DISSERTATION

INFLUENCE OF ABIOTIC AND BIOTIC FACTORS ON THE RESPONSE OF BENTHIC MACROINVERTEBRATES TO METALS

Submitted by

Peter Michael Kiffney

In partial fulfillment of the requirements

for the degree of Doctor of Philosophy

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY PETER M. KIFFNEY ENTITLED "INFLUENCE OF ABIOTIC AND BIOTIC FACTORS ON THE RESPONSE OF BENTHIC MACROINVERTEBRATE COMMUNITIES TO METALS" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

INFLUENCE OF ABIOTIC AND BIOTIC FACTORS ON THE RESPONSE OF STREAM INVERTEBRATE COMMUNITIES TO METALS

Stream ecologists are well aware that chemical, biological, and physical characteristics of lotic systems vary spatially and temporally. With this in mind, I designed a series of experiments and field studies to examine the role of spatial variation in stream benthic macroinvertebrate communities in response to metals. Specifically, I tested the hypothesis that stream invertebrate communities from pristine streams of different size and altitude varied in their response to metals. To evaluate how metals affected biotic interactions, I manipulated invertebrate density, predation intensity, and metals in stream microcosms. Using stream invertebrate communities, I also designed an experiment and field survey to identify reliable bioindicators of metal contamination in western streams.

Results from microcosm experiments and field studies showed that benthic invertebrate populations from high-altitude streams were more sensitive to the effects of metals than invertebrate populations from low-altitude streams. For example, Baetis sp. and Rhithrogena hageni from Little Beaver Creek (LBC), Colorado, (high-altitude stream) were significantly more sensitive to zinc than the same species from the South Fork of the Poudre River (SFP) (low-altitude stream) in stream microcosms. Results from field surveys showed that densities of most groups of aquatic insects (e.g., Ephemeroptera, Plecoptera, Trichoptera) were lower at high-altitude metal contaminated streams than those same groups at low-altitude streams. Other factors, such as variation in water temperature or nutrient concentrations between high- and low-altitude streams, could be

responsible for these differences. However, because invertebrate responses were similar under controlled and field conditions, I hypothesize that differences in abundances between reference and contaminated locations was a result of metals.

To determine if body size contributed to the variation in sensitivity of insects to metals, size measurements were made on species collected from LBC (high-altitude) and SFP (low-altitude). Measurements were also made on species from control and metal-treated stream microcosm. Most species were smaller at LBC (high-altitude stream) than the same species from SFP (low-altitude stream). For example, the mayfly Baetis tricaudatus and the caddisfly Arctopsyche grandis were significantly larger at SFP than LBC. In addition, insect body size was larger in metal-dosed microcosms than in controls. Brachycentrus sp., B. tricaudatus, R. hageni, Ephemerella infrequens, and P. badia were significantly larger in metal-treated microcosms than in controls. Logistic regression indicated survival in metal-dosed microcosms was less for small individuals than for larger individuals of the same species. These results suggest that some insect species from high-altitude streams were smaller than those from low-altitude streams, and that survival was greater for larger lifestages. Thus the variation in response of macroinvertebrates between different altitude streams observed in earlier studies may be due to differences in body size.

The effects of low levels of metals (half the chronic levels of Cd, Cu, and Zn) on some species varied in relation to invertebrate density (low and high density) and invertebrate predation (no predators added and predators added). The abundance of Hydropsyche sp. was significantly lower in metal-dosed, high density treatments than in control, high density treatments. Moreover, the effects of an invertebrate predator (Hesperoperla pacifica) on Hydropsyche sp. was significantly greater in metal-dosed microcosms than in controls. These results suggest that metals interact with biotic factors to influence stream invertebrate community structure, and that effects occurred at metal concentrations lower than chronic criteria value.

Toxicity experiments in stream microcosms showed that the abundance and species richness of aquatic insects were significantly reduced at 1x, 5x and 10x the United States Environmental Protection Agency chronic levels of cadmium, copper, and zinc (1x=1.1, 5.0, and 110 μg/L Cd, Cu, and Zn, respectively). Mayflies were the most sensitive group, as the abundance of Baetis sp. and Rithrogena hageni were significantly reduced in the 1x treatment. The response of Drunella grandis was size dependent, as small lifestages were significantly more sensitive than large lifestages. Stoneflies were also affected, but their response was more variable with abundances of some species (Pteronarcella badia) being reduced in the 1x treatment, whereas other species were unaffected (Sweltsa sp.). Heptageniid mayflies were consistently less abundant downstream of sources of metal contamination in the Arkansas and Eagle rivers, whereas the response of other measures were more variable. For instance, species richness and total density were greater at a metal-contaminated site on the Arkansas River compared with an upstream reference site. Therefore, results from this experiment and field survey suggest that changes in abundance of heptageniid mayflies may provide a reliable indicator of metal-contamination in western streams.

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GENERAL INTRODUCTION

Many streams in western North America are contaminated by metals from historic and active mining operations (Moore et al. 1991, Wilkinson 1992). Surveys of benthic communities are frequently employed to differentiate reference, contaminated, and recovery sites in streams receiving metals (Winner et al. 1980, Chadwick et al. 1986, Clements et al. 1988, Rosenberg and Resh 1993, Clements 1994). Typically, this approach involves a comparison of sites upstream and downstream of pollutant sources. These sites should be similar in all respects except the presence of contaminants. Unfortunately, this rarely occurs as physical, chemical, and biological characteristics of streams vary naturally from upstream to downstream (Vannote et al. 1980, Minshall et al. 1985). This longitudinal variation in community structure may be especially problematic in western streams, where structural and functional changes occur over relatively short distances (Ward 1986).

An additional confounding factor is that similar taxa from different environmental regimes may vary in their response to anthropogenic disturbance (Vannote et al. 1980, Poff and Ward 1990). For example, Fisher (1977) showed that an estuarine species of phytoplankton was more resistant to a chemical stressor than the same species from an oceanic environment. It was hypothesized that estuarine organisms were more tolerant because they evolved under more variable environmental regimes. In high-gradient western streams, environmental conditions, such as water temperature and discharge can change over short distances. These changes may affect insect life histories, and therefore sensitivity to anthropogenic inputs. For example, an insect from a high-altitude stream may be smaller within the same lifestage, or at a different lifestage, than the same species from a low-altitude stream in response to different temperature regimes. It is well established that smaller organisms are more sensitive to contaminants than larger lifestages of the same species (Powlesland and George 1986). Thus, it is important to consider natural variability in community structure and differential sensitivity of organisms to contaminants when deciding the ecological risk of contaminants.

Although there is theoretical (Menge and Sutherland 1987, Menge and Olson 1990, Dunson and Travis 1991) and empirical (Walde 1986, Peckarsky et al. 1990, Warner et al. 1993) evidence that the relative importance of species interactions in structuring communities shifts along environmental gradients, little effort has been devoted to studying this relationship by stream ecologists. In the only study to date that tested effects of metals on biotic interactions in stream invertebrate communities, Clements et al. (1989) observed that net-spinning caddisflies were more vulnerable to an invertebrate predator in metal-dosed stream microcosms. To better understand the effects of contaminants on stream invertebrate communities, it is not only important to evaluate the direct effects of toxicants, but also the indirect effects.

Single-species laboratory experiments can provide a strong link between a contaminant and a biological response because environmental factors are controlled (Norton et al. 1992). Although control of confounding factors is important in assessing the effects of contaminants on aquatic organisms, single-species tests do not always predict the response of natural communities in the field to contaminants (Cairns 1983, Kimball and Levin 1985). The use of stream microcosms and mesocosms containing indigenous stream organisms offers a practical alternative to the lack of ecological realism of single-species tests and the problems associated with field surveys. The most powerful and efficient method of gaining knowledge in environmental research is to combine field surveys with controlled experimentation (Eberhardt and Thomas 1991). Results from such an approach would produce reliable bioindicators of environmental contamination.

This research examined the influence of abiotic (stream altitude) and biotic factors (invertebrate density and predation) on the response of stream invertebrate communities to metals. An additional goal of this research was to produce reliable bioindicators of metal contamination in western streams through controlled experiments and field surveys. My thesis is presented as three major blocks of research in five chapters. The first two chapters address variation in metal-sensitivity of benthic invertebrate communities from streams of different size and altitude, while the third chapter presents a mechanism that may explain differences in metal-sensitivity observed. In the second section (Chapter IV), I present results from a controlled experiment that examined the main and interactive effects of invertebrate density, predation, and metals on invertebrate community structure in stream microcosms. The last chapter (Chapter V) describes an experiment and field survey that evaluated the response of benthic invertebrate communities to metals to rigorously define bioindicators of metal-contamination in western streams. A common theme throughout this work is that ecological effects of contaminants are complex, and to assess the response of indigenous organisms to toxicants a more ecologically realistic approach than that currently in practice is required. If ecologically realistic experiments using indigenous organisms are combined with rigorously designed field surveys that have specific hypotheses, a more accurate assessment of the effects of contaminants on natural systems will be achieved.

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CHAPTER I

STRUCTURAL RESPONSES OF BENTHIC MACROINVERTEBRATE COMMUNITIES TO ZINC

Introduction

The River Continuum Concept (RCC) proposed by Vannote et al. (1980) predicts that diversity and structure of stream macroinvertebrate communities shift with progression downstream in response to changes in environmental conditions. Specifically, species richness and the proportion of certain functional groups vary with stream size. Because headwater and mid-order stream macroinvertebrate communities differ in composition and species richness (Vannote et al. 1980, Minshall et al. 1985), it is likely that response to stress will also vary from upstream to downstream. For example, Minshall et al. (1980) proposed that small, headwater streams in undisturbed catchments are more resistant to physical disturbance than intermediate-sized streams. Recently, it has been hypothesized that biotic responses to anthropogenic disturbance may differ depending on historical patterns of natural environmental variability and disturbance (Poff and Ward 1990). Although it is uncertain whether sensitivity to perturbation will increase or decrease along a longitudinal stream gradient, I hypothesize that response of benthic communities to anthropogenic stress will vary among locations.

Observed changes in structural characteristics in aquatic insect communities along stream gradients have important practical implications for assessing effects of contaminants. Surveys of benthic communities are frequently employed to delineate among reference, contaminated and recovery sites in streams receiving pollutants (Winner et al. 1980, Clements et al. 1988a). Typically, this approach involves comparison of sites upstream and downstream of an effluent source. Ideally, these sites should be similar in all respects except the presence of contaminants. However, natural variation in structural characteristics from upstream to downstream may confound results of biomonitoring studies. Often there is some degree of uncertainty whether observed changes in species richness or species composition is due to contaminants or simply a consequence of natural variation.

Longitudinal variation in environmental conditions can influence population distributions and life history characteristics of macroinvertebrates, which in turn may modify the response of stream insect communities to anthropogenic disturbance (Poff and Ward 1990). For example, some researchers have shown that effects of contaminants on aquatic organisms vary among age classes (Luoma and Carter 1991). In this research, I compared the effects of Zn on communities of aquatic insects collected from a third - order and fourth - order stream to test the hypothesis that community responses to Zn will vary among stream orders.

Methods

Study Sites

Artificial substrates were colonized at two sites on the Cache la Poudre River (CLP) system approximately 50 km west of Fort Collins, Colorado. This catchment is located in north central Colorado in the Southern Rocky Mountain physiographic province. The Laramie and Medicine Bow mountain ranges form the western boundary of the basin. The CLP joins the South Platte River east of Fort Collins and has a total mainstem length of 192 km (USFS 1980). The two field sites, Little Beaver Creek (LBC) and the Big South Fork of the Poudre River (SFP), are third and fourth - order streams, respectively, of the CLP catchment (Figure 1.1). Riparian canopy at both sites consists of willows (Salix sp.), ponderosa pine (Pinus ponderosa), and quaking aspen (Populus tremuloides).

These streams are distinctly different in physical characteristics (Table 1.1). The LBC field site is at a higher elevation, encompasses a smaller drainage area, and has a denser riparian canopy. LBC is also a much narrower and shallower stream than SFP. Furthermore, based on qualitative estimates, overall substrate size is larger and relative embeddeness is higher at LBC.

Experimental System

The artificial substrates consisted of 10 x 10 x 6 cm plastic trays filled with air-dried pebbles and small cobbles (2-6 cm diameter) providing a surface area of approximately 0.01 m^2. Details of the trays have been published previously (Clements et al. 1988a, 1988b, Clements et al. 1989). Thirty-four trays were placed in riffle sections of both LBC and SFP from 20 September to 20 October 1991. Trays were retrieved by placing a 100-μm mesh net directly downstream to prevent loss of organisms. Contents of trays from SFP (n=5) and LBC (n=7) were washed through a 350- μm sieve. Only five trays from SFP were used because some trays were lost as a result of high flows. Organisms retained were preserved in 100% ethanol in the field. These samples served as estimates of initial macroinvertebrate abundance and community composition. The

Figure 1.1. Map of the Cache la Poudre River, Colorado catchment. Field sites (LBC and SFP) where macroinvertebrates were collected are designated in italics.

