenoid and chlorophyll concentrations to decrease over time, while in Hibernia Lake concentrations increased (Figures 3.10 and 3.11, Tables 3.6 and 3.7). In the carotenoids, within-lake responses to UVR manipulations were similar across all pigment types (Figure 3.10). In Leach Lake, removing both UVA and UVB radiation led to significant decreases in carotenoid concentrations (MANOVA and individual ANOVA tests; Figure 3.10, Table 3.6). In Honeymoon Lake, carotenoids were again reduced in the presence of UVR, although there was a strong interaction with time, caused by a dip in carotenoid concentrations under the PAR treatment on the second sampling occasion (Figure 3.10, Table 3.6). Although individual contrasts suggest that carotenoid concentrations tended to be higher in the PAR treatment, this effect was non-significant at an adjusted p-value (Figure 3.10, Table 3.6). In Hibernia Lake, the effect of light treatment on carotenoid concentrations showed no significant, common pattern

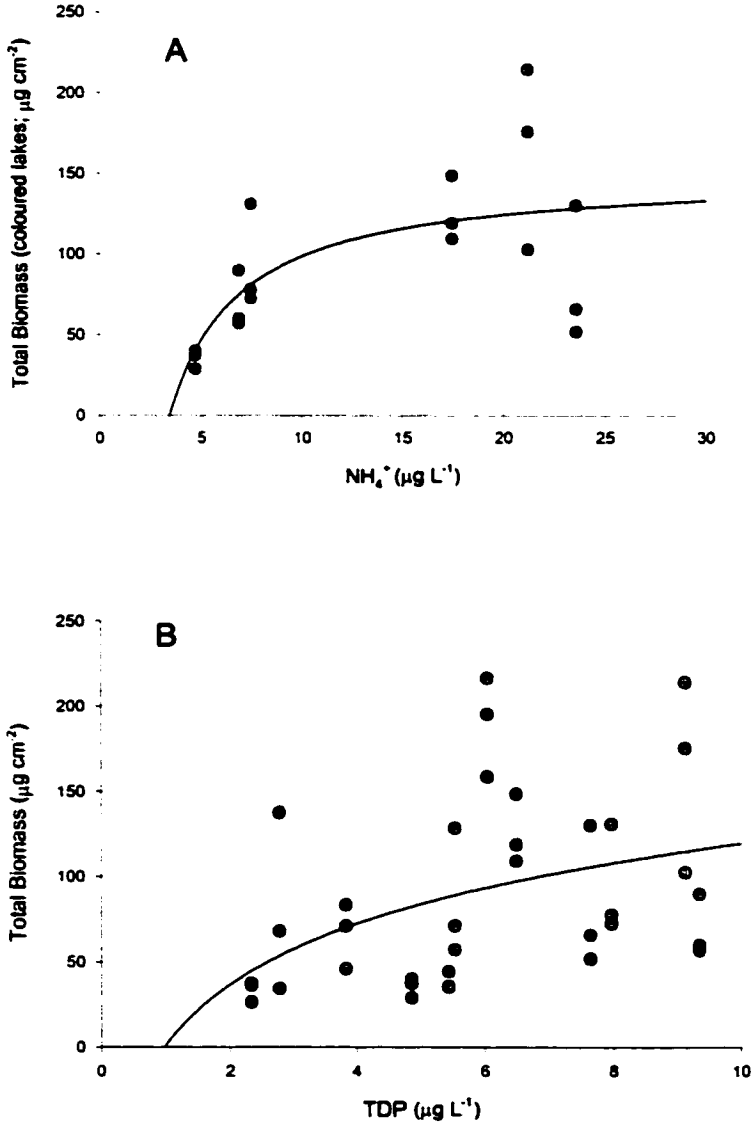


Figure 3.7: Relationship between total biomass and dissolved nitrogen and phosphorus concentrations in four study lakes in Jasper National Park, Alberta.
(A) $Biomass_{(coloured\ lakes)} = 149.97 - (515.348/NH_4^+)$ r^2=0.43, p=0.0031.
(B) Biomass = 0.952 + 51.5518log(TDP), r^2= 0.165, p=0.0141.

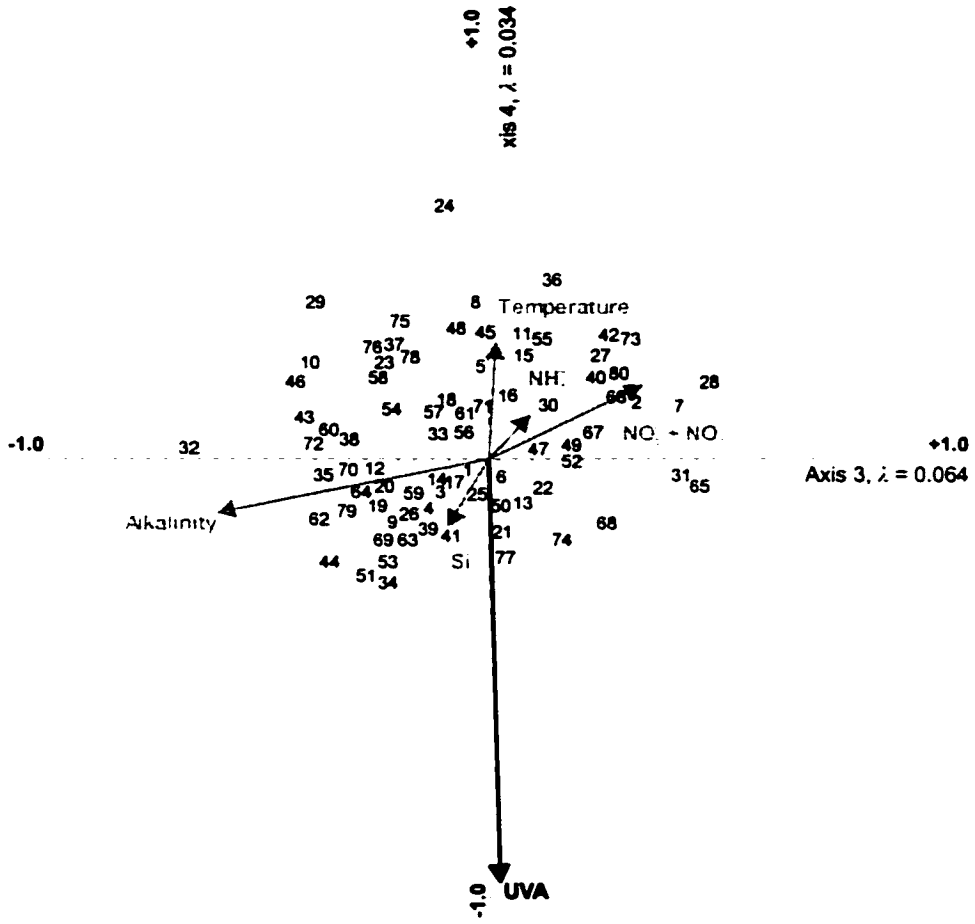


Figure 3.8: Correlation biplot of third and fourth ordination axes for algal species and environmental variables for four lakes in Jasper National Park, Alberta. Numbers corresponding to algal taxa are as in Figure 3.5.

