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Changes in dissolved organic material determine exposure of stream benthic communities to UV-B radiation and heavy metals: implications for climate change

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Abstract

Changes in regional climate in the Rocky Mountains over the next 100 years are expected to have significant effects on biogeochemical cycles and hydrological processes. In particular, decreased discharge and lower stream depth during summer when ultraviolet radiation (UVR) is the highest combined with greater photo-oxidation of dissolved organic materials (DOM) will significantly increase exposure of benthic communities to UVR. Communities in many Rocky Mountain streams are simultaneously exposed to elevated metals from abandoned mines, the toxicity and bioavailability of which are also determined by DOM. We integrated field surveys of 19 streams (21 sites) along a gradient of metal contamination with microcosm and field experiments conducted in Colorado, USA, and New Zealand to investigate the influence of DOM on bioavailability of heavy metals and exposure of benthic communities to UVR. Spatial and seasonal variation in DOM were closely related to stream discharge and significantly influenced heavy metal uptake in benthic organisms. Qualitative and quantitative changes in DOM resulting from exposure to sunlight increased UV-B (290–320 nm) penetration and toxicity of heavy metals. Results of microcosm experiments showed that benthic communities from a metal-polluted stream were tolerant of metals, but were more sensitive to UV-B than communities from a reference stream. We speculate that the greater sensitivity of these communities to UV-B resulted from costs associated with metal tolerance. Exclusion of UVR from 12 separate Colorado streams and from outdoor stream microcosms in New Zealand increased the abundance of benthic organisms (mayflies, stoneflies, and caddisflies) by 18% and 54%, respectively. Our findings demonstrate the importance of considering changes in regional climate and UV-B exposure when assessing the effects of local anthropogenic stressors.

Keywords: benthic macroinvertebrates, biogeochemistry, compound perturbations, dissolved organic matter, mining pollution, photo-oxidation, Rocky Mountain streams, stream discharge, UV-B radiation

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Introduction

Because investigations of anthropogenic disturbances in aquatic ecosystems are typically limited to a single stressor, we generally lack the ability to predict how communities will respond to compound perturbations. Paine *et al*. (1998) reviewed responses of communities subjected to a variety of stressors, including El Niño events, severe storms, exotic species, wildfires, deforestation, and hypoxia. These researchers noted that while communities exposed to a single disturbance generally recovered, the addition of natural or anthropogenic perturbations impeded recovery and often forced systems to a new stable state. We believe this

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situation is likely to occur in contaminated systems that are simultaneously experiencing effects of global climate change and increased exposure to ultraviolet radiation (UVR), particularly UV-B (290–320 nm). While considerable research has been devoted to understanding direct effects of these stressors on aquatic ecosystems, much less is known about how global change will interact with contaminants to structure aquatic communities.

Increased levels of atmospheric CO_2 and associated climate change are expected to have significant impacts on riparian vegetation, biogeochemical cycles, and hydrological processes in watersheds within the Rocky Mountain region (Baron *et al.*, 2000). Because most (60–85%) of the annual precipitation in Colorado's mountains occurs as snow (Baron *et al.*, 1992), alterations in winter snow pack and the timing of spring runoff have important hydrological and biogeochemical implications. In particular, climate-induced alterations in stream discharge in this region are likely to influence the quality and quantity of allochthonous dissolved organic material (DOM). Baron *et al.* (2000) reported that a fundamental change in snow accumulation would occur with a 4 °C warming scenario, allowing snow melt to continue and soils to remain wet throughout much of the winter. Continuously wet soils and altered spring snow melt patterns enhance the flushing of shallow soil and litter where most DOM is produced (Hornberger *et al.*, 1994; Neff & Asner, 2001). Climate change in the Rocky Mountain region is likely to reduce the seasonal pulse of DOM due to continuous loss through winter, resulting in lower levels of DOM during low flow, summer conditions.

Superimposed on these climate-related changes in stream discharge and biogeochemical processes are the increased levels of UV-B resulting from loss of stratospheric ozone. Reduced primary production, shifts in community composition, and alterations in trophic structure are associated with UV-B exposure (Smith *et al.*, 1992; Vincent & Roy, 1993; Bothwell *et al.*, 1994; Williamson, 1995; Kiffney *et al.*, 1997a). UV-B exposure, which increased significantly since the 1970s (Kerr & McElroy, 1993; World Meteorological Organization, 1998), will likely remain elevated over the next several decades because of reduced ozone levels (McKenzie *et al.*, 2007). Because of shallow depth and naturally low levels of UVR-attenuating DOM, aquatic communities in Rocky Mountain streams are subjected to intense levels of UV-B radiation during summer (Kiffney *et al.*, 1997a). In addition, because UV-B increases by approximately 20% for each 1000 m in elevation, alpine and subalpine ecosystems are at considerable risk (Sommaruga, 2001).

Interactions among DOM, UV-B, and heavy metals

High elevation streams in the Southern Rocky Mountain Ecoregion offer a unique opportunity to examine potential interactions among climate change, UV-B, and heavy metal pollution. Heavy metal contamination from historic mining operations is ubiquitous in the West and widely regarded as a major environmental stressor in Rocky Mountain streams (Clements *et al.*, 2000; Niyogi *et al.*, 2001; Cain *et al.*, 2004). Seven of the fifteen US EPA 'Superfund' sites in Colorado are associated with mining activities, and heavy metals from approximately 19 000 abandoned mines affect over 2600 km of Colorado's streams (Colorado Department of Health, 1992). A survey of 79 randomly selected streams in Colorado's Southern Rocky Mountain Ecoregion showed that approximately 23% of these sites were degraded by heavy metal contamination (Clements *et al.*, 2000). Extrapolating these results to the larger population of streams in this region, these data suggest that a significant number of Colorado streams are simultaneously subjected to intense UV-B and heavy metal pollution. Surprisingly, there is little information on the direct effects of UV-B in these streams and even less is known about the potential interactions between UV-B and heavy metals.