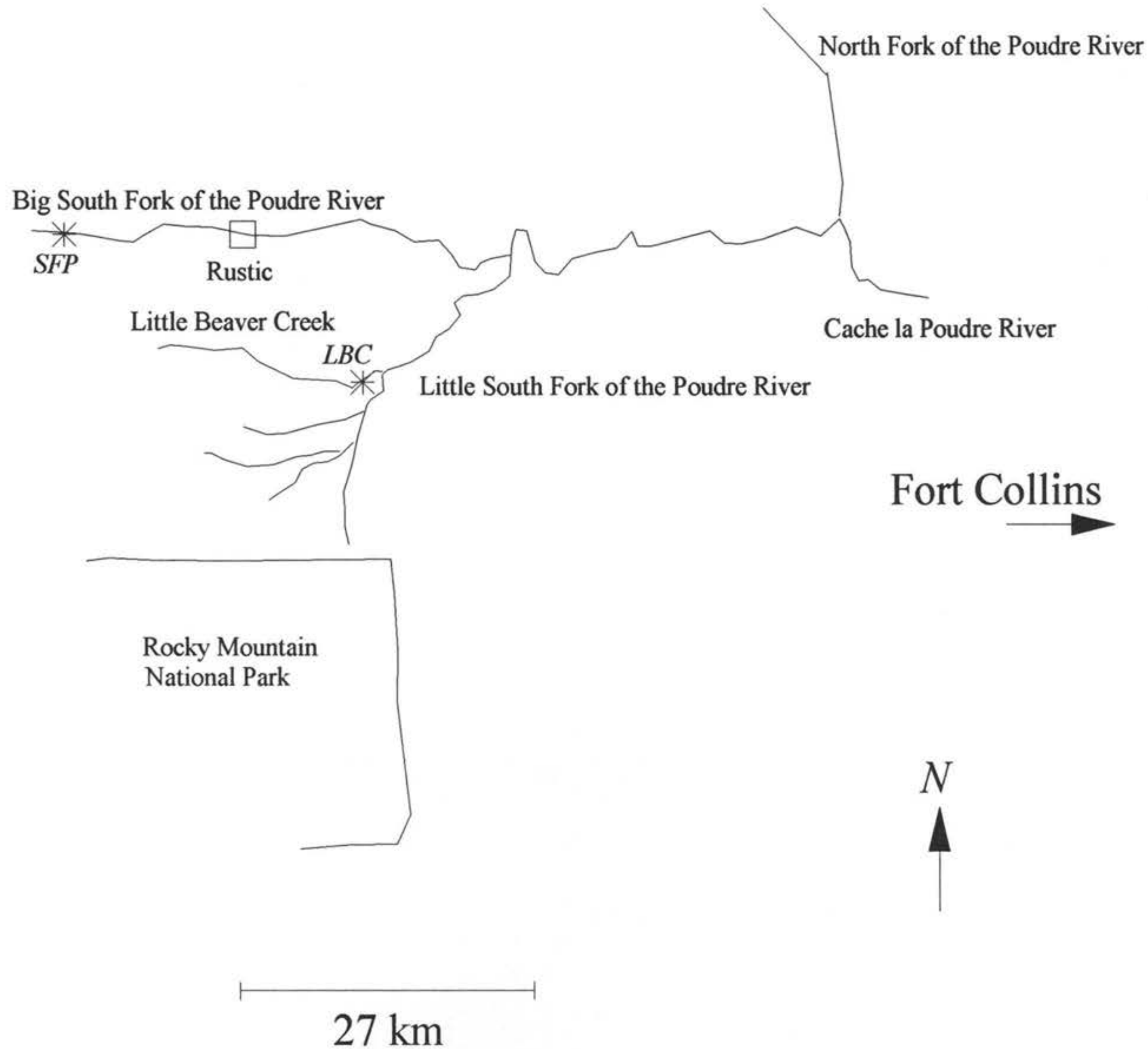

North Fork of the Poudre River
Big South Fork of the Poudre River
SFP
Rustic
Little Beaver Creek
LBC
Cache la Poudre River
Little South Fork of the Poudre River
Fort Collins
Rocky Mountain
National Park
N
27 km

Table 1.1. Comparison of physical and chemical characteristics of natural stream sites (Little Beaver Creek (LBC) and South Fork of the Poudre (SFP)), and indoor experimental streams.

	Natural		Experimental	
	LBC	SFP	Control	Treated
Temperature C°	4	9	14	14
D. O. (mg/l)	11	9	8.3	8.3
Current (cm/s)	18	10	30	30
Conductivity (m℧)	0.05	0.05	0.9	0.9
Hardness (mg/l $CaCO_3$)	10	24	380	380
Alkalinity (mg/l $CaCO_3$)	20	30	280	280
pH	7.5	7.9	8.3	8.3
[Zn] (μg/l)	BD[1]	BD	BD	130
Depth (cm)	10-30	20-40	10	10
Width (m)	1.5	15	0.18	0.18
Drainage area (km^2)	31	512	NA[2]	NA
Altitude (m)	2545	2320	NA	NA

[1] Below detection of analytical equipment.

[2] Not applicable.

remaining 24 trays from each stream were randomly assigned to plastic coolers (four trays per cooler) filled with stream water. Each of the 12 coolers was aerated by an airstone connected to 12-volt air pump.

Trays were transferred to fiberglass experimental streams (76 x 46 x 14 cm) at Colorado State University. Trays (n=4) in each cooler were randomly assigned to a stream (n=12 streams). Each oval, flow-through stream received chilled (Frigid Units Inc., Toledo, Ohio), aerated well water at a rate of 0.5 L/min. Turnover time in the 13-L streams was approximately 26 min, and water depth in each stream was 10 cm. Current was provided by a paddlewheel at an average current of 30 cm/s. A 12L:12D photoperiod was maintained with cool-white fluorescent lights.

After a 48-h acclimation period, each experimental stream was randomly assigned to one of two treatments: 0 and 150 μg/l Zn (target concentration). This target concentration was used because it is comparable to the chronic toxicity value for Zn based on water quality characteristics of our source water (Colorado Department of Health) and to values measured at natural metal-contaminated streams in Colorado (Kiffney and Clements 1993, Clements 1994). Peristaltic pumps delivered stock solutions of $ZnSO_4$ from separate 20-L acid washed carboys into each treated stream at a rate of 5 ml/min. Therefore, the final experimental design consisted of each experimental stream receiving four trays from one of two sites and two levels of Zn treatment. After seven days, the four trays in each stream were pooled and washed into a 350-μm sieve, and this sample represented one replicate. All organisms were preserved in 100% ethanol. Organisms were identified to species level when possible, except chironomids, which were identified to tribe.

Temperature, alkalinity, hardness, dissolved oxygen, and conductivity were determined when trays were collected from LBC and SFP (APHA 1985). These parameters were also measured on day 0, 2, 4, and 7 in experimental streams. Current was measured at each site with a General Oceanics (Miami, FL) digital flow meter at the beginning and end of the 30 d colonization period. Historic discharge data were obtained from the USGS Water Resources Survey Papers. Annual water temperature data for LBC and SFP were obtained from Dr. Kurt Fausch (Colorado State University) and Colorado Division of Wildlife (CDOW), respectively.

Total concentrations of zinc, copper, and cadmium were measured on water samples collected from field (n=1), control (n=6) and dosed (n=6) experimental streams (n=3) on day 0. Additional water samples were collected from experimental streams on days 2, 4 and 7. All samples were analyzed using flame atomic absorption spectrophotometry IL (Instrumentation Laboratory, Franklin, MA) Video 22 Dual Channel Flame Atomic Absorption Spectrophotometer. All water samples for metals analysis were acidified with HNO_3 to pH < 2. Metal (Cd, Cu and Zn) standards were also analyzed to determine precision of our chemical analysis.

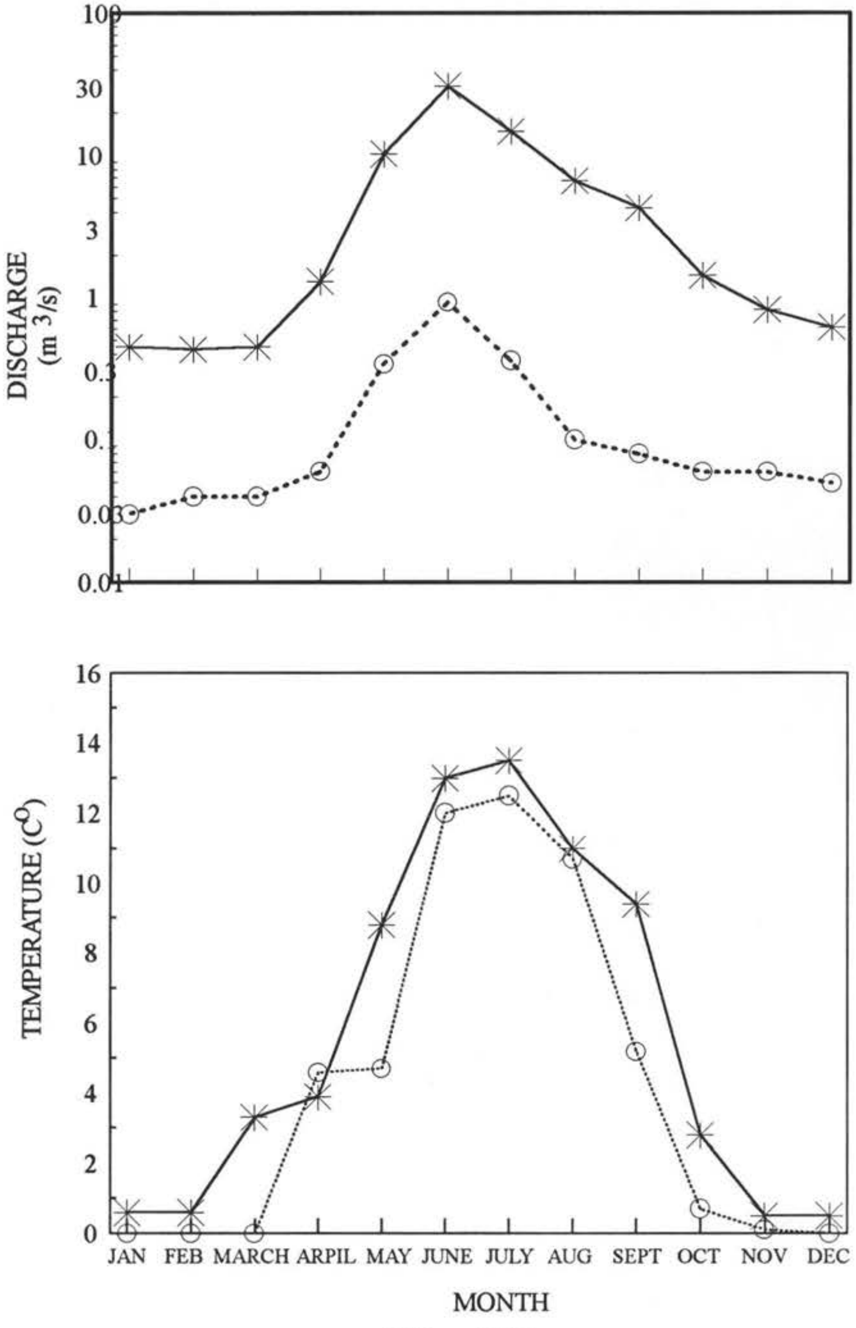
Statistical Analysis

A two-way treatment structure, consisting of stream order and metal level, and a completely randomized design were used. Specifically, there were three 0 μg/l Zn (i.e., control) and three 150 μg/l Zn (i.e., treated) experimental streams for each stream location. A two-way analysis of variance (ANOVA) was used to test the main effects of stream order, metal level, and the interaction between stream order and metal level. Effects on community-level parameters, such as number of taxa, number of individuals, and abundance of major orders at both sites were examined. Similar analyses were conducted on population-level measurements, which in this study represented densities of dominate genera (i.e., >1% relative abundance) collected at both sites. With the exception of two mayfly taxa, a rather coarse presentation of taxonomic resolution is presented. A one-way ANOVA was conducted on these parameters to compare: (1) community composition of trays collected from both sites at the end of the colonization period and trays in control experimental streams on day 7, and (2) community structure of control experimental streams from each site on day 7. A ln(n+1) transformation was used to normalize count data. All statistical analyses were performed using a PC-version of Statistical Analysis Systems (SAS 1985). A significant difference was determined to exist at a $p < 0.05$.

Results

Most water quality characteristics (i.e., hardness, alkalinity, pH, conductivity, D. O., and [Zn]) were similar between field sites (Table 1.1). An exception was water temperature, which was lower at LBC at the end of the colonization period. Seasonally, annual mean water temperatures are cooler at LBC compared to SFP (Figure 1.2). Flow

Figure 1.2. Mean monthly discharge (1960-1968) and water temperature (1991) for LBC and SFP.


DISCHARGE (m 3/s)
100
30
10
3
1
0.3
0.1
0.03
0.01
TEMPERATURE (C^{O})
16
14
12
10
8
6
4
2
0
JAN FEB MARCH ARPIL MAY JUNE JULY AUG SEPT OCT NOV DEC
MONTH
SFP LBC

regimes were also dissimilar, as SFP exhibited higher flows compared to LBC.

Temperature, current, water hardness, alkalinity, conductivity, and pH were higher in experimental streams compared to field streams (Table 1.1). The mean concentrations of Zn, Cd, and Cu in field and control (n=6) streams were below detection (i.e., 5, 5, and 10 μg/l, respectively). The mean concentration ($\pm$ 1 SE, n=24) of Zn measured in treated streams was 130 $\pm$ 3 μg/l.

