DISSERTATION

THE INFLUENCE OF ULTRAVIOLET-B RADIATION ON BENTHIC COMMUNITIES IN ROCKY MOUNTAIN STREAMS WITH DIFFERENT METAL EXPOSURE HISTORIES

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY ROBERT EARL ZUELLIG ENTITLED "THE INFLUENCE OF ULTRAVIOLET-B RADIATION ON BENTHIC COMMUNITIES IN ROCKY MOUNTAIN STREAMS WITH DIFFERENT METAL EXPOSURE HISTORIES" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

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ABSTRACT OF DISSERTATION

THE INFLUENCE OF ULTRAVIOLET-B RADIATION ON BENTHIC COMMUNITIES IN ROCKY MOUNTAIN STREAMS WITH DIFFERENT METAL EXPOSURE HISTORIES

Increased levels of atmospheric CO_2 and associated global climate changes over the next 100 years are expected to have significant impacts on riparian vegetation, biogeochemical cycles and hydrologic processes in the Rocky Mountain region. While considerable research has been devoted to understanding these direct impacts of global warming, much less is known about the interactions between climate change and other forms of anthropogenic disturbance.

Contamination from historic mining operations is common in many streams in Colorado and is recognized as a major environmental problem. Pollution from historic mining operations coupled with physicochemical characteristics of Rocky Mountain streams (e.g. shallow, low dissolved organic carbon, high elevation) that increase exposure of benthic communities to UVB (280 to 320 nm) provide an opportunity to examine how UVB interacts with metals contamination to structure benthic communities. I integrated a series of UVB addition experiments

conducted in stream microcosms with a large-scale UVB removal experiment to test the hypothesis that the influence of UVB is greater on benthic communities from metal-polluted streams compared to reference streams.

Fully understanding the influence UVB radiation in aquatic systems requires estimates of UVB dose. Although radiometric equipment is available for quantifying UVB dose, it is expensive and often impractical for continuous use at remote sites. I applied polysulfone (PSF) dosimetry to measure UVB at the water surface in conjunction with estimates of UVB attenuation and stream depth measurements to determine UVB dose to benthic communities in 12 Colorado Rocky Mountain streams over a 60-day period. Average cumulative UVB intensity near the water surface ranged between 12 J/cm^2 to 66 J/cm^2, while the average amount of UVB reaching the streambed ranged between 25 -71% of UVB measured at the water surface. Nearly 80% of the variation in the amount of UVB reaching the streambed was explained by dissolved organic carbon concentration (DOC). These results indicate that some benthic communities in high elevation Rocky Mountain streams are subject to levels of UVB that are over 70% of surface measurements.

Microcosm experiments involved short-term exposure (7-10d) of natural benthic macroinvertebrate communities collected from reference and metal-contaminated sites to lamp-generated UVB. In all cases measures of abundance decreased in UVB-treated streams compared to controls. However, effects of UVB addition on the total abundance of mayflies, Heptageniidae, and *Baetis bicaudatus* was significantly greater in communities from metal-polluted sites compared to those from reference sites. The field experiment involved removing UVB for 60 days from portions of the streambed at 12 separate 1^{st} – 4^{th} order stream sites along a Zn gradient. Median Zn concentrations at these sites ranged from 5 and 530 µg/L. Results of the field experiment indicated that the removal of UVB significantly increased total abundance and the abundance of grazers, mayflies, caddisflies, Orthocladiinae midges, and the mayfly *Baetis bicaudatus* compared to controls. Although grazer abundance was significantly greater in UVB removal treatments, no treatment differences were found in algal biomass or Heptageniidae abundance. The metals gradient may have masked the chlorophyll *a* and Heptageniidae response to UVB removal, as these endpoints are influenced by metal concentration. Alternatively, increased grazing under the UVB removal treatments may have limited algae accrual. As with the microcosm experiments, effects of UVB removal were generally greater at metal-polluted sites than reference sites.

This research incorporated the first large-scale field experiment to investigate the direct influence of UVB on benthic communities. Results from both laboratory and field experiments demonstrated that benthic communities in Rocky Mountain streams were negatively influenced by UVB radiation. Benthic response in both laboratory and field experiments was comparable to other studies, but our results demonstrate that benthic communities in Colorado Rocky Mountain streams are negatively influenced by UVB radiation and that communities subjected to long-term metal exposure are more sensitive to UVB than reference communities. As a consequence, the effects of increased UVB radiation reaching the earth's surface may be more severe than previously considered in systems receiving multiple stressors.

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CHAPTER 1

ESTIMATING UVB EXPOSURE TO BENTHIC COMMUNITIES IN ROCKY MOUNTAIN STREAMS USING POLYSULFONE DOSIMETRY

ABSTRACT

Fully understanding the influence of solar ultraviolet-B (UV-B; 280 to 320 nm) radiation in aquatic systems requires estimates of UVB dose. Although radiometric equipment is available for quantifying UVB dose, it is expensive and often impractical for continuous use at remote sites. We applied polysulfone (PSF) dosimetry to measure UVB at the water surface in conjunction with estimates of UVB attenuation and stream depth measurements to determine UVB dose to benthic communities in 12 Colorado Rocky Mountain streams. Average daily surface measurements of UVB using PSF dosimetry were reasonably comparable to radiometric measurements taken at a permanent UVB monitoring station over the same 60-day period averaged over a 7-year period. Average cumulative UVB intensity near the water surface at each site over a 60-day period ranged between 12 J/cm^2 to 66 J/cm^2, while the average amount of UVB reaching the streambed ranged between 25 -71% of UVB measured at the water surface. Nearly 80% of the variation in the amount of UVB reaching the streambed was explained by dissolved organic carbon concentration. These results indicate that some benthic communities in high elevation Rocky Mountain streams are subject to levels of UVB that are over 70% of surface measurements. Expected changes in biogeochemical cycling, riparian vegetation, and hydrologic cycles associated with climate change may place aquatic communities of this region at risk of damaging levels of UVB exposure.

INTRODUCTION

The interest in solar ultraviolet radiation (UVR; 280 to 400nm) and its influence on aquatic ecosystems has increased tremendously over the past 15 years. Much of this interest is related to the deleterious effects of increased UVB radiation (UV-B; 280 to 320 nm) resulting from stratospheric ozone depletion. Dramatic changes in primary production, community composition, and trophic structure have been reported following exposure to background levels of UVB (Smith et al. 1992; Vincent and Roy 1993; Bothwell et al. 1994; Williamson 1995; Kiffney et al. 1997). Further understanding the influence of UVB radiation on stream benthic communities will require integrated measures of UVB exposure that are comparable to other studies. Most researchers typically use either radiometric techniques to estimate instantaneous exposure or time-integrated measurements (dosimetry) to estimate UVB dose. Integrated measurements are arguably more environmentally relevant than instantaneous measurements because they actually quantify UVB dose that can be used to compare across studies. Instantaneous radiometric measurements can be accumulated over time to give an integrated measurement of dose; however, these methods are generally impractical and cost-prohibitive for large-scale field studies, especially when monitoring several sites simultaneously. Additionally, radiometers require a power source and are not readily portable, making them impractical for continuous use at remote field sites. Reliable fixed station radiometers are available but are expensive to operate and maintain especially for large-scale field studies.

Several dosimetry methods for estimating UVB dose have been developed over the last 30 years. Relatively recently, Geographic Information Systems models are used to incorporate fixed station UVB data to estimate dose at point locations away from the fixed station radiometers (Diamond et al 2005). Other systems rely on a biological response in bacteria (Karentz and Lutze 1990), solutions with DNA molecules (Boelen et al. 1999), or on a chemical response in polymer films such as polysulfone (Davis et al. 1976). Polysulfone (PSF) dosimetry has been employed in a wide variety of UVB monitoring applications, including assessments of the effects of UVB exposure on human skin (Webb et al. 1990), plants (Parisi and Wong 1994) and shallow marine environments (Dunne 1999). Researchers generally agree that PSF dosimetry is a reliable method for estimating UVB dose (Davis et al. 1976, Kollias et al. 1988, Diffey 1989, Krines 2001, Kollias et al. 2003). Polysulfone dosimetry has several advantages over instantaneous measurements as it is relatively inexpensive, integrates exposure over time, and can be used at multiple locations simultaneously. In this paper, we apply this relatively well-developed low-cost method in conjunction with direct UVB vertical attenuation measurements to estimate UVB dose to benthic communities in 12 Rocky Mountain streams. Additionally, we quantify the influence of dissolved organic carbon (DOC) and water depth on UVB attenuation in these systems.

METHODS

PSF Calibration

Generally, the methods described in Diffey (1989) were used to estimate UVB dose in our study. First, we developed a relationship between changes in absorbance of the PSF and UVB after exposure to ambient sunlight. Commercially available PSF film (100 μm thickness) obtained from Westlake Plastics Company (Lenni, PA, USA) was cut into 1 cm x 6 cm strips that fit snugly into a 1 cm x 1 cm x 5 cm quartz cuvette. Paired (exposed and control) PSF dosimeters were deployed on a flat, white surface parallel to the ground between 10:00 am and 2:00 pm mountain standard time during full sun conditions. Exposures at increasingly long intervals from 50 min to 8.5 hrs were made as changes in absorbance decrease with exposure time. Exposures longer than the four-hour window (10:00 am to 2:00 pm) were made on consecutive days. During exposures, dosimeters were covered by Teflon™ filters (1 mm x 2 cm x 6 cm) for cosine correction. Cosine correction of the Teflon™ filter adjusts for errors due to daily changes in the angle of the sun and resulting solar spectrum. Control dosimeters were placed on the same surface but placed in a paper envelope to prevent exposure to UVB radiation. Concurrent UVB radiation intensities were measured with a calibrated radiometer (International Light model 1400, Newburyport, Massachusetts) using a broadband UV detector (SUD 240) fitted with cosine correction quartz and Teflon™ diffusers (W#8186 and T#17733) and a UV-B-1 filter (#24246; detector range: 265-332 nm, peak at 300 nm). Readings were logged to a personal computer every 0.5 seconds and processed using Virtual Light software (LabView 4 Run-Time Version 4.1, International Light, National Instruments). Control and exposed dosimeters were placed in quartz cuvettes and the change in absorbance at 330 nm was measured on a UV-visible spectrophotometer (Shimadzu UV160U, Tokyo, Japan). The difference in absorbance between control and exposed dosimeters (ΔA_{330nm}) was regressed against the natural log of corresponding time-integrated UVB readings from the radiometer. The resulting regression equation was used to estimate UVB dose based on changes in PSF absorbance.

Maximal Deployment Period of PSF Dosimeters

We determined that the maximum absorbance of our PSF dosimeters at 330 nm was 2.5 absorbance units. Because we were estimating UVB simultaneously at several sites over a large geographic region, it was necessary to determine the maximum deployment period for the PSF so they could be changed out before maximal absorbance was reached. To determine the maximal deployment period, we placed PSF strips covered with Teflon™ filters (1 mm thickness) at three field sites located in the Snowy Range Mountains of Wyoming. Elevations at these sites ranged between 2256 m and 3078 m above sea level. Absorbance at 330 nm was measured at three time intervals over a seven-day period. The number of days until maximum absorbance was reached was determined by plotting the change in absorbance units by exposure duration.