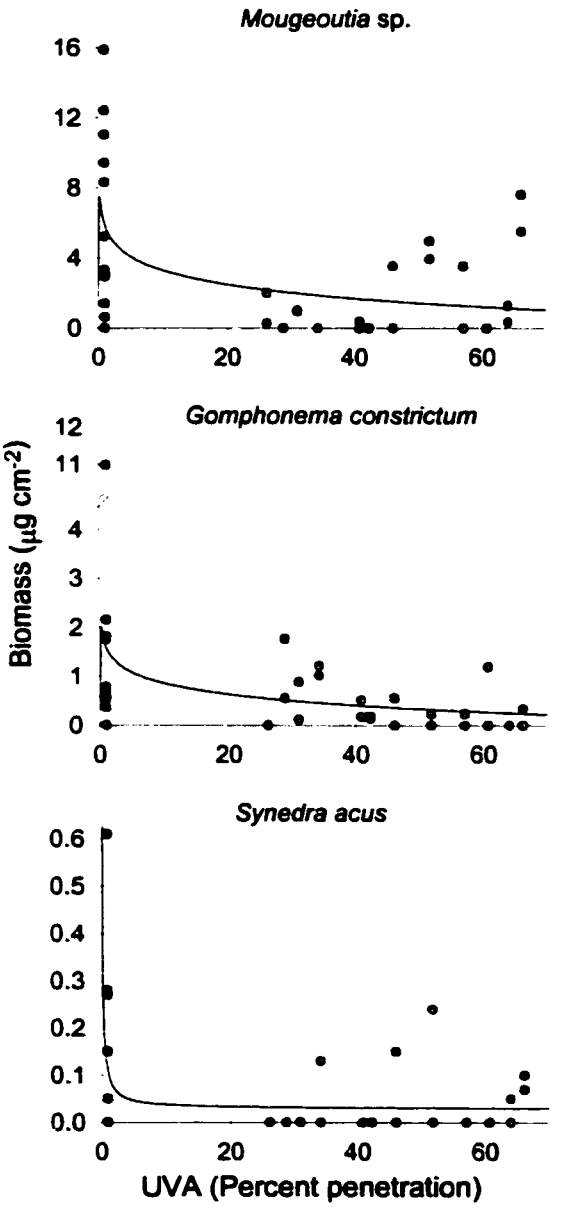


Figure 3.9: Negative relationship between *Mougeotia* sp. (Biomass = 5.928 – 1.151lnUVA, r^2=0.269, p=0.001), *Gomphonema constrictum* (Biomass = 1.59 – 0.324lnUVA, r^2=0.100, p=0.058), and *Synedra acus* (Biomass = 0.0289 + (0.0841/UVA), r^2=0.100, p=0.060) and UVA radiation in four study lakes in Jasper National Park, Alberta.

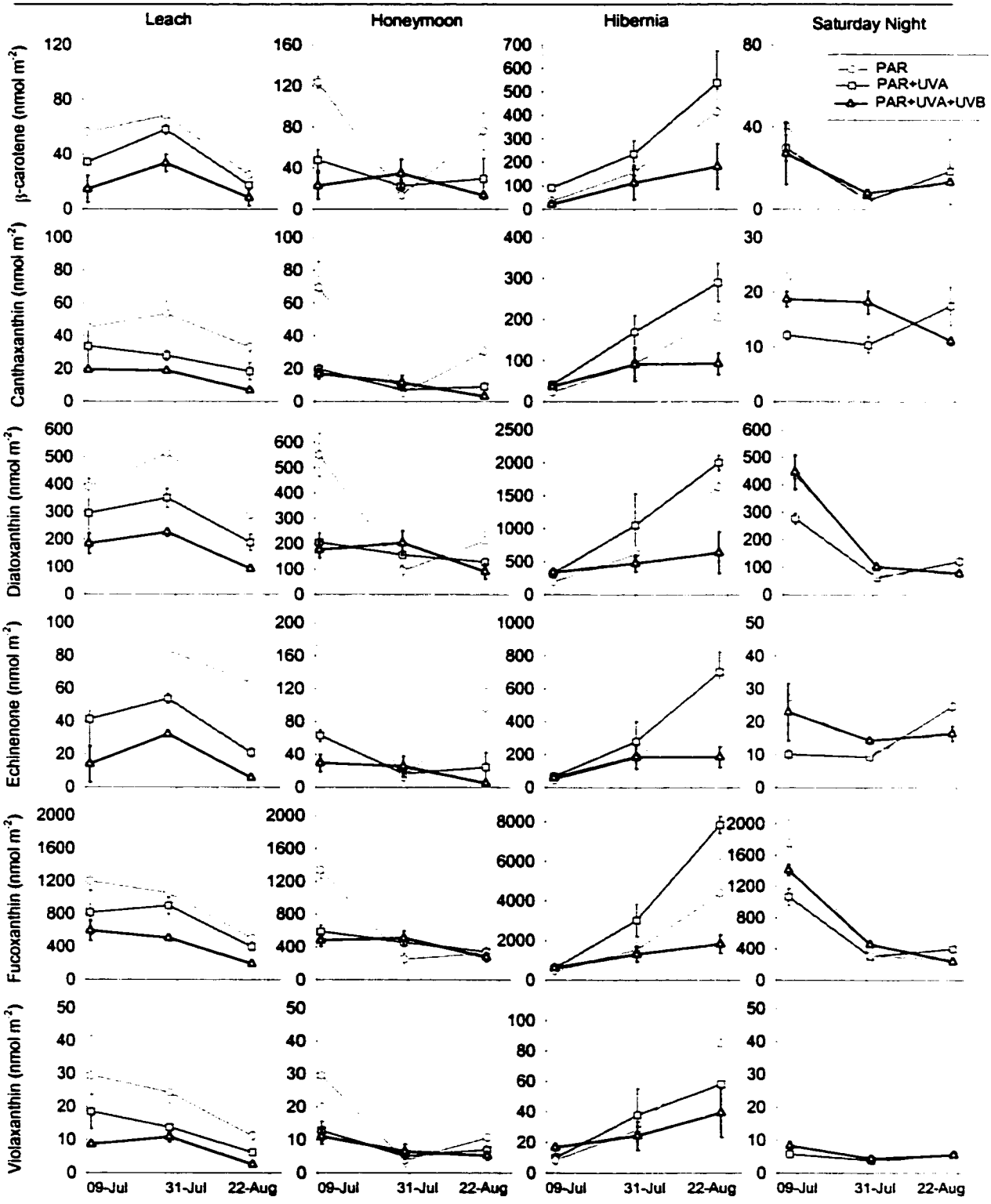


Figure 3.10: Mean molar concentrations (mean ± 1 standard error, $n = 2$) of carotenoid pigments under 3 different UVR regimes, in four study lakes in Jasper National Park, Alberta.

