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The influence of metal exposure history and ultraviolet-B radiation on benthic communities in Colorado Rocky Mountain streams

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Abstract. Interest in understanding the influence of ultraviolet-B (UVB; 280–320 nm) radiation in aquatic ecosystems has increased since the early 1990s. Pollution from historic mining operations coupled with physicochemical characteristics of Rocky Mountain streams that increase exposure of benthic communities to UVB provided an opportunity to examine how UVB interacted with heavy metal contamination to structure stream communities. We integrated a series of UVB addition experiments done in stream microcosms with a large-scale UVB shading experiment to test the hypothesis that effects of UVB were greater on benthic communities from metal-polluted streams than from reference streams. Microcosm experiments involved short-term exposure (7–10 d) of natural benthic macroinvertebrate communities collected from reference and metal-contaminated sites to lamp-generated UVB. In all cases, abundance decreased in UVB-treated streams compared to controls. Moreover, effects of UVB addition were significantly greater on communities from metal-polluted sites than from reference sites. The field experiment involved shading portions of the streambed from UVB for 60 d at 12 streams along a Zn gradient. Median Zn concentration at these sites ranged between 5 and 530 μg/L, and mean UVB reaching the streambed varied from 6.5 to 29.0 J/cm^2. Results of the field experiment indicated that removal of UVB significantly increased total macroinvertebrate abundance and abundance of grazers, mayflies, caddisflies, Orthocladiinae midges, and the mayfly *Baetis bicaudatus* compared to controls. Grazer abundance was significantly greater in UVB removal treatments compared to controls, but UVB removal had no effect on algal biomass. As with the microcosm experiments, the effects of UVB removal on benthic communities were generally greater at metal-polluted sites than at reference sites. We speculate that the energetic cost of regulating metals might inhibit the ability of some organisms to repair efficiently DNA damaged by 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 are reference communities. As a consequence, the effects of increased UVB radiation reaching the earth's surface might be more severe than previously considered in systems receiving multiple stressors.

Key words: UVB, metals, macroinvertebrates, tolerance, streams, Colorado.

Decreasing levels of stratospheric O_3 consequent to discharge of chlorofluorocarbons have been measured for ~2 decades. These reduced levels of O_3 have been correlated with increased levels of ultraviolet-B radiation (UVB; 280–315 nm) reaching the earth's surface (Kerr and McElroy 1993, Michaels et al. 1994). UVB is a small component of the sun's energy, but it can have deleterious effects on aquatic communities (Smith et

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al. 1992, Vincent and Roy 1993, Williamson 1995, Vinebrooke and Leavitt 1999). Considerable research has been devoted to understanding the influence of ultraviolet radiation (UVR) on aquatic ecosystems (Smith et al. 1992, Bothwell et al. 1994, Williamson 1995, Kiffney et al. 1997a, Vinebrooke and Leavitt 1999, Kelly et al. 2003), but much less is known about how UVB might interact with other forms of anthropogenic disturbance (Liess et al. 2001, Duquesne and Liess 2003).

Experiments shading UVB from streams or artificial channels have shown substantial changes in community composition (Kiffney et al. 1997a), herbivore density (DeNicola and Hoagland 1996), trophic structure (Bothwell et al. 1994), and invertebrate biomass (Kelly et al. 2003). Likewise, results of UVB addition experiments have included increases in invertebrate drift (Kiffney et al. 1997a), 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 generally is considered the primary factor influencing UVB penetration in oligotrophic systems (Scully and Lean 1994, Xenopoulos and Schindler 2001). Rocky Mountain streams typically are shallow, have naturally low levels of DOM, and are located at high elevations. Therefore, aquatic communities in open-canopied 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 Colorado streams and is recognized as a major environmental problem. Heavy metals from ~10,000 abandoned mines affect >2600 km of Colorado's high-elevation streams (Colorado Department of Public Health and Environment 1992). Clements et al. (2000) estimated that ~25% of the headwater streams in Colorado are affected by heavy metal pollution. Extrapolation of 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 suggests that a large number of Colorado streams are subjected to both intense UVB and heavy metal pollution.

The response of Rocky Mountain benthic communities to heavy metal contamination is well documented in the literature. Changes in community composition (Clements 1994, 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. 2007). 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. 2007), 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 and susceptibility to additional stressors (Weis 2002).

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 at a relatively large spatial scale. We used a series of UVB addition experiments conducted in stream microcosms and a large-scale field study involving the removal of UVB in 12 streams along a metal pollution gradient to assess the direct effects of UVB radiation on benthic communities. We predicted that communities with a history of exposure to heavy metals would be more susceptible to UVB than would 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 by comparing results of 5 similar microcosm experiments conducted over a 9-y period (1995–2004). Natural benthic communities were collected from metal-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 [WT]) 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 contamination on benthic communities for >2 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 US Environmental Protection Agency Superfund Site (Voynick 1984). Recently, metal concentrations entering the UAR from LMDT and CG have decreased significantly because of remediation that began in 1992 (Clements 2004). Based on the last 16 y of field sampling at UAR sites, maximum total Zn concentrations have ranged from 425 to 8624 μg/L (Table 1). Although metal concentrations in the UAR have been reduced, benthic communities in this stream probably

TABLE 1. Characteristics of 5 ultraviolet-B (UVB) addition experiments done 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 = not measured.