PSF Dosimetry at Field Sites

To measure UVB at the water surface, three paired (control and exposed) PSF dosimeters were deployed in 12 1st to 4th order streams (2535-3304 masl) in the Rocky Mountains of Colorado (Table 1). Polysulfone dosimeters were attached to a section of plastic pipe 60 cm above the surface of the water. The plastic pipe was anchored to the stream bottom using a concrete block surrounded by stream cobble. Control dosimeters were stored in a labeled manila envelope housed in a plastic bag to protect it from light exposure and moisture. The exposed dosimeter was placed on top of the plastic bag and covered with a piece of 1 mm thick Teflon™ (2 x 6 cm) for cosine correction and anchored to the top of the plastic pipe. Paired dosimeters were collected and a new set deployed every 5-6 days from 28 July 2003 to 26 September 2003. Additionally, three corresponding stream depth measurements ($\pm$1 cm) were taken at fixed points adjacent to each set of dosimeters when they were collected. Dosimeter absorbance at 330 nm was measured as described above and the equation generated from the calibration data was used to estimate UVB dose at the water surface. Additionally, we compared our estimates of UVB reaching the water surface to observed intensities at a permanent monitoring station operated by the United States Department of Agriculture (see http://UV-B.nrel.colostate.edu/UV-B/home_page.html, Bigelow et al. 1998) located at Steamboat Springs, Colorado (N 40° 27' 01", W 106° 43' 48", 3220 masl). The Mean UVB dose value used for this comparison was averaged over the same 60-day period from data collected at the USDA site.

It should be noted that Dunne (1999) developed the methodology applied in this paper to use PSF dosimetry in underwater environments. We chose not to deploy PSF below the surface because of concerns about light attenuation by algal growth on the dosimeters, equipment loss due to swift currents in high gradient reaches typical of Rocky Mountain streams, and because we could not visit each site more frequently than every 5-6 days. For these reasons we integrated PSF dosimetry with UVB attenuation in water to estimate dose to benthic organisms.

Integrating Attenuation Coefficients and Estimating UVB to the Streambed

Ultraviolet-B attenuation coefficients were calculated on three occasions over the sampling period for each stream reach using a hand held radiometer (International Light model 1400, Newburyport, Massachusetts) equipped with a broadband UV detector (SUD 240) fitted with cosine correction quartz (W#8186 and T#17733) and a UV-B-1 filter (#24246; detector range: 265-332 nm). Ultraviolet-B attenuation was measured in the deepest flowing water (at least 40-cm) adjacent to the paired dosimeters at each site. On each occasion, three UVB measurements were taken every 2 cm starting at the water surface and ending at the streambed between 10:00 am and 2:00 pm in full sun conditions. The downward irradiance of UVB intensity at a given depth was calculated for each stream reach using the following equation (Kirk 1994):

$$\ln E_z = \ln E_0 - K_d (z)$$

where z is stream depth, E_z is the UVB intensity at z, E_0 is the UVB intensity at the surface, and K_d is the vertical attenuation coefficient for UV-B. Based on this relationship, the vertical attenuation coefficient ($-K_d$) is determined as the slope of the relationship between ln UVB intensity in water and depth. K_d can then be used to estimate the percent of UVB measured at the surface with PSF dosimetry that is reaching the streambed at each stream reach.

Dissolved Organic Carbon and UVB at the streambed

We measured dissolved organic carbon (DOC) concentrations on four occasions that bracketed the study period (30 June, 5 August, 6 September, and 10 October) to determine how DOC concentrations were related to the amount of UVB reaching the streambed in these systems. Dissolved organic carbon (DOC) is defined as the portion of organic C that can pass through a 0.7 μm filter (Kaplan 1994) and usually comprises approximately half of total DOM. During each visit, one water sample was collected directly from each stream near the paired dosimeters using a 60 mL syringe fitted with a collection tube and 0.7 μm pore size glass fiber filter. Water samples were filtered in the field and preserved with hydrochloric acid at a pH of 2.0 in airtight amber glass vials with Teflon™ septa and stored at 4°C. Preserved water samples were analyzed for DOC concentration using a Shimadzu Model 5050A Total Organic Carbon Analyzer.

Quality assurance and quality control (QA/QC) measures included field blanks (-0.08 –0.700 mg/L), field replicates, laboratory spikes (90-100%), acid blanks, nanopure blanks and field blanks (± 0.316 mg/L), internal control solutions, and external control solutions. Less than 5% of all QA/QC values fell outside these bounds. Details of QA/QC procedures are described in Prusha (2002). Dissolved organic carbon values from periods that deviated strongly from base flow conditions (e.g., DOC samples collected within a 24 hour period after a rainfall event) where not used to calculate the relationship between DOC and the percent UVB reaching the streambed. As a result, three out of 48 samples were discarded for this analysis.

RESULTS

Results of our initial dosimeter calibration showed a strong relationship between PSF ΔA_{330nm} and UVB dose (Fig. 1). Additional dose estimates calculated from the calibration equation when compared with concurrent radiometric measurements at the same location were within 10 % of the radiometer values. Our PSF dosimeters reached their maximal ΔA_{330nm} after 7 days so we determined 5 to 6 days to be a conservative deployment period. The use of thicker Teflon™ for cosine correction could extend the deployment period but would require PSF calibration with Teflon™ of the same thickness. The manufacturing of PSF polymers is not standardized for this scientific use so the calibration equation used in this study would likely change among PSF production runs or among manufacturers with slightly different formulations. Thus, each new lot of PSF likely requires the development of a new calibration equation. Developing the calibration curve took approximately 30 days because the PSF was exposed between 10:00 am and 2:00 pm during full sun conditions. The calibration process may take longer in areas that have constant cloudy conditions.

Average surface levels of UVB estimated at our 12 fi eld sites ranged from 12 J/cm^2 to 66 J/cm^2 (Fig. 2). Although surface estimates of UVB were higher than UVB reaching the streambed (6 J/cm^2 to 29 J/cm^2), benthic communities were still exposed to relatively high levels of UVB in many streams. Between 25 and 71% of UVB measured at the surface reached the streambed (Fig. 2). The amount of UVB attenuated in these streams was closely related to the concentration of DOC, explaining 78% of the variation in the amount of surface UVB reaching the streambed (Fig. 3).

Average values of UVB intensities measured by PSF dosimetry over the 60-day period were relatively comparable to intensities measured at the USDA permanent UVB monitoring station in Steamboat Springs, Colorado (Fig. 2). At most sites, UVB estimates were within the error of measurement at the USDA monitoring station and the variability measured at each stream site. Average UVB values at West Tennessee Creek, the Arkansas River, the East Fork of the Arkansas River, and at 10 Mile Creek were over twice as high as those measured at Steamboat. Interestingly, these four sites are geographically close and receive full sun for the majority of the day. These four sites are at lower elevations than the steamboat station but in general the surrounding areas also receive less rainfall (see http://www.ocs.orst.edu/prism/). Although not measured in this study, it is likely that increased cloud cover associated with increased regional precipitation at the steamboat site explains some of the difference between the monitoring station and PSF measurements.

DISCUSSION

We integrated PSF dosimetry with estimates of UVB attenuation in water to determine UVB streambed exposure for 12 Rocky Mountain streams over a 60-day period. Our estimates of UVB at the water surface were mostly comparable to USDA monitoring station data. In comparison to the USDA data, some sites received much less UV-B, whereas others received over double (Fig 2). Many factors can significantly influence UVB exposure at a specific location. These include changes in cloud cover, the presence of UVB absorbing particles and aerosols, elevation, nearby landscape features, and canopy cover, of which some can change exposure by as much as 80% (Tsay and Stammnes 1992, Parsi and Wong 1994, Blumthaler et al. 1997, Diamond et al. 2005). Deploying PSF dosimeters simultaneously incorporated these local characteristics that influenced UVB exposure at our sites. Taking into consideration the many sources of error and the inherent variability associated with estimating UVB exposure, our estimates seem reasonable in comparison with the Steamboat data and are certainly realistic relative across the sites studied herein. Along the gradient of surface level exposure, sites with the highest estimates received full sun for the majority of the day whereas sites with the lowest estimates were partially shaded by either large landscape features or vegetative canopy.

Percent UVB estimated at the streambed was variable across sites. Typically, small, high elevation streams in the Rocky Mountains are relatively shallow and clear. Despite shallow depths and optical clarity, UVB reaching the streambed was sometimes less than 30% at some sites (Figs. 2). These differences suggest surface measurements of UVB alone are not appropriate for estimating UVB dose to benthic organisms in shallow, optically clear, Rocky Mountain streams. Additionally, these results indicate that some benthic communities in typical high elevation Rocky Mountain streams are at risk of receiving high levels of UV-B. Some benthic communities were likely exposed to over 70% of UVB that was reaching the water surface at some sites (Fig. 3).

Dissolved organic matter (DOM) absorbs UVB radiation and its attenuation of UVB can be the main determinant of UVB penetration in natural waters (Kirk 1994, Marguerite *et al.* 2001). In these systems, DOC concentration explained nearly 80% of the variation in the estimated UVB dose to benthic communities. The remaining variability is possibly due to differences in absorbance of UVB by DOM from different sources with varied histories of photooxidation (Brooks In Press) and biodegradation (Moore et al. 2003, Certini et al. 2004) prior to sample collection. Because of shallow depth, high elevation, and naturally low levels of UVB attenuating dissolved organic material (DOM), some aquatic communities in

Rocky Mountain streams are subjected to intense levels of UVB radiation. Increased levels of atmospheric CO_2 and associated global climate changes over the next 100 years are expected to have significant impacts on riparian vegetation, biogeochemical cycles, and hydrologic processes in the Rocky Mountain region (Baron et al. 2000). Changes in biogeochemical cycles and riparian vegetation will likely influence DOM quality and quantity in high elevation streams. Summer time stream flows are expected to decrease in snowmelt driven basins in response to climate change (Lettenmair and Gan 1990, Rango 1995, Baron et al. 2000). A decrease in stream flow during summer when UVB is at its highest could have substantial implications for aquatic communities in this region. This coupled with expected climate induced changes in riparian vegetation that will likely influence DOM quantity will likely further increase UVB exposure in these systems.

Our evaluation demonstrates the value of PSF dosimetry for estimating UVB dose in aquatic environments, particularly where numerous, simultaneous measurements are needed and relative dose along the gradient measured is important. When similar concerns are appropriate for other studies, the method described herein provides a reasonable integrated measure of UVB dose in streams that have varying canopy cover, cloud cover, aspect, surrounding landscape features, and concentrations of DOC. This low-cost method allows researchers to reasonably estimate UVB exposure across large geographic regions simultaneously without the need to purchase or maintain several sets of expensive radiometric equipment.

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TABLES

Table 1. Stream order, basin size, location, and elevation of the twelve stream sampling locations in the Colorado Rocky Mountains.