Interactions among DOM, heavy metals, and UV-B in streams are likely because natural organic materials simultaneously affect metal bioavailability and penetration of UV-B (Fig. 1). The ability of DOM to bind metals and attenuate light varies with source (e.g. allochthonous vs. autochthonous) and chemical composition (McKnight *et al.*, 1983, 2001). Qualitative and quantitative changes in DOM resulting from climate-induced variation in hydrology and biogeochemistry (Schindler *et al.*, 1996; Pienitz & Vincent, 2000) have the potential to increase metal bioavailability and UV-B exposure in

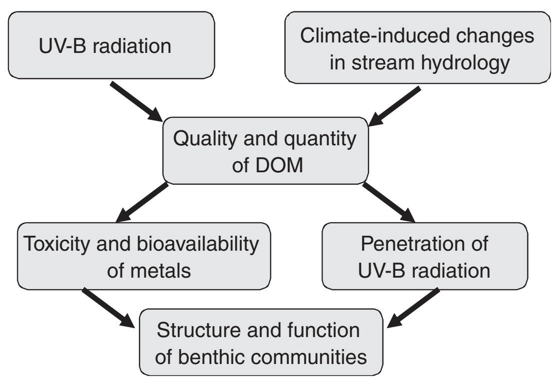

Fig. 1 Conceptual model showing the influence of UV-B radiation and stream hydrology on dissolved organic materials and its effects on heavy metal bioavailability and UV-B exposure in benthic communities.

stream communities (Brooks *et al*., 2007). Furthermore, DOM is degraded by exposure to UV-B through a process known as photo-oxidation, thereby increasing UV-B penetration and metal bioavailability (De Haan, 1993; Morris & Hargreaves, 1997; Larson *et al*., 2007).

We tested the hypothesis that climate-induced changes in stream hydrology that are predicted for Rocky Mountain streams will alter the quality and quantity of DOM, thereby increasing the exposure of benthic communities to UV-B radiation and heavy metals. We integrated field surveys of 19 streams along a gradient of metal contamination with microcosm and field experiments to examine the direct and interactive effects of UV-B radiation and heavy metals on macroinvertebrate communities. Our findings suggested that responses of benthic communities to these compound perturbations are complex, interactive, and could not be predicted based on our understanding of the individual stressors.

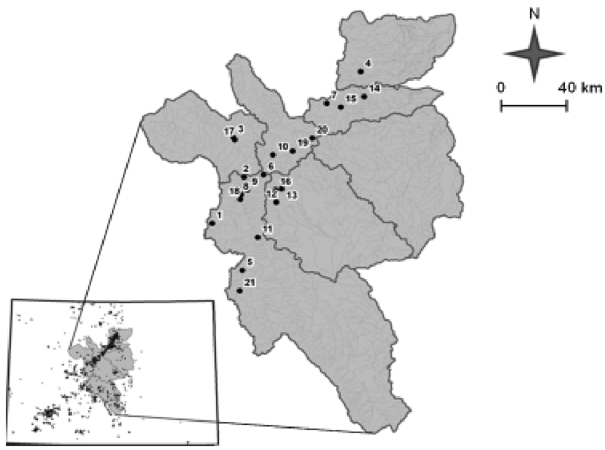

Fig. 2 Map showing watershed boundaries and locations of the 21 field monitoring sites in Colorado (see Table 1 for site names). Inset map of Colorado shows the distribution of abandoned mines in the study area.

Materials and methods

Study sites

Field monitoring and UV-B exclusion experiments were conducted in streams within the Southern Rocky Mountain Ecoregion of Colorado. To provide a broader context for our study and to quantify UV-B effects in a region where levels are dramatically elevated, we also conducted UVR exclusion experiments on the North Island of New Zealand. For a variety of reasons, UV-B levels in New Zealand are considerably greater than those measured at equivalent latitudes in the northern hemisphere (McKenzie *et al*., 1999).

For the field monitoring study, we selected 21 sites (mean elevation = 2897 m) along a gradient of metal contamination within several of the major watersheds in Colorado (Fig. 2). Streams in this region drain high elevation catchments with sparse soil development. The sites were characterized by relatively steep gradients, open canopies, and predominately cobble substrate. Typical landcover in these basins consists of bare rock, open meadow, and coniferous forest. Riparian vegetation is dominated by willow (*Salix* spp.) and thinleaf alder (*Alnus tenuifolia*). Snow and ice cover these streams for up to 6 months of the year, followed by a typical snow melt-dominated hydrograph where peak annual discharge coincides with peak snow melt.

Field monitoring

The objectives of our field-monitoring program were to (1) characterize the relationship between stream discharge and DOM; (2) examine spatial and seasonal variation in DOM, heavy metals, and UV-B exposure; and (3) quantify the influence of DOM, stream discharge, and other physicochemical characteristics on metal bioaccumulation in benthic macroinvertebrates. To characterize seasonal variation and measure the relationship between discharge and DOM, we sampled each of the 21 sites every 3–4 weeks from April 11 to October 12, 2003. To estimate discharge in streams without permanent gauges, we measured current velocity (Marsh-McBirney Flow Mate, Fredrick, MD, USA) and depth at 20 cm intervals across the stream channel. Stream discharge ($m^3 s^{-1}$) was calculated using the continuity equation (discharge = area × current velocity). Water samples for analysis of dissolved organic carbon (DOC; a surrogate measure that comprises approximately half of the DOM in a sample), were filtered (0.45 μm) in the field, acidified with hydrochloric acid, and stored in baked amber glass containers at 4 °C. DOC was measured in the laboratory within 24 h using a Shimadzu TOC-5050A total organic carbon analyzer. Routine water quality parameters (temperature, conductivity, and pH) were measured in the field. Water samples (1 L) for determination of hardness and alkalinity were analyzed in the laboratory using standard titration procedures. Water samples (15 mL) for determination of heavy metals (Cd, Cu, and Zn) were filtered in the field through a 0.45 μm filter and preserved with nitric acid to a pH of <2. Metals were analyzed using flame or furnace atomic adsorption spectrophotometry (Varian Model FS 220).

To examine metal bioavailability and the influence of DOC on metal uptake, net-spinning caddisflies (Hydropsychidae: *Arctopsyche grandis*) were collected in spring and fall 2003, from 16 stations along a gradient of metal contamination. *Arctopsyche* is a relatively large, widely distributed caddisfly common in the Rocky Mountain streams. Because *Arctopsyche* is tolerant of heavy metals, we were able to obtain organisms from reference and most metal-contaminated streams. Organisms were collected using a D-frame net and transported to the laboratory on ice. Caddisflies were dried to a constant weight at 50 °C, cooled to room temperature, and weighed to the nearest 0.1 mg. Samples were digested in analytical grade nitric acid (1.0 mL), after which hydrogen peroxide (100 μL) was added to each sample to complete the digestion process. Samples were diluted to a final volume of 7 mL with glass-distilled water and analyzed for heavy metals using atomic absorption spectrophotometry.