Table 3.6: Multivariate and two-way ANOVA results for the effects of ultraviolet radiation (UVR) on log-transformed taxa-specific carotenoid concentrations in four study lakes in Jasper National Park, Alberta. Reported are degrees of freedom, F statistics and p-values for $n = 2$ replicates. Results significant at adjusted p-value of 0.0102 for two-way ANOVAs, 0.0170 for MANOVA contrasts, and a p-value of 0.05 for MANOVA are highlighted in bold. The Hotelling-Lawley F statistic is used in the MANOVA test.

		Lake							
		Leach		Honeymoon		Hibernia		Saturday Night	
	df	F	p	F	p	F	p	F	p
Canthaxanthin									
UVR	2	27.8922	**0.0001**	9.1668	**0.0067**	5.7092	0.0251	1.3673	0.3030
Time	2	11.3603	**0.0035**	20.7242	**0.0004**	34.0545	**<.0001**	5.0011	0.0346
UVR x Time	4	1.1668	0.3871	8.0122	**0.0049**	1.9631	0.1841	6.4897	**0.0097**
Diatoxanthin									
UVR	2	18.9139	**0.0006**	3.4581	0.0769	4.0171	0.0566	1.2802	0.3241
Time	2	11.3998	**0.0034**	11.0025	**0.0038**	20.5628	**0.0004**	115.5023	**<.0001**
UVR x Time	4	0.3171	0.8596	7.2379	**0.0068**	2.3528	0.1318	4.2776	0.0327
Echinenone									
UVR	2	14.4926	**0.0015**	7.8671	0.0106	4.4139	0.0461	1.0139	0.4008
Time	2	4.8415	0.0374	9.6890	**0.0057**	44.8175	**<.0001**	3.3348	0.0825
UVR x Time	4	0.9827	0.4636	4.6131	**0.0266**	2.4871	0.1179	1.7754	0.2178
Fucoxanthin									
UVR	2	12.9430	**0.0022**	0.7158	0.5146	8.6950	**0.0079**	0.1445	0.8674
Time	2	19.4526	**0.0005**	19.6427	**0.0005**	53.4082	**<.0001**	171.3765	**<.0001**
UVR x Time	4	0.4168	0.7928	7.6672	**0.0057**	3.0310	0.0771	7.6058	**0.0058**
Violaxanthin									
UVR	2	24.9126	**0.0002**	1.0465	0.3903	0.1112	0.8960	1.6560	0.2441
Time	2	27.4526	**0.0001**	12.5172	**0.0025**	19.8209	**0.0005**	18.2677	**0.0007**
UVR x Time	4	1.0338	0.4409	2.2566	0.1428	1.7134	0.2305	5.7184	0.0143
MANOVA									
UVR	2	6.9081	**0.0058**	3.5900	**0.0414**	2.6397	0.0911	3.4326	**0.0467**
Time	2	6.0062	**0.0090**	10.6341	**0.0013**	10.3305	**0.0015**	307.6504	**<.0001**
UVR x Time	4	0.9323	0.5674	4.7928	**0.0022**	1.0762	0.4534	5.4849	**0.0011**
MANOVA CONTRASTS									
PAR vs. PAR + UVA + UVB		>	**0.0037**	=	0.0268	NSD		=	0.0220
PAR vs. PAR + UVA		=	0.0827	=	0.0375			=	0.0935
PAR + UVA vs. PAR + UVA + UVB		=	0.0504	=	0.6428			=	0.2895

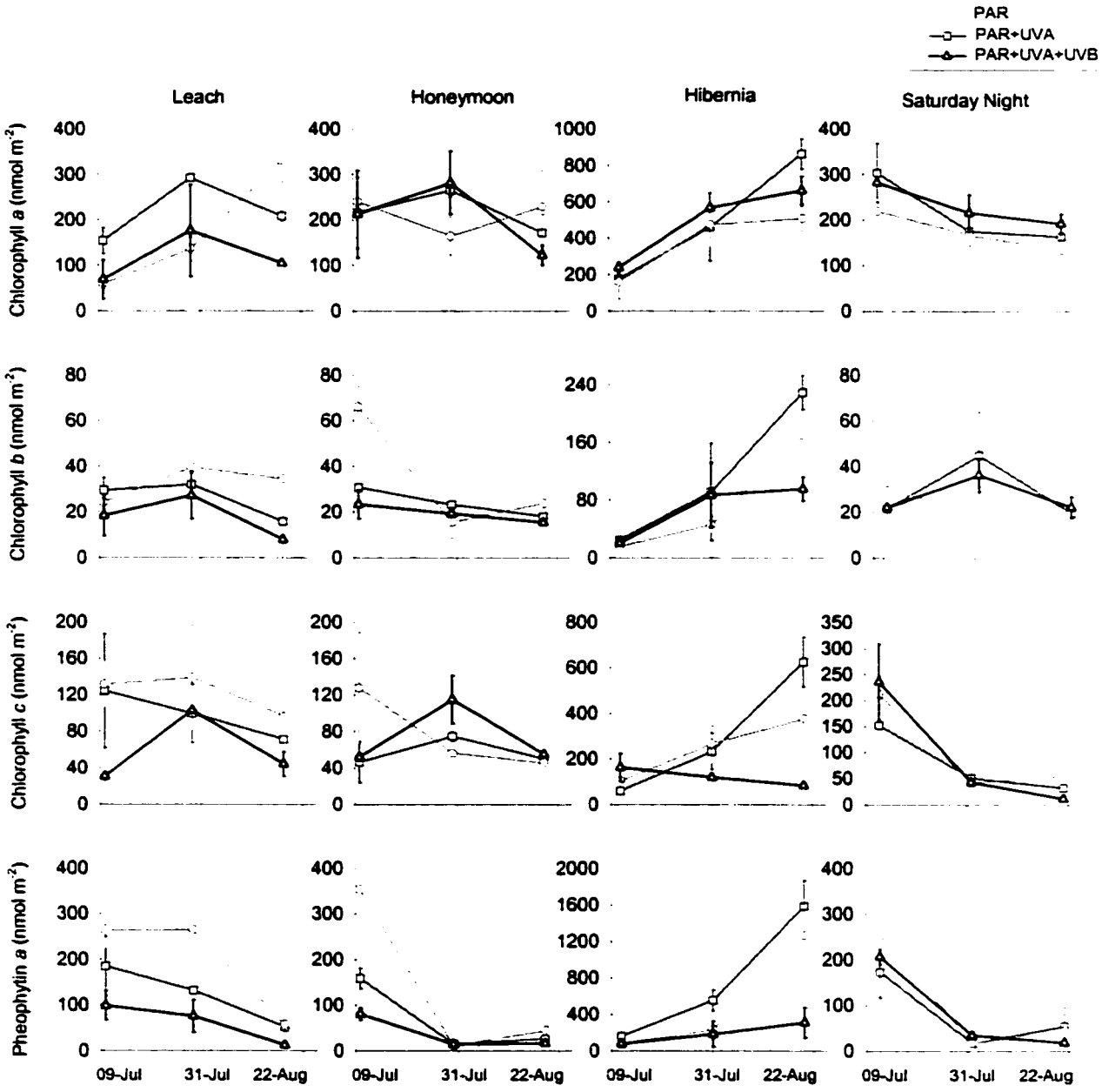


Figure 3.11: Mean molar concentrations (mean ± 1 standard error, $n = 2$) of chlorophyll pigments under 3 different UVR regimes, in four study lakes in Jasper National Park, Alberta.