Site	Stream Order	Basin Size (ha)	Latitude	Longitude	Elevation (m)
Arkansas River	4	29230	N39°13'19"	W106°21'14"	2909
Buckskin Gulch	2	2131	N39°17'00"	W106°04'12"	3207
Chalk Creek	2	5055	N38°42'07"	W106°20'58"	3052
Clear Creek	3	21762	N39°45'00"	W105°39'45"	2535
East Fork Arkansas River	2	9825	N39°16'21"	W106°18'20"	3221
Four Mile Creek	1	2236	N39°12'20"	W106°06'28"	3304
French Gulch	1	2326	N39°28'50"	W106°01'04"	3011
Middle Cottonwood Creek	2	3353	N38°48'53"	W106°20'02"	3021
Mosquito Creek	3	3100	N39°16'23"	W106°06'37"	3275
Ten Mile Creek	2	8103	N39°28'48"	W106°01'07"	3050
West Clear Creek	3	6920	N39°46'21"	W105°45'38"	2901
West Tennessee Creek	2	2459	N39°20'49"	W106°19'54"	3090

FIGURES

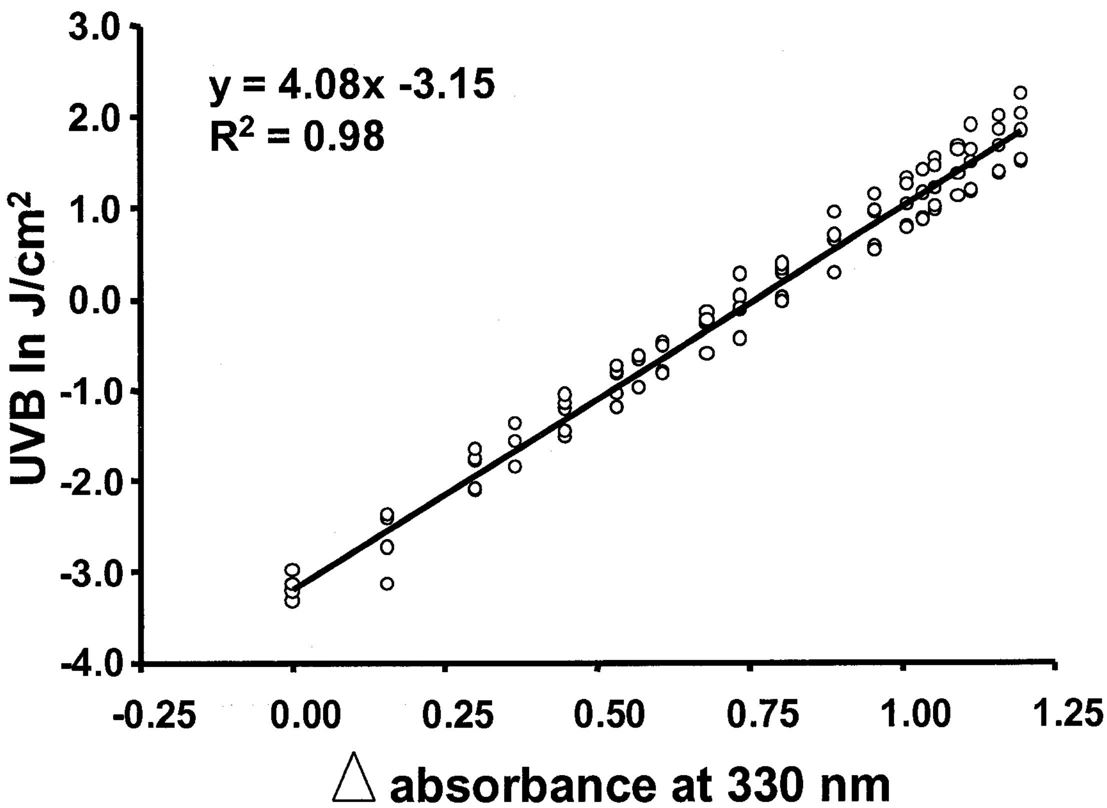

Figure 1. Regression of the natural log of J/cm^2 with PSF ΔA_{330nm}. The resulting equation y = 4.08x - 0.98 was used to convert the PSF ΔA_{330nm} to the natural log of UVB dose (J/cm^2). The solid line represents the average calibration of all trials.

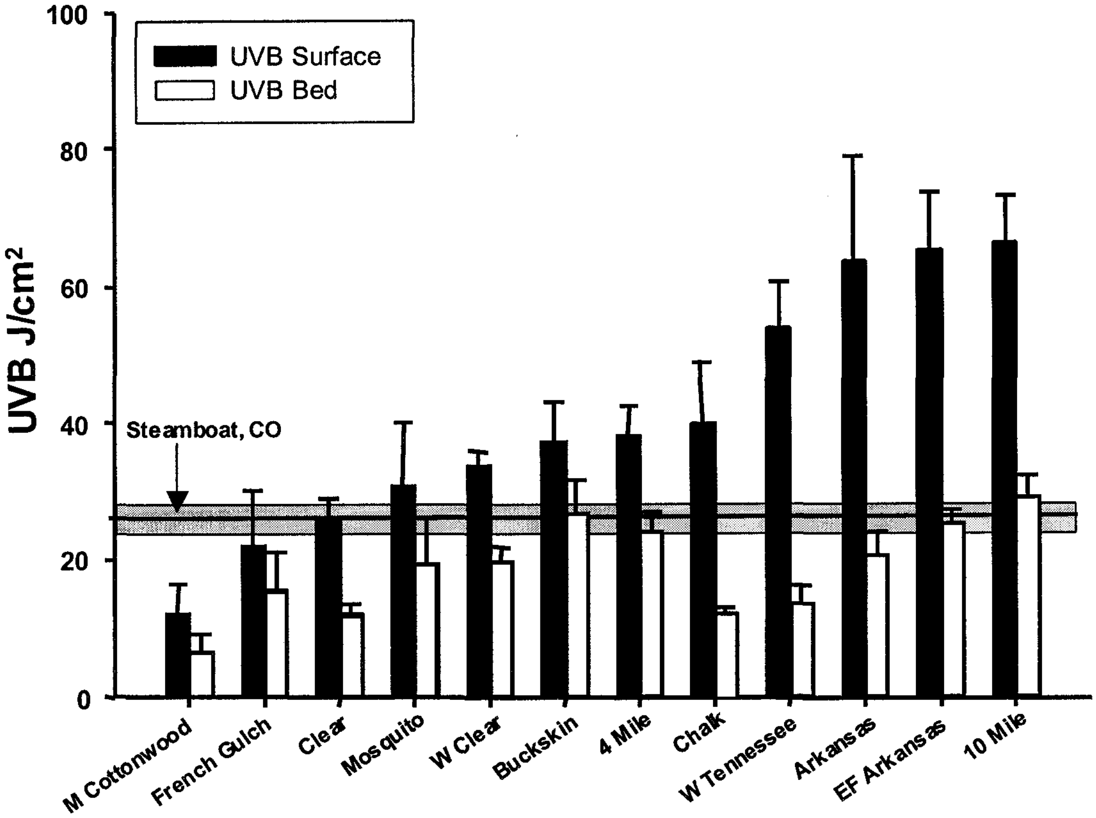

Figure 2. Mean of UVB ($J/cm^2 \pm 1$ SD) 60-day dose at the surface and at the streambed of 12 Colorado Rocky Mountain streams in comparison to USDA UVB monitoring data at Steamboat Springs, Colorado collected over the same time period. Open bars represent estimated means of UVB at the streambed. Solid bars represent means of UVB reaching the water surface at each site. The solid horizontal line represents USDA mean UVB J/cm^2 over the same 60-day period (26 July – 27 September) at the Steamboat Springs site and the gray box represents estimated measurement error. See Lantz et al. (1999) for details on measurement error at USDA monitoring sites.

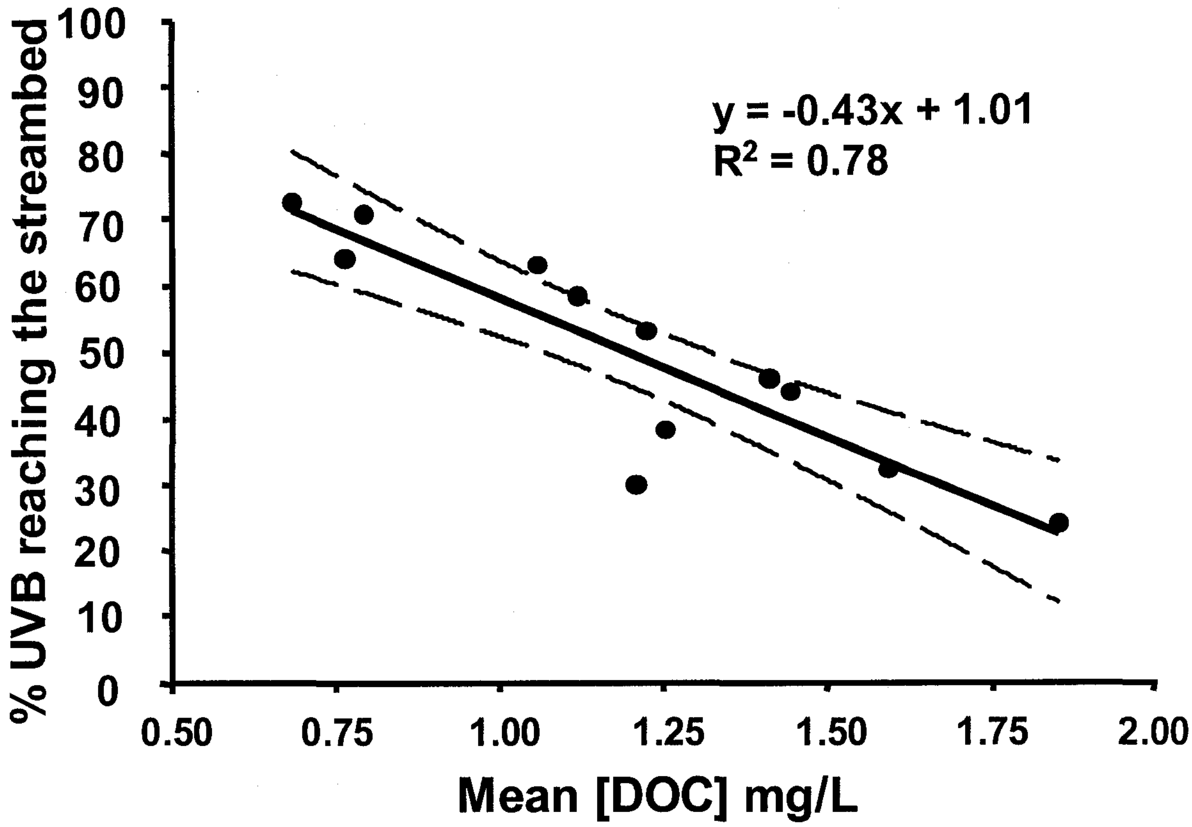

Figure 3. Relationship between percent UVB (J/cm2) reaching the streambed and mean DOC concentration from Late July to mid October 2002 for 12 Colorado Rocky Mountain streams. Dashed lines are 95% confidence intervals for the regression.

CHAPTER 2

METAL EXPOSURE HISTORY AND THE INFLUENCE OF ULTRAVIOLET-B RADIATION ON BENTHIC COMMUNITIES IN COLORADO ROCKY MOUNTAIN STREAMS

ABSTRACT

Interest in understanding the influence of ultraviolet-B (UVB [280-320 nm]) radiation in aquatic systems has accelerated since the early 1990's. Pollution from historic mining operations coupled with physicochemical characteristics of Rocky Mountain streams that increase exposure of benthic communities to UVB provide an opportunity to examine how UVB interacts with metals contamination to structure stream benthic communities. We integrated a series of UVB addition experiments conducted in stream microcosms between 1995 and 2004 with a large-scale UVB shading experiment to test the hypothesis that effects of UVB are greater on benthic communities from metal-polluted streams compared to reference streams. Microcosm experiments involved short-term exposure (7-10d) of natural benthic macroinvertebrate communities collected from reference and metal-contaminated sites to lamp-generated UVB. In all cases measures of abundance decreased in UVB-treated streams compared to controls. Moreover, effects of UVB addition were significantly greater in communities from metal-polluted sites compared to those from reference sites. The field experiment involved shading UVB for 60 days from portions of the streambed at 12 separate 1^{st} – 4^{th} order stream sites along a Zn gradient. Median Zn concentrations at these sites ranged from 5 and 530 µg/L and mean UVB reaching the streambed varied from 6.5 to 29.0 J/cm^2. Benthic communities at some sites received over 70% of UVB measured at the water surface. Results of the field experiment indicated that the removal of UVB significantly increased total benthos

abundance and the abundance of grazers, mayflies, caddisflies, Orthocladiinae midges, and the mayfly *Baetis bicaudatus*, compared to controls. Although grazer abundance was significantly greater in UVB removal treatments, no treatment differences were found in algal biomass or heptageniid mayfly abundance. Heptageniid mayflies are primarily grazers in these systems, which may be influenced by changes in algal biomass. However, the metals gradient may have masked the chlorophyll *a* and Heptageniidae response to UVB removal, as these endpoints are influenced by metal concentration. Alternatively, increased grazing under the UVB removal treatments may have limited algae accrual. As with the microcosm experiments, effect size of UVB removal was generally greater at metal-polluted sites than reference sites. We speculate that the energetic cost of regulating metals may inhibit the ability of the organism to efficiently repair damaged DNA due to UVB exposure. Our results demonstrate that benthic communities in Colorado Rocky Mountain streams are negatively influenced by UVB radiation and that communities subjected to long-term metal exposure are more sensitive to UVB than reference communities. As a consequence, the effects of increased UVB radiation reaching the earth's surface may be more severe than previously considered in systems receiving multiple stressors.