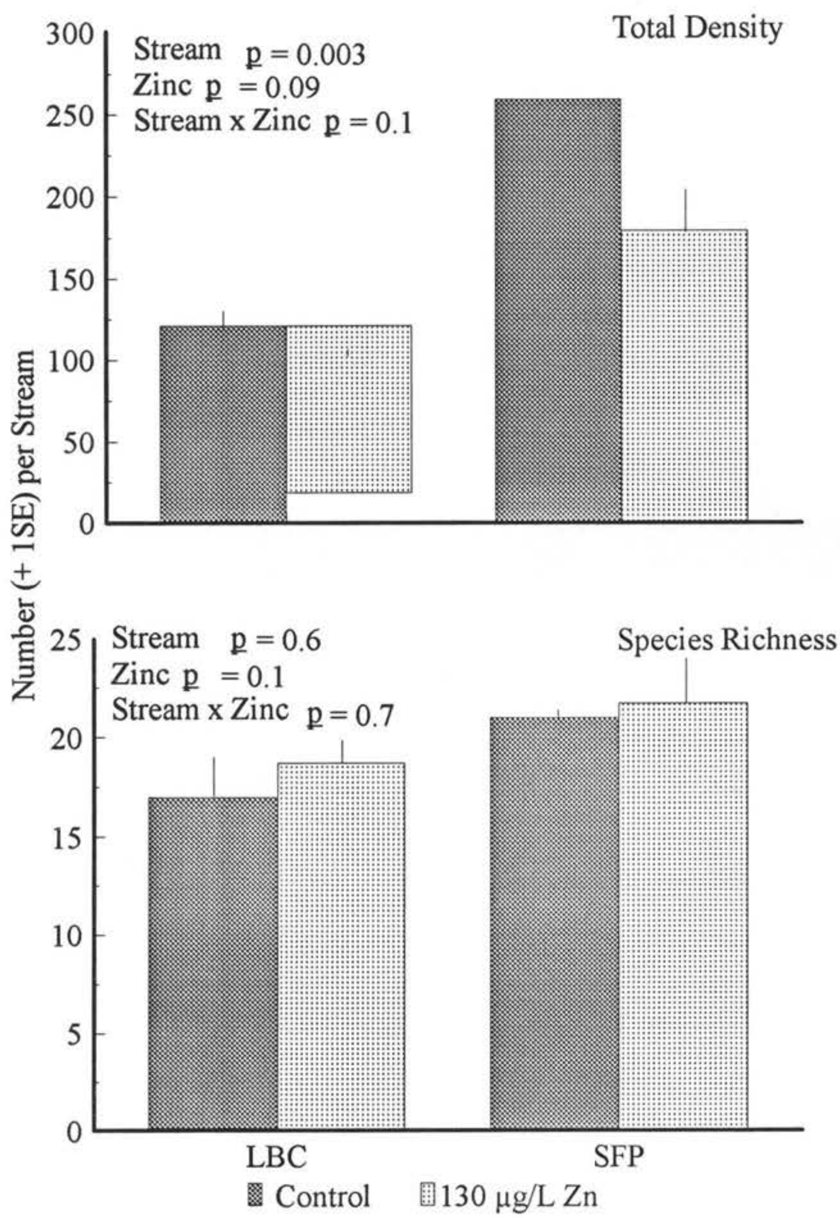
There were no statistically significant differences in community structure between trays collected from field sites at the end of the colonization period and those in experimental streams. Control streams were dominated by ephemeropterans (mayflies), plecopteans(stoneflies), and trichopterans (caddisflies) (Table 1.2). Densities of stoneflies, mayflies, caddisflies, chironomids, and total number of individuals were greater on SFP trays.

The main effects of 130 μg/l Zn and stream order, and the interaction between stream order and Zn had statistically significant effects at both the community and population-level. At the community-level, there were significantly more individuals in SFP experimental streams than in LBC streams, while there were no differences in the number of taxa between streams (Figure 1.3). The number of individuals was more affected at SFP, but this decrease in density was not statistically significant. The abundance of mayflies was affected by Zn, stream order, and the Zn x stream order interaction (Figure 1.4). Mayflies from both sites were reduced in Zn-treated streams, but effects of Zn were greater in LBC streams. Although mayfly density was significantly greater in SFP streams, it was reduced by 35% in SFP compared with 63% in LBC treated streams. Densities of

Table 1.2. Mean (± 1 SE) macroinvertebrate abundance of major groups, total number of individuals, and number of taxa collected from substrate-filled colonization trays in control experimental streams (n=3 for LBC and SFP) after 7 days.

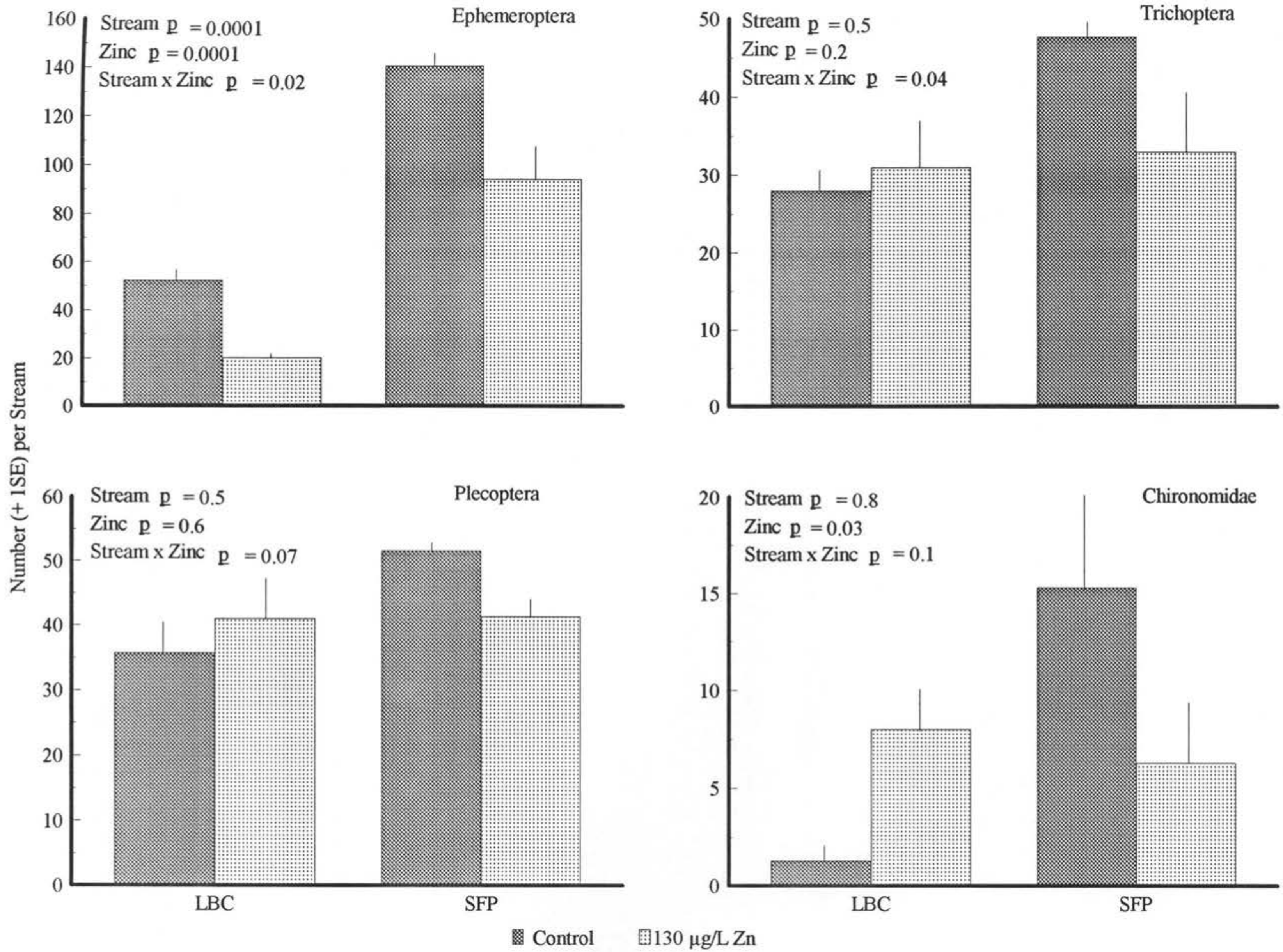
	LBC	SFP	p-value
Ephemeroptera	54.0 (4.9)	140.7 (6.6)	0.0007
Plecoptera	34.0 (5.5)	52.0 (2.6)	0.06
Trichoptera	28.0 (3.8)	39.0 (1.9)	0.07
Diptera	1.7 (0.7)	1.0 (0.6)	0.4
Chironomidae	1.3 (0.7)	16.0 (7.2)	0.03
Total Individuals	121.0 (10.6)	295.0 (1.3)	0.001
Number of Taxa	17.0 (2.5)	21.0 (0.6)	0.2

Figure 1.3. Comparison of mean total density and species richness ($\pm$ 1SE) from LBC and SFP in control and Zn-treated stream microcosms.



Total Density
Stream p = 0.003
Zinc p = 0.09
Stream x Zinc p = 0.1
300
250
200
150
100
50
0
Number (+ 1SE) per Stream
Species Richness
Stream p = 0.6
Zinc p = 0.1
Stream x Zinc p = 0.7
25
20
15
10
5
0
LBC
SFP
Control
130 μg/L Zn

Figure 1.4. Comparison of mean density ($\pm$ 1SE) of Ephemeroptera, Plecoptera, Trichoptera, and Chironomidae from LBC and SFP in control and Zn-treated stream micrcocosms.


Number (+ 1SE) per Stream
Ephemeroptera
Stream p = 0.0001
Zinc p = 0.0001
Stream x Zinc p = 0.02
Trichoptera
Stream p = 0.5
Zinc p = 0.2
Stream x Zinc p = 0.04
Plecoptera
Stream p = 0.5
Zinc p = 0.6
Stream x Zinc p = 0.07
Chironomidae
Stream p = 0.8
Zinc p = 0.03
Stream x Zinc p = 0.1
LBC
SFP
LBC
SFP
Control
130 µg/L Zn

chironomids, plecopterans and trichopterans were greater in LBC Zn-treated streams than in controls, whereas densities of these groups decreased in SFP dosed streams; however, the Zn x stream order interaction was not statistically significant for these measures except for the trichopteran abundance.

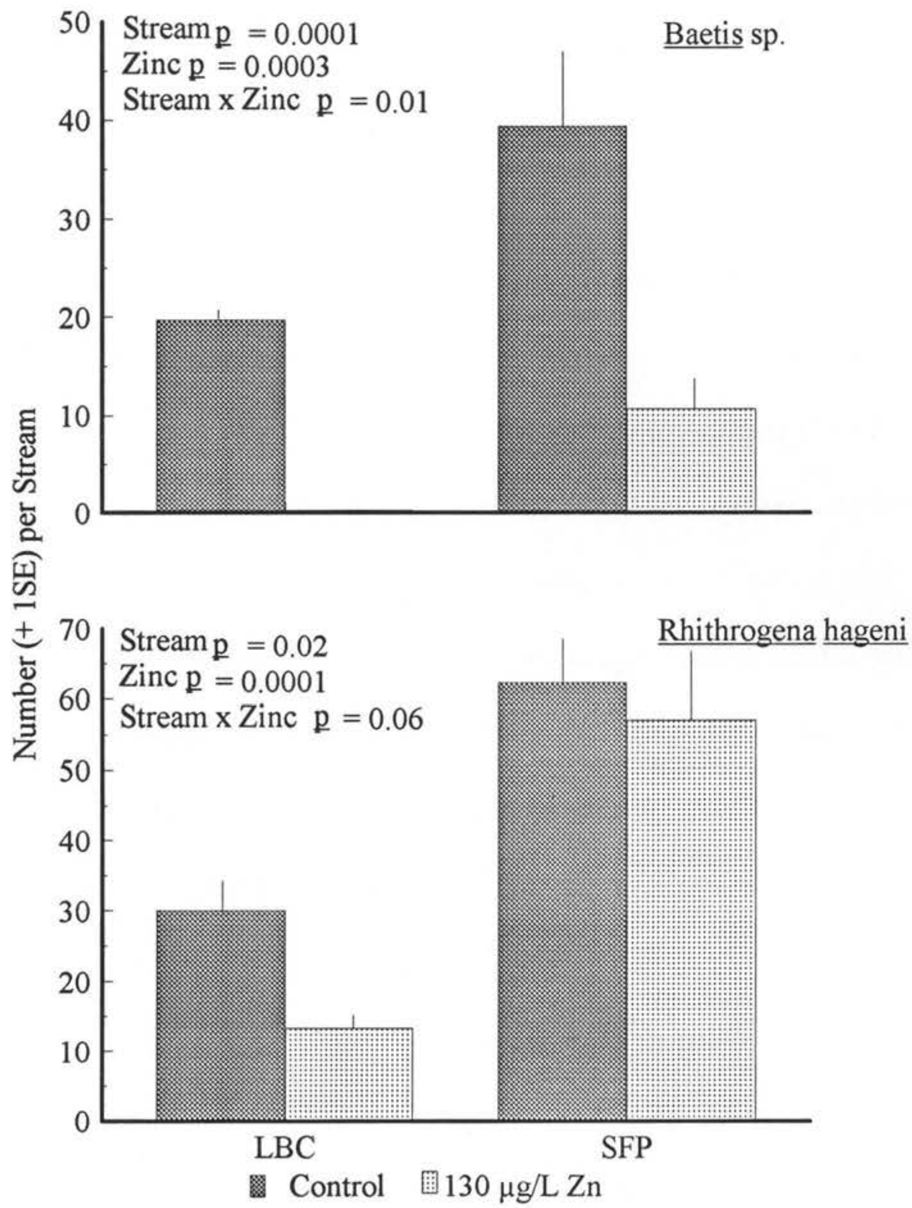
At the population level, two mayfly taxa collected from both sites appeared to differ in their response to Zn (Figure 1.5). Densities of Baetis sp. and Rhithrogena hageni were significantly greater in SFP streams. Both were significantly reduced by Zn, but effects varied with stream order. Densities of Baetis sp. were reduced by 73% and 99% in SFP and LBC dosed streams, respectively. Although, densities of R. hageni were reduced by 8% in SFP treated streams and 43% in LBC treated streams, the Zn x stream order interaction for R. hageni was not quite statistically significant ($p=0.06$).

Discussion

This experiment was the first attempt to determine if aquatic insect communities from different stream orders within the same catchment varied in response to contaminants. The results presented here provide evidence to suggest that insect communities from LBC (third - order) and SFP (fourth - order) varied in their sensitivity to Zn. For example, mayflies from LBC were more sensitive to low levels of Zn (130 μg/l) than mayflies from SFP.