Starting date of experiment:	10 October 1995			14 August 2000	15 August 2003	10 September 2003	17 September 2004	
Stream	Arkansas River		Trap Creek	Cache la Poudre River	West Tennessee Creek	Arkansas River	Arkansas River	
Site	AR1	AR5	TC	PR	WT[a]	AR3[a]	AR2	AR3
Type	Polluted	Polluted	Reference	Reference	Reference	Polluted	Polluted	Polluted
Latitude (N)	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 (W)	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 (μg/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
Length of experiment (d)	7	7	7	10	10	10	12	12
n (control, treatment)	3, 3	2, 3	2, 3	4, 4	4, 4	4, 4	4, 4	4, 4
UVB measurements	SPEC	SPEC	SPEC	NM	PSF	PSF	PSF	PSF
UVB exposure (h/d)	4	4	4	6	10	10	8	8
UVB dose (J/cm^2)[b]	3.9	3.9	3.9	NM	4.8	4.9	2.5	2.3
Ambient UVB (J/cm^2)[c]	2.7	2.6	2.8	4.1	4.1	3.1	3.1	3.1

[a] Data from Kashian et al. (2007)

[b] Estimates were measured at the surface

[c] Ambient elevation-adjusted UVB data from a permanent monitoring station operated by the US Department of Agriculture at Steamboat Springs, Colorado (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

have been exposed to elevated metal concentrations for >125 y. WT also is located in the UAR basin, but this site does not have a history of metal contamination. Total Zn at WT ranges between 5 and 32 μg/L (Table 1). The other 2 reference sites were located ~161 km north and west of the Leadville sites in the Cache la Poudre River basin (Cache la Poudre River [PR] and Trap Creek [TC]). Zn concentrations at these sites were below detection (10 μg/L) at the time of the experiments. Elevation at all sites ranged between 2335 and 3046 m asl (Table 1). Additional details about the UAR sites are described in Clements and Kiffney (1995) and details about the PR 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 × 10 × 6 cm) filled with gravel and small cobbles were anchored to the streambed at each site between mid-July and early September and were left to colonize for 35 to 50 d (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, Colorado) (Kiffney et al. 1997b). This facility houses 18 oval experimental streams (76 × 46 × 14 cm) located in a greenhouse that filters 97% of incoming solar UV radiation (280–400 nm), while allowing the transmission of ~50 to 80% of photosynthetically active radiation (400–500 nm; Kiffney et al. 1997a). Water delivered to the streams is obtained directly from a nearby reservoir that has physicochemical characteristics similar to those of unpolluted Rocky Mountain streams (Clements 1999). Water temperature during these greenhouse experiments is relatively constant because the water source is taken from a constant elevation in the lower portion of the nearby reservoir. Current in each stream is provided by paddlewheels and depth is regulated by standpipes that are covered with fine mesh during each experiment 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 ~4 cm below the water surface. After a 24- to 48-h acclimation period, UVB radiation was generated by one 50.8-cm fluorescent sunlamp (UVB-313; National Biological, Twinsburg, Ohio) suspended directly above the trays and within 15 cm of the water surface in each treated stream. UVB exposure times ranged between 4 and 10 h/d centered on solar noon (Table 1). Lamp-generated UVB dose was measured for the duration of each experiment using either polysulfone dosimetry (Davis et al. 1976) or a Spectroradiometer (Model 754; Optronics, Orlando, Florida) (Kiffney et al. 1997a). These values were compared to average UVB over the same time period recorded at a long-term monitoring site in Steamboat Springs, Colorado (lat 40°27′01″N, long 106°43′48″W; 3220 m asl) (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 shown previously to respond to UVB enhancement or removal experiments (Bothwell et al. 1994, Kiffney et al. 1997a, b, Kelly et al. 2001) were examined. Response variables included total macroinvertebrate abundance and total abundance of Ephemeroptera, Trichoptera, heptageniid mayflies, and Orthocladiinae midges. In addition, effects on the mayfly *Baetis bicaudatus* were tested because abundance of this species was great enough across all microcosm experiments to provide a meaningful statistical interpretation. Last, the total abundance of scrapers and grazers (as defined by Vieira et al. 2006) was determined in each sample. These groups feed on algal material, and as a result, have been shown to respond indirectly to UVB (Bothwell et al. 1994, DeNicola and Hoagland 1996).

The magnitude of effects were compared 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 as a 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 metal-polluted streams.

UVB removal field experiment

We measured the effects of UVB removal on benthic communities across a gradient of metal contamination in 12 Rocky Mountain streams in central Colorado. Streams of this region drain high-elevation basins with sparse soil development. Typical land cover in these basins consists of bare rock, alpine meadow, and coniferous forest. Riparian vegetation is dominated by low-growing willow (*Salix* spp.) and thinleaf alder (*Alnus tenuifolia*). Snow and ice covers these streams for up to 7 mo of the year. This period is followed by a typical snowmelt-driven hydrograph where peak annual stream flow coincides with peak snowmelt. Zn is the primary contaminant in these streams (Clements et al. 2000). Therefore, stream reaches were selected across an average annual Zn concentration 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 stream reaches (100–200 m) that minimized the variability associated with elevation (2500–3000 m asl), stream order (1^{st}–4^{th}), substrate size (predominantly large cobble), ambient UVB exposure during midday (open-canopy conditions between 1000 hours and 1400 hours), and riffle depth at base flow (<20 cm) (Table 2).