INTRODUCTION

Decreasing levels of stratospheric ozone (O_3) as a result of the discharge of chlorofluorocarbons (CFCs) have been measured for about two decades. These reduced levels of ozone have been correlated with increased levels of ultraviolet-B radiation (UVB, 280-315 nm) reaching the earth's surface (Michaels et al. 1994, Kerr and McElroy 1993). Although UVB is a small component of the sun's energy, it can have deleterious effects on aquatic communities (Smith et al. 1992, Vincent and Roy 1993, Williamson 1995, Vinebrooke and Leavitt 1999). While considerable research has been devoted to understanding the influence of ultraviolet radiation on lentic and marine ecosystems (Smith et al. 1992, Williamson 1995, Vinebrooke and Leavitt 1999), much less is known about how UVB exposure affects stream benthic communities or how UVB might interact with other forms of anthropogenic disturbance.

Experiments shading UVB from streams or artificial channels have shown substantial changes in community composition (Kiffney et al. 1997b), herbivore density (DeNicola and Hogland 1996), trophic structure (Bothwell et al. 1994), and invertebrate biomass (Kelly et al. 2003). Likewise, UVB addition experiments have reported increases in invertebrate drift (Kiffney et al. 1997b), reduced survival (McNamara and Hill 1999) and shifts in diatom community composition (Rader and Belish 1997). The concentration of dissolved organic matter (DOM) in the water column is the principle factor influencing UVB exposure in oligotrophic systems (Scully and Lean 1994, Xenopoulos and Schindler 2001). Because

Rocky Mountain streams are typically shallow, have naturally low levels of DOM, and are high in elevation, aquatic communities in open-canopied stream reaches are subjected to intense levels of UVB radiation during summer.

Rocky Mountain streams offer a unique opportunity to examine how UVB exposure might interact with additional anthropogenic stressors to influence benthic communities. Contamination from historic mining operations is common in many streams in Colorado and is recognized as a major environmental problem. Heavy metals from approximately 10,000 abandoned mines affect over 2,600 km of Colorado's high elevation streams (Colorado Department of Public Health and Environment 1992). Clements et al. (2000) estimated that approximately 25% of mountain headwater streams in Colorado are degraded by heavy metal pollution. Extrapolating the results from Clements et al. (2000) and estimates from the Colorado Department of Public Health and Environment (1992) to the entire population of headwater streams in the region, we suggest that a large number of Colorado streams are subjected to both intense UVB and heavy metal pollution. Surprisingly, there is little information on the effects of UVB in these high elevation streams and even less is known about how UVB and heavy metals might interact to cause greater or lesser than additive effects on benthic communities.

The response of Rocky Mountain benthic communities to heavy metal contamination is well documented in the literature. Changes in community composition (Clements 1994, Clements 2004), growth and secondary production (Carlisle and Clements 2003), and genetic diversity (Beaty et al. 1998) have been reported. In addition, long-term exposure to metals can increase benthic community tolerance to metals (Clements 1999, Kashian et al. in press). Despite their greater tolerance to metals, these same communities are reported to be more susceptible to other biotic and abiotic stressors, including stonefly predation (Clements 1999) acidic pH (Courtney and Clements 2000), and UVB (Kashian et al. in press), compared to communities from reference streams. Studies examining prior exposure to contaminants in fish and other invertebrates have revealed a similar connection between increased tolerance to contamination and the susceptibility of populations to additional stressors (Weis 2002 and citations within).

The extent of metal contamination in Rocky Mountain streams coupled with the susceptibility of benthic communities to high levels of UVB provides an opportunity to investigate the interactions between UVB exposure and heavy metal pollution on a large scale. We used a series of UVB addition experiments conducted in laboratory stream microcosms and a large-scale field study involving the removal of UVB in 12 Colorado streams to assess the direct effects of UVB radiation on benthic communities. We also investigated the influence of long-term exposure to heavy metal pollution on benthic community susceptibility to UVB. We predicted that communities with a long-term history of elevated metals exposure would be more susceptible to UVB than communities from reference streams.

METHODS

UVB Addition Experiments in Stream Microcosms

We assessed the effects of UVB addition on benthic communities with differing heavy metal exposure histories from Rocky Mountain streams by comparing four similar microcosm experiments conducted over a nine-year period (1995 – 2004). Natural benthic communities were colonized from polluted and reference sites and exposed to lamp-generated UVB in the laboratory (Table 1). The metal polluted sites (AR1, AR2, AR3, AR5) and one of the reference sites (West Tennessee Creek) were located in the Upper Arkansas River Basin (UAR) near Leadville, Colorado. The UAR has been the focus of research investigating the long-term influence of heavy metal exposure to benthic communities for over two decades (Roline 1988, Clements 1994, Beaty et al. 1998, Courtney and Clements 2000, Clements 2004). Historically, large amounts of heavy metals (mainly Zn) entered the UAR via the Leadville Mine Drainage Tunnel (LMDT) and from California Gulch (CG), a U. S. Environmental Protection Agency's Super Fund Site (Voynick 1984). Recently, metal concentrations entering the UAR from the LMDT and CG have significantly decreased due to remediation that began in 1992 (Clements 2004). Based on the last 16 years of field sampling at UAR sites, maximum total Zn concentrations have ranged from 425 to 8624 ug/L (Table 1). Although metals input into the UAR has been reduced, benthic communities from the UAR have likely been exposed to elevated metal concentrations for well over 125 years. West Tennessee Creek (WT) was also located in the UAR Basin, but this site does not have a history of metal contamination. Total Zn concentration at WT ranged between 5 and 32 µg/L. The other two reference sites were located approximately 161 km north and west of the Leadville sites in the Cache la Poudre River Basin (Cache la Poudre River, and Trap Creek). Concentrations of Zn at these sites were below 10 µg/L at the time of the experiments. Elevation at all sites ranged between 2320 and 3046 masl. Additional details about the UAR sites are described in Clements and Kiffney (1995) and details regarding the Cache la Poudre River site are provided in Courtney and Clements (2000).

Experimental stream microcosm system - Natural macroinvertebrate communities were collected for each experiment by methods described previously (Clements et al. 1989, Kiffney and Clements 1996). In general, colonization trays (10 x 10 x 6 cm) filled with gravel and small cobbles were anchored to the streambed at each site between mid July and early September and left to colonize for 35 to 50 days (Table 1). Benthic assemblages colonizing these trays are similar to those found on the natural substrate (Kiffney and Clements 1994, 1996, Courtney and Clements 2000). After the colonization period, groups of trays (3-4) were removed from the stream, placed in aerated coolers, and transported to the Stream Research Laboratory at Colorado State University (Fort Collins, CO) (Kiffney et al. 1997a). This facility houses 18 oval experimental streams (76 x 46 x14 cm) located in a greenhouse that filters 97% of incoming solar UV radiation (280-400 nm), while allowing the transmission of approximately 50-80% of photosynthetically active radiation (PAR) (400-500 nm;

Kiffney et al. 1997b). Water delivered to the streams is obtained directly from a nearby reservoir that has similar physicochemical characteristics as unpolluted Rocky Mountain streams (Clements 1999). Current in each stream is provided by paddlewheels and depth is regulated by standpipes that were covered during each experiment with fine mesh to prevent the loss of drifting organisms.

For each experiment, the contents of each cooler were placed in a separate experimental stream and the streams were randomly assigned to a treatment (control or UVB exposed). Depth was maintained in each stream so that the tops of the trays were within 4 cm below the water surface. After a 24-to 48-hour acclimation period, UVB radiation was generated by one SF 20 lamp (UVB-313; National Biological, Twinsbury, OH) suspended directly above the trays within 15 cm of the water surface in each treated stream. UVB exposure times ranged between 4 and 10 hours per day bracketing solar noon (Table 1). Lamp-generated UVB dose was measured for the duration of each experiment by using either polysulfone dosimetry (Davis et al. 1976) or an Optronics Model 754 Spectroradiometer (Kiffney et al. 1997b). These values were compared to average UVB over the same time period recorded at a long-term monitoring site in Steamboat Springs, Colorado (N 40° 27' 01", W 106° 43' 48", 3220 masl) (see http://UV-B.nrel.colostate.edu/UV-B/home_page.html, Bigelow et al. 1998). Ambient values reported in Table 1 were corrected for altitudinal differences between the Steamboat site and field sites where benthic communities were collected for each experiment (Blumthaler et al. 1997). The Steamboat data were not corrected for additional sources of UVB variability such as topographic features, canopy cover, and cloud cover.

At the end of the experiment, the trays from a single stream were combined to form a sample and the contents rinsed through a 335-µm mesh sieve and preserved in ethanol. All organisms were removed from organic debris in an enamel pan and identified and enumerated under a dissecting microscope. Organisms were mostly identified to genus or species except Chironomidae, which were identified to subfamily.

Data analysis - Community metrics that have been previously shown to respond to UVB enhancement or removal experiments (Bothwell et al. 1994, Kiffney et al 1997a, Kiffney et al 1997b, Kelly et al. 2001) were examined in this study. Response variables included total macroinvertebrate abundance and total abundance of mayflies (Ephemeroptera), caddisflies (Trichoptera), heptageniid mayflies, and Orthocladiinae midges (Diptera: Chironomidae). Additionally, we tested for effects on the mayfly *Baetis bicaudatus* because abundance of this species was large enough across all microcosm experiments to provide a meaningful statistical interpretation. Finally, we calculated the total abundance of scrapers and grazers (as defined by Vieira et al. 2006) in each sample. These groups feed on algal material, and as a result have been shown to indirectly respond to UVB (Bothwell et al. 1994, DeNicola and Hoagland 1996).

We compared the magnitude of effects between microcosm experiments using benthic communities with a known history of heavy metal contamination (n=5) and those from reference sites (n=3). Standardized mean difference (Hedges' *d*) was used in this analysis as the measure of effect size, representing the mean difference between treatment and control standardized by the pooled standard deviation of both treatments (Gurevitch and Hedges 1993). Hedges ' *d* is often used in weighted meta-analysis when comparing results across several similar experiments (Gurevitch and Hedges 1999). Metrics (±95% confidence intervals) were plotted for each response variable and examined. The Mann-Whitney U test was used to test for significant ($p < 0.05$) differences in effect size of UVB addition on benthic communities from reference and polluted streams.

UVB Removal Field Experiment

We measured the effects of UVB removal on benthic communities across a gradient of metals contamination in 12 Rocky Mountain streams in central Colorado. Streams of this region drain high elevation watersheds with sparse soil development. Typical landcover in these basins consists of bare rock, alpine meadow, and coniferous forest. Riparian vegetation is dominated by species of willow (*Salix* spp.) and thinleaf alder (*Alnus tenuifolia*). Snow and ice covers these streams for up to seven months of the year, followed by a typical snowmelt driven hydrograph where peak annual stream flow coincides with peak snowmelt. Zinc is the primary metal contaminant in these streams (Clements et al. 2000), therefore, sites were selected across an average annual Zn gradient ranging from below detection (10 μg/L) to 377 μg/L (Clements and Kiffney 1995, Clements et al. 2000, Carlisle and Clements 2003). Additional effort was made to select sites that minimized the variability associated with elevation (2500-3000 masl), stream order (1^{st} to 4^{th}), substrate size (large cobble dominant), ambient UVB exposure (mostly open canopy), and riffle depth at base flow (< 20 cm) (Table 2).