Total density and species richness were greater in SFP experimental streams. SFP is a larger stream with greater discharge and temperature extremes than LBC. According to the River Continuum Concept (RCC) (Vannote et al. 1980), species richness of aquatic

Figure 1.5. Comparison of mean density of Baetis sp. and Rhithrogena hageni (± 1SE) from LBC and SFP in control and Zn-treated stream microcosms.


Stream p = 0.0001
Zinc p = 0.0003
Stream x Zinc p = 0.01
Baetis sp.
50
40
30
20
10
0
Number (+ 1SE) per Stream
Stream p = 0.02
Zinc p = 0.0001
Stream x Zinc p = 0.06
Rhithrogena hageni
70
60
50
40
30
20
10
0
LBC
SFP
Control
130 µg/L Zn

insect communities increases in medium-sized rivers because a larger number of species are exposed to optimum temperature conditions. Other researchers have also shown that species richness and abundance in western, mountain streams increase from headwater to mid-order streams (Minshall et al. 1985, Ward 1986).

The increase in densities of chironomids, plecopterans and trichopterans from LBC is unusual, but similar trends have been observed in other studies. Utilizing a similar experimental system, Clements et al. (1988a) observed higher densities of Orthocladiini in streams dosed with Cu and Zn than in control streams. It was hypothesized that this increase was a result of early instars passing through the sieve during the initial stages of the experiment. These organisms then grew and were retained on later sampling occasions. Despite the unique responses of chironimids, plecopterans, and trichopterans from LBC to Zn, none of the statistical tests detected significant treatment effects.

These experimental results support the findings of other researchers who have reported that mayflies are generally more sensitive to heavy metals than stoneflies, caddisflies, and chironomids (Winner et al. 1980, Wiederholm 1984, Clements et al. 1988a, Clements et al. 1989). Baetis sp. and Rhithogena hageni have been reported to be sensitive to heavy metals in western mountain streams (Peckarsky and Cook 1981, Leland et al. 1986), eastern streams and experimental streams (Clements et al. 1988a, 1988b, Clements et al. 1989). It is possible that the historical spatial and temporal variability of LBC and SFP influenced biotic response to Zn. Poff and Ward (1990) hypothesized that communities and populations from more naturally variable environments will exhibit greater resistance and persistence than those from less variable systems. For example, phytoplankton species from a highly variable estuarine environment were more resistant to PCB than the same species from a less variable oceanic environment (Fisher 1977).

Alternatively, the apparent greater sensitivity of mayflies from LBC may be the result of a synergistic interaction between water temperature and Zn. Water temperature the day trays were collected was 4° C at LBC and 9° C at SFP, whereas, the experimental stream temperature was 14° C. Because of the large volume of water flowing through each laboratory stream, it was impossible to chill the water to approximate field temperatures. Clements et al. (1988b) observed Baetis sp. was more sensitive to heavy metals in experimental streams during the summer and attributed this increased sensitivity to higher water temperatures. Thus, temperature may have interacted with Zn to cause greater effects on insects from LBC than from SFP. Nevertheless, there were no statistical differences in community or population structure in control streams compared with trays used for initial community structure, suggesting temperature alone did not cause mortality. Furthermore, organisms from LBC are typically exposed to daily maximum water temperatures (5-14° C) during October (Dr. K. Fausch, unpublished results), which is within the range of water temperatures of our experimental streams.

An additional factor contributing to the greater effect of Zn on mayflies from LBC than SFP may be a result of species composition. For example, we had difficulty identifying Baetis to the species level. At both sites, Baetis was at an early stage of development which makes precise identification of this taxon difficult (Dr. B. Kondratieff, personal communication). In Rocky Mountain streams, there are two common Baetis species, B. tricaudatus and B. bicaudatus, which have both been collected at LBC and

SFP. B. bicaudatus appear to be the dominate species at LBC, while B. tricaudatus is more common at SFP (Kiffney, personal observation). Thus, the observation that there was a larger decrease in the densities of mayflies from LBC compared to SFP may be a result of differences in the sensitivity of the two Baetis species to Zn.

Apparent differences in sensitivity to Zn between populations of Baetis sp. and R. hageni from LBC and SFP may be attributed to life history factors. Populations of Baetis sp. and R. hageni from SFP were larger and at a later stage of development compared to LBC populations (Kiffney, personal observation). This may be a result of the warmer annual temperatures observed at SFP. As was mentioned earlier, macroinvertebrates at early stages of development may be more sensitive to metals compared to later instars (Luoma and Carter 1991). In a recent experiment, early instars of Drunella grandis were significantly more sensitive to heavy metals than later instars (Kiffney, unpublished results).

These results suggest that aquatic insect communities from different sites along a stream gradient respond differentially to Zn. The mechanism of this variation in response is uncertain, but it appears that stream location may influence the effects of heavy metals on macroinvertebate communities. These data also have important practical implications for stream biomonitoring studies. Specifically, natural longitudinal and seasonal variability of stream macroinvertebrate communities may be confounding factors in biomonitoring studies, because responses of aquatic insects to contaminants may differ with location and time of year. In addition, stream biomonitoring studies are hampered by the difficulty of locating appropriate reference sites, especially in western streams where heavy metals contaminate entire watersheds. The experimental system presented here offers an approach to account for some of these confounding factors and may be successful in predicting benthic community responses to heavy metals. Thus, using stream microcosms allows a researcher to manipulate environmental conditions to address the factor of interest. For instance, in this study I was concerned with whether insects from different stream orders varied in their response to metal exposure. Although water quality characteristics of our experimental system were different from natural Rocky Mountain streams, these results can still be compared to natural streams contaminated by heavy metals. In preliminary comparisons, I observed that benthic community composition of natural metal-contaminated streams and experimental streams dosed with metals were similar. Another unique advantage of this experimental stream system is that it can be used to examine how natural variation in environmental and ecological conditions influences the response of aquatic insect communities to contaminants. Experimentally testing these interactions are important in that they provide a bridge between aquatic toxicology and community ecology.

The apparent differences in susceptibility between headwater and mid-order stream macroinvertebrate communities to zinc has important implications in the design and implementation of remediation procedures of metal-impacted western streams. I suggest that predicting the response of Rocky Mountain benthic macroinvertebrate communities to heavy metals may depend on the location of the effluent along the stream longitudinal gradient.

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CHAPTER II

EFFECTS OF METALS ON STREAM MACROINVERTEBRATES FROM DIFFERENT ALTITUDES

Introduction

Sensitivity to anthropogenic disturbance can vary among species within a locale (Kiffney and Clements 1994a, 1994b), and is likely to vary among populations, communities, and ecosystems of different geographical areas (Resh et al. 1988). For example, the influence of acid precipitation on stream organisms may vary depending on community composition and characteristics of underlying bedrock (Resh et al. 1988). Trophic state may also influence a community's response to disturbance (Steinman et al. 1992, Lozano and Pratt 1994). Resistance of periphyton communities to chlorine exposure was greater in laboratory streams with greater amounts of biomass (Steinman et al. 1992). Thus, there is theoretical and empirical evidence that suggests the response of aquatic organisms to anthropogenic disturbance may depend upon chemical, physical, and biological characteristics of that system.

Surveys of benthic communities are frequently employed to differentiate reference (clean), contaminated, and recovery sites in streams receiving pollutants (Winner et al. 1980, Clements et al. 1988, Rosenberg and Resh 1993, Clements 1994). Typically, this approach involves comparison of sites upstream and downstream of a pollutant source. These sites should be similar in all respects (e.g., substrate, discharge, water temperature) except for the presence of contaminants. Unfortunately, this rarely occurs as physical, chemical, and biological characteristics of streams vary naturally from upstream to downstream (Vannote et al. 1980, Rosenberg and Resh 1993). As a result of this natural variation, there is often some uncertainty whether observed changes in species richness or species composition at polluted sites are due to contaminants (Clements 1994). An additional confounding factor is that similar taxa from different environmental regimes may vary in their response to disturbance (Poff and Ward 1990). For example, estuarine organisms have been shown to be more resistant to chemical stressors than similar taxa from oceanic environments (Fisher 1977, Hyland et al. 1985, Tedengren et al. 1988). Therefore, natural variability in community structure and differential sensitivity of organisms to contaminants are factors that should be considered when evaluating the effects of anthropogenic disturbance on stream organisms.

Life history and population genetics are two possible mechanisms that may contribute to the differential sensitivity of the same aquatic insect species to chemical contaminants (Poff and Ward 1990). Life history traits may be important because individuals from a high-altitude stream may be smaller than individuals of the same species from a lower-altitude stream within the same lifestage (Markarian 1980, Wise 1980, Rader and Ward 1990). It is also possible that an insect species from a high-altitude stream may be at an earlier, smaller lifestage than the same species from a lower-altitude stream. Regardless, it has been shown that smaller individuals of aquatic insects are more sensitive to contaminants than larger individuals of the same species (Powlesland and George 1986, Diamond et al. 1992, Kiffney and Clements, 1994b). Greater tolerance may also be attributed to greater phenotypic and genotypic diversity of organisms from more variable environments compared with similar species from more stable environments (Levins 1965, Poff and Ward 1990).

One of the most serious water quality problems in western North America is metal pollution of streams (Moore et al. 1991, Kiffney and Clements 1993). It has been estimated that acid mine drainage has degraded 1,950 km of Colorado streams (Wilkinson 1992), including high-altitude, headwater streams and lower-altitude, larger streams (Clements, unpublished results). Although there is theoretical support for the notion that communities in streams of different size and different environmental regimes may respond differentially to disturbance (Vannote et al. 1980, Minshall et al. 1983, Poff and Ward 1990, Kiffney and Clements 1994b), there has been little attempt to assess this difference. If there are differences between locations, agencies responsible for managing water resources should account for this variation when establishing criteria for contaminants. For example, differences in lifestage among stream altitude may influence an organism's sensitivity to contaminants.

To accurately estimate the ecological risk of contaminants on natural populations, it is necessary to evaluate how habitat may influence the biological response to contaminants. In this study, I compared the responses of macroinvertebrate assemblages collected from two streams of different size and altitude to metals. Macroinvertebrates from a third-order (sensu Strahler 1957) (2545 m above means sea level) and fourth-order stream (2320 m) were exposed to metals in stream microcosms. To compare laboratory results to field responses, I measured effects of metals on macroinvertebrate assemblages in metal-polluted streams located at different altitudes.

Methods

Experimental System

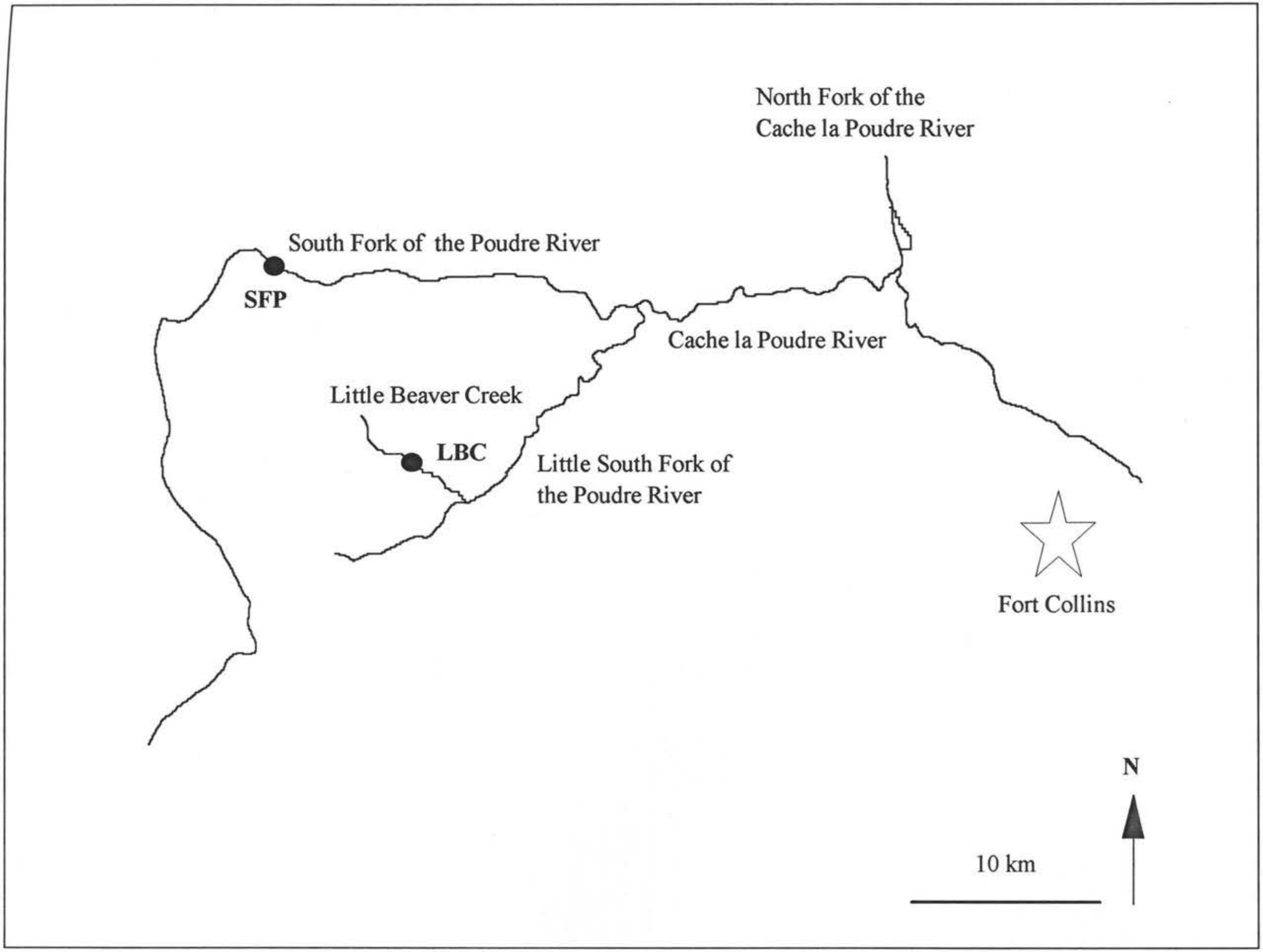
Artificial substrates were placed at two sites on the catchment of the Cache la

Poudre River, Colorado, approximately 40 km west of Fort Collins, CO (Figure 2.1). The two sites, Little Beaver Creek (LBC) and the South Fork of the Poudre River (SFP), are third-order and fourth-order streams (Table 2.1), respectively.