Field experimental system.—Three replicate 1.0×2.0-m polyvinyl chloride (PVC) frames were placed in riffle areas at similar depths along each stream reach. A greenhouse filter material that blocked UVB (Klerk's Plastic Products Manufacturing, Richburg, South Carolina) from the streambed was placed over ½ ($1.0\ m^2$) of each frame (treatment), while the other ½ remained uncovered (control). The structures were anchored in the streambed with cement blocks and arranged so the filter material was set 10 cm above the water surface. The experiment began in late July 2003 and ran for 60 d.

At the end of the experiment, invertebrate samples were collected using a $0.1\text{-}m^2$ Hess sampler equipped with 350-μm-mesh 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 subsampled until 300 organisms (±10%) were removed from the sample following methods described by Moulton et al.

Table 2. Habitat and physical characteristics of the 12 field sites used in the ultraviolet-B (UVB) removal experiment. Sites are ordered left to right along the Zn concentration gradient reported in Table 3.

Characteristic	Middle Cottonwood 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
Latitude (N)	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′
Longitude (W)	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′
Elevation (m asl)	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
Median particle size (mm)	90	180	128	128	180	128	90	128	154	165	90	128
Mean depth at baseflow (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

(2000). All organisms were processed and identified in a manner consistent with the microcosm experiments. In addition, algal biomass was estimated as the concentration of chlorophyll *a* using the top rock scrape method (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.—A suite of physicochemical variables (dissolved organic C [DOC], Zn, Cd, major anions, major cations, pH, alkalinity, hardness, temperature, conductivity, dissolved O_2) were measured to characterize water chemistry at each site. Water samples were collected bimonthly from May through June to characterize snowmelt runoff and then monthly from July through October 2003. Water samples for analysis of DOC were filtered (0.7 μm) in the field, acidified with 2N HCl, and stored in baked amber glass containers at 4°C. A Shimadzu TOC-5050A total organic C analyzer equipped with an ASI-5000A auto sampler (Shimadzu, Columbia, Maryland) was used to measure DOC. Water samples for determination of total recoverable metals (Cd, Cu, Zn) were preserved by acidification with HNO_3 to a pH <2.0. 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, Wyoming (USEPA 1994). Water temperatures were recorded hourly over the course of the experiment using data loggers (Optic Stow-Away®; Onset Computer Corp., Pocasset, Massachusetts). Details of quality assurance/quality control procedures for DOC and metal analyses are described in Prusha (2002). Total UVB dose at the water surface was determined over the course of the experiment using polysulfone dosimetry (Davis et al. 1976). To estimate UVB dose to benthic communities, these surface estimates were integrated with UVB attenuation coefficients (K_d; calculated on 3 occasions during full-sun conditions between 1000 h and 1400 h) and stream depth (measured every 5–6 d) for each PVC structure. K_d integrates depth over time and allows calculation of % UVB reaching the streambed vs the amount of UVB at the water surface (Kirk 1994). UVB measurements taken to calculate K_d were made in the deepest locations along the stream reach that were similar in character to the areas where the structures were placed.

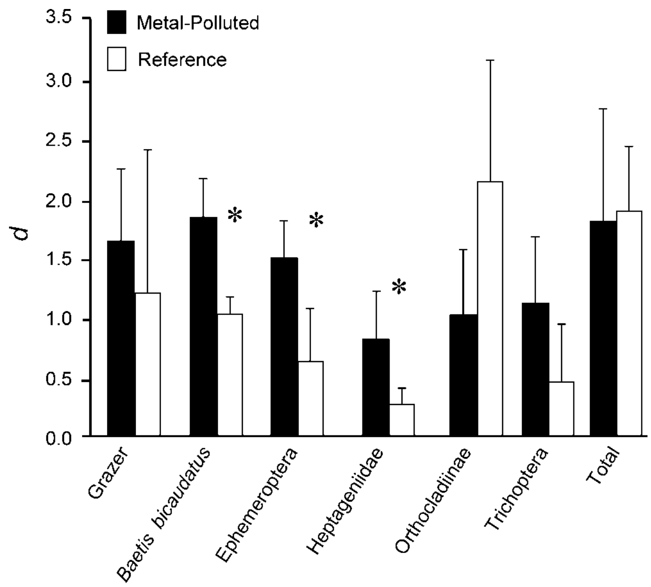

FIG. 1. Standardized mean difference (d) (+95% confidence intervals) of selected abundance metrics for ultraviolet B (UVB) addition experiments conducted in the laboratory with benthic communities from reference sites (n = 3) and with those that have an elevated metals exposure history (n = 5). * = $p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

Data analysis.—The effect of UVB removal was examined on same suite of macroinvertebrate abundance metrics evaluated in the microcosm experiments. Two-way factorial analysis of variance (ANOVA) was used to test for main effects of UVB removal (treatment), site, and the treatment × site interaction (SPSS® 2004, version 13; SPSS, Chicago, Illinois). A significant interaction term was interpreted as an indication that the effect of UVB removal was dependent upon site. Analysis was done on $\log_{10}(x)$-transformed data when necessary to meet assumptions of homogeneity of variance and normality. Hedges' d for each site was calculated as described above for community metrics that showed a significant UVB removal effect.