Field experimental system – Three replicate 1.0 x 2.0 m PVC frames were placed in riffle areas at similar depths at each site. A greenhouse filter material that blocked UVB (Klerk's Plastic Products Manufacturing Inc., Richburg, SC) from the streambed was placed over half of each frame ($1 m^2$, treatment), while the other half remained uncovered ($1 m^2$, control). The structures were anchored to the streambed with cement blocks and arranged so the PVC frame was set 10 cm above the surface of the water at the time of installation. The experiment began in late July 2003 and ran for 60 days. Although field sites were accessible by road, substantial effort was necessary to conduct this experiment (0.8 km of PVC pipe and 1815 kg of cement blocks for frames and 14000 km of travel for maintenance).

At the end of the experiment, invertebrate samples were collected using a 0.1 m^2 Hess sampler equipped with 350 µm mesh collection net from each paired treatment and control at each structure. Samples were rinsed through a 350 µm sieve in the field and preserved in 80 % ethanol. In the laboratory, organisms were sub-sampled until 300 organisms (±10%) were removed from the sample following methods described by Moulton et al. (2000). All organisms were processed and identified in a manner consistent to the microcosm experiments.

Additionally, algal biomass was estimated as the concentration of chlorophyll *a* using the top rock scrape method described in Moulton et al. (2002). Chlorophyll *a* samples were processed in the laboratory using extraction and analytical methods described in Biggs and Kilroy (2000).

Physiochemical characteristics – We measured a suite of physicochemical variables (dissolved organic carbon, Zn, Cd, major anions, major cations, pH, alkalinity, hardness, temperature, DO) to characterize water chemistry at each site. Water samples were collected bimonthly from May through June to characterize snowmelt runoff and monthly from July through October 2003. Water samples for analysis of dissolved organic carbon (DOC) were filtered (0.7 μm) in the field, acidified with 2N hydrochloric acid, and stored in baked amber glass containers at 4°C. A Shimadzu TOC-5050A total organic carbon analyzer equipped with an ASI-5000A auto sampler was used to measure DOC. Water samples for determination of total recoverable metals (Cd, Cu, Zn) were preserved by acidification with nitric acid to a pH of < 2. Samples for anions were filtered in the field through a 0.45 μm filter, and stored in glass containers at 4°C. Metals, anions, and cations were analyzed using flame and furnace atomic absorption spectrophotometry (major cations and trace metals; Ca^{2+}, Mg^{2+}, Na^{2+}, K^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}) or ion chromatography (major anions; Cl^-, F^-, NO_3^-, PO_4^{3-}, SO_4^{2-}) at the University of Wyoming Red Buttes Environmental Biology Laboratory near Laramie (U.S. Environmental Protection Agency 1994). Water temperature measurements were recorded hourly over the course of the experiment using data loggers (Optic Stow-Away®, Onset Computer Corp., Pocasset, Massachusetts). Details of QA/QC procedures for DOC and metals analyses are described in Prusha (2002). Routine water quality parameters (dissolved oxygen, conductivity, hardness, alkalinity, pH, and temperature) also were measured at each site visit. Additionally, we estimated total UVB dose to the streambed (Table 3) over the course of the experiment using polysulfone dosimetry (Davis et al. 1976). We integrated vertical UVB attenuation coefficients (K_d, calculated on three occasions) and stream depth (measured every 5-6 days) for each PVC structure over the course of the study to measure UVB dose at the water surface. UVB attenuation (K_d) integrates depth over time and allows the calculation of percent UVB reaching the streambed vs the amount of UVB at the water surface (Kirk 1994).

Data analysis - We examined the same suite of abundance metrics evaluated in the microcosm experiments plus the concentration of chlorophyll *a*. We used a two-way factorial Analysis of Variance (ANOVA) to test for main effects of UVB removal (treatment), site, and the treatment x site interaction. We interpreted a significant interaction term as an indication that the effect of UVB removal was dependent upon site. Analysis was performed on log-transformed data when necessary to meet assumptions of homogeneity of variance or normality. For community metrics that showed a significant UVB removal effect we calculated the standardized mean difference (effect size *D*) as described above for each site (without correcting for sample size). Effect size of UVB removal for the statistically significant metrics was plotted along a gradient of Zn concentration associated with each site. These plots were examined to determine if effect size of UVB removal was consistently larger at sites with high Zn concentration compared to sites with low Zn concentration.

The concentration of dissolved organic matter (DOM) in the water column is the primary factor that influences UVB penetration in aquatic systems (Scully and Lean 1994). To illustrate this relationship in headwater Rocky Mountain streams under base flow conditions, we regressed percent UVB reaching the streambed versus mean DOC concentration. DOC values from periods that deviated strongly from base flow conditions (e.g., DOC samples collected within a 24-hour period after a rainfall event (3 out of 48 samples) were removed from this analysis. ANOVA and regression analysis were conducted using SPSS® (2004) statistical software.

RESULTS

Laboratory Experiments

Lamp-generated UVB dose - Overall, lamp-generated UVB in the microcosm experiments was comparable to elevation-corrected values recorded at Steamboat Springs, Colorado (Table 1). Mean cumulative lamp-generated UVB at the water surface in the experimental streams ranged between 2.3 and 4.9 J/cm^2 compared to ambient levels (2.6 - 4.1 J/cm^2 over the same time period.

Details about the spectral composition of the lamp-generated UVB irradiance are provided in Kiffney et al. (1997a).

Effects of UVB addition on invertebrate communities from reference and polluted sites - Effect size (standardized mean difference *D*) of UVB addition was generally large for most response variables at both reference and polluted sites (Fig. 1). In all cases measures of abundance decreased in UVB treatments compared to controls. Moreover, mean effect size was significantly greater at the polluted sites than at the reference sites for three of the seven community metrics we examined. Trends in the remaining metrics also suggested that UVB exposure had greater effects at metal polluted sites. The largest differences in effect size of UVB addition between reference and metal polluted sites were observed for mayflies. The effect size of UVB addition for the total abundance of mayflies ($Z = 2.24$, $P = 0.025$), Heptageniidae ($Z = 1.96$, $P = 0.0450$), and *B. bicaudatus* ($Z = 2.24$, $P = 0.025$) was significantly greater at the polluted sites compared to reference sites. Across all experiments, the mayfly assemblage was dominated by *B. bicaudatus*, *Diphetor hageni, Ephemerella dorothea infrequens*, and *Rhithrogena* spp., which accounted for 84% of mayfly abundance. In contrast, differences between reference and polluted sites were not significant for total abundance ($Z = 0.15$, $p = 0.8815$) and the abundance of grazers ($Z = 0.45$, $P = 0.6547$), caddisflies ($Z = 1.34$, $P = 0.1797$), and Orthocladiinae midges ($Z = -1.64$, $P = 0.1010$).

Field Experiment

Physiochemical characteristics of field sites - Physiochemical characteristics of the field sites showed considerable variation among streams (Table 3). Conductivity, alkalinity, SO_4^{2-}, Ca^{2+}, K^+, and Na^+ concentrations were highest at 10 Mile Creek. Conductivity, pH, alkalinity, hardness, NO_3^-, SO_4^{2-}, Ca^{2+}, Mg^{2+}, and Zn^{2+} were lowest at West Tennessee Creek. Median Zn concentrations ranged from below detection (5 µg/L) at West Tennessee and Middle Cottonwood Creeks to 530 µg/L at Chalk Creek. Median DOC values ranged between 0.8 at French Gulch to 2.5 mg/L at West Tennessee Creek. Ultraviolet-B radiation reaching the streambed over the 60-day experiment varied among field sites and ranged from 6.5 J/cm^2 at Middle Cottonwood Creek to 29.1 J/cm^2 at 10 Mile Creek. Dissolved organic carbon concentration accounted for 78% of the variation between UVB at the water surface and the amount of UVB reaching the streambed at these sites ($R^2 = 0.78$, $F_{1, 10} = 36.26$, $P = 0.0001$, Fig. 2). In general, UVB levels at the streambed were high, and sites with low DOC received as much as 70% of the surface UVB at the streambed. Zinc was the predominant contaminant in streams where metals were present. We separated the 12 sites into two groups based on Zn concentration using a hardness adjusted criterion value of 60 µg/L as a cutoff for identifying contaminated sites (U.S. EPA 2002). Sites having a hardness adjusted criterion value below 60 µg/L Zn were considered reference and those above 60 µg/L were considered metal-polluted.

Effects of UVB Removal on benthic communities - Results of two-way ANOVA indicated that the removal of UVB significantly increased abundance of most groups (Fig. 3; Table 4). Specifically, the removal of UVB increased total abundance ($P = 0.0009$) and the abundance of grazers ($P < 0.0001$), mayflies ($P < 0.0001$), caddisflies ($P = 0.0146$), Orthocladiinae midges ($P = 0.0012$), and the mayfly *B. bicaudatus* ($P = 0.0415$). There were no significant effects of UVB removal on Heptageniidae abundance ($P = 0.1380$) or chlorophyll *a* concentration ($P = 0.9600$). Grazer abundance was strongly related to chlorophyll *a* under the UVB removal treatments (Pearson Correlation $r = -0.80$, $P = 0.001$) and ambient conditions (Pearson Correlation $r = -0.75$, $P = 0.001$).

Site effects were significant for most response variables (Table 4), but this was expected because the sites were selected along a gradient of metal contamination, which is known to cause changes in benthic community composition (Clements 2004, Clements 1994). There were no significant interactions between UVB removal and site for the community metrics evaluated.

Effects of UVB removal along the Zn gradient - Metrics that showed a significant response to the removal of UVB from the two-way ANOVA (total abundance and the abundance of grazers, mayflies, caddisflies, Orthocladiinae, and *Baetis bicaudatus*) were plotted along a Zn gradient to determine if effect of UVB removal increased as metal concentration increased. Overall, effect size of UVB removal at sites above 60 µg/L Zn was generally greater than at the sites below 60 µg/L Zn for all metrics except caddisfly abundance (Fig. 4). Both increases and decreases in abundance occurred in response to UVB removal at sites with low Zn concentrations. In contrast, abundance consistently increased when UVB was removed at metal-polluted sites. One important exception to this pattern was overall responses at French Gulch, a relatively polluted stream (median Zn = 392 µg/L). Effect size of UVB removal at French Gulch was low for all metrics except Orthocladiinae abundance. Interestingly, average UVB reaching the streambed at French Gulch was also the lowest of all metal-polluted sites (10.1 J/cm^2) (Table 3), which was likely due to partially shading by the incised channel and dense streamside vegetation at this site.

DISCUSSION

Effects of UVB addition experiments in stream microcosms

Community metric values were consistently reduced under UVB treatments regardless of the experiment and effect size was larger for communities with a history of metals contamination. Despite the interest in how aquatic systems respond to increases in UVB, laboratory experiments using stream macroinvertebrate communities are scarce in the literature (Kiffney et al. 1997a, McNamara and Hill 1999). These few studies showed apparent increases in the abundances of drifting *Baetis* mayflies, caddisflies, and blackflies in experimental streams (Kiffney et al. 1997a) and how certain species (*Diphetor hageni*, *Corynoneura taris*, *Elimia clavaeformis*, and *Physella gyrina*) are differentially susceptible to UVB exposure (McNamara and Hill 1999). We selected a suite of abundance metrics previously found to respond to UVB manipulation in aquatic ecosystems (Bothwell et al. 1994, Kiffney et al 1997a, Kiffney et al 1997b, Kelly et al. 2001). Results of our UVB addition experiments showed a relatively strong response for most of the metrics we evaluated and that abundance was consistently reduced under UVB exposure. In particular, total abundance and the abundance of mayflies, *B. bicaudatus,* grazers and Orthocladiinae was reduced by 40-75%, corresponding to relatively large effect sizes. These results were generated from eight sites associated with 2 major river basins in Colorado. Community (total abundance), trait (grazers), and species level (*Baetis bicaudatus*) measurements were highly sensitive to UVB addition in these experiments. The consistency of these results is notable and provides evidence that benthic communities are highly sensitive to UVB addition regardless of site history in Colorado Rocky Mountain streams.