Quantification of UV-B exposure in the field

UV-B attenuation was measured in the field on three occasions (June, August, and September 2003) at the 12 sites where UVR removal experiments were conducted. We quantified UV-B with an International Light radiometer (model 1400) equipped with a broadband UV detector and fitted with cosine correction quartz and a UV-B-1 filter. This device shows maximum responsiveness at 290 nm, and then falls off by approximately 50% at 270 and 300 nm (Ryer, 1998). To determine the influence of DOC on UV-B attenuation at our field sites we measured changes in UV-B with depth. UV-B measurements ($n = 3$) were taken during midday (10:00 hours to 14:00 hours) every 2 cm between the water surface and streambed under full sun conditions. Downward irradiance of UV-B at a given depth was calculated for each stream using Eqn (1):

$$\ln E_z = \ln E_0 - K_\mathrm{d}(z), \tag{1}$$

where z is the stream depth, E_z is UV-B intensity at z, E_0 is UV-B intensity at the surface, and K_d is the vertical attenuation coefficient. We estimated seasonal variation in UV-B flux to benthic communities at our field sites by combining results of the above relationship with our measures of stream depth and regional measures of surface UV-B obtained from a fixed monitoring station in Steamboat Springs, CO (http://uvb.nrel.colostate.edu/UVB/home_page.html).

Effects of UV-B photo-oxidation on DOM and metal bioavailability

We quantified the effects of photo-oxidation on DOC concentration, UV-B absorption, and metal bioavailability in the laboratory. Details of these experimental procedures have been published previously (Brooks *et al.*, 2007). Briefly, natural DOM was collected from six sites during spring peak and summer base flow conditions in 2003. Water samples were passed through a series of filters (10, 5, 1, and 0.2 μm) and an H^+ cation-exchange resin that removed >95% of all metals. DOM was concentrated using portable reverse osmosis (PROS/2S, RealSoft) and concentrates were stored in the dark at 4 °C in borosilicate bottles.

Photo-oxidation experiments were conducted by irradiating DOM solutions in a solar simulator (Atlas Suntest CPS+) under a full-spectrum 1.0 kW xenon lamp for 0, 4, 8, and 24 h. The dose of UV-B after 24 h irradiation corresponded to approximately 4 days of exposure at our field sites. We estimated the effects of irradiation on DOC concentration and specific UV absorption (SUV*a*). As a direct measure of photochemical effects on metal–DOM complexation, we titrated $Cu(NO_3)_2$ into replicate ($n = 2$–3) control and irradiated (24 h) solutions of DOM and recorded $\{Cu^{2+}\}$ with ion-selective (Cu-ISE; Orion Research, model 9429) and double-junction (Ag/AgCl; Orion Research, model 09-02) electrodes. We chose Cu for these experiments because its binding stability with organic molecules is relatively high among the transition metals (Stumm & Morgan, 1996). For metals bound less strongly than Cu, we would expect even greater effects of UV-B on metal complexation. Therefore, evaluating photochemical effects on Cu provided a conservative estimate of how UV-B would affect DOM complexation relative to other weakly bound metals.

Stream microcosm experiments

Two separate microcosm experiments were conducted to assess the effects of UV-B radiation and heavy metals on benthic communities collected from a reference and a metal-polluted stream. Details of these experiments have been published previously (Kashian *et al.*, 2007). Benthic communities were collected from West Tennessee Creek (reference) and the Arkansas River (metal polluted) using plastic trays (10 × 10 × 6 cm) filled with pebble and small cobble that were colonized for 40 days. Previous research has shown that communities established on these trays are similar to those collected from the natural substrate (Kiffney & Clements, 1996; Clements, 1999; Courtney & Clements, 2000). After colonization, the trays were removed, placed in insulated containers (four trays per container), and transferred to the Stream Research Laboratory at Colorado State University, Fort Collins, CO. The experimental streams in this facility receive untreated water directly from a nearby reservoir. We have previously used this facility to quantify species-specific differences in sensitivity to metals (Kiffney & Clements, 1994), compare the effects of metals on communities collected from different locations (Kiffney & Clements, 1996; Medley & Clements, 1998), and quantify the effects of UV-B radiation (Kiffney *et al*., 1997b; Kashian *et al*., 2004), acidification (Courtney & Clements, 1998, 2000), and stonefly predation (Clements, 1999) on benthic communities. For experiments conducted with the metal-polluted communities, colonization trays were distributed among 16 stream microcosms ($n = 4$) which were randomly assigned to one of four treatments (control, metals only, UV-B only, metals + UV-B) in a 2×2 factorial design (metals $\times$ UV-B exposure). The experimental design for the reference community was identical except that one additional replicate ($n = 5$) was used for the metal and metal + UV-B treatments. Stock solutions of metals ($CdCl_2$, $CuSO_4$, and $ZnSO_4$) were delivered to each treated stream from separate 20 L carboys using peristaltic pumps. Mean concentrations measured in metal-treated streams ($3.8\,\mu g\,L^{-1}$ Cd + $19.4\,\mu g\,L^{-1}$ Cu + $437.0\,\mu g\,L^{-1}$ Zn) were representative of those previously observed in the Arkansas River (Clements, 2004). UV-B treatments were achieved using a single SF 20 lamp (313 nm; National Biological, Twinsbury, OH, USA) suspended 15 cm above the water surface. The level of UV-B at the water surface in treated microcosms was $5.9\,J\,cm^{-2}$.

After 10 days, the four trays from each stream were combined and contents were rinsed through a 350 μm mesh sieve. In the laboratory, organisms were sorted and all individuals (except chironomids) were identified to genus or species. The biological response variables measured in these experiments included macroinvertebrate drift, community metabolism, and community composition (Kashian *et al*., 2007). Here, we report the effects of heavy metals and UV-B on the total abundance of Ephemeroptera, Plecoptera, and Trichoptera (EPT abundance), organisms know to be sensitive to both stressors (Kelly *et al*., 2003; Clements, 2004).

Responses to UVR removal in New Zealand outdoor experimental streams

To determine the effects of UVR removal on natural benthic communities in New Zealand, we conducted UVR exclusion experiments in outdoor stream channels during February 2005 (midsummer in the Southern Hemisphere). Experiments were conducted at the Whatawhata Research Centre, a biological reserve located near Hamilton, New Zealand. A description of the experimental stream facility has been published previously (Quinn *et al*., 1997). Natural river water from the Mangaotama Stream was delivered through a headbox to 12 stream channels ($20\,cm \times 2.4\,m$). Depth (40 mm), current velocity ($0.08 \pm 0.01\,m\,s^{-1}$) and discharge ($1.3 \pm 0.1\,L\,s^{-1}$) were controlled by manipulating the channel slope and the rate of water delivery from the headbox. Before the start of the experiment, each channel was filled with pebble and small cobble substrate collected from the Mangaotama Stream. The 12 stream channels were then randomly assigned to three treatments ($n = 4$): no UVR, delayed UVR, and full UVR. The no UVR and delayed UVR channels were covered with a plastic greenhouse film (Model K50, Klerk's Plastic Product Manufacturing Inc., Richburg, SC, USA) that excluded 99% of the incident UV-B and a significant portion of the UV-A (up to 380 nm), but allowed the passage of 89% of the photosynthetically active radiation (PAR). Filter material was removed from the delayed UVR treatments after 15 days, thereby exposing these communities to UVR only during the second half of the experiment. Benthic macroinvertebrate communities were collected from each stream on days 15 and 30 using a small ($0.022\,m^2$) Surber sampler. Samples were washed through a 350 μm mesh sieve and processed in the laboratory as described above.