Zn was the predominant contaminant where metals were present. Therefore, the 12 sites were separated into 2 groups based on a hardness-adjusted Zn concentration criterion of 60 μg/L (USEPA 2002). Reference sites were defined as streams with a mean Zn concentration <60 μg/L, and metal-polluted sites were defined as streams where Zn concentration ≥60 μg/L. Effect size (d) of metrics that showed a significant UVB removal effect were plotted along the Zn gradient and examined to determine whether d of UVB removal was consistently larger at sites with high Zn concentrations than at sites with low Zn concentrations. A 1-way ANOVA was used to test for differences in d for each metric between metal-polluted and reference sites.

The concentration of DOM in the water column is the primary factor that influences UVB penetration in aquatic systems (Scully and Lean 1994). The relationship between % UVB reaching the streambed and DOC under baseflow conditions was examined using linear regression (SPSS® 2004). DOC values from periods that deviated strongly from baseflow conditions (e.g., DOC samples collected within a 24-h period after a rainfall event) were removed from this analysis. This censoring affected only 3 of 48 samples.

Results

Microcosm experiments

Lamp-generated UVB dose.—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) measured over the same period. Details about the spectral composition of the lamp-generated UVB irradiance are provided in Kiffney et al. (1997b).

Effects of UVB addition on invertebrate communities from reference and polluted sites.—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 d was significantly greater at the metal-polluted sites than at reference sites for 3 of the 7 community metrics. Trends in the remaining metrics also suggested that UVB exposure had greater effects on communities from metal-polluted sites than from reference sites. The largest differences in d of UVB addition between reference and metal-polluted sites were observed for Ephemeroptera. d of UVB addition for total abundance of Ephemeroptera ($Z = 2.24$, $p = 0.025$), Heptageniidae ($Z = 1.96$, $p = 0.0450$), and *B. bicaudatus* ($Z = 2.24$, $p = 0.025$) was significantly greater at the metal-polluted sites compared to reference sites. Across all experiments, the Ephemeroptera assemblage was dominated by *B. bicaudatus*, *Diphetor hageni*, *Ephemerella dorothea infrequens*, and *Rhithrogena* spp. Together, these taxa accounted for 84% of total Ephemeroptera abundance. In contrast, differences between reference and metal-polluted sites were not significant for total macroinvertebrate abundance ($Z = 0.15$, $p = 0.8815$), the abundance of grazers ($Z = 0.45$, $p = 0.6547$), Trichoptera ($Z = 1.34$, $p = 0.1797$), and Orthocladiinae midges ($Z = -1.64$, $p = 0.1010$).

TABLE 3. Physiochemical characteristics of the 12 field sites where the ultraviolet-B (UVB) removal experiment was done. 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 concentration gradient. DOC = dissolved organic C.

Characteristic	Middle Cottonwood 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 (μS/m)	87	31	163	195	641	168	136	165	157	147	135	83
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	25.2	29.2	24.2	11.9	26.7	19.4	20.7	10.1	12.1

Field experiment

Physiochemical characteristics of field sites.—Physiochemical characteristics of the field sites showed considerable variation among streams (Table 3). Conductivity, 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 WT. Median Zn concentrations ranged from below detection (5 μg/L) at WT and Middle Cottonwood Creek to 530.9 μg/L at Chalk Creek. Median DOC values ranged from 0.8 mg/L at Buckskin Gulch and French Gulch to 2.5 mg/L at WT. UVB radiation reaching the streambed over the 60-d experiment varied among field sites and ranged from 6.5 J/cm^2 at Middle Cottonwood Creek to 29.2 J/cm^2 at 10 Mile Creek. DOC concentration accounted for 78% of the variation between UVB at the water surface and the amount of UVB reaching the streambed at these sites ($y = -0.43x + 1.01$, $R^2 = 0.78$, $F_{1,10} = 36.26$, $p = 0.0001$). In general, estimated UVB levels at the streambed were high, and sites with low DOC received as much as 70% of the surface UVB.

Effects of UVB removal on benthic communities.—The 2-way ANOVA indicated that removal of UVB significantly increased the abundance of most groups (Table 4, Fig. 2A–H). Specifically, removal of UVB increased the abundances of grazers ($p = 0.0014$; Fig. 2A), the mayfly *B. bicaudatus* ($p = 0.0415$; Fig. 2B), Ephemeroptera ($p = 0.0136$; Fig. 2D), Orthocladiinae midges ($p = 0.0012$; Fig. 2E), Trichoptera ($p = 0.0146$; Fig. 2F), and total abundance ($p = 0.0009$; Fig. 2G). UVB removal had no significant effects on Heptageniidae abundance ($p = 0.1380$; Fig. 2C) or chlorophyll *a* concentration ($p = 0.9627$; Fig. 2H). Grazer abundance was strongly related to chlorophyll *a* under both 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 result was expected because the sites were selected along a gradient of metal contamination, which is known to cause changes in benthic community composition (Clements 1994, 2004). There were no significant interactive effects of UVB removal and site on the community metrics evaluated.

Effects of UVB removal along the Zn gradient.—Metrics that showed a significant response to removal of UVB based on 2-way ANOVA (total abundance and the abundance of grazers, Ephemeroptera, Trichoptera, Orthocladiinae, and *B. bicaudatus*) were plotted along a Zn concentration gradient to determine if effect of UVB removal increased with metal concentration. *d* of UVB removal at metal-polluted sites was generally

TABLE 4. Results of the 2-way analysis of variance for macroinvertebrate abundance and chlorophyll *a* concentration for the main effects of site, ultraviolet-B (UVB) removal, and for the site × UVB removal interaction for 12 Rocky Mountain streams in central Colorado. Significant *p*-values indicating UVB treatment effects are shown in bold. df for site and site × UVB interaction were 11, 47; df for UVB removal were 1, 47.