Although UVB dose in our microcosm experiments was comparable to ambient levels of solar UVB at the water's surface in open canopy conditions, irradiance certainly differed as the wavelength spectrum of lamp-generated UVB differs from natural sunlight (Kiffney et al. 1997a, McNamara and Hill 1999). The lamps used in these studies produce a spectrum with higher energy at shorter wavelengths (290 – 312 nm) compared to natural sunlight. Because the spectrum of these lamps is weighted in the shorter wavelengths they likely produce a greater biologically effective dose per unit energy than natural UVB (Behrenfeld et al. 1993). Because susceptibility to UVB exposure is dependent on both total energy received and irradiance (McNamara and Hill 1999), biological effects observed in the laboratory using UVB lamps should be interpreted cautiously. Although lamp-generated UVB does not perfectly reproduce the actual solar spectrum, we consistently observed decreases in abundance across a range of average doses (2.3 to 4.8 J/cm^2). Kiffney et al. (1997) used the Diffey action spectra (McKinley and Diffey 1987) to calculate Diffey erythemal weighted irradiance for the same lamps used in this study. Although, the spectra of these lamps are weighted in the shorter wavelengths, Kiffney et al. (1997) found that Diffey erythemal weighted irradiance of the lamps was similar to that of natural sunlight (3.18×10^{-5} W^{-1} cm^{-2} for natural sunlight vs 2.78×10^{-5} W^{-1} cm^{-2} for the lamps). These experiments provided preliminary evidence that some insects from metal polluted streams were more sensitive to UVB that those from unpolluted sites. The consistency across these experiments provided useful information regarding specific responses of benthic communities to UVB exposure that was further tested in the field.

Effects of UVB removal experiments in the field

Most of what is known about the effects of UVB on stream benthic communities is a result of removal experiments conducted over the last 13 years. These studies have collectively shown that manipulating ambient levels of ultraviolet radiation (UVR [300-380]) can cause changes in trophic structure (Bothwell et al. 1994), herbivore density (DeNicola and Hogland 1996), community composition and colonization dynamics (Kiffney et al. 1997b, Donahue and Schindler 1998, Kelly et al. 2001, Kelly et al. 2003), biomass (Kelly et al. 2003), and mayfly grazing behavior (Johansson and Nystrom 2004).

Several of these studies also reported that greatest effects were observed near the end of the UVB removal experiments (30 to 91 days) (Kiffney et al. 1997b, Kelly et al. 2001, Kelly et al. 2003). In an experiment conduced in a Rocky Mountain stream, Kiffney et al. (1997b) observed significant increases in total abundance and the abundance of mayflies, caddisflies, and Heptageniidae in UVB removal treatments. Results from our study conducted in 12 separate 1st – 4th order Rocky Mountain streams also showed increases in total abundance and the abundance of mayflies and caddisflies after 60 d of UVB removal. However, in contrast to Kiffney et al. (1997b), Heptageniidae mayflies and chlorophyll *a* biomass did not increase in our UVB removal treatments. Our sites were selected across a gradient of metal contamination, which may have masked the chlorophyll *a* and Heptageniidae response to UVB removal as these endpoints are known to be influenced by metals (Carlisle and Clements 2003, Clements et al. 2002).

Alternatively, increased grazing under the UVB removal treatments may have limited algae accrual. Several studies have shown direct effects of UVR manipulation on algal biomass (Bothwell et al. 1993, Kiffney et al 1997b, McNamara and Hill 1999, Kelly et al 2003). Short-term exclusion of UVR typically increases algal biomass; however, increased grazing pressure can offset the positive effects of UVR removal after longer periods of time (Bothwell et al. 1994, Kelly et al. 2001). The magnitude of grazing effects on algal biomass depends on the relative susceptibility of algae and grazers to UVB. If UVR removal significantly increases grazer abundance, algal biomass can be substantially lower compared to ambient conditions (Bothwell et al 1994, Kelly et al. 2003, but see Hill et al. 1997). Across all sites we found significantly more grazers in our UVB removal treatments compared to ambient conditions, but found no differences in algal biomass. Observed chlorophyll *a* concentrations were inversely related to grazers and were similar in ambient and UVB removal treatments. Kelly et al. (2003) reported that the interaction between grazers and algal biomass was dependent on canopy cover and that greatest effects of UVB removal were observed under partial canopy. We observed the greatest effect of UVB removal on grazers at Middle Cottonwood Creek, a site with the lowest levels of UVB at the streambed due to canopy cover (6.46 J/cm^2 over 60 days). Algal biomass in UVB removal treatments at this site was 34% lower than under ambient light, suggesting that grazers inhibited algal accrual. However, this relationship was not consistent across all sites, possibly because of differences in the composition of grazer assemblages (Kelly et al. 2003). For example, at French Gulch the dominant grazer was the cased caddisfly *Oligophlebodes minutus* whereas the dominant grazer at Middle Cottonwood Creek was *Baetis bicaudatus.*

We hypothesize that grazer-algal interactions likely varied among streams because different invertebrate grazers have differential sensitivities to UVR (Hill et al. 1997, McNamara and Hill 1999, Kelly et al. 2003, Johansson and Nystrom 2004) and metals (Clements et al. 2000). Furthermore, enhanced UVB can alter algae biomass and diversity in open canopied sections of Rocky Mountain streams (Rader and Belish 1997), thus altering specific food types available for foraging and ultimately altering trophic dynamics.

Effects of UVB on invertebrate communities with varying histories of heavy metal exposure

Benthic communities with a past history of metals exposure were more susceptible to UVB exposure than naive communities, especially mayflies. Studies investigating combined effects of UVB and other stressors are rare in the literature. Kashian et al. (In press) collected natural benthic communities from two of the sites used in this study (WT and AR3; Table 1) and exposed them to UVB and metals in stream microcosms. They reported that benthic communities subjected to long-term metal pollution were more tolerant to metals but more sensitive to UVB than communities from a reference stream. The most dramatic effects of UVB at the metal-polluted site in the Kashian et al. study were observed for total mayfly abundance (primarily *Baetis bicaudatus*), which was reduced by 41% compared to controls by UVB exposure. Results of our field and microcosm experiments were similar and showed that the mayfly *B. bicaudatus* from polluted sites were consistently more susceptible to UVB than organisms from reference sites. Our study enhanced the design of Kashian et al. (In press) by analyzing their data combined with additional microcosm experiments in conjunction with corresponding field experiments to better understand the susceptibility of benthic communities with varying metal exposure histories to UVB.

Baetis bicaudatus is widely distributed in high elevation Colorado Mountain streams and can be the dominant mayfly in terms of abundance (McCafferty et al. 1993). Baetid mayflies are highly mobile and feed by collecting detritus and browsing algae (Wilzbach 1990, Vieira et al. 2006 and citations within) from the tops of rocks during the day (Allan et al. 1986). Although this behavior routinely exposes *B. bicaudatus* to naturally high levels of UVB typical of shallow, high-elevation streams, this species was quite sensitive to UVB exposure, especially in streams with a history of elevated metals contamination. Abundance of *B. bicaudatus* and other baetids are generally greatest in areas of high algal biomass (Wallace and Gurtz 1986, Richards and Minshall 1988). We speculate that the benefit of utilizing abundant food resources on the tops of rocks during the day outweighs the damage caused by UVB. Although it would be difficult to measure, *B. bicaudatus* might benefit from partial shading from UVB by algae on substrate patches with highest algal biomass. Also, their high mobility may protect them from expose to UVB for long periods of time while foraging. We suggest the greater sensitivity to UVB at contaminated sites may be related to a cost associated with increased tolerance to metals.

The cost of tolerance – Enhanced tolerance to heavy metals in populations with a prior history of exposure has been demonstrated in algae (Stokes et al 1973, Niederlehner and Cairns 1992) invertebrates (Bryan and Humnmerstone 1971, Fraser et al 1978, Wentsel et al. 1978, Maltby 1991, Clements 1999) and fish (Weis et al. 1981, Diamond et al. 1991). Physiological acclimation and genetic adaptation are two general explanations for enhanced tolerance (Klerks and Levinton 1989, Niederlehner and Carins 1992). Although the ability to adapt or acclimate to metals is beneficial at polluted sites, there may be additional energetic costs. Organisms expending energy on eliminating, sequestering, or binding metals may have less energy available for other physiological functions, including the ability to tolerate additional stressors (Sibley and Calow 1989). There are a variety of examples in the literature suggesting that there is a cost to enhanced tolerance to contaminants. Weis and Weis (1989) demonstrated a decreased tolerance to salinity in populations of estuarine dwelling killifish that had a past history of metals exposure. Postma (1995) found metal-tolerant midges reared in the absence of metals continued to exhibit low growth and high mortality rates, indicating that costs can continue even after the stressor is removed. Additionally, Durou et al. (2005) found lower energy reserves in polychaetes previously exposed to metals compared to unexposed populations and Marchand et al. (2004) showed reduced fecundity and poorer general condition of flounder in metal-exposed populations.

Additional studies specific to the Arkansas River system have shown that metal-polluted communities are more susceptible to additional stressors such as acidification (Courtney and Clements 2000), UVB radiation (Kashian et al. in press) and predation by stoneflies (Clements 1999) compared to communities from reference sites. Previous findings coupled with our results suggest energetic and other physiological costs of acclimation and adaptation to chronic levels of metal pollution may increase susceptibility of aquatic organisms to additional stressors. Aquatic insects mainly regulate metals through the production of the metal-binding protein metallothionein (Hare 1992). Metallothionein is a low molecular weight protein that functions in both metal homeostasis and detoxification (Roesijadi 1992). Harrahy (2000) observed elevated levels of metallothionein and lower growth rates in baetid mayflies from some of the metal-polluted sites used in our study (AR1 and AR5). A similar mechanism may be responsible in the Courtney and Clements (2000) study where Arkansas River communities were more tolerant to metals than reference communities but more susceptible to acidic pH. Although the precise mechanism is unknown, we speculate that the energetic cost of producing large amounts of metallothionein to regulate metals may inhibit the ability of organisms to efficiently repair damaged DNA due to UVB exposure.

Implications

Results of this study have important implications given the characteristics of Rocky Mountain streams and the magnitude of metals pollution in Colorado and other Rocky Mountain states. Dissolved organic matter (DOM) absorbs UVB radiation and can be the main determinant of UVB penetration in natural waters (Kirk 1994). Because of shallow depth, high elevation, and naturally low levels of UVB-attenuating DOM, our results showed that aquatic communities in Rocky Mountain streams are subjected to intense levels of UVB radiation, in some instances exceeding 70% of UVB at the water surface, especially where DOC is low. Predicted effects of climate change over the next 100 years in the Rocky

Mountain region are expected to have significant impacts on riparian vegetation, biogeochemical cycles, and hydrologic processes (Baron et al. 2000). These changes in biogeochemical cycles and riparian vegetation will likely lower DOM quantity when UVB is highest, increasing UVB exposure to benthic communities. Moreover, summer stream flows are expected to decrease in snowmelt driven basins in response to climate change (Lettenmaier and Gan 1990, Rango 1995, Baron et al. 2000). A decrease in stream flow during summer when UVB is highest, coupled with decreases in climate-induced changes in DOM quantity, could have substantial implications for aquatic communities in this region, especially at sites with a history of metals contamination.