Table 3.7: Multivariate and two-way ANOVA results for the effects of ultraviolet radiation (UVR) on log-transformed chlorophyll concentrations in four study lakes in Jasper National Park, Alberta. Reported are degrees of freedom, *F* statistics and p-values for $n = 2$ replicates. Results significant at adjusted p-value of 0.0127 for two-way ANOVAs, 0.0170 for MANOVA contrasts, and a p-value of 0.05 for MANOVA are highlighted in bold. The Hotelling-Lawley *F* statistic is used in the MANOVA test.

		Lake							
		Leach		Honeymoon		Hibernia		Saturday Night	
	df	*F*	p	*F*	p	*F*	p	*F*	p
Chlorophyll *a*									
UVR	2	4.0936	0.0544	0.1338	0.8722	1.1877	0.3485	2.8196	0.1120
Time	2	6.3448	0.0191	1.0725	0.3821	16.2285	**0.0010**	8.1816	**0.0094**
UVR x Time	4	1.5191	0.2761	1.1035	0.4118	0.4666	0.7593	0.2699	0.8901
Chlorophyll *b*									
UVR	2	4.7578	0.0389	3.0805	0.0957	0.7310	0.5080	0.3929	0.6861
Time	2	4.3045	0.0488	11.2952	**0.0035**	20.8492	**0.0004**	1.8706	0.2092
UVR x Time	4	0.9924	0.4592	3.9870	0.0394	0.7054	0.6079	1.2896	0.3436
Chlorophyll *c*									
UVR	2	5.3377	0.0296	0.7794	0.4873	5.4051	0.0287	2.4130	0.1449
Time	2	2.0189	0.1887	2.0326	0.1869	12.0251	**0.0029**	52.1311	**<.0001**
UVR x Time	4	1.4521	0.2941	2.4477	0.1218	8.7537	**0.0036**	4.7040	0.0252
Pheophytin *a*									
UVR	2	14.4024	**0.0016**	10.1380	**0.0050**	6.2808	0.0196	0.1328	0.8773
Time	2	24.0839	**0.0002**	131.9528	**<.0001**	15.5476	**0.0012**	24.8403	**0.0002**
UVR x Time	4	0.7963	0.5566	5.3409	0.0175	0.7953	0.5572	1.1682	0.3866
MANOVA									
UVR	2	4.6494	**0.0134**	1.9682	0.1563	2.4075	0.0969	0.7764	0.6329
Time	2	5.1601	**0.0093**	21.6247	**<.0001**	5.7314	**0.0064**	27.0423	**<.0001**
UVR x Time	4	0.5419	0.8881	1.4145	0.2378	1.7417	0.1284	1.5964	0.1690
MANOVA CONTRASTS									
PAR vs. PAR+UVA+UVB		>	**0.0142**	NSD		NSD		NSD	
PAR vs. PAR+UVA		=	0.2425						
PAR+UVA vs.		=	0.0191						

(MANOVA, Figure 3.10, Table 3.6). However, fucoxanthin concentrations were significantly higher under the PAR + UVA treatment (Figure 3.10, Table 3.5; Tukey-Kramer; PAR = PAR + UVA + UVB, p=0.4829; PAR < PAR + UVA, p=0.0450; PAR + UVA > PAR + UVA + UVB, p=0.0072). In Saturday Night Lake, MANOVA indicates a suppression of carotenoid concentrations under UVA and UVB exposure (Table 3.6). However, this trend was not reflected in individual ANOVA results (Table 3.6).

The trends in the chlorophyll pigments were much less pronounced than for the carotenoids. In Leach Lake, chlorophyll concentrations decreased significantly under UVB exposure (Figure 3.11, Table 3.7). In Honeymoon Lake, although pheophytin *a* concentrations decreased significantly under UVB exposure (Figure 3.11, Table 3.7; Tukey-Kramer; PAR > PAR + UVA + UVB, p=0.0038; PAR = PAR + UVA, p=0.1474; PAR + UVA = PAR + UVA + UVB, p=0.0903), MANOVA indicates no significant overall effect of UVR exposure on chlorophyll concentrations (Figure 3.11, Table 3.7). No other significant effects of UVR on chlorophyll concentrations were found (Figure 3.11, Table 3.7).

Algal pigment ratios

The ratio of carotenoids to chlorophyll *a* decreased significantly with UVR exposure in Honeymoon, Hibernia and Saturday Night Lakes (MANOVA; Figure 3.12, Table 3.8). This change was driven by decreases in the relative concentrations of β-carotene in Honeymoon and Hibernia Lakes, fucoxanthin in Hibernia and Saturday Night Lakes, and violaxanthin in Saturday Night Lake (Figure 3.12, Table 3.8). In Honeymoon and Saturday Night Lakes, this ratio appears to be greatest in the PAR treatment, while in Hibernia Lake, ratios in the PAR + UVA treatment are greater than in the PAR + UVA + UVB treatment (Figure 3.12, Table 3.8). Although this general trend also occurs in Leach Lake, the result is not statistically significant (Figure 3.12, Table 3.8).