Artificial substrates consisted of 10 x 10 x 6- cm plastic trays (0.01 m^2 surface area) filled with air-dried pebbles and small cobbles (2-6 cm diameter). Previous studies have demonstrated that macroinvertebrate assemblages colonizing trays were similar to those collected from surrounding natural substrate (Clements et al. 1989), although, densities were generally higher on trays (Kiffney and Clements, 1994b). Thirty-two trays were placed in riffle sections of LBC and SFP from 18 July 1992 to 1 September 1992, then retrieved by placing a 100- μm net directly downstream to prevent loss of organisms. Twenty-four trays from each stream were randomly assigned to plastic coolers (four trays per cooler) filled with stream water. Each of the 12 coolers was aerated by an airstone connected to a 12- volt air pump. The remaining 8 trays were preserved in the field to obtain an initial estimate of macroinvertebrate abundance.

Trays were transferred to 12 oval, fiberglass experimental streams (76 x 46 x 14-cm) located at Colorado State University in Fort Collins. Previous studies have shown that there is little mortality during this transfer (Kiffney, unpublished results). Trays (four) were randomly assigned to a stream. Each 13-L stream received new, aerated, chilled, dechlorinated tap water at rate of 0.5 L/min giving a turnover time of approximately 26

Figure 2.1. Cache la Poudre drainage where experimental animals were collected. LBC=Little Beaver Creek (small, high-altitude) and SFP=South Fork of the Poudre River (large, low-altitude).



North Fork of the
Cache la Poudre River
South Fork of the Poudre River
SFP
Cache la Poudre River
Little Beaver Creek
LBC
Little South Fork of
the Poudre River
Fort Collins
N
10 km

Table 2.1. Physical and chemical characteristics of Little Beaver Creek (LBC), the South Fork of the Poudre River, and experimental control ($\underline{n}$=3 for each location) and metal-treated ($\underline{n}$=3 for each location) microcosms during September 1-11, 1994 experiment.

	Field		Microcosms	
	LBC	SFP	Control	Metal-Treated
Altitude (m)	2545	2320	NA[1]	NA
Width (m)	4.0	20	0.018	0.018
Longitude	106° 32'	105° 42'	105° 03'	105° 03'
Latitude	40° 32'	40° 42'	40° 35'	40° 35'
Depth (cm)	20	30	10	10
Canopy Cover (%)	49	15	NA	NA
Current Velocity (cm/s)	52	60	30	30
Temperature (°C)	9	11	12	12
Dissolved Oxygen (mg/L)	9.0	9.2	9.1	9.1
pH	7.6	7.5	7.7	7.7
Conductivity (μmhos/s)	50	50	50	50
Hardness (mg/L)	14	16	36	36
[Cd] μg/L	BD[2]	BD	BD	5.5 $\pm$0.7 a[3]
[Cu] μg/L	BD	BD	BD	40.0$\pm$ 3.0 b
[Zn] μg/L	BD	BD	BD	480 $\pm$25 c

[1] Not applicable.

[2] Below detection of analytical equipment (5, 5, and 10 μg/L for Zn, Cd, and Cu, respectively).

[3] Mean ($\pm$ 1SE; $\underline{n}$=12) metal concentrations in water collected from experimental streams. Values followed by a lower-case letter are statistically different from control concentrations at $\underline{p}$<0.0001.

min. Water depth in each stream was 10 cm. Current was provided by a paddlewheel at an average velocity of 30 cm/s. A 13 light:11 dark photoperiod was maintained with cool-white fluorescent lights.

After a 48 h acclimation period, each experimental stream was randomly assigned to one of two treatments (three replicates per treatment): control and metal-treated. Metal concentrations in treated streams were 5.5, 60, and 550 μg/L Cd, Cu, and Zn, respectively. These values were also approximately 5X federal chronic toxicity values considered protective of freshwater aquatic life (EPA 1980a, 1980b, 1980c) for water of less than 50 mg/L $CaCO_3$, and were similar to levels measured at moderately-polluted sites at the Arkansas River near Leadville, CO (Kiffney and Clements 1993, Clements 1994). Peristaltic pumps delivered a stock solution of $CdCl_2$, $CuSO_4$, and $ZnSO_4$ from separate 20- L acid-washed carboys into each dosed stream at a rate of 5 ml/min. The final experimental design was of a 2 x 2 factorial design with assemblages from two sites (LBC and SFP) and two metal treatments (control and metal-treated) for a total of 12 experimental units each containing four artificial substrates.

After 10 d, the four trays in each stream were pooled, washed into a 355 μm sieve, and retained organisms were preserved in 100% ethanol. Samples were sorted in the laboratory in white enamel pans and all organisms except chironomids were identified to genus or species. Chironomids were identified to subfamily or tribe (Merritt and Cummins 1984).

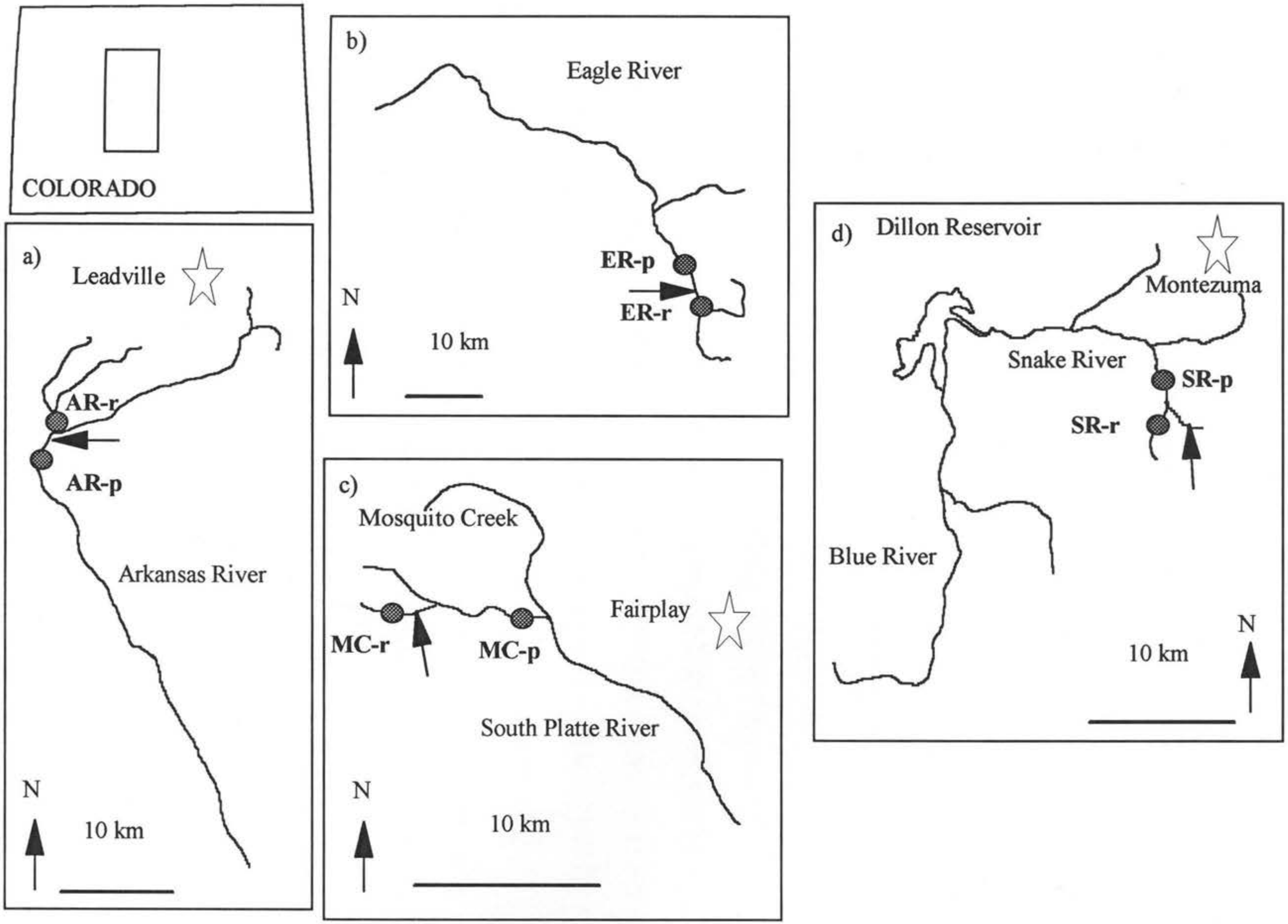
Water samples were collected from LBC and SFP ($\underline{n}$=1) when trays were removed from the streams, and on days 0, 2, 4, 8, and 10 in experimental microcosms. Alkalinity and hardness were determined in the laboratory by titration (APHA 1989), conductivity with a conductivity meter (VWR Scientific Model 1054), and pH with a Chemcadet meter. Dissolved oxygen and water temperature were measured in field and laboratory streams with an O_2 meter (Yellow Springs Instrument Company model 51B). Samples for metals analyses were acidified with reagent-grade HNO_3 to pH < 2. Metals were measured on an IL 22 video dual-channel flame atomic absorption spectrophotometer using standard methods (method #3111 A, APHA 1989), and presented as total (suspended + dissolved) metals.

Current velocity was measured with a digital meter (General Oceanics 2030R). Stream depth and stream channel width were measured at three random points at each site. Canopy cover was estimated at each transect where stream width was determined.

Field Surveys

To compare laboratory results to those measured in the field, I used biological, chemical, and physical data from four sites on four Colorado streams collected during August 1992. The Arkansas River, near Leadville, flows through a high mountain basin between the Sawatch and Mosquito mountain ranges in central Colorado (Figure 2.2a). The Eagle River, a tributary to the Colorado River, originates at the north side of Tennessee Pass (Figure 2.2b). Mosquito Creek, located approximately 5 km north of Fairplay, originates on the eastside of the Sawatch Range and eventually flows into the South Platte River (Figure 2.2c). The Snake River, which drains into Dillon Reservoir, flows from the west side of the Continental divide near Montezuma (Figure 2.2d).

Figure 2.2. Location of metal-polluted field streams. a) Arkansas River (low metal, low-altitude), b) the Eagle River (high metal, high-altitude), c) Mosquito Creek (low metal, high-altitude), and d) the Snake River (high metal, high-altitude). Sample sites are indicated by closed circles. The letters -r and -p represent reference and metal polluted sites, respectively. Arrows indicate the approximate location of the effluent.


COLORADO
a)
Leadville
AR-r
AR-p
Arkansas River
N
10 km
b)
Eagle River
ER-p
ER-r
N
10 km
c)
Mosquito Creek
Fairplay
MC-r
MC-p
South Platte River
N
10 km
d)
Dillon Reservoir
Montezuma
Snake River
SR-p
SR-r
Blue River
N
10 km

Substrate of these streams consists predominantly of gravel-cobble, and riffles and runs comprise the majority of stream habitat. Riparian canopy consists of willow (Salix spp.), quaking aspen (Populus tremuloides), ponderosa pine (Pinus ponderosa), lodgepole pine (Pinus contorta), alder (Alnus tenuifolia), and assorted grasses and sedges.

A priori, one metal-polluted site and one non-polluted site were selected from each of the four streams for statistical analyses based on the following criteria: (1) similarity in concentrations of metals in water at polluted sites, and (2) variation in size of stream and altitude at which metals entered. For example, one metal-polluted site from Mosquito Creek (altitude=3115 m) and the Arkansas River (altitude=2898 m) were compared for statistical analysis, because concentrations of metals were similar ([Zn]=168 and 181 μg/L) but the sites differed in size (i.e., width and depth) and altitude (Table 2.2). Similar criteria were used to select sites on the Snake River and Eagle Rivers ([Zn]=364 and 407 μg/L and altitudes of 3060 m and 2405 m). In the text below, Mosquito Creek and Arkansas River are described as "low-metal" streams, and the Eagle River and the Snake River as "high-metal" streams. Reference sites (i.e., non-polluted sites) were located immediately above the source of metal discharge on each site, and have been used historically as reference locations.

Benthic macroinvertebrate samples (n=5) were collected at reference and polluted sites using a 0.1 m^2 Hess sampler (355 μm mesh net and substrate was disturbed to approximately 10 cm depth). Samples were washed through 355 μm sieve and retained organisms were preserved in 70% ethanol, then sorted and identified as described previously. Water samples (n=1) for chemical analyses, and physical measurements were

Table 2.2. Physical and chemical characteristics of Mosquito Creek (MC), the Arkansas River (AR), the Snake River (SR), and the Eagle River (ER)) during August 1992. MC and SR were small, high-altitude streams, whereas AR and ER were large, low-altitude streams.