Abundance	Source	*F*	*p*
Grazer	Site	15.38	<0.0001
	UVB removal	6.52	**0.0014**
	UVB removal × site	0.51	0.8864
Baetis bicaudatus	Site	11.97	<0.0001
	UVB removal	4.39	**0.0415**
	UVB removal × site	0.51	0.8864
Heptageniidae	Site	28.98	<0.0001
	UVB removal	2.28	0.1380
	UVB removal × site	1.07	0.4080
Ephemeroptera	Site	25.00	<0.0001
	UVB removal	6.58	**0.0136**
	UVB removal × site	1.19	0.3175
Orthocladiinae	Site	25.55	<0.0001
	UVB removal	11.85	**0.0012**
	UVB removal × site	1.12	0.3701
Trichoptera	Site	10.61	<0.0001
	UVB removal	6.43	**0.0146**
	UVB removal × site	0.75	0.6860
Total	Site	19.52	<0.0001
	UVB removal	12.70	**0.0009**
	UVB removal × site	0.91	0.5344
Chlorophyll *a*	Site	8.15	<0.0001
	UVB removal	0.01	0.9627
	UVB removal × site	0.70	0.7293

greater than at reference sites (Fig. 3A–F) and was significantly different for Orthocladiinae ($p = 0.0160$; Fig. 3B) and *B. bicaudatus* ($p = 0.0290$; Fig. 3C). Both increases and decreases in abundance occurred in response to UVB removal at reference sites. In contrast, abundance consistently increased when UVB was removed at metal-polluted sites. One important exception to this pattern was observed at French Gulch, a heavily polluted stream (median Zn concentration = 392 μg/L). *d* of UVB removal at French Gulch was low for all metrics except Orthocladiinae abundance. Average UVB reaching the streambed at French Gulch was the lowest of all metal-polluted sites (10.1 J/cm^2) (Table 3), a result that probably was a consequence of partial shading caused by the incised channel and dense streamside vegetation at this site.

Discussion

Effects of UVB addition experiments in stream microcosms

Results of the microcosm experiments showed that abundances of the selected macroinvertebrate groups were reduced in UVB treatments and that the magnitude of these effects was consistently larger for communities with a history of metals contamination. Despite considerable interest in how aquatic systems respond to UVB, controlled laboratory experiments investigating effects of UVB on stream macroinvertebrate communities are relatively uncommon in the literature (Kiffney et al. 1997b, McNamara and Hill 1999). These previous studies showed apparent increases in the drift of mayflies, caddisflies, and black flies in experimental streams (Kiffney et al. 1997b) and that certain species (*D. 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, b, Kelly et al. 2001). Results of our UVB addition experiments showed a relatively strong response for most of the metrics and that abundance was consistently reduced under UVB exposure. In particular, total macroinvertebrate abundance and the abundance of Ephemeroptera, *B. bicaudatus*, grazers, and Orthocladiinae were reduced by 40 to 75%, corresponding to relatively large *d* values. These experimental results were generated from 8 different sites within 2 major river basins in Colorado. Community (total abundance), trait (grazer abundance), and species-level (*B. bicaudatus*) measurements responded to UVB addition. The consistency of these results is notable and provides evidence that benthic communities in Colorado Rocky Mountain streams are highly sensitive to UVB addition.

Total UVB dose in our microcosm experiments was comparable to ambient levels under open-canopy conditions, but the wavelength spectrum of our lamp-generated UVB probably differed from natural sunlight (Kiffney et al. 1997b, McNamara and Hill 1999). Lamps used in these studies are likely to produce a greater biologically effective dose than natural UVB (Behrenfeld et al. 1993) because they produce a spectrum with higher energy at shorter wavelengths (290–312 nm). Biological effects observed in the laboratory using UVB lamps should be interpreted cautiously because effects of UVB exposure are dependent on total energy and irradiance (McNamara and Hill 1999). Although lamp-generated UVB does not perfectly reproduce the natural solar spectrum, we consistently observed decreases in abundance across a range of doses (2.3–4.8 J/cm^2). Kiffney et al. (1997b) used the Diffey action spectra (McKinley and Diffey 1987) to calculate Diffey-erythemal-weighted irradiance for the lamps used in our study. Although the spectra of these lamps are weighted toward the shorter wavelengths, Kiffney et al. (1997b)