Responses to UVR removal in the field

To examine the responses of benthic communities to UVR and heavy metals in the field, we conducted UVR exclusion experiments in 12 natural streams along a gradient of Zn contamination ($1–377\,\mu g\,L^{-1}$ Zn) and DOC concentration ($0.7–3.8\,mg\,L^{-1}$). Details of the experimental design and methods have been reported previously (Zuellig *et al*., 2008). Experiments were conducted for 60 days beginning in late summer, 2003. Replicate ($n = 3$) UVR exclusion filters ($1\,m^2$) constructed from plastic greenhouse film were attached to PVC frames and suspended 20 cm above the stream surface. Controls consisted of $1\,m^2$ open frames without UVR filter material. Because these experiments were conducted in relatively swift-flowing reaches of each stream, we assumed that physical and chemical characteristics (e.g. stream temperature, water quality) other than UVR levels were similar between open frame controls and treatments. After 60 days, benthic macroinvertebrates were collected using a $0.1\,m^2$ Hess sampler and preserved in 80% ethanol. In the laboratory, macroinvertebrate samples were processed and enumerated as described above.

Statistical analyses

All statistical analyses were conducted using SAS/STAT (SAS Institute, Cary, NC, USA). Where necessary, data were log-transformed (ln + 1) to satisfy assumptions of normality and homogeneity of variance. We quantified the relationship between UV-B attenuation (K_d) and

DOC concentration in the field using linear regression (PROC REG). Effects of solar irradiation on UV absorption and the relationship between cupric ion activity (p{Cu2 + }) and total Cu concentration (p[Cu]T) were analyzed using two-way (treatment × season) ANOVA (PROC ANOVA) and general linear models (PROC GLM), respectively. Responses of benthic communities to metals and UV-B in the stream microcosm experiments were analyzed using two-way (metals × UV-B) ANOVA (PROC ANOVA). Effects of UVR removal on EPT abundance in the New Zealand and Colorado experiments were determined using one-way and two-way (treatment × stream) ANOVA, respectively. In the Colorado field experiments, we also calculated effect size (Cohen's *d*) for each stream based on the mean difference in EPT abundance between UVR removal treatments and controls divided by the pooled standard deviation (Cohen, 1988). Cohen's *d* is an index commonly used in meta-analysis to characterize the relative magnitude of treatment effects. Values greater than 0.8 (or < -0.8) are considered to be 'strong effects' (Cohen, 1988).

Results

Field monitoring

Mean concentrations of Zn, the predominant metal measured in all streams, exceeded the US EPA hardness-adjusted water quality criterion value at 11 of the 21 sites and were greatest at French Creek, Snake River, and Chalk Creek (Fig. 3a). Concentrations of Cd and Cu were either below detection or rarely approached toxic concentrations at any site. Factors that influence bioavailability and toxicity of heavy metals were also variable among streams. For example, because of low levels of DOC, alkalinity, and pH (Table 1; Fig. 3b), heavy metals in the Snake River are considerably more bioavailable than in streams with similar concentrations. Mean DOC concentration varied approximately 4 × among the 21 sites, and was significantly correlated with stream discharge ($r = 0.54$; $P < 0.0001$). Highest DOC concentrations in these snow melt-dominated watersheds were measured in late spring, preceding peak discharge by 2–3 weeks (Fig. 4a) in most streams. The smaller secondary peak in DOC concentration observed in late summer resulted from local and isolated thunderstorms in several watersheds.

Levels of surface UV-B recorded at a permanent monitoring station in Steamboat Springs, CO ranged from 1.7 to 5.0 J cm^{-2} (Fig. 4b). Measures of vertical attenuation coefficients (K_d) showed that 25–71% of the surface UV-B reached the streambed in summer. We also observed a highly significant relationship between UV-B attenuation and DOC concentration

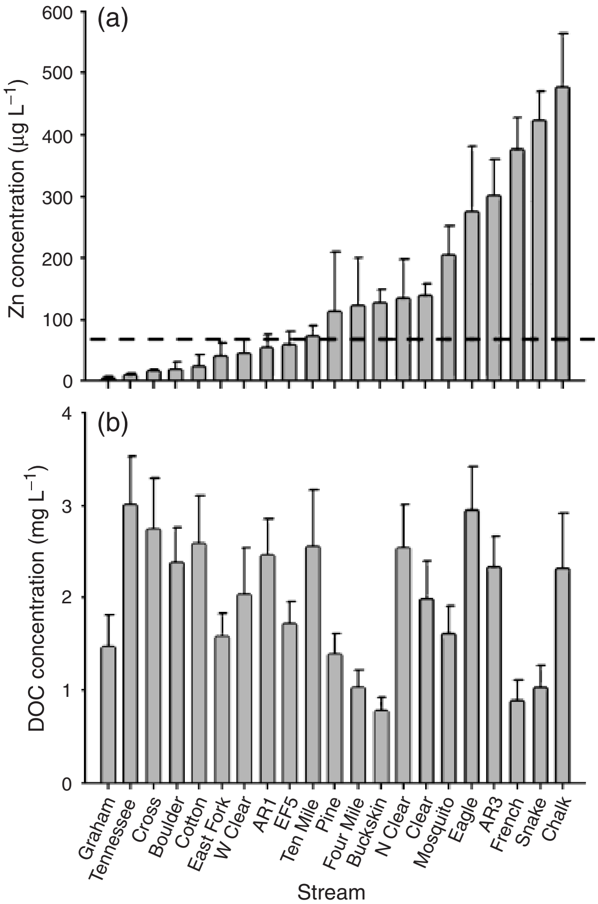

Fig. 3 Annual mean (±SE) concentration of Zn (a) and DOC (b) measured at 21 monitoring stations in Colorado. Samples were collected every 3–4 weeks from April 18 to October 11, 2003. Sites are arranged along a gradient of Zn concentration. The dashed horizontal line in the upper panel represents the approximate water quality criterion value for Zn in these streams.