Conclusions

Large-scale studies addressing the influence of UVB radiation on stream benthic communities are rare, despite the importance of understanding the effects of increased radiation reaching the earth's surface on stream ecosystems. Our study is the first large-scale field experiment to investigate the direct influence of UVB on benthic communities. Our results from both laboratory and field experiments demonstrated that benthic communities in Rocky Mountain streams were negatively influenced by UVB radiation. Benthic response in these series of experiments was comparable to other studies, but our results suggest that communities subjected to long-term metal exposure were more sensitive to UVB than reference communities. Given the characteristics inherent to Rocky Mountain streams that increase UVB exposure to benthic communities (e.g.

swallow, clear, high elevation) and the extent of heavy metal pollution in the Rocky Mountains, the implications of increased UVB radiation reaching the earth's surface may be more severe than previously considered, especially for stream communities already subjected to other stressors.

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TABLES

Table 1. Characteristics of UVB addition experiments conducted in stream microcosms between 1995 and 2004. SPEC = lamp-generated UVB values measured with a spectroradiometer; PSF = lamp generated UVB values estimated using polysulfone dosimetry; NM = lamp generated UVB not measured.

Experiment Characteristics								
	Arkansas River	Arkansas River	Trap Creek	Cache la Poudre River	West Tennessee Creek	Arkansas River	Arkansas River	Arkansas River
Site Abbreviation	AR1	AR5	TC	PR	WT	AR3	AR2	AR3
Type	Polluted	Polluted	Reference	Reference	Reference	Polluted	Polluted	Polluted
Latitude North	39 15.42	39 07.69	40 33.39	40 41.96	39 19.08	39 13.22	39 13.35	39 13.22
Longitude West	106 20.63	106 18.68	105 49.32	105 42.67	106 20.25	106 21.35	106 21.41	106 21.35
Elevation (m)	3000	2835	3046	2335	3025	2909	2930	2909
[Zn] (ug/L)	10-425	56-1040	<10	<10	5-32	54-8624	12-426	54-8624
Colonization Period (d)	40	40	40	35	40	50	45	45

Start Date of Experiment	10-Oct-95	10-Oct-95	10-Oct-95	14-Aug-00	15-Aug-03	10-Sep-03	17-Sep-04	17-Sep-04
Length of Experiment (d)	7	7	7	10	10	10	12	12
Number of Control Replicates	3	2	2	4	4	4	4	4
Number of Treatment Replicates	3	3	3	4	4	4	4	4
UVB Measurements	SPEC	SPEC	SPEC	NM	PSF	PSF	PSF	PSF
UVB exposure (hr/d)	4	4	4	6	10	10	8	8
UVB Dose (J/cm^2)	3.9	3.9	3.9	NM	4.8	4.9	2.5	2.3
Ambient UVB [1] (J/cm^2)	2.7	2.6	2.8	4.1	4.1	3.1	3.1	3.1

[1] Ambient elevation-adjusted UVB data from a permanent monitoring station operated by the U. S. Department of Agriculture at Steamboat Springs, Colorado (see http://UV-B.nrel.colostate.edu/UV-B/home_page.html, Bigelow et al. 1998). Data are estimates of UVB striking the water surface at the associated field site.

Table 2. Physical characteristics of the 12 sites used in the UVB removal experiment. Sites are ordered left to right along the Zn gradient reported in Table 3.

	Site and Basin Characteristics											
	Middle Cotton wood Creek	West Tennessee Creek	West Clear Creek	East Fork Arkansas River	10 Mile Creek	4 Mile Creek	Clear Creek	Buckskin Gulch	Mosquito Creek	Arkansas River	French Gulch	Chalk Creek
Site Latitude North	38 48.53	39 19.08	39 46.21	39 16.21	39 28.48	39 12.20	39 45.00	39 17.00	39 16.23	39 13 19	39 28.50	38 42.07
Site Longitude West	106 20.02	106 20.25	105 45.38	106 18.20	106 01.07	106 06.28	105 39.45	106 04.12	106 06.37	106 21.14	106 01.04	106 20.58
Site Elevation (m)	3021	3090	2901	3221	3050	3304	2535	3207	3275	2909	3011	3052
Stream Order	2	2	3	2	2	1	3	2	3	4	1	2

Riffle Mean Particle Size (mm)	106	199	125	130	199	135	101	156	170	190	117	139
Riffle Depth at Base Flow (m)	0.14	0.18	0.20	0.15	0.20	0.16	0.18	0.14	0.20	0.20	0.15	0.20
Mean Wetted Width (m)	4.63	3.98	6.10	5.40	7.60	4.65	11.65	3.15	6.28	8.40	3.60	5.75
Median Discharge (m^3/s)	0.28	0.17	1.03	0.08	0.80	0.21	2.20	0.23	0.28	1.27	0.16	0.35
Basin Area (km^2)	33.5	24.6	69.2	98.2	81.1	22.4	217.6	21.3	31.0	292.3	23.3	50.6
Basin Slope %	19.7	14.0	23.8	17.8	18.6	20.0	22.5	21.9	18.9	13.3	19.2	19.7

Table 3. Physiochemical characteristics of the 12 field sites where the UVB removal experiment was conducted. Values represent medians (except where indicated) from samples collected bimonthly from May through June and monthly from July through October 2003. Sites are ordered left to right along the Zn gradient.

	Physiochemical Characteristics											
	Middle Cotton wood Creek	West Tennessee Creek	West Clear Creek	East Fork Arkansas River	10 Mile Creek	4 Mile Creek	Clear Creek	Buckskin Gulch	Mosquito Creek	Arkansas River	French Gulch	Chalk Creek
Conductivity (uS/m)	86.6	31.3	163.2	195.20	641.0	168.0	136.6	165.5	157.3	147.9	135.3	83.9
Average Temp (°C)	8.1	11.0	8.9	10.3	9.1	7.1	11.7	7.5	8.4	11.2	6.8	8.9
pH	7.3	7.2	7.2	7.8	7.2	7.9	7.6	7.7	7.8	7.8	7.5	7.3
Alkalinity (mg/L)	47.5	13.5	19.5	74.5	31.0	102.6	38.8	44.2	59.9	45.5	51.3	20.5

Hardness (mg/L)	53.0	16.5	76.5	107.5	241.0	126.0	63.0	95.0	96.5	95.0	78.3	44.0
DOC (mg/L)	2.3	2.5	1.6	1.5	1.9	0.9	1.6	0.8	1.4	2.2	0.8	1.8
Cu^{2+} (µg/L)	0.3	0.9	0.5	0.4	0.8	1.6	0.8	0.9	0.9	0.6	0.2	0.5
Cd^{2+} (µg/L)	0.02	0.04	0.09	0.08	0.67	0.18	0.41	0.55	0.34	1.27	1.05	1.47
Fe (µg/L)	51.9	189.5	23.3	68.8	9.2	15.8	127.7	8.8	57.4	100.1	19.5	71. 6
Zn^{2+} (µg/L)	5.0	5.0	36.4	38.6	49.7	52.9	128.3	142.0	174.9	334.6	391.8	530.9
UVB (J/cm^2) Streambed	6.5	13.6	19.6	25.160	29.160	24.2	11.9	26.7	19.4	20.7	10.1	12.1

Table 4. Results of the two-way analysis of variance for selected abundance metrics and Chlorophyll *a* concentration for the main effects of site, UVB removal, and for the site x UVB removal interaction for 12 Rocky Mountain streams in central Colorado. Significant *P* values indicating treatment effects are in bold type.

Abundance	**Source**	***$F_{1,11}$* value**	***p - value***
Grazer	Site	15.38	< 0.0001
	UVB Removal	6.52	**0.0014**
	UVB Removal x Site	0.51	0.8864
Baetis bicaudatus	Site	11.97	< 0.0001
	UVB Removal	4.39	**0.0415**
	UVB Removal x Site	0.51	0.8864
Heptageniidae	Site	28.98	< 0.0001
	UVB Removal	2.28	0.1380
	UVB Removal x Site	1.07	0.4080
Mayfly	Site	25.00	< 0.0001
	UVB Removal	6.58	**0.0136**
	UVB Removal x Site	1.19	0.3175
Orthocladiinae	Site	25.55	< 0.0001
	UVB Removal	11.85	**0.0012**
	UVB Removal x Site	1.12	0.3701
Caddisfly	Site	10.61	< 0.0001

	UVB Removal	6.43	**0.0146**
	UVB Removal x Site	0.75	0.6860
Total	Site	19.52	< 0.0001
	UVB Removal	12.70	**0.0009**
	UVB Removal x Site	0.91	0.5344
Chlorophyll *a*	Site	8.15	< 0.0001
	UVB Removal	0.01	0.9627
	UVB Removal x Site	0.70	0.7293

FIGURES

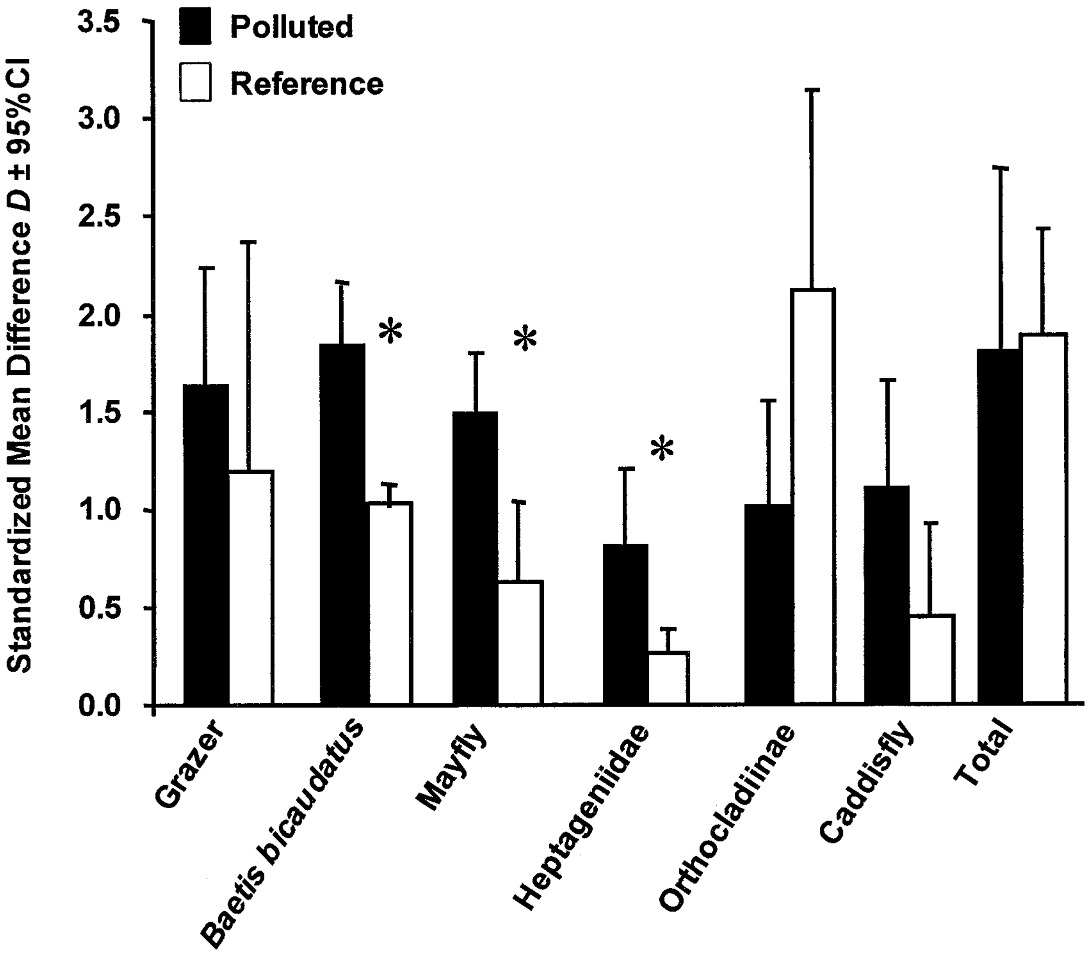

Figure 1. Standardized mean difference (*D*) ± 95% confidence intervals of abundance metrics for UVB addition experiments conducted in the laboratory with benthic communities from reference sites (n = 3) and with those that have an elevated metals exposure history (n = 5). * = $P < 0.05$ based on Mann-Whitney U test.