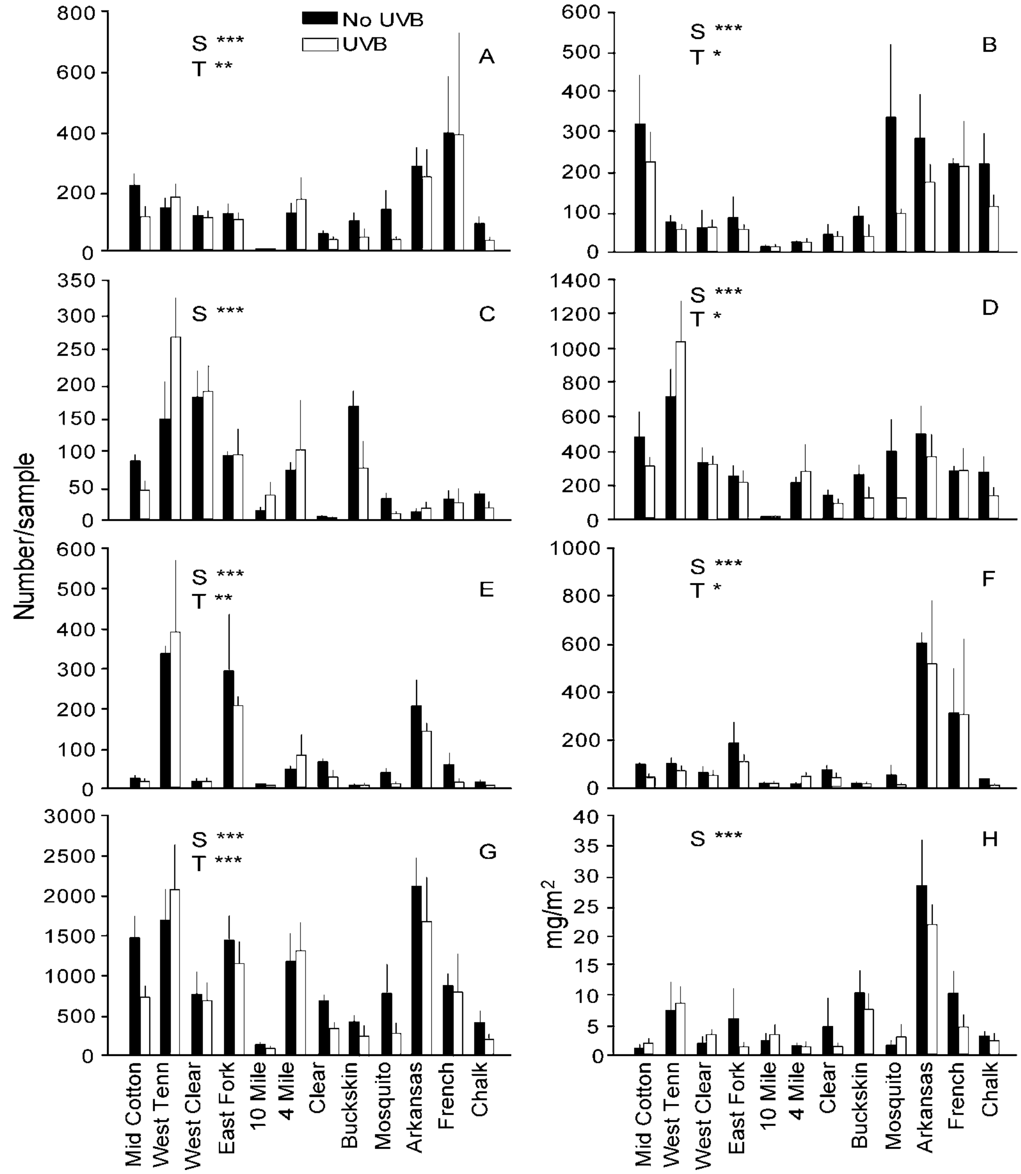

FIG. 2. Mean (+1 SE) abundances of grazers (A), *Baetis bicaudatus* (B), Heptageniidae (C), total Ephemeroptera (D), Orthocladiinae (E), total Trichoptera (F), total macroinvertebrate abundance (G), and chlorophyll *a* (H) in ultraviolet-B (UVB) removal and control treatments in 12 Rocky Mountain streams, Colorado. Sites are ordered from left to right along an increasing Zn concentration gradient. Results are given for the 2-way factorial analysis of variance (site [S] and treatment [T]). The site × treatment interaction term was not significant. See Table 4 for details of statistical analysis. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Mid Cotton = Middle Cottonwood, West Tenn = West Tennessee.

found that Diffey-erythemal-weighted irradiance of the lamps was similar to that of natural sunlight (3.18×10^{-5} W/cm^2 for natural sunlight vs 2.78×10^{-5} W/cm^2 for the lamps). More important, these experiments provide evidence that insects from metal-polluted streams are more sensitive to UVB than are those from unpolluted sites. The consistency of results across these experiments provides useful information regarding specific responses of benthic communities to UVB exposure, and this information supports the results of our field experiments.

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 y. These studies have shown collectively that manipulating ambient levels of ultraviolet radiation (UVR) can