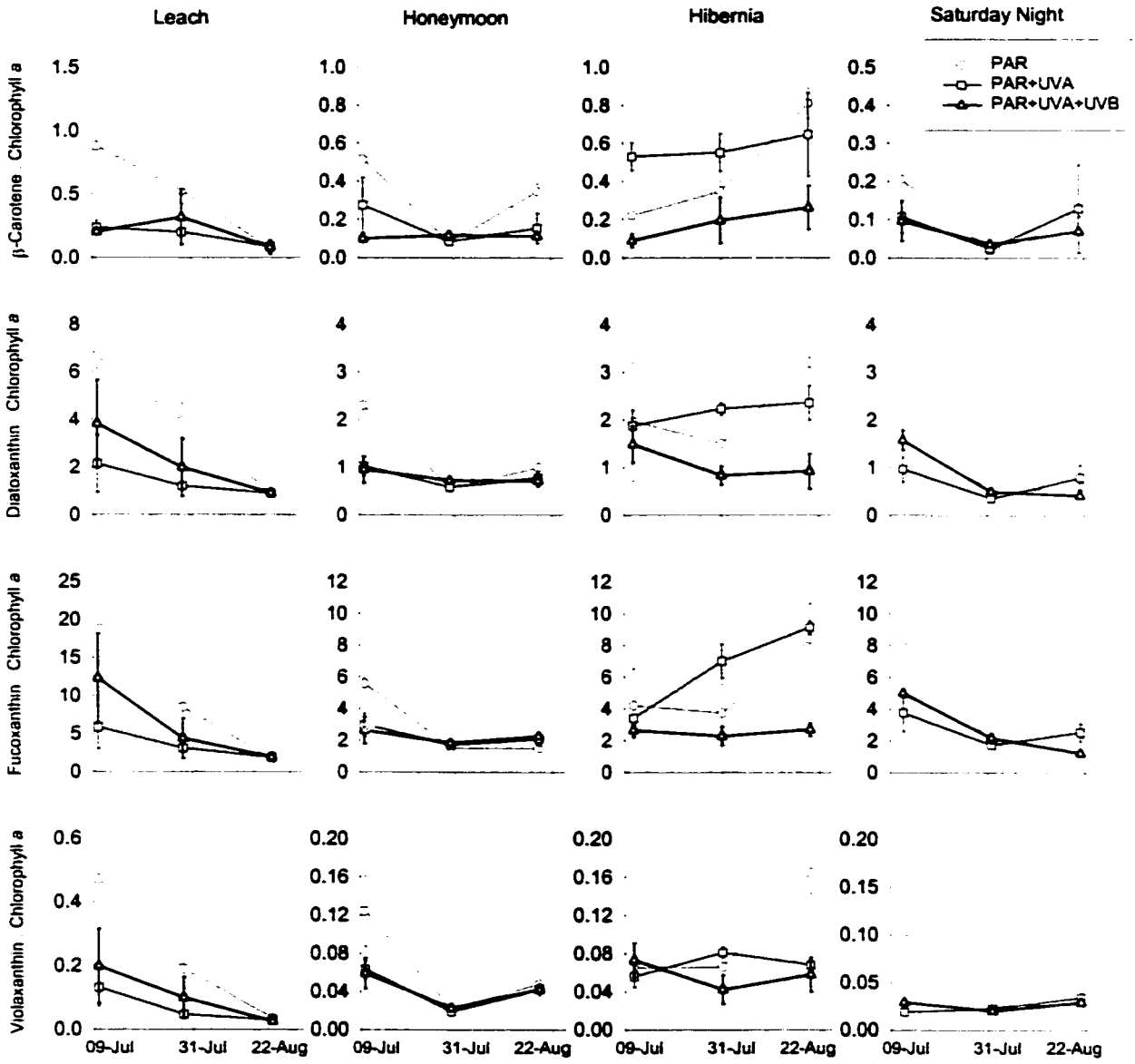


Figure 3.12: Mean molar ratio (mean ± 1 standard error, $n = 2$) of algal carotenoid to chlorophyll *a* ratios under 3 different UVR regimes, in four study lakes in Jasper National Park, Alberta.

Table 3.8: Multivariate and two-way ANOVA results for the effects of ultraviolet radiation (UVR) on chlorophyll *a*:carotenoid ratios in four study lakes in Jasper National Park, Alberta. Reported are degrees of freedom, *F* statistics and p-values for $n = 2$ replicates. Results significant at adjusted p-value of 0.0127 for two-way ANOVAs, 0.0170 for MANOVA contrasts, and a p-value of 0.05 for MANOVA are highlighted in bold. The Hotelling-Lawley *F* statistic is used in the MANOVA test.

		Lake							
		Leach		Honeymoon		Hibernia		Saturday Night	
	df	*F*	p	*F*	p	*F*	p	*F*	p
β-carotene: chlorophyll *a*									
UVR	2	1.9412	0.1991	5.7277	0.0249	11.815	**0.0030**	0.1002	0.9056
Time	2	9.6667	**0.0057**	7.1201	0.0140	4.1208	0.0536	4.2555	0.0500
UVR x Time	4	0.7166	0.6014	3.1793	0.0690	0.7901	0.5600	0.3043	0.8680
Diatoxanthin: chlorophyll *a*									
UVR	2	1.7997	0.2200	1.9317	0.2004	3.1069	0.0942	1.5976	0.2548
Time	2	7.7822	**0.0109**	12.2024	**0.0027**	0.2892	0.7555	40.1552	**<.0001**
UVR x Time	4	0.7207	0.5991	2.2625	0.1421	0.9416	0.4827	4.3779	0.0307
Fucoxanthin: chlorophyll *a*									
UVR	2	0.4818	0.6327	0.1637	0.8514	3.9110	0.0599	6.5149	**0.0039**
Time	2	17.6206	**0.0008**	13.0542	**0.0022**	1.6219	0.2503	20.4460	**<.0001**
UVR x Time	4	1.2315	0.3635	3.2820	0.0640	0.5933	0.6764	7.5483	**<.0001**
Violaxanthin: chlorophyll *a*									
UVR	2	4.2191	0.0510	0.2496	0.7843	1.5363	0.2667	9.2998	**0.0065**
Time	2	30.1019	**0.0001**	26.4795	**0.0002**	0.9409	0.4255	10.0231	**0.0051**
UVR x Time	4	0.6691	0.6295	0.4597	0.7639	1.3523	0.3235	10.8546	**0.0017**
MANOVA									
UVR	2	2.5385	0.0845	3.1236	**0.0476**	4.1004	**0.0205**	6.5149	**0.0039**
Time	2	8.3637	**0.0015**	6.3882	**0.0042**	2.6564	0.0750	20.4460	**<.0001**
UVR x Time	4	1.6127	0.1638	2.3423	**0.0423**	0.9100	0.5720	7.5483	**<.0001**
MANOVA CONTRASTS									
PAR vs. PAR + UVA + UVB		NSD		=	0.0292	=	0.1328	=	0.0255
PAR vs. PAR + UVA				=	0.0364	=	0.1271	>	**0.0060**
PAR + UVA vs. PAR + UVA + UVB				=	0.9459	>	**0.0097**	=	0.0267

DISCUSSION

The role of UVR in structuring epilithic communities

Our results suggest that UVR may not be of primary importance in controlling algal taxonomy, total biomass, and distribution in the epilithon of montane lakes. Our analysis of the partitioning of major taxonomic groups under differential UVR exposure revealed no consistent taxonomic shifts. This contrasts with several other benthic studies, which have found the proportion of diatoms to decrease in the presence of UVR (Vinebrooke and Leavitt 1996, Francoeur and Lowe 1998, Vinebrooke and Leavitt 1999). Decreased diatom abundance is often predicted in freshwater systems because of their relative inability to produce photoprotective pigments (Roy 2000, but see, e.g., Zudaire and Roy 2001), and poor capacity for repair in comparison to other groups, such as the Cyanobacteria (Quesada and Vincent 1997).