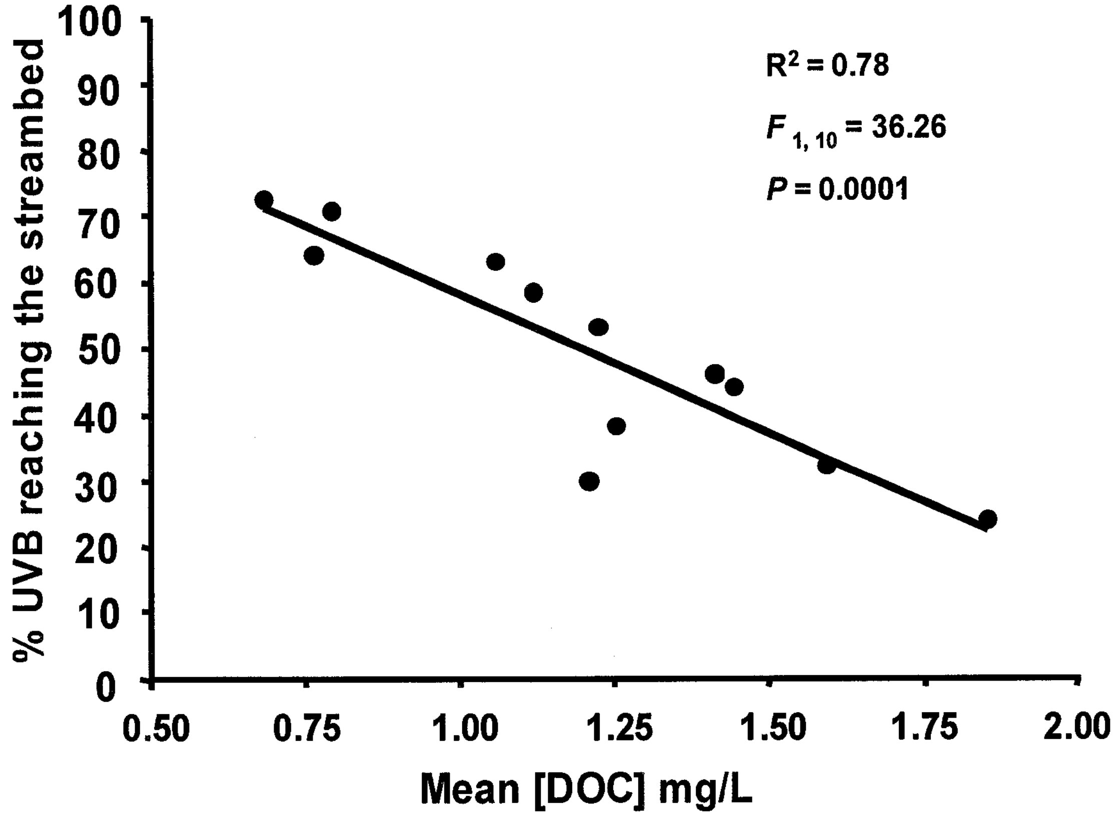

Figure 2. Relationship between percent UVB reaching the streambed and mean DOC concentration from late July to mid October 2002 for 12 Colorado Rocky Mountain streams.

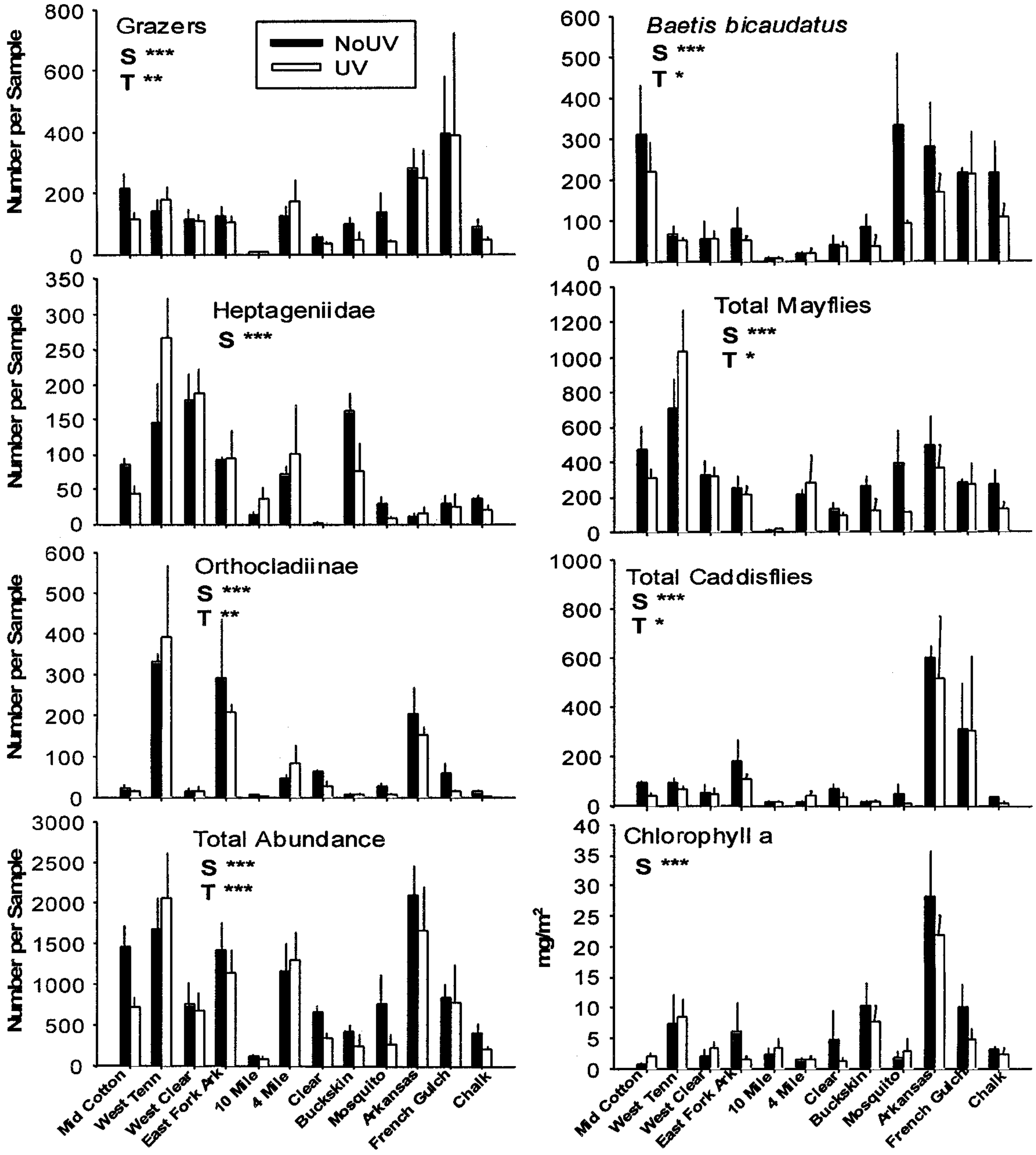

Figure 3. Responses of benthic communities to UVB removal from 12 Rocky Mountain streams, Colorado. Sites are ordered from left to right along an increasing Zn gradient. Results of two-way factorial ANOVA showing effects of site (S) and treatment (T). There was no site by treatment interaction. See Table 4 for details of statistical analysis. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

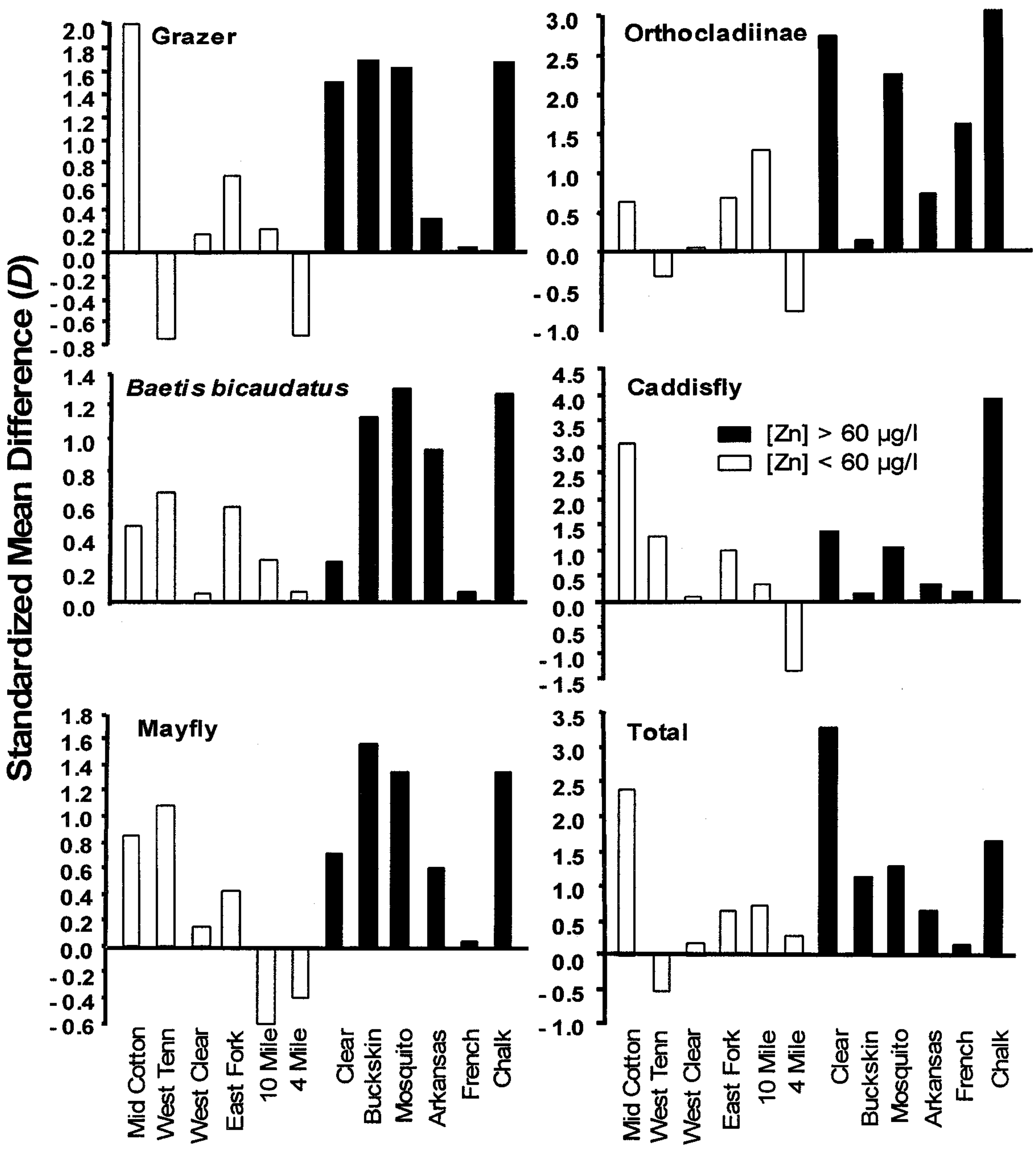

Figure 4. Standardized mean difference (*D*) of abundance metrics that showed a significant response to UVB removal from the field experiment. Sites are ordered from left to right along an increasing Zn gradient. Positive *D* values indicate an increase in abundance due to UVB removal, whereas negative values indicate a decrease in abundance due to UVB removal.