($K_d = 4.39[\text{DOC}] - 1.78$; $r^2 = 0.78$; $P < 0.0001$), indicating that UV-B exposure to benthic communities was especially high in streams with low levels of DOC. Our estimates of seasonal variation in UV-B flux showed that the levels of UV-B reaching the streambed were lowest during late spring when stream depth and DOC concentrations were greatest. UV-B levels at the streambed gradually increased over the summer as a result of greater incident UV-B, lower discharge, and reduced DOC concentration.

Bioaccumulation of metals in the caddisfly *A. grandis* reflected seasonal and spatial variation in Zn, DOC, and other physicochemical characteristics of these streams (Fig. 5). Results of general linear models analysis (PROC GLM; $r^2 = 0.79$, $P < 0.0001$) showed that Zn concentrations in caddisflies were significantly higher in May than in October ($P = 0.0476$) and were greatly elevated at metal-polluted stations, particularly Chalk Creek and

Table 1 Mean physicochemical characteristics of the 21 field monitoring sites (see Fig. 2) in Colorado

Station	Conductivity ($\mu S\,cm^{-1}$)	Temperature (°C)	pH	Hardness ($mg\,L^{-1}$)	Alkalinity ($mg\,L^{-1}$)	Discharge ($m^3\,s^{-1}$)
1. Graham Gulch	73.3	3.8	7.58	38.8	37.6	0.21
2. Tennessee Creek	30.5	7.8	7.22	17.1	13.8	0.37
3. Cross Creek	45.1	6.4	6.85	21.6	12.1	2.12
4. Boulder Creek	36.9	7.8	7.24	17.6	13.6	2.41
5. Cottonwood Creek	90.9	4.4	7.42	52.1	46.9	0.48
6. East Fork of the Arkansas River	110.4	6.1	7.29	67.3	32.1	0.37
7. West Clear Creek	173.1	6.8	7.13	76.4	20.1	1.75
8. Arkansas River (AR1)	130.1	10.1	7.73	73.5	49.3	2.43
9. Arkansas River (EF5)	199.1	10.5	7.89	111.9	70.6	1.33
10. Ten Mile Creek	551.4	6.4	7.23	301.8	31.1	1.39
11. Pine Creek	82.6	6.5	7.40	45.3	36.4	0.39
12. Four Mile Creek	190.4	6.9	7.76	127.3	102.4	0.28
13. Buckskin Creek	167.6	7.7	7.61	91.9	46.3	0.30
14. North Clear Creek	79.1	6.7	7.17	37.1	20.0	0.76
15. Clear Creek	168.4	9.9	7.51	68.4	36.9	4.19
16. Mosquito Creek	158.1	9.0	7.76	99.6	60.1	0.48
17. Eagle River	120.9	7.0	7.55	68.8	56.8	5.27
18. Arkansas River (AR3)	171.6	9.6	7.70	92.0	50.9	2.45
19. French Creek	135.3	5.7	7.45	75.1	50.9	0.36
20. Snake River	116.3	5.9	5.65	96.5	2.3	0.54
21. Chalk Creek	86.9	4.5	7.22	43.9	21.4	0.75

Data were collected every 3–4 weeks from April 18 to October 11, 2003.

the Eagle River. Zn bioaccumulation in *A. grandis* was positively related to Zn in water ($P<0.0001$) and negatively associated with DOC concentration ($P<0.0040$).

Photo-oxidation effects on DOC, UV-B absorbance, and metal toxicity

Exposure of natural DOM to UV-B radiation decreased DOC concentration by 10–20% (mean $= 13 \pm 1\%$), but effects did not differ between spring and summer ($P = 0.8400$). Photo-oxidation significantly ($P = 0.0285$) reduced specific UV-B absorbance (SUV*a*) of DOM by an average of $22 \pm 3\%$ (mean $\pm$ SE) in 24 h exposures (Fig. 6a); however, most of this reduction occurred within the first 8 h of irradiation. UV-B absorbance of DOM was also significantly ($P<0.0005$) greater in spring than summer. These alterations in DOM quality and quantity influenced the bioavailability of heavy metals. Photo-oxidation of DOM significantly ($P<0.0001$) increased the concentration of $\{Cu^{2+}\}$, the most toxic form of Cu (Fig. 6b). There was also evidence that photo-oxidation effects on DOM were significantly greater in spring than summer ($P = 0.0105$).

Combined effects of UV-B and metals on benthic communities in stream microcosms

Regardless of site history, benthic communities were adversely affected by metals in the stream microcosm experiments (Fig. 7); however, the magnitude of these effects was much greater in communities from the reference site compared with the metal-polluted site. Relative to the controls, total EPT abundance of communities from the reference site was reduced by 57% in metal treatments compared with 26% for communities from the metal-polluted site. In contrast to the effects of metals, significant responses to enhanced UV-B were limited to the metal-polluted site. Total EPT abundance of communities from the metal-polluted site was reduced by 17% in the UV-B treatments compared with the no UVR treatments ($P = 0.0002$). The metals $\times$ UV-B interaction term was not significant for either the reference ($P = 0.3304$) or metal-impacted ($P = 0.7198$) community, indicating that UV-B exposure did not enhance the effects of metals on EPT abundance.

Community responses to removal of UVR in New Zealand and Colorado

The removal of UVR significantly increased the abundance of EPT taxa ($P = 0.0172$) in the New Zealand outdoor stream channels (Fig. 8). Although there were no differences among treatments after 15 days, EPT abundance was 32–84% greater in the no UVR treatment compared with the delayed and full UVR treatments after 30 days. Because of the relatively short duration of these experiments, differences among

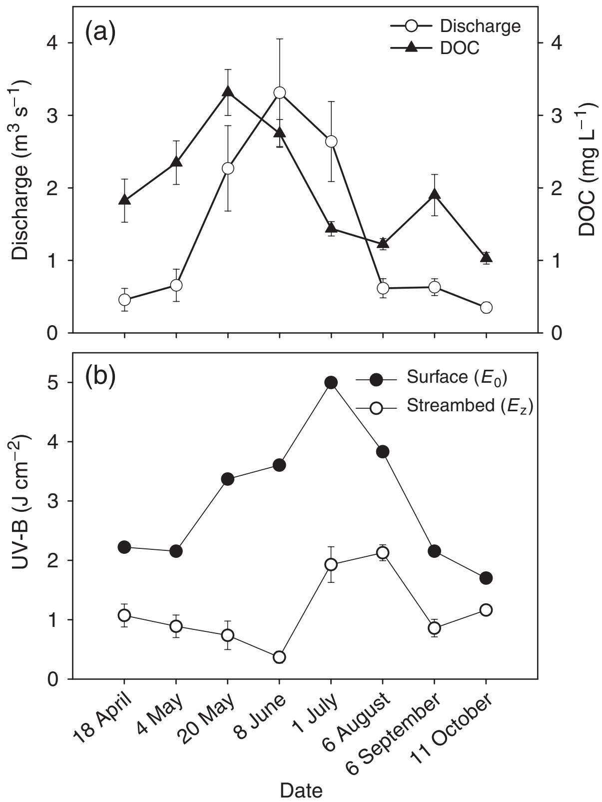

Fig. 4 Seasonal variation (mean ± SE) in stream discharge, DOC concentration (a), and UV-B radiation (b) in 21 Colorado streams sampled between April and October, 2003. Measures of surface UV-B (E_0) were obtained from a fixed monitoring station in Steamboat Springs, Colorado, and represent the cumulative weekly dose at this site. Streambed UV-B (E_z) was estimated from Eqn (1) based on E_0, vertical attenuation (K_d), and stream depth (z).