We also found no overall biomass response to differential UVR treatment in our study. Although biomass was higher under the PAR treatment in some cases (most noticeably in the two coloured lakes; Figure 3.4), this trend was not universal through time. Decreases in algal biomass with UVR exposure have often been observed in both attached and planktonic communities (Worrest et al. 1981a, Vinebrooke and Leavitt 1996, Wängberg et al. 1996, Santas et al. 1998). However, several studies have also shown UVR to be unimportant (Vinebrooke and Leavitt 1998, Kaczmarska et al. 2000, Xenopoulos 2001), or of secondary importance (Kelly 2001, Xenopolous et al. in press) in structuring these communities.

In our experimental communities, ordination analysis suggests that nutrient concentrations, and not light quality, are most important for structuring epilithic assemblages. Ordination showed nitrogen, silica and alkalinity concentrations to be important determinants of algal distribution. Further analysis by univariate regression suggests that phosphorus may also be an important predictor of algal biomass. Cyanobacteria and chlorophyte abundance was significantly negatively related to silica concentrations (Figure 3.7). These taxa were most abundant in Honeymoon Lake, where silica concentrations were lowest (Figures 3.3 and 3.5). Unlike the pattern commonly

found in eutrophied systems, silica and phosphorus concentrations were highly correlated to one another (Table 3.3). Despite the fact that it was not included in our ordination, TDP, rather than Si, best described fluctuations in total biomass between sites (Figure 3.7). Biomass measurements were also highly related to nitrogen concentrations in our coloured lakes (Figure 3.7). However, *Epithemia argus*, *Rhopalodia* sp., and *Denticula elegans* showed little relationship with nitrogen concentration. Species of these genera are known to form endosymbiotic relationships with nitrogen-fixing Cyanobacteria, thus allowing them to thrive under very low nitrogen conditions (DeYoe et al. 1992, Graham and Wilcox 2000). These species were either rare or absent in Honeymoon Lake, where nitrogen concentrations were not as low as in other lakes (Figure 3.2).

Such strong correlations between nutrients and algal biomass are not unexpected in these oligotrophic systems: concentrations of both nitrogen and phosphorus were extremely low in our study lakes (Wetzel 1993). Epilithic C:P ratios $\geqslant 600$, and C:N ratios as high as 20 (Chapter 2) further suggest that algae of the epilithon are highly nutrient limited in these lakes (Hillebrand and Sommer 1999, Frost and Elser in press). In fact, it may be that nutrient limitation is the cause of the poor response to UVR in our study communities. Several studies have found the presence of nutrient stress to preclude strong inhibition by UVR in planktonic algae (Behrenfield et al. 1994, Xenopoulos et al. in press). Other benthic investigations have found the abundance of species of the Epithemiaceae, such as the endosymbiotic hosts observed in our study, to be strongly inhibited by UVR, despite otherwise weak UVR responses in the algal community as a whole (Francoeur and Lowe 1998, Watkins et al. 2001). We did not find such a response in our study. However, these species were most abundant in our more coloured lakes, where penetration of UVR to the depth of our experimental incubations may not have been substantial enough to effect such taxonomic shifts.

The epilithic algae of our study lakes did exhibit some inhibition in the presence of UVR, suggesting that light quality may indeed have represented a secondary stress in our systems. Combined exposure to both UVA and UVB decreased epilithic community diversity, as assessed by the Shannon-Wiener index. UVR-induced decreases in both richness (Vinebrooke and Leavitt 1996), and diversity (Santas et al. 1998) in benthic

algae have been found in other studies. In our analyses, only decreases in diversity were significant, while decreases in richness were not. In contrast to Simpson's index, which is most sensitive to changes in abundant species, the Shannon-Wiener diversity index reflects changes in rare species most strongly (Peet 1974). Thus, our results suggest that decreases in community evenness due to changes in the abundance of rare species occurred more strongly than decreases in absolute richness under UVR exposure.

Closer investigation of our ordination analysis further indicates that subtle, species-specific shifts may have occurred as a result of UVR exposure. Several species were found to have reduced biomass in the presence of UVA radiation. Of these, *Mougeotia* sp. was the most strongly inhibited. This species is traditionally expected to increase in the presence of UVR: it has been found to increase to large numbers in acidified lakes (Turner et al. 1995), in which acid-mediated bleaching of dissolved organic carbon causes substantial increases in water clarity (Schindler and Curtis 1997). Other studies, however, have found decreased *Mougeotia* sp. biomass in response to UVR exposure (Kaczmarska et al. 2000), while filamentous algae have been shown to be specifically sensitive to UVR (Xiong et al. 1996).

Although the biomass of diatoms as a whole did not decrease with exposure to UVR, both *Gomphonema constrictum* and *Synedra acus* were found to decrease with increasing UVA radiation, suggesting that certain diatoms in our study communities may in fact display increased sensitivity to UVR. Other species of these genera have been found to increase in the absence of UVR (Vinebrooke and Leavitt 1996, Francoeur and Lowe 1998). *Synedra acus* is a small-celled organism (~100 μm^3), which would traditionally be expected to decrease under UVR exposure (Garcia-Pichel 1994). Furthermore, *Gomphonema* sp. is often stalked (Graham and Wilcox 2000), while *Synedra acus* has an upright aspect. These traits are also indicative of UVR susceptibility, because of decreased shading of those species that may extend above the epilithic matrix (Vinebrooke and Leavitt 1998). In contrast, *Achnanthes minutissima* was found to have no relationship with UVA radiation, despite previous findings that this species tends to decline strongly under UVR stress (Bothwell et al. 1993, Vinebrooke and Leavitt 1996).

In our ordination analysis, neither UVB nor PAR was found to be a significant predictor of algal biomass and distribution. Although UVB is more energetic (and thus, potentially more damaging) than UVA, it may be that fluxes of UVB to the benthos were too low to be of significance, due to low solar influx, and high attenuation of UVB in the water column (Scully and Lean 1994). Several other studies have found UVA to be the major photoinhibitor of algal populations (Kelly 2001, Bothwell et al. 1993, Milot-Roy and Vincent 1994), while models of UVR-induced damage in the phytoplankton predict that UVB should be of minor importance because of these low fluxes (Cullen and Neale 1994). The exclusion of PAR from our model may simply indicate that our systems were not light limited. However, this result may also have been caused by the strong correlation between PAR and temperature, which was included in our model.

The effect of UVR on epilithic pigments

In contrast to the insignificant UVR-specific response observed for our count-based estimates of algal biomass, epilithic pigment concentrations were markedly depressed in the presence of UVR. This finding was much more striking for carotenoid concentrations than for the chlorophylls, and, as a result, carotenoid to chlorophyll *a* ratios also decreased under UVR exposure (Tables 3.5-3.7, Figures 3.10-3.12).