	Low Metals				High Metals			
	MC-r[1]	MC-p[2]	AR-r	AR-p	SR-r	SR-p	ER-r	ER-p
Altitude (m)	3511	3115	2910	2898	3206	3060	2670	2405
Longitude	107° 07'	107° 07'	106° 22'	106° 22'	106° 50'	106° 50'	106° 25'	106° 22'
Latitude	39° 17'	39° 17'	39° 13'	39° 10'	39° 30'	39° 30'	39° 37'	39° 37'
Width (m)	3.0	4.6	6.8	10.7	2.8	4.4	6.4	16
Depth (cm)	11	25	30	30	14	30	30	40
Canopy Cover (%)	11	25	17	3	31	15	56	20
Current Velocity (cm/s)	65.3	52.5	66.0	93.0	52.3	76.1	65	79
Temperature (°C)	11	16	15	15	10	11	11	14
Dissolved Oxygen (mg/L)	7.6	7.2	7.6	7.6	7.6	7.5	8.4	8.0
pH	7.8	8.3	8.0	7.9	7.5	6.8	8.5	8.0
Conductivity (μmhos/s)	40	155	150	170	65	100	180	145
Hardness (mg/L)	18	96	92	100	36	52	122	84
[Cd] μg/L	BD[3]	BD	BD	BD	BD	BD	BD	BD
[Cu] μg/L	BD	BD	BD	BD	BD	BD	BD	BD
[Zn] μg/L	BD	168	26	181	13	364	BD	407

[1] Reference sites.

[2] Metal-polluted sites.

[3] Below detection of analytical equipment (see Table 3.1).

taken at each site as described above.

Statistical Analysis

For experimental and field results, a two-way analysis of variance was used to test the main effects of stream location and metal treatment, and the stream x metal interaction on number of taxa, number of individuals, EPT species richness (total number of taxa of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)), and abundance of those same three groups of insects. Although species composition in field and experimental streams were similar (Kiffney and Clements 1994a and 1994b, Clements and Kiffney, unpublished manuscript), for the most part it was impractical to make statistical comparisons of specific taxa (i.e., genus or species) because of variation in density among streams. A few taxa were collected at high enough densities at Mosquito Creek and the Arkansas River to allow comparisons of responses between these streams.

A statistically significant difference was accepted if $p < 0.05$. Statistical analyses were performed on ln(n+1) transformed data, as plots of residuals indicated heterogeneity of variances among treatments. Values of species richness were not transformed since plots of residuals indicated no relationship between means and variances. All statistical analyses were conducted using Statistical Analysis System's PROC GLM (SAS 1988).

Results

Physical and Chemical Characteristics

LBC and SFP were physically distinct sites, as LBC was shallower and narrower, had more canopy cover, and was approximately 225 m higher than SFP. Mean annual discharge and water temperature were also lower at LBC compared to SFP (Kiffney and Clements 1994a). Chemical characteristics were similar in water of LBC and SFP, and experimental streams, except for the presence of metals in dosed streams (Table 2.1). Concentrations of Cd, Cu, and Zn in water were significantly higher in metal-dosed stream microcosms compared to reference microcosms ($p < 0.0001$ for all metals).

Water hardness was lower at the Mosquito Creek reference (MC-r) site compared with MC-p and both AR sites (Table 2.2). Altitude at MC was 200-600 m higher than AR sites. MC was also smaller and had more canopy cover than AR.

Water hardness, conductivity, and pH were lower in the Snake River than in the Eagle River (Table 2.2). The SR sites were located at a higher-altitude (390-800 m) and were smaller than the ER sites, which had a denser canopy.

Stream Macroinvertebrates

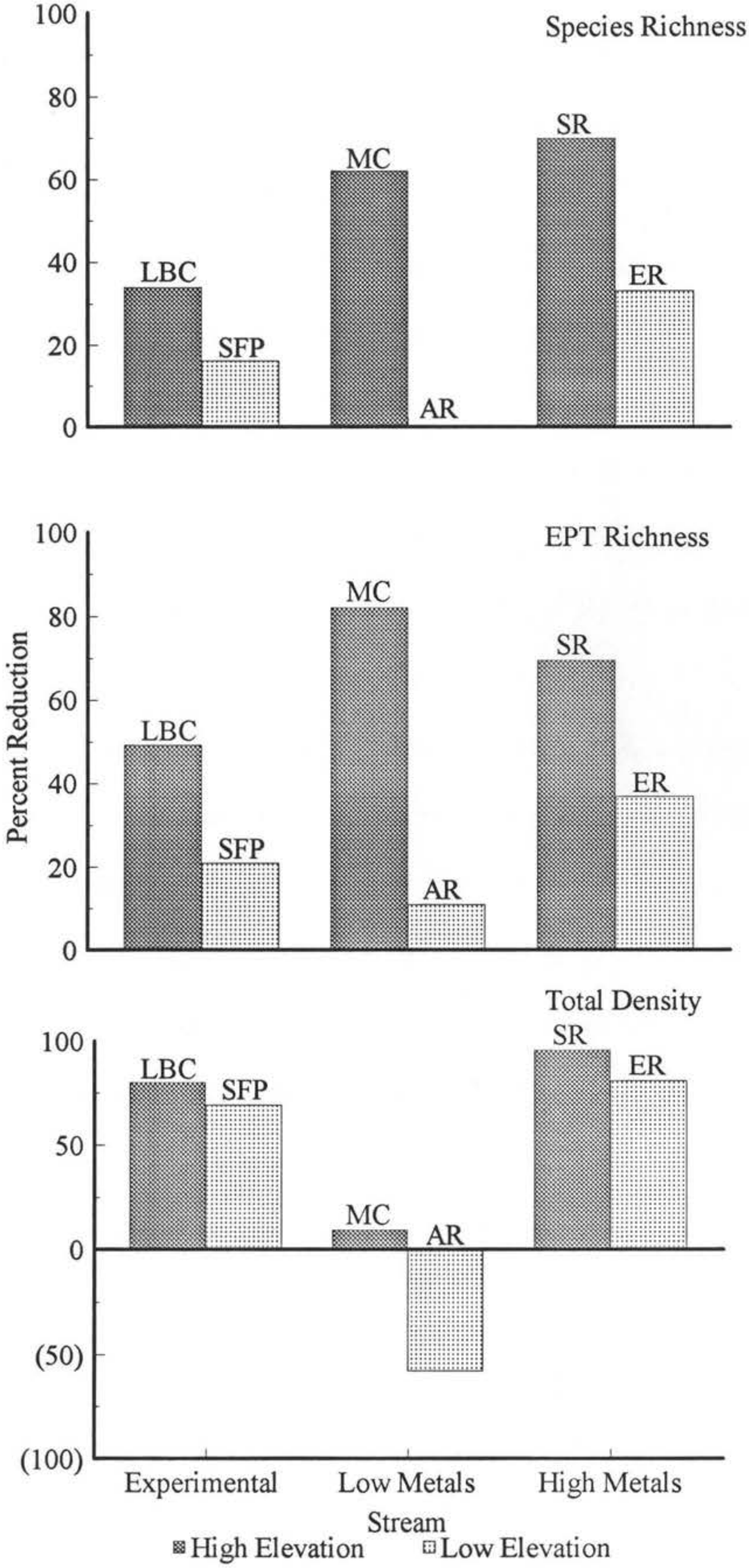
There were significant differences in macroinvertebrate assemblages between low- and high-altitude streams. In general, total abundance, EPT richness, abundance of mayflies, and abundance of caddisflies were significantly greater at large, low-altitude control sites than in small, high-altitude control sites (Table 2.3).

In all instances, effects of metals were greater on macroinvertebrate assemblages from small, high-altitude streams. Species richness, EPT species richness, and total abundance were reduced more in LBC metal-polluted experimental streams and at MC and SR metal-polluted field sites (Figure 2.3) relative to low-altitude sites. Stream x

Table 2.3. P-values from F-tests in a two-way ANOVA that tested the significance of stream location, metal treatment, and the stream x metals interaction on invertebrate assemblages in experimental streams, and from low (Mosquito Creek and Arkansas River) and high (Snake River and Eagle River) metal streams.

		Factor	
Variable	Stream	Metals	Stream x Metals
Total Abundance			
Experimental Streams	0.0001	0.0001	0.03
Low Metal Streams	0.003	0.30	0.10
High Metal Streams	0.0001	0.0001	0.0001
Species Richness			
Experimental Streams	0.36	0.0008	0.23
Low Metal Streams	0.0001	0.0001	0.0001
High Metal Streams	0.0001	0.0001	0.0003
EPT Richness			
Experimental Streams	0.04	0.003	0.06
Low Metal Streams	0.0001	0.0001	0.0001
High Metal Streams	0.0001	0.0001	0.002
Mayfly Abundance			
Experimental Streams	0.0002	0.0001	0.009
Low Metal Streams	0.0001	0.0001	0.0001
High Metal Streams	0.0001	0.0001	0.02
Stonefly Abundance			
Experimental Streams	0.46	0.0007	0.06
Low Metal Streams	0.08	0.001	0.0003
High Metal Streams	0.22	0.0001	0.03
Caddisfly Abundance			
Experimental Streams	0.0001	0.0001	0.01
Low Metal Streams	0.0001	0.99	0.0001
High Metal Streams	0.0001	0.0001	0.34

Figure 2.3. Percent reduction in species richness, EPT richness, and total macroinvertebrae abundance in experimental streams, and at low metal and high metal field streams. LBC=Little Beaver Creek, SFP=South Fork of Poudre River, MC=Mosquito Creek, AR=Arkansas River, SR=Snake River, ER=Eagle River.


Species Richness
LBC
SFP
MC
AR
SR
ER
EPT Richness
Percent Reduction
Total Density
100
80
60
40
20
0
50
(50)
(100)
Experimental
Low Metals
High Metals
Stream
High Elevation
Low Elevation

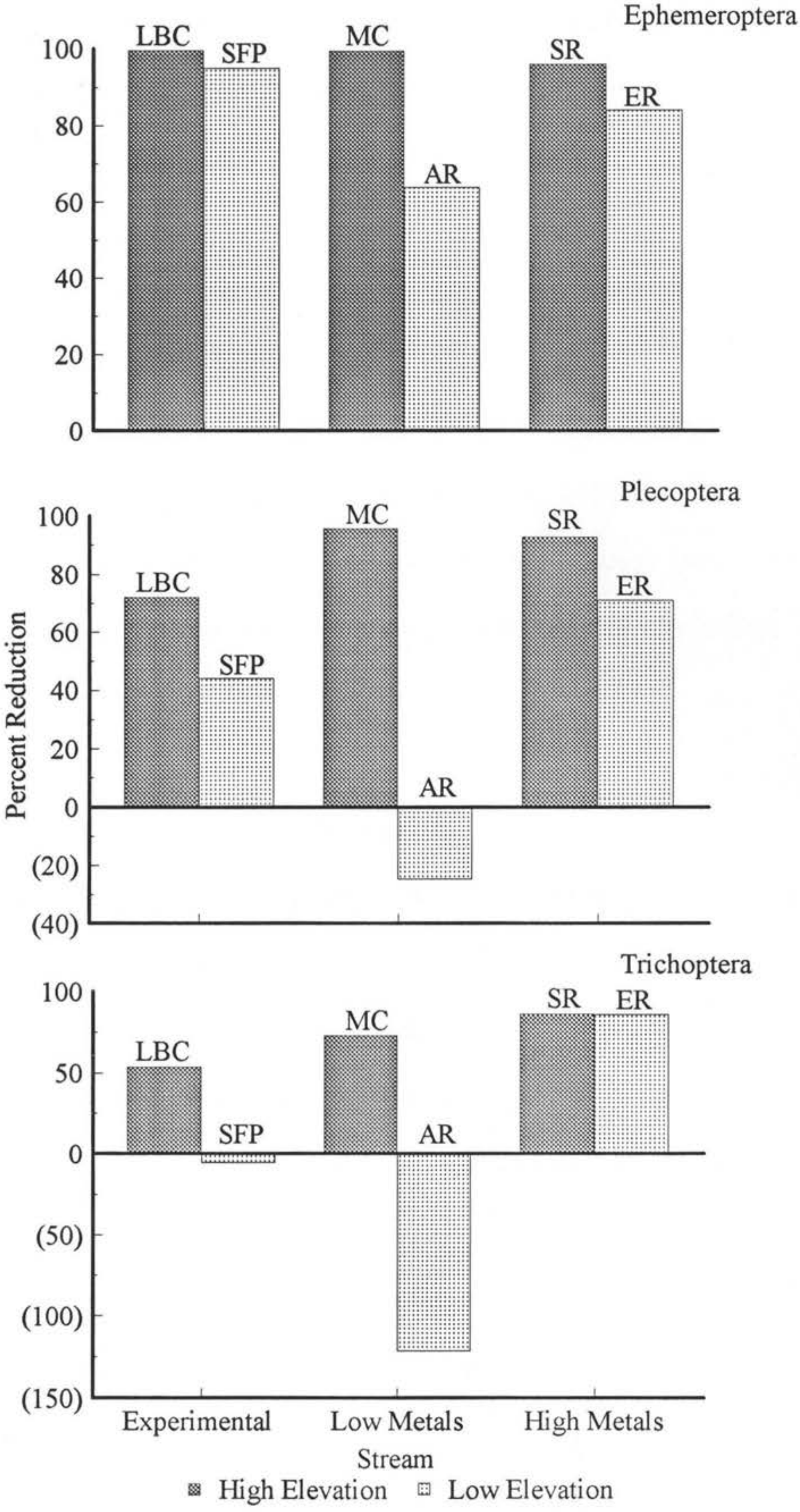
metals interactions were statistically significant ($p < 0.05$) for all parameters, except for number of taxa ($p=0.23$) and EPT richness ($p=0.06$) in experimental streams and total density at low metal streams (MC vs. AR, $p=0.1$) (Table 2.3).

The response of mayflies to metals differed depending upon stream location, as shown by interaction terms for experimental streams (LBC vs. SFP, $p=0.009$) and field sites (MC vs. AR, $p=0.0001$; SR vs. ER, $p=0.02$) (Table 2.3). This variation was reflected by a greater relative reduction in densities of mayflies at high-altitude, metal-polluted sites compared to mayflies from low-altitude sites. At metal-polluted field sites, mayfly density was reduced by 96 and 99% at high-altitude sites (SR-p and MC-p) compared with 84 and 64% at low-altitude sites (ER-p and AR-p) (Figure 2.4).