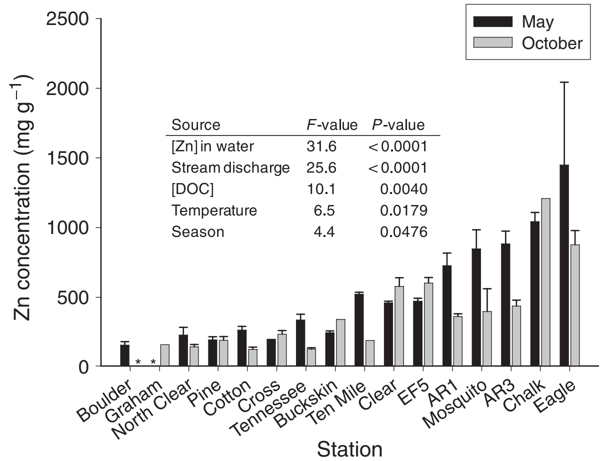

Fig. 5 Mean (±SE) Zn concentration measured in the caddisfly *Arctopsyche grandis* (Trichoptera) collected from 16 streams in May and October 2003. Inset shows results of general linear model analysis (PROC GLM) indicating the influence of Zn concentration in water, discharge, DOC concentration, water temperature, and season on metal uptake. Asterisk (*) indicates organisms not collected.

treatments were likely a result of lower recruitment and greater emigration of organisms in the delayed and full UVR streams. Interestingly, we observed greater effects at the end of the experiment in the delayed UVR treatments, in which filters were removed from the stream channels after 15 days, compared with the full UVR treatments.

Effects of UVR removal on benthic communities at the 12 Colorado field sites were variable among streams (Fig. 9a). However, the most frequently observed pattern was increased EPT abundance in the UVR exclusion treatments compared with controls. Cohen's *d*, an index of effect size, showed strong positive effects ($d > 0.8$) of UVR removal at five of the twelve streams and strong negative effects ($d < -0.8$) at one stream (Fig. 9b). Results of two-way ANOVA (stream × UVR treatment) showed that across all 12 streams, EPT abundance increased by approximately 18% when UVR was removed ($P = 0.0052$). As expected, there was a highly significant difference in EPT abundance among streams ($P < 0.0001$); however, there was no evidence of an interaction between UVR removal and stream ($P = 0.4624$).

Discussion

DOC, UV-B penetration, and metal bioavailability

The inverse relationship between DOC concentration and UV-B penetration in aquatic ecosystems is well established in literature (Williamson *et al*., 1996; Morris & Hargreaves, 1997; Sommaruga, 2001; Frost *et al*., 2005). However, because most of this research has been conducted in lentic or marine ecosystems, our understanding of interactions between DOC and UV-B in streams is limited (Frost *et al*., 2005). DOC concentration in our study was a major determinant of UV-B penetration, explaining 78% of the variation in K_d values among streams. Our laboratory experiments showed that qualitative and quantitative changes in DOC resulting from photo-oxidation likely increased the exposure of benthic communities to UV-B. Kelly *et al*. (2001) reported sharp increases in UV-B effects on benthic communities when DOC was reduced from 5.0 to 2.0 mg L^{-1}. Similarly, Williamson *et al*. (1996) observed dramatic increases in UV penetration at DOC concentrations <2.0 mg L^{-1}. In the present study, the mean concentration of DOC across seasons was <3.0 mg L^{-1} in all streams. DOC concentrations generally declined

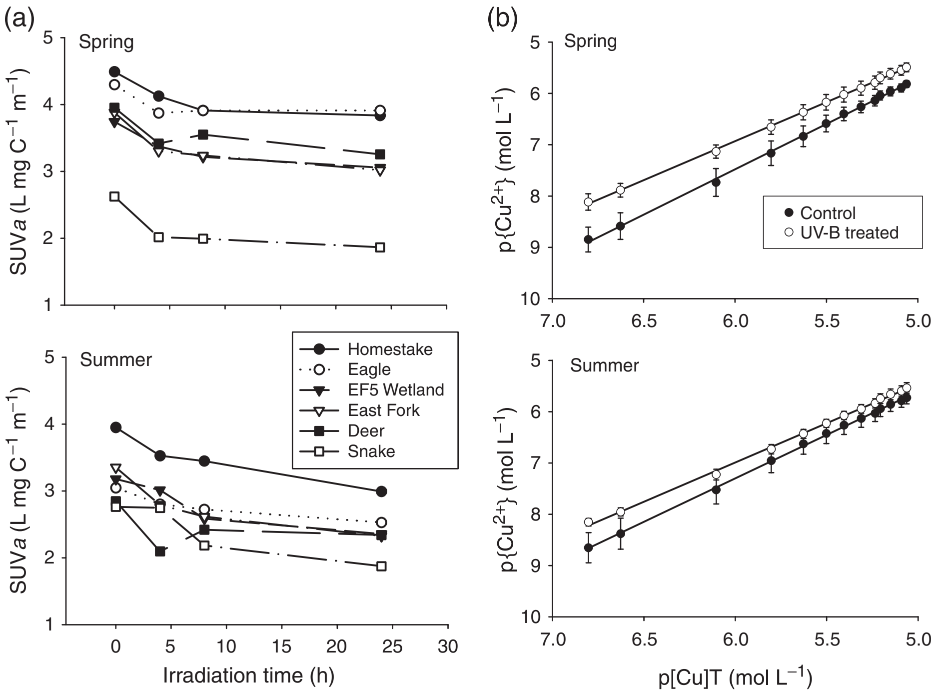

Fig. 6 Effects of photo-oxidation on UV-B absorption and Cu toxicity measured in spring and summer 2003. (a) Changes in the specific UV absorption (SUV*a*) of dissolved organic materials (DOM) after 0, 4, 8, and 24 h exposure to UV-B in a solar simulator. (b) Relationship between the negative logs of cupric ion activity (p{Cu^{2+}}) and total Cu concentration (p[Cu]T) during titration of Cu into control and irradiated (24 h) DOM solutions.