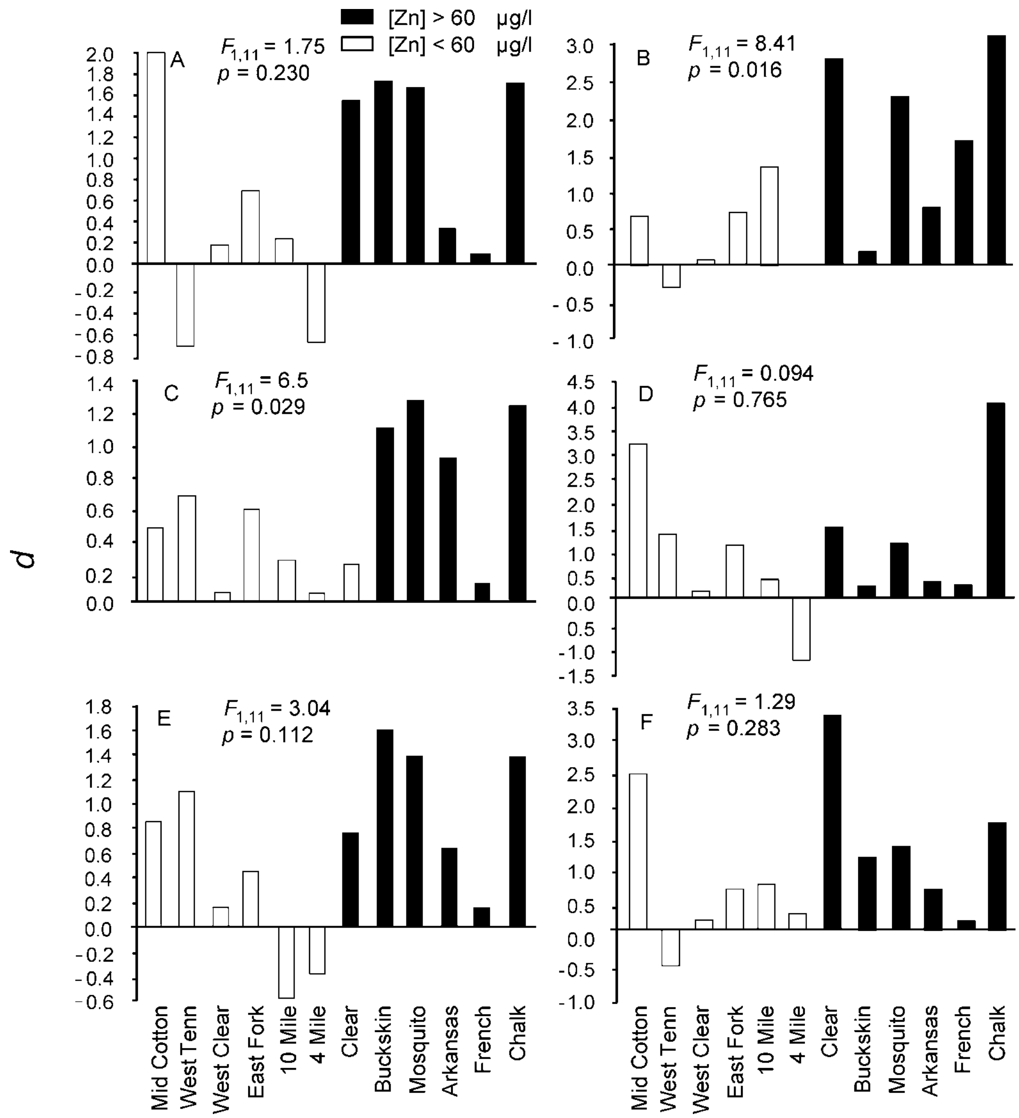

FIG. 3. Standardized mean difference (*d*) of abundance metrics that showed a significant response to ultraviolet-B (UVB) removal from the field experiment. Sites are ordered from left to right along an increasing Zn concentration gradient. Positive *d* values indicate an increase in abundance caused by UVB removal, whereas negative values indicate a decrease in abundance caused by UVB removal. A.—Grazers. B.—Orthocladiinae. C.—*Baetis bicaudatus*. D.—Trichoptera. E.—Ephemeroptera. F.—Total abundance. CI = confidence interval. Mid Cotton = Middle Cottonwood, West Tenn = West Tennessee.

cause changes in trophic structure (Bothwell et al. 1994), herbivore density (DeNicola and Hoagland 1996), community composition and colonization dynamics (Kiffney et al. 1997a, Donahue and Schindler 1998, Kelly et al. 2001, 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 experiments (30–91 d) (Kiffney et al. 1997a, Kelly et al. 2001, 2003), demonstrating the importance of time scale when assessing UVB effects. Results from our study conducted in 12 separate 1st- to* 4th-order Rocky Mountain streams showed that UVB removal resulted in significant increases for 6 of the 7 macroinvertebrate metrics that we examined. The strength and consistency of these responses are especially noteworthy given that our UVB filters shaded a relatively small portion of the streambed. We believe that variation in the magnitude of UVB effects among the 12 streams was most probably a result of metal contamination (see below) and DOC concentration, which ranged from 0.8 to 2.5 mg/L. Because the amount of UVB that reached the streambed was closely related to DOC concentration, relatively minor differences in DOC among streams could alter exposure to benthic communities.

In contrast to a previous study in a Rocky Mountain stream (Kiffney et al. 1997a), abundance of heptageniid mayflies and chlorophyll *a* biomass did not increase in our UVB removal treatments. Our sites were selected across a gradient of metal contamination that could have obscured the response of chlorophyll *a* and Heptageniidae to UVB removal because these 2 endpoints are known to be influenced by metals (Clements et al. 2002, Carlisle and Clements 2003). Alternatively, increased grazing pressure in UVB removal treatments might have limited algae accrual. Several studies have shown direct effects of UVR manipulation on algal biomass (Bothwell et al. 1993, Kiffney et al. 1997a, McNamara and Hill 1999, Kelly et al. 2003). Short-term exclusion of UVR typically increases algal biomass, but 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 UVB removal significantly increases grazer abundance, then 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, significantly more grazers were found in our UVB removal treatments compared to ambient conditions, but no differences were found in algal biomass. Observed chlorophyll *a* concentrations were inversely related to grazer abundances 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. In our study, the greatest effect of UVB removal on grazers was observed at Middle Cottonwood Creek, the site with the lowest levels of UVB at the streambed (6.46 J/cm^2 over 60 d). 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). We hypothesize that grazer–algae interactions probably 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 affect algal biomass and diversity in open-canopy sections of Rocky Mountain streams (Rader and Belish 1997), thus altering resource quality available to grazers and potentially modifying trophic dynamics.