Stoneflies from small, high-altitude streams were also affected more by metals than stoneflies from lower-altitudes. The stream x metals interaction was not significant for experimental streams ($p=0.06$), whereas the interaction term was significant for low metal streams (MC vs. AR, $p=0.0003$) and high metal streams (SR vs. ER, $p=0.03$) (Table 2.3). Stonefly densities were reduced by 73% in LBC metal-polluted microcosms compared with 44% in SFP microcosms. Similarly, relative reductions in stonefly densities were greater at high-altitude, metal-polluted sites (SR-p and MC-p) compared to lower-altitude, metal-polluted sites (ER-p and AR-p) (Figure 2.4).

Abundances of caddisflies were reduced at all high-altitude, metal-polluted sites; however, the response at low-altitude sites was variable (Figure 2.4). Caddisfly densities were greater in SFP metal-polluted streams (+6%) and at AR-P (+57%) compared with dosed microcosms and AR-r, whereas densities were reduced at ER-p (-80%). These

Figure 2.4. Percent reduction in abundance of Ephemeroptera, Plecoptera, and Trichoptera in experimental streams, and at low metal and high metal field sterams (see Figure 2.3 for further details).



Ephemeroptera
Plecoptera
Trichoptera
LBC
SFP
MC
AR
SR
ER
100
80
60
40
20
0
(20)
(40)
50
(50)
(100)
(150)
Percent Reduction
Experimental
Low Metals
High Metals
Stream
High Elevation
Low Elevation

dissimilar responses were manifested by statistically significant stream x metals interactions (p=0.01) in microcosms and at low metal streams (MC versus AR, p=0.0001) (Table 2.3).

Some dominant taxa (i.e., > 1% total relative abundance) from Mosquito Creek and the Arkansas River were abundant enough to make comparisons feasible at the taxon level. Abundance of three mayfly taxa was reduced more at the high-altitude site (MC-p) than at the low altitude site (AR-p) (p=0.002 for Baetis sp. (Ephemeroptera:Baetidae), p=0.005 for Drunella sp. (Ephemeroptera:Ephemerellidae), p=0.03 for Epeorus sp. (Ephemeroptera:Heptageniidae)) (Table 2.4, Figure 2.5). This trend was also evident for chloroperlid (Plecoptera:Chloroperlidae) stoneflies (p=0.002), and the caddisfly Rhyacophila sp. (Trichoptera:Rhyacophilidae) (p=0.04).

Discussion

These results support the hypothesis that effects of metals were greater on macroinvertebrates from small, high-altitude streams compared with those from large, low-altitude streams. To my knowledge, this is the first study to compare experimental and field responses to metals of stream macroinvertebrate assemblages from different locations. Previously, Kiffney and Clements (1994a) reported that mayflies from a small, high-altitude stream (Little Beaver Creek (LBC)) were more sensitive to zinc relative to mayflies from a larger, lower-altitude stream (South Fork of the Poudre River (SFP)). There are a number of possible interrelated mechanisms that may explain the differences that were found.

Table 2.4. P-values from F-tests in a two-way ANOVA that tested the significance of stream location, metal treatment, and the stream x metals interaction on invertebrate taxa from Mosquito Creek (high-altitude stream) and the Arkansas River (low-altitude stream).

		Factor	
Variable	Stream	Metals	Stream x Metals
Baetis sp.	0.0001	0.0001	0.0002
Drunella sp.	0.1	0.003	0.005
Epeorus sp.	0.7	0.0004	0.03
Chloroperlidae	0.1	0.02	0.002
Rhyacophila sp.	0.001	0.4	0.04

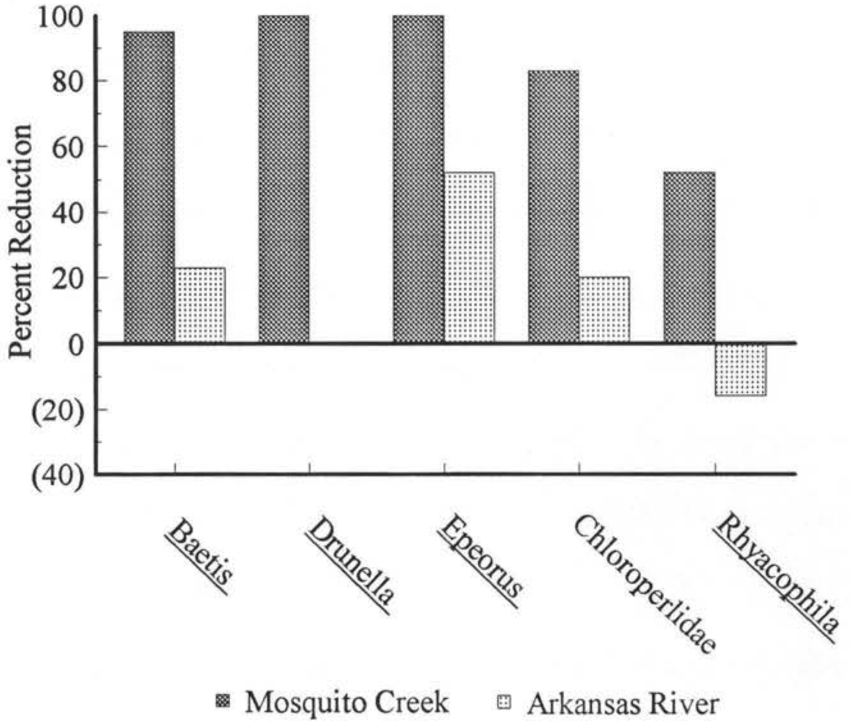


Figure 2.5. Percent reduction in abundance of Baetis sp., Drunella sp., Epeorus sp., Chloroperlidae, and Rhyacophila sp. from high- (Mosquito Creek) and low- (Arkansas River) altitude sites at low metal streams.

The first possible explanation, and one I consider most likely, was that differences in metal-sensitivity were affected by organism size. Water temperature is an important variable influencing body size of aquatic insects. Because water temperatures of high mountain streams are intimately coupled with air temperature (Ward 1992), lower annual water temperatures were expected at higher-altitude sites. Little Beaver Creek (LBC), Mosquito Creek (MC), and the Snake River (SR) were approximately 200 to 800-m higher than the South Fork of the Poudre (SFP), the Arkansas River (AR), and the Eagle River (ER). These differences in altitude correspond to a 1.3-4.0° C colder air temperature at the high-altitude streams (Ward 1992). Evidence for this was observed at LBC and SFP where LBC's annual water temperature was 1.8° C colder than SFP (Kiffney, unpublished results).

The relationship between size and water temperature in aquatic insects is complex, and a number of hypotheses have been proposed to explain this relationship (Ray 1960, Sweeney and Vannote 1978, Vannote and Sweeney 1980, Hawkins 1986, Hogue and Hawkins 1991). Most researchers agree that larval growth depends largely on the total length of time available for growth (Sweeney 1984); therefore, insects from high-altitude, cold streams should have a slower physiological developmental rate compared with insects from low-altitude, warmer streams (Vannote and Sweeney 1980). Moreover, the potential for small individuals to grow during rising vernal temperatures is reduced relative to large individuals, because a greater proportion of assimilated energy is required for maintenance (Sweeney and Vannote 1980). Some researchers have observed that aquatic insects from high-altitude, colder sites were smaller (Anderson and Cummins 1979, Markarian 1980, Wise 1980, Rader and Ward 1990), whereas others have noted insects from colder streams were larger (Hogue and Hawkins 1991). Preliminary research has shown that some taxa from LBC (high-altitude) were smaller than those from SFP (low-altitude). For instance, Baetis tricaudatus and Arctopsyche grandis (Trichoptera:Hydropsychidae) were significantly smaller at LBC compared to SFP during the period of this study (B. tricaudatus; t-test, $p=0.0001$, and A. grandis; t-test, $p=0.03$). Although it was not determined if these species were at the same lifestage, my results do show that the same species varied in its size depending upon altitude. Rader and Ward (1990) observed that B. tricaudatus was smaller at a high-altitude (2593 m) site than a lower-altitude site (2426 m) site. They suggested that the water temperature regime at the high-altitude site was less than optimal with respect to growth for this species. These results provide some evidence to suggest that organisms from small, high-altitude Rocky Mountain streams were smaller than the same species from larger, lower-altitude streams.

If colder water temperatures at high-altitude streams (LBC, MC, and SR) during this study resulted in smaller body size by limiting larval developmental rate or influencing phenology, then these smaller taxa may be more sensitive to metals than similar species at lower-altitudes (SFP, AR, and ER). The greater sensitivity of small individuals to contaminants compared to large individuals of the same species is well documented (Powlesland and George 1986, Diamond et al. 1992). In a previous experiment, it was observed that the average size of Drunella grandis (Ephemeroptera:Ephemerellidae) was larger in metal-dosed microcosms compared with controls (Kiffney and Clements 1994b). Similar results were observed in this study for Brachycentrus sp.

(Trichoptera:Brachycentridae) and Pteronarcella badia (Plecoptera:Pteronarycidae), as these taxa were larger in metal-dosed microcosms than in controls (Brachycentrus; t-test, p=0.04, and P. badia; t-test, p=0.0001). Thus, these data suggest that smaller individuals were more likely to be negatively affected by metals than large individuals of the same species in stream microcosms. Increased sensitivity of small individuals may result from larger surface area:volume ratios, higher initial lipid content, or a greater weight specific metabolism which would facilitate uptake of toxicants (Powlesland and George 1986).

A second possible explanation is that abiotic factors, such as water hardness or alkalinity, may have caused the differential response of stream macroinvertebrates from different locations to metals observed in this study. The greater sensitivity of macroinvertebrates from SR (small, high-altitude) relative to ER (large, low-altitude) could be a result of lower water hardness at SR sites, which increases metal bioavailability and hence toxicity (Prosi 1981, Sprague 1984). However, differences in water hardness could not account for differential responses observed in experimental streams and at low metal sites. Effects of metals on macroinvertebrates from LBC were greater than from SFP, even though these organisms were subjected to similar environmental regimes in stream microcosms. Additionally, water hardness at MC (small, high-altitude) and AR (large, low-altitude) was similar, whereas macroinvertebrate densities were reduced more at MC.

Differences in environmental regimes at high- and low-altitude sites may be a third factor important in determining the response of stream macroinvertebrates to metals. Other researchers have noted the influence of location on sensitivity to contaminants in aquatic organisms. For instance, the greater resistance of estuarine organisms to contaminants compared with similar oceanic taxa may be due to greater variability of environmental conditions in estuaries (Fisher 1977, Hyland et al. 1985, Tedengren et al. 1988). Poff and Ward (1990) propose that spatial and temporal characteristics of the physical environment affect an organism's response to disturbance, and that organisms from variable environments should be more tolerant to anthropogenic disturbance than organisms from constant environments. Populations from more variable environments may be more tolerant because they exhibit a greater range of phenotypes and genotypes allowing the population to persist when faced with anthropogenic stress (Poff and Ward 1990). Annual and diel variation in the thermal regime of temperate streams generally increases from headwater to mid-order reaches (Smith 1972, Vannote and Sweeney 1980, Ward 1986, Ward 1992). The relatively greater effect of metals on macroinvertebrates from small, high-altitude streams observed in this study may be a result of these organisms evolving under more constant temperature regimes, and thus possessing less genetic and/or phenotypic diversity than populations from large, low-altitude streams.

Although benthic invertebrate community composition between sites was similar, subtle differences may have played a role in the variation in responses we observed. For example, species richness of stoneflies and caddisflies generally increases from upstream to downstream in mountain rivers (Ward 1986). Species belonging to these groups have been shown to be more tolerant to the effects of metals than mayflies (Chadwick et al. 1986, Leland et al. 1989, Kiffney and Clements 1994a, 1994b). Thus, the greater effect of metals on invertebrate assemblages from low-altitude streams may have been due to a greater representation of metal-tolerant stoneflies and caddisflies.

Whatever the mechanism(s) explaining the differences in responses that were observed, I suggest that my results have important implications for managing the quality of the nation's surface waters. Water quality criteria for metals are based primarily on levels of water hardness or alkalinity (EPA 1980a, 1980b, 1980c) using organisms that are not typically found in mountain streams. Results from this research suggest that this approach is simplistic and ignores other factors, such as variation in species composition, size, and/or phenology of indigenous organisms that may influence response to contaminants (Kiffney and Clements, 1994b). In previous experiments, I observed significant decreases in the abundance of macroinvertebrates when exposed to Cd, Cu, and Zn at criteria concentrations (Kiffney and Clements, 1994b). Moreover, in this study invertebrate assemblages from small, high-altitude streams were 12-85% more sensitive to metals than assemblages from large, low-altitude streams. I recommend that state and federal agencies responsible for water quality should incorporate these differences in metal-sensitivity of indigenous aquatic organisms into the process of setting metals criteria. For instance, our results indicate that macroinvertebrate assemblages from Rocky Mountain headwater streams are more sensitive to the effects of metals than assemblages from lower-altitude streams. A number of mechanisms may explain these differences, but I hypothesize that insect body size is the most important factor affecting an insect's response to metals.

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CHAPTER III

RESPONSE OF STREAM MACROINVERTEBRATES TO METALS: IS BIGGER BETTER?

Introduction

Body size is an important variable influencing many aspects of the ecology, physiology, and survival of organisms (Miller et al. 1988, Scrimgeour et al. 1994, Wooten 1994). Body size can influence an animal's behavioral interactions (Flecker et al. 1988), survival (Werner 1984), and life history traits (Scrimgeour et al. 1994). Scrimgeour et al. (1994) observed that the effects of predators on food selection by the mayfly Baetis tricaudatus (Ephemeroptera:Baetidae) was greater for small than for large larvae. The predaceous stonefly Hesperoperla pacifica (Plecoptera:Perlidae) exhibited size-selective predation as small stoneflies preferred small prey, whereas large stoneflies preferred large prey (Allan et al. 1987). Fishery biologists have also explored ecological factors that influence larval survival, because this lifestage may be the most vulnerable to mortality (Werner 1984, Post and Evans 1989, Miller et al. 1988, Rice et al. 1993). For instance, Miller et al. (1988) noted that predator capture success was highly size-dependent, as small larval fish were more vulnerable to predators than large ones.