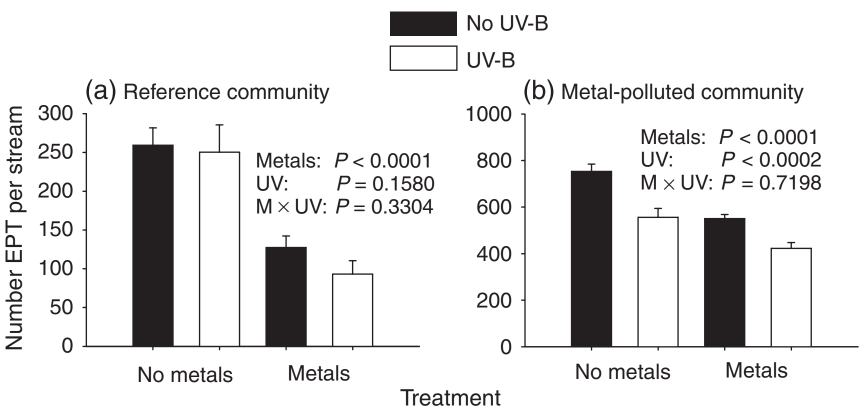

Fig. 7 Individual and combined effects of metals and UV-B on the total abundance of EPT (Ephemeroptera, Plecoptera, and Trichoptera) organisms in stream microcosms. Experiments were conducted using benthic communities collected from a reference site (a) and a metal impacted site (b). Mean (±SE) number of EPT organisms in each treatment and results of two-way (metals × UV-B) ANOVA are shown.

and stream depth decreased throughout the late spring and early summer. In contrast, UV-B levels remained elevated during this period. These results suggest that relatively modest changes in DOC resulting from natural photo-oxidation will increase UV-B penetration and exposure to benthic communities in these shallow, clear-water streams.

Our laboratory experiments and field monitoring studies also indicated that photo-oxidation of DOC increased the toxicity and bioavailability of heavy metals. Consistent with previous studies (Prusha & Clements, 2004), we observed that DOC concentration was inversely associated with heavy metal uptake in benthic organisms. The inverse relationship between DOC and

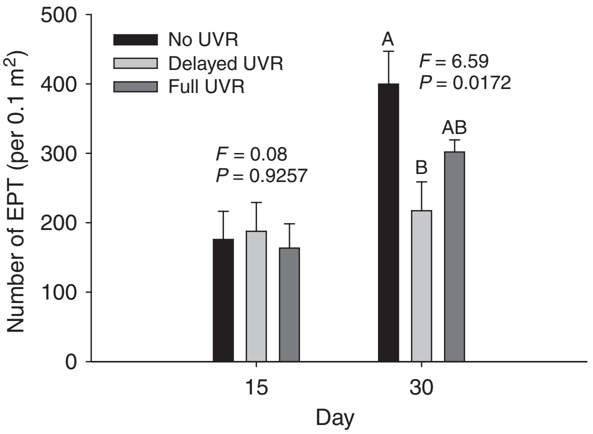

Fig. 8 Effects of UVR exclusion on abundance of EPT (Ephemeroptera, Plecoptera, and Trichoptera) organisms in outdoor experimental streams, New Zealand. Benthic communities in delayed UVR treatments were shielded during the first 15 days of the experiment and then exposed to full UVR during the final 15 days. Results of one-way ANOVA are also shown. Means with the same letter are not significantly different based on Ryan–Einot–Gabriel–Welsch multiple range test.

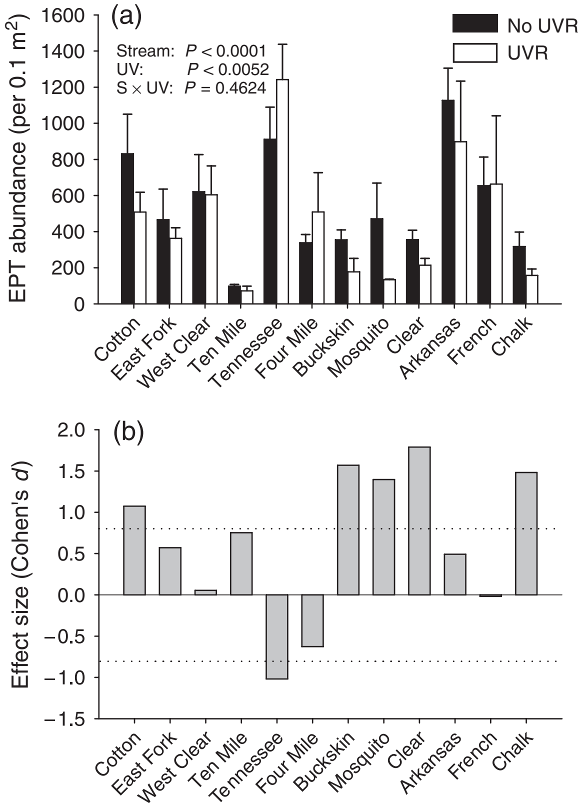

Fig. 9 Effects of UVR exclusion on the abundance of EPT (Ephemeroptera, Plecoptera, and Trichoptera) organisms in 12 Colorado streams. Experiments were conducted for 60 days from late summer to early fall 2003. (a) Mean (± SE) number of EPT in no UVR and UVR treatments. Results of two-way ANOVA (stream × UVR treatment) are also shown. (b) Effect size (Cohen's *d*), calculated as the mean difference between no UVR and UVR treatments divided by the pooled standard deviation. Values above or below the dotted lines are considered to represent relatively 'strong' effects (Cohen, 1988).

metal toxicity is well documented, and studies have shown that heavy metals are less bioavailable in systems with high levels of DOC (MacRae *et al*., 1999). Our experimental results demonstrated that photo-oxidation of DOC decreased complexation with Cu, resulting in a greater fraction of $\{Cu^{2+}\}$, the most toxic Cu species. Winch *et al*. (2002) reported similar effects of UV-B on metal bioavailability and speculated that photo-oxidation observed during long-term exposure in the field would be much greater than changes in the laboratory. Taken together, our laboratory and field studies indicate that photo-oxidation of DOC will increase both UV-B exposure and heavy metal bioavailability in shallow Rocky Mountain streams. Because photo-oxidation will be greatest in mid-summer when DOC concentrations and stream depths are lowest, we predict that the combined effects of heavy metals and UV-B will be greatest during this period.