Although both chlorophylls and carotenoids are often used as biomass indicators (Millie et al. 1993), UVR has been shown to change the pigment content of the cell. Under intense, short-term exposure, UVR has been shown to reduce cellular algal carotenoid and chlorophyll concentrations (Döhler and Buchmann 1995, Döhler and Haas 1995, Döhler 1998). However, carotenoids are important photoprotective compounds. They act as antioxidants (Roy 2000) and, through the xanthophyll cycle (Demers et al. 1991), to minimize UVR-specific damage. Accordingly, and in contrast to our results, long-term studies generally show increases in carotenoids, or in carotenoid: chlorophyll ratios, in chronically UVR-stressed algal cells (Ben-Amotz et al. 1989, Paerl et al. 1993, Goes et al. 1994).

There are several potential explanations for the discrepancy between count- and pigment-based estimates of algal biomass, and the atypical pigment response observed in

our study. First, our pigment results could indicate biomass increases in our UVR-shielded algal communities, which have not been mirrored in our taxonomic data because of incorrect or highly variable counts. This explanation, however, seems unlikely. Such an increase in growth can not explain our observed decrease in carotenoid: chlorophyll ratios under UVR exposure. Furthermore, care was taken to only count live cells, through the use of a staining procedure that allows for verification of cells live at collection, even in the diatoms (Owen et al. 1978). Similarly, although pico-plankton sized cells are easily missed in algal counts, we have found no report of these cells in the benthos. Thus, errors due to missed or wrongly counted cells were likely rare. In fact, further investigation reveals a systematic discrepancy between count-based estimates of algal biomass and pigment concentrations, suggesting that variable taxonomic counts can also not explain our observations. Although statistical analyses are not possible on these data due to a lack of replication, carotenoid: biomass ratios are consistently highest in our UVR-shielded treatments in both Leach and Honeymoon Lakes (Figure 3.13). The trend for chlorophyll: biomass ratios, however, is much less clear.

A shift in pigment concentrations within the cell is a second possible explanation for our observations. Again, however, this seems unlikely. In chlorophytes and Cyanobacteria, carotenoid concentrations have been well documented to rise in response to acute UVR exposure (Buckley and Houghton 1976, Goes et al. 1994). Although no such response for the diatom carotenoids (fucoxanthin, diatoxanthin) has been observed, photobleaching of chlorophylls often results in increased carotenoid: chlorophyll ratios, which is likely also photoprotective (Vernet 2000). Of the pigments analysed in this study, only violaxanthin is known to decrease under UVR stress, through its participation in the xanthophyll cycle (Schubert et al. 1994). Clearly, the universal decrease of carotenoid concentrations under UVR exposure observed in our study is unexpected at the cellular level.

Finally, changes in grazing pressure under differential UVR exposure may account for our observations. When grazed, carotenoids have been shown to be much less susceptible to degradation than chlorophylls (Poister et al. 1999). Chlorophylls, conversely, have been shown to undergo extensive photobleaching once senescence

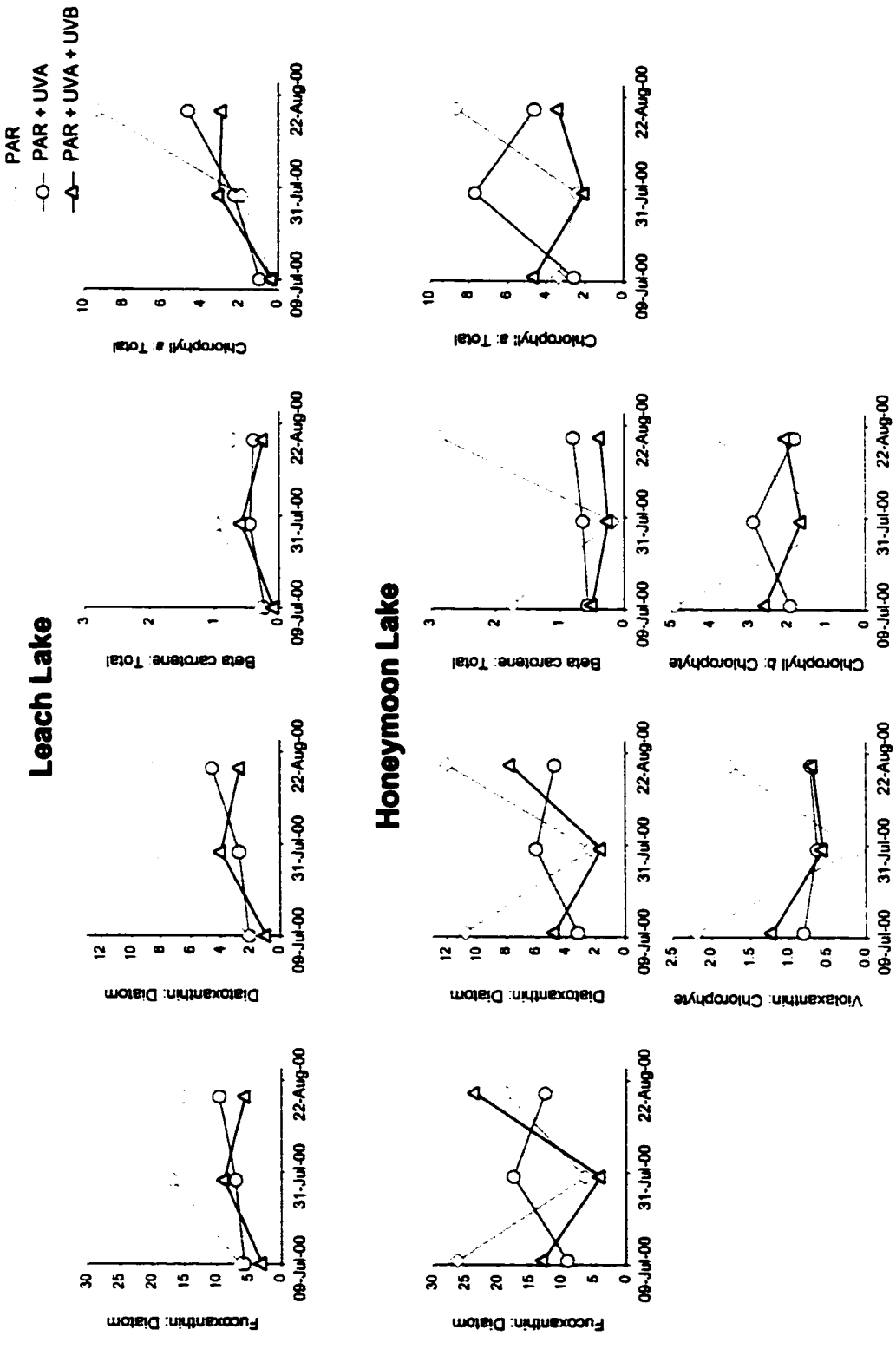


Figure 3.13: Ratios of algal pigments to their representative taxa in Leach (top row) and Honeymoon (bottom two rows) Lakes in Jasper National Park, Alberta. Ratios are nmoles of pigment per μg biomass; total = total biomass for all taxonomic groups.

occurs (Welschmeyer and Lorenzen 1985, Carpenter et al. 1986, Cuddington and Leavitt 1999). Thus, high grazing pressure would be expected to increase carotenoid: chlorophyll ratios, by maintaining relatively high carotenoid concentrations, relative to chlorophylls. Increased algal growth rates, and commensurate increases in pigment production, would be expected to be reflected most strongly in carotenoid signatures.