Early lifestages of many invertebrates and fish are also more sensitive to contaminants than later lifestages (Howarth and Sprague 1978, Green et al. 1986, Naylor et al. 1990, Stuhlbacher et al. 1993). Kiffney and Clements (1994a) found that early lifestages of the stream invertebrate Drunella grandis (Ephemeroptera:Ephemerellidae) were more sensitive to heavy metals than later lifestages. Greater sensitivity of small individuals may result from large surface area:volume ratios, higher initial lipid content, or a greater weight-specific metabolism which may facilitate uptake of toxicants (Sprague 1985, Powlesland and George 1986).

I previously found that benthic invertebrates from small, high-altitude streams were more sensitive to heavy metals than those from larger, lower-altitude streams (Kiffney and Clements 1994b). I hypothesized that greater metal-sensitivity of high-altitude populations may be due to a greater proportion of small individuals. Although these experiments were designed to test the influence of altitude on benthic invertebrate response to metals, by examining preserved samples from these experiments I was able to determine the influence of body size on sensitivity to metals. I also present evidence that benthic insects from high-altitude streams were smaller than the same species from lower-altitude streams, and that this difference may explain the variation in metal-sensitivity described earlier (Kiffney and Clements 1994b).

Methods

Experimental System

To evaluate the effects of metals on benthic invertebrate communities along an altitudinal and longitudinal gradient, artificial substrates were placed at three sites on the Cache la Poudre River, Colorado catchment west of Fort Collins (Figure 3.1). The three sites, Little Beaver Creek (LBC), the South Fork of the Poudre River (SFP), and the mainstem of the Cache la Poudre (CLP) are third, fourth, and sixth-order streams (sensu Strahler 1957) (Table 3.1), respectively. Mean stream width (wetted channel), depth, and canopy cover were determined at three randomly selected locations at each field site. Relative canopy cover was determined by measuring the distance riparian vegetation extended over the stream and dividing this value by stream width (i.e., wetted channel).

Figure 3.1. Map of the Cache la Poudre, Colorado catchment. LBC=Little Beaver Creek, SFP=South Fork of the Poudre River, and CLP=Cache la Poudre River.

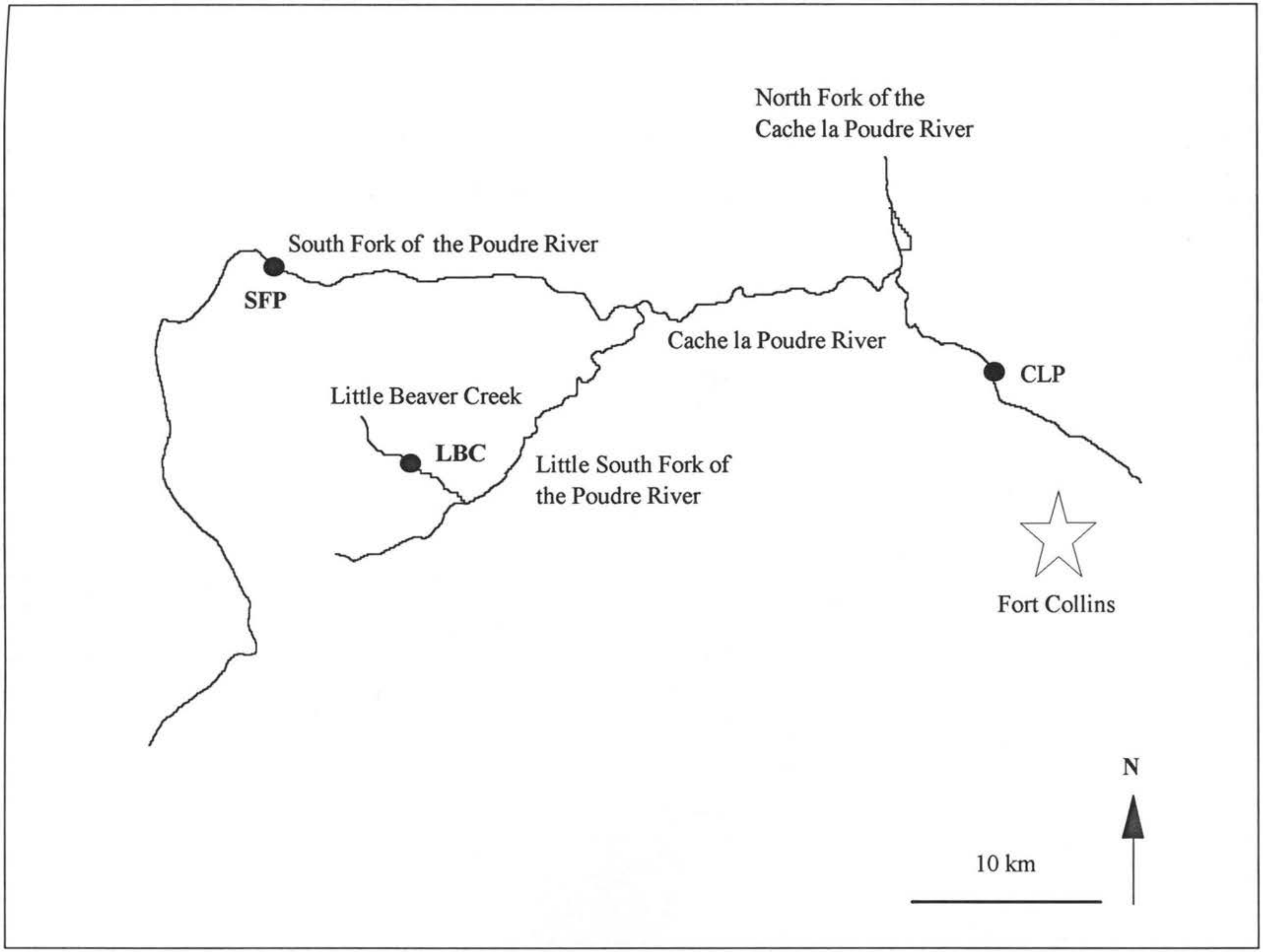

North Fork of the
Cache la Poudre River
South Fork of the Poudre River
SFP
Cache la Poudre River
CLP
Little Beaver Creek
LBC
Little South Fork of
the Poudre River
Fort Collins
N
10 km

Table 3.1. Physical and chemical characteristics of Little Beaver Creek (LBC), the South Fork of the Poudre River, and Cache la Poudre River (CLP).

	LBC	SFP	CLP
Elevation (m)	2545	2320	1650
Drainage Area (km^2)	31	512	2745
Stream Order	3rd	4th	6th
Width (m)	4.0	20	28
Depth (cm)	20	30	40
Canopy Cover (%)	49	15	<1
Annual Discharge (m^3/sec)	0.18	6.1	7.4
Annual Temperature (C^0)	4.1	5.8	7.8
Annual Degree-Days (C^0)	1525	2080	2737
pH	7.6	7.5	7.7
Conductivity (μmhos/s)	48	52	56
Hardness (mg/L)	14	21	36

The artificial substrates consisted of 10 x 10 x 6- cm plastic trays (0.01 m^2 surface area) filled with air-dried pebbles and small cobbles (2-6 cm diameter). Trays were placed in riffle sections of LBC and SFP from 18 July 1992 to 1 September 1992, and in riffles of SFP and CLP from 1 October 1993 to 6 November 1993. Trays were transferred to 12 experimental streams (76 x 46 x 14- cm) at Colorado State University, Fort Collins (Kiffney and Clements 1994a, 1994b, Kiffney, unpublished results). Further details of this experimental approach have been published previously (Kiffney and Clements 1994a, 1994b).

After a 48-h acclimation period, each experimental stream was randomly assigned to one of two treatments (three replicates per treatment): control or metal-treated (mixture of Cd, Cu, and Zn). Metal concentrations in treated microcosms were generally similar to levels measured at metal-polluted sites at the Arkansas River near Leadville, CO (Kiffney and Clements 1993, Clements 1994). Specifically, target concentrations were 5X the chronic values for Cd, Cu, and Zn (1X=1.1, 12.0, and 110 μg/L Cd, Cu, and Zn, respectively) in the first experiment (September 1992), and equal to the chronic values for these metals in the second experiment (November 1993; EPA 1980a, 1980b, 1980c).

After 10 d, the four trays in each stream were pooled, washed into a 355 μm sieve, and organisms retained were preserved in 100% ethanol. Samples were sorted in the laboratory in white enamel pans, and all organisms were identified to genus or species (Merritt and Cummins 1984).

Head capsule widths (widest point) and body lengths (tip of head to end of abdomen) of insects in control and metal-treated streams were measured to the nearest

0.05 mm using an ocular micrometer mounted to a model SZ60 Olympus dissecting microscope. To determine if insect size differed between field sites, I measured insects collected from Hess samples (0.1 m^2 area) and additional trays. Only populations with densities greater than 20 individuals per sample were included in these analyses. Regression analysis showed that head capsule widths were highly correlated with body lengths ($p < 0.01$); therefore, only head capsule measurements are presented.

Water samples were collected from each site for water chemistry and metal analysis when trays were collected. Alkalinity and hardness were determined in the laboratory by titration (APHA 1989). Conductivity was analyzed with a conductivity meter (VWR Scientific Model 1054) and pH with Chemcadet meter. Dissolved oxygen and water temperature were measured in field and laboratory streams with an O_2 meter (Yellow Springs Instrument Company model 51B). All samples for metals analyses were acidified with reagent-grade HNO_3 to pH < 2. Samples were analyzed on an IL 22 video dual channel flame atomic absorption spectrophotometer using standard methods (method #3111 A, APHA 1989). Mean stream temperature for LBC was based on thermograph data collected during 1991-1992 (Gowan and Fausch 1993), whereas water temperature for SFP was based on measurements collected during 1991-1992 by the Colorado Division of Wildlife (A. Ganek, unpublished results). Temperature data for the CLP site was obtained from the United States Geological Survey (USGS) for water year 1992-1993 (C. M. Tate, unpublished results). Estimates of annual degree-days (with 0° C as developmental threshold) for these sites were based on monthly average temperatures. Flow records for LBC, SFP, and CLP were obtained from USGS water records.

Statistical Analysis

To determine if head capsule width was different among field sites and between treatments, PROC NPAR1WAY and a Wilcoxon two-sample test was performed (SAS 1990a). The null hypothesis for this test was that the two samples come from populations having the same statistic of location (Sokal and Rohlf 1981). Logistic regression (PROC LOGISTIC) was used to examine the relationship between the percent survival (i.e., survival rate = number of individuals per size class surviving in metal-treated streams relative to control streams) and size class, and to derive parameter estimates (slope and intercept). This procedure fits linear logistic regression models to binary response data (e.g., survived or died) by the method of maximum likelihood (SAS 1990a). A statistically significant, negative slope would indicate that invertebrate survival increased with increased body size. Rather than arbitrarily choosing size classes, we used SAS's PROC CHART to determined midpoints (SAS 1990b). This procedure determines midpoints based on the algorithm by Terrell and Scott (1985).

Results

Mean stream width and mean depth, drainage area, stream order, mean annual discharge, mean water temperature, and annual degree-days (LBC and SFP, 1991-1992; CLP, 1992-1993) increased from upstream to downstream, whereas canopy cover decreased (Table 3.1). Most water quality parameters, such as hardness, alkalinity, and conductivity were similar among locations. Metal concentrations at these sites were consistently below detection (Kiffney and Clements 1994a, 1994b). Water quality of control experimental streams was similar to that of field sites (Kiffney and Clements 1994b, Kiffney, unpublished results).

Most species collected from field sites at densities high enough to make comparisons (> 20 individuals at both locations) were larger at low-altitude streams than at high-altitude streams. Baetis tricaudatus (Ephemeroptera:Baetidae; p=0.0001) and Arctopsyche grandis (Trichoptera:Hydropsychidae; p=0.002) were significantly larger at SFP compared with LBC. Although Brachycentrus sp. (Trichoptera:Brachycentridae) was also larger at SFP, this difference was not statistically significant (p=0.7) (September 1992; Figure 3.2). Rhithrogena hageni (Ephemeroptera:Heptageniidae; p=0.0001) and B. tricaudatus (p=0.002) were significantly larger at CLP, the lowest altitude site, compared with SFP (November 1993; Figure 3.2).

Exposure to heavy metals in microcosms affected size distributions of most taxa. In almost all comparisons, mean insect size was larger in metal-treated streams than in controls, although these differences were not always statistically significant. For instance, mean head capsule width of Hesperoperla pacifica (p=0.06), A. grandis (p=0.09), and Brachycentrus sp. (p=0.02) from LBC (September 1992; Figure 3.3) was larger in metal-dosed streams than in control streams. Body size of B. tricaudatus (p=0.04), R. hageni (p=0.009), and Pteronarcella badia (Plecoptera:Pteronarcyidae) (p=0.0001) (September 1992; Figure 3.3) were also larger in metal-dosed microcosms. This trend in body size was also observed in insects from the lowest altitude stream (CLP), as B. tricaudatus (p=0.001), R. hageni (p=0.3), and Ephemerella infrequens (Ephemeroptera:Ephemerellidae) (p=0.0001) were larger in metal-dosed streams than in

Figure 3.2. Mean ($\pm$ 1SE) head capsule width of invertebrates collected from streams at different altitudes (LBC, 2545 meters above mean sea level; SFP=2320 m; CLP=1650 m) during September 1992 (LBC and SFP) and November (SFP and CLP).