Effects of UV-B on benthic communities

Although considerable effort has been devoted to understanding the effects of UV-B on marine and freshwater plankton (Smith *et al*., 1992; Williamson *et al*., 1999; Mostajir *et al*., 1999; Day & Neale, 2002; Allen & Smith, 2002), much less is known about responses of benthic communities in streams (Bothwell *et al*., 1994; Kiffney *et al*., 1997a, b; Kelly *et al*., 2001, 2003). The experiments conducted by Bothwell *et al*. (1994) demonstrated that some patterns observed in pelagic ecosystems may not be applicable to benthic communities. Results of our microcosm and field experiments were generally consistent with these previous studies and indicated that UV-B had a significant influence on the structure of benthic communities. Elimination of natural levels of UVR (up to 380 nm) from experimental stream channels in New Zealand and in 12 separate Rocky Mountain streams increased EPT abundance by 54% and 18%, respectively.

Microcosm experiments demonstrated that benthic communities subjected to long-term heavy metal pollution in the Arkansas River were generally more tolerant to metals but more sensitive to UV-B radiation compared with communities from a nearby reference stream. The greater sensitivity of Arkansas River communities to UV-B is in agreement with the model proposed by Paine *et al*. (1998), which predicted that responses of ecosystems subjected to multiple perturbations would differ from those resulting from a single stressor. Our results suggest a potential tradeoff between metal tolerance and UV-B sensitivity. Greater tolerance of Arkansas River benthic communities to metals has likely resulted from both population-level responses (e.g. acclimation and adaptation) and shifts in community composition (Clements, 1999; Courtney & Clements, 2000; Kashian *et al*., 2007). The underlying mechanisms responsible for the greater sensitivity of Arkansas River communities to UV-B are uncertain, but previous researchers have speculated that adaptation or acclimation to one set of environmental stressors may increase the susceptibility to novel stressors (Antonovics *et al*., 1971; Wilson, 1988; Clements, 1999). Levinton *et al*. (2003) concluded that the rapid loss of resistance to heavy metals in oligochaetes after cleanup of a metal-contaminated site resulted from a tradeoff between adaptation to Cd and reproductive fitness. Regardless of the specific mechanism for the Arkansas River communities, these results highlight the importance of previous exposure to contaminants and demonstrate that community composition can significantly influence responses to anthropogenic disturbance.

We believe that the neglect of benthic communities in UV-B research is a result of logistical challenges associated with manipulating UV-B in lotic ecosystems and the assumption that rapid attenuation of UVR limits exposure in benthic habitats. Indeed, in streams with relatively high DOC ($>10\,\mathrm{mg\,L^{-1}}$) benthic communities receive little UV-B exposure (Frost *et al*., 2005). In contrast to these results, our study provides evidence that benthic communities in Rocky Mountain streams are likely exposed to very high levels of UV-B radiation. Because of shallow depth and naturally low levels of DOC, as much as 70% of the UV-B measured at the surface reached the streambed in these systems during summer. Unlike planktonic organisms, periphyton communities and many benthic taxa are relatively sessile and unable to avoid exposure to UV-B. Even mobile macroinvertebrate taxa may be exposed to intense UV-B while grazing on periphyton in shallow, clear streams. We conclude that UV-B exposure poses a significant threat to benthic organisms in Rocky Mountain streams.

Effects of climate-induced alterations in stream hydrology on DOC and UV-B

Results of this research suggest that UV-B radiation and climate-induced changes in stream hydrology may interact in complex ways to influence DOM, UV-B exposure, and heavy metal bioavailability in Rocky Mountain streams. Warmer and drier conditions have been associated with reduced DOM export and increased UVR penetration in other regions (Schindler *et al*., 1990, 1996). DOC concentration in our study was positively correlated with stream discharge, reflecting both seasonal and spatial variation. Reduced concentrations of DOC resulting from changes in climate, hydrology, and/or acidification are considered to be more important determinants of UVR exposure in aquatic ecosystems than the loss of stratospheric ozone (Williamson *et al*., 1996; Pienitz & Vincent, 2000). Regional models of climate warming for the western USA predict significant declines in snow pack and substantial shifts in the timing of peak snow melt (Barnett *et al*., 2005; Mote *et al*., 2005). Earlier spring runoff will result in lower stream depth and reduced DOC concentrations in summer when UV-B levels and potential for photo-oxidation are greatest.

We believe that seasonal variation in DOC and UV-B penetration observed in our study provides a reasonable depiction of how these watersheds will respond to future climate-induced alterations in stream hydrology. In a UV-B exclusion experiment conducted in the nearby Cache la Poudre River, Kiffney *et al*. (1997a) observed that the effects of UV-B removal only occurred during late summer, low-flow conditions when DOC concentration and depth were lowest. Similarly, late summer decreases in stream depth and DOM resulted in greater UV exposure in a British Columbia stream (Kelly *et al*., 2003). Based on our measures of UV-B attenuation, stream depth, and DOC (Fig. 4b) we estimate that relatively modest (25%) reductions in stream depth and DOC concentration in early summer when surface flux is greatest could increase UV-B to the streambed by 1.6–2.6 $\times$.

Conclusions

Because ecosystem responses to global environmental stressors such as climate change and UV-B radiation are often complex and nonadditive (Shaw *et al*., 2002), an improved understanding of stressor interactions is critical. We believe that changes in DOC represent the most likely mechanism by which compound perturbations will affect benthic communities in metal-polluted streams. Climate-induced changes in biogeochemical cycles, hydrological processes, and vegetation predicted for the Rocky Mountain region (Baron *et al*., 2000) will likely reduce DOM concentrations in streams. Decreased water depth during summer when UV-B is highest coupled with increased photo-oxidation and alterations in DOM concentration will significantly increase the exposure of benthic communities to the combined effects of UV-B and heavy metals. The biotic ligand model, a theoretical approach that predicts toxicity based on interactions among trace metals, DOC, and other ligands, is being developed by the US Environmental Protection Agency to establish site-specific water quality criteria for metals (USEPA, 2003). Because of the potential for photo-oxidation, we believe that conservative estimates of metal–DOM complexation should be used when developing these criteria in streams subjected to UV-B radiation. Finally, results of microcosm and field experiments demonstrated that benthic communities responded to both the enhancement and removal of UV-B radiation. Greater UV-B sensitivity of the metal-impacted Arkansas River community may have implications for other Colorado streams because of the large number of watersheds in the region that are contaminated by mining pollution (Clements *et al*., 2000). These findings support the hypothesis that responses to multiple perturbations are often not additive and that superimposing stressors such as enhanced UV-B on disturbed communities may result in ecological surprises (Paine *et al*., 1998).

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