Such high carotenoid: chlorophyll ratios, and increased carotenoid concentrations occurred in our UVR-shielded treatments, when compared to non-shielded communities. Furthermore, high levels of both algal growth and grazing pressure may have occurred in the absence of UVR. Despite the fact that chironomid abundance was not included in our ordination as a significant descriptor of algal distribution, counts show that invertebrate numbers increased in our UVR-shielded study communities (Chapter 2). Although increases in algal growth rates are also expected to occur in the absence of UVR, this increase in grazing pressure would have dampened biomass accrual, perhaps to a point where algal biomass was not different between treatments, as observed in our study. Thus, differential pigment degradation with grazing may have masked the typical UVR-pigment response in our benthic communities.

Clearly, our study contrasts with those that have found increasing carotenoid: chlorophyll ratios under UVR exposure. Unlike our study, most have occurred in the pelagic zone, where grazed cells can quickly sediment out of the water column (Carpenter et al. 1986), or in simplified *ex-situ* benthic communities, in which the influence of grazers is negligible or absent. In *in-situ* benthic communities, however, it appears that grazing and the accumulation of detrital material can decouple measures of pigment and biomass accrual. This decoupling has been found in several non-UV benthic studies (Havens et al. 1999, Baulch 2002), further suggesting that pigment-based measures of algal biomass, and biomass-specific pigment estimations, may be inaccurate in the benthos.

In our study of four montane lakes, we found little effect of UVR on algal community composition. Species diversity, but not richness, was altered and of our eighty observed algal species, three decreased significantly under UVR exposure. Algal pigments, however, did decrease under UVR stress, indicating a decoupling between

these two metrics. Although analyses of photosynthetic pigments do suggest a certain amount of sensitivity to UVR in our benthic systems, it appears that nutrients are much more important in structuring these epilithic algal communities than are variations in light quality.

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CHAPTER FOUR: GENERAL CONCLUSIONS

The results of this study suggest that ultraviolet radiation (UVR) may be of secondary importance in structuring montane lacustrine communities. In Chapter 3, I show that variations in nutrient concentrations, and not UVR, are most important for controlling algal species distribution in my study lakes. In Chapter 2, I show that of my four experimental communities, only one displayed a strong response to UVR amendments.

Although my study is the first to show the secondary importance of UVR in montane systems, recent investigations in boreal (Watkins et al. 2001, Xenopoulos 2001), and alpine (Vinebrooke and Leavitt 1998) lakes have also found UVR to be a secondary factor in structuring algal communities. Experimental nutrient amendment studies have shown that UVR exposure is less important under conditions of nutrient stress in structuring phytoplankton communities (Behrenfield et al. 1994, Xenopoulos et al. in press). My study is the first to carry these conclusions to benthic communities, or to show that UVR can be secondary to nutrient stress using only ambient nutrient conditions.

In pelagic communities, different UVR-specific responses between lakes have been documented (Kazckmarka et al. 1999, Xenopoulos 2001). These studies suggest that inter-lake differences occur as a result of differences in exposure history between lakes. Given the similar clarity of several of my study lakes, and similar UVR exposure in my experimental communities, differences in exposure history in my study systems seem an unlikely explanation for their different UVR-specific responses.

Where epilithic communities did respond to UVR exposure, however, notable direct and indirect effects were observed. In Chapter 2, I show that UVR can directly decrease both epilithic standing crop and invertebrate colonization, and increase epilithic food quality (through decreasing carbon: nutrient ratios, and increasing dry-mass specific PUFA concentrations). I also present evidence that this increase in food quality may have implications for higher trophic levels; that is, it may increase grazer growth rates. These results contrast with the traditional prediction that UVR should decrease food

quality (Hessen et al. 1997). Decreased carbon to phosphorus ratios under UVR exposure have been reported elsewhere, as a result of decreased carbon acquisition under UVR exposure (Watkins et al. 2001, Xenopoulos et al. in press). UVR-induced shifts in food quality with respect to PUFAs and carbon to nitrogen ratios, however, have not been documented. The results of my study suggest that in the benthos, high accumulation of carbon and other detrital material may be especially important for decreasing food quality under low levels of UVR exposure.

This study further underscores the necessity of *in situ* research for understanding how stressors will affect complex ecological systems. As has been demonstrated previously, even the best-designed laboratory and mesocosm studies often miss ecosystem components that are unrecognized for their importance (Schindler 1998). This is illustrated in Chapter 3, where decreases in pigment concentrations under UVR exposure were not accompanied by commensurate shifts in the algal community. This result has not been reported previously, and is hypothesized to occur because of differential grazing pressure, caused by variations in UVR exposure, decoupling pigment and biomass accrual. Had this experiment been performed in a controlled *ex-situ* environment, where only one trophic level was investigated, both this result and the importance of food-web interactions in this system would have been missed. The results of this study also stress the importance of performing experiments in several locations. Clearly, had these studies been conducted in only one lake, spurious conclusions might have been reached about the role of UVR in montane freshwaters. Despite the apparent similarity of my study systems, results from the four lakes were widely divergent.

Future research directions

These experiments suggest several areas of future research. First, although we clearly show that UVR can increase food quality through its effect on carbon to nutrient ratios, and ratios of eicosapentanoic acid to total benthic dry weight, our feeding experiment suggests that other parameters may also be important for controlling the effect of UVR on food quality. Recently, the importance of biochemicals other than poly-unsaturated fatty acids for consumer nutrition has been suggested (von Elert and

Wolffrom 2001). Our study suggests that future research in this direction may yield important results. Tightly coupled with this is the clear need for repeated, more rigorously replicated, feeding experiments such as those performed in this study.

Second, experiments to investigate how algal pigments and biomass may decouple in benthic systems appear to be warranted. Here, I hypothesize that differential grazing pressure brought about by stresses such as UVR may be important in altering pigment signatures. A better understanding of how pigments may reflect taxonomic composition in the benthos, and what factors may alter this relationship is important both for those studies that wish to use pigments to infer taxonomic biomass (Millie et al. 1993), and to understand pigment-based physiological processes in the benthos.

Finally, these experiments emphasize that not all organisms, or environments, respond analogously to similar UVR exposure levels. In this study, I suggest that both nutrient concentrations and the resident community structure may be important in regulating these differences. Furthering our understanding of how and why different ecosystems will respond to UVR stress is crucial in understanding the magnitude of its impact in years to come.

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