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

Results of microcosm and field experiments showed that benthic communities with a history of metal exposure are more susceptible to UVB than are naive communities. Studies investigating combined effects of UVB and other stressors are rare in the literature. Kashian et al. (2007) collected natural benthic communities from 2 of the sites used in our 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 of 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. (2007) study were observed for total mayfly abundance (primarily *B. bicaudatus*), which was reduced by 41% in UVB treatments.

Baetis bicaudatus is widely distributed in Rocky 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 is quite sensitive to UVB exposure, especially in streams with a history of elevated metals. 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 using abundant food resources on the surface of rocks during the day outweighs the damage caused by intense UVB exposure. Previous research conducted in pelagic areas of lentic ecosystems has described the important tradeoff between grazing in high-quality habitats and avoiding intense UVB (Williamson 1995), but few studies have reported this phenomenon in benthic habitats. To avoid UVB exposure, *Baetis* might limit grazing activity to areas with partial shading during certain times of the day. Also, their high mobility might protect them from exposure to UVB for long periods of time while foraging.

Results of our field and microcosm experiments supported the hypothesis that *B. bicaudatus* from polluted sites were consistently more sensitive to UVB than were *B. bicaudatus* from reference sites. The greater sensitivity of *B. bicaudatus* to UVB at contaminated sites might be a cost associated with gaining an increased tolerance to metals.

The cost of tolerance.—Enhanced tolerance to heavy metals in populations with a history of exposure has been demonstrated in algae (Stokes et al. 1973, Niederlehner and Cairns 1992), invertebrates (Bryan and Hummerstone 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 2 general explanations for enhanced tolerance to metals (Klerks and Levinton 1989, Niederlehner and Carins 1992). The ability to adapt or acclimate to metals is beneficial at polluted sites, but there might be additional energetic costs. Organisms expending energy on eliminating, sequestering, or binding metals might have less energy available for other physiological functions, including the ability to tolerate additional stressors (Sibly and Calow 1989). Several examples in the literature report a cost associated with enhanced tolerance to contaminants. Weis and Weis (1989) demonstrated decreased tolerance to salinity in populations of estuarine killifish with a history of metals exposure. Postma et al. (1995) found that 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. In addition, 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 poor general condition of flounder in metal-exposed populations.

Previous studies conducted using communities from the Arkansas River have shown that organisms from metal-polluted sites are more susceptible to additional stressors such as acidification (Courtney and Clements 2000), UVB radiation (Kashian et al. 2007), and predation by stoneflies (Clements 1999) compared to organisms from reference streams. These findings coupled with our results suggest that energetic and other physiological costs of acclimation and adaptation to chronic levels of metal pollution might increase susceptibility of aquatic organisms to additional stressors. Aquatic insects regulate metals mainly through the production of metallothionein (Hare 1992), 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 might have been involved in a study in which Arkansas River communities were more tolerant to metals but more susceptible to acidic pH than reference communities (Courtney and Clements 2000). The precise mechanism is unknown, but we speculate that the energetic cost of producing metallothionein to regulate metals might inhibit the ability of organisms to repair DNA efficiently when it has been damaged by UVB exposure.

Implications

Our results have important implications for Rocky Mountain streams, given their unique physicochemical characteristics and the magnitude of metals pollution in this region. Aquatic communities in Rocky Mountain streams are subjected to intense levels of UVB radiation, in some instances >70% of UVB at the water surface, because of shallow depth, high elevation, and naturally low levels of UVB-attenuating DOM. Predicted effects of climate change in the Rocky Mountain region over the next 100 y are expected to have significant impacts on riparian vegetation, biogeochemical cycles, and hydrologic processes (Baron et al. 2000). Changes in biogeochemical cycles and riparian vegetation probably will decrease DOM quantity in summer when UVB is highest, thereby increasing benthic community exposure to UVB. Moreover, summer stream flow and water depth are expected to decrease in snowmelt-driven watersheds in response to climate change (Lettenmaier and Gan 1990, Rango 1995, Baron et al. 2000). Reduced stream discharge during summer when UVB is highest, coupled with climate-induced decreases in DOM quantity, might have significant consequences for aquatic communities in this region, especially at sites with a history of metals contamination.

In conclusion, studies assessing the influence of UVB radiation on benthic communities are uncommon despite the importance of understanding the effects of increased UVR on stream ecosystems. Our study is the first large-scale field experiment to investigate the direct effects 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. Responses of benthic communities in these experiments were comparable to other studies and showed that communities subjected to long-term metal exposure were more sensitive to UVB than were communities from reference streams. Given the characteristics inherent to Rocky Mountain streams that increase UVB exposure to benthic communities and the extent of heavy metal pollution in this region, the effects of increased UVB radiation reaching the earth's surface might be more severe than previously considered, especially for stream communities already subjected to other stressors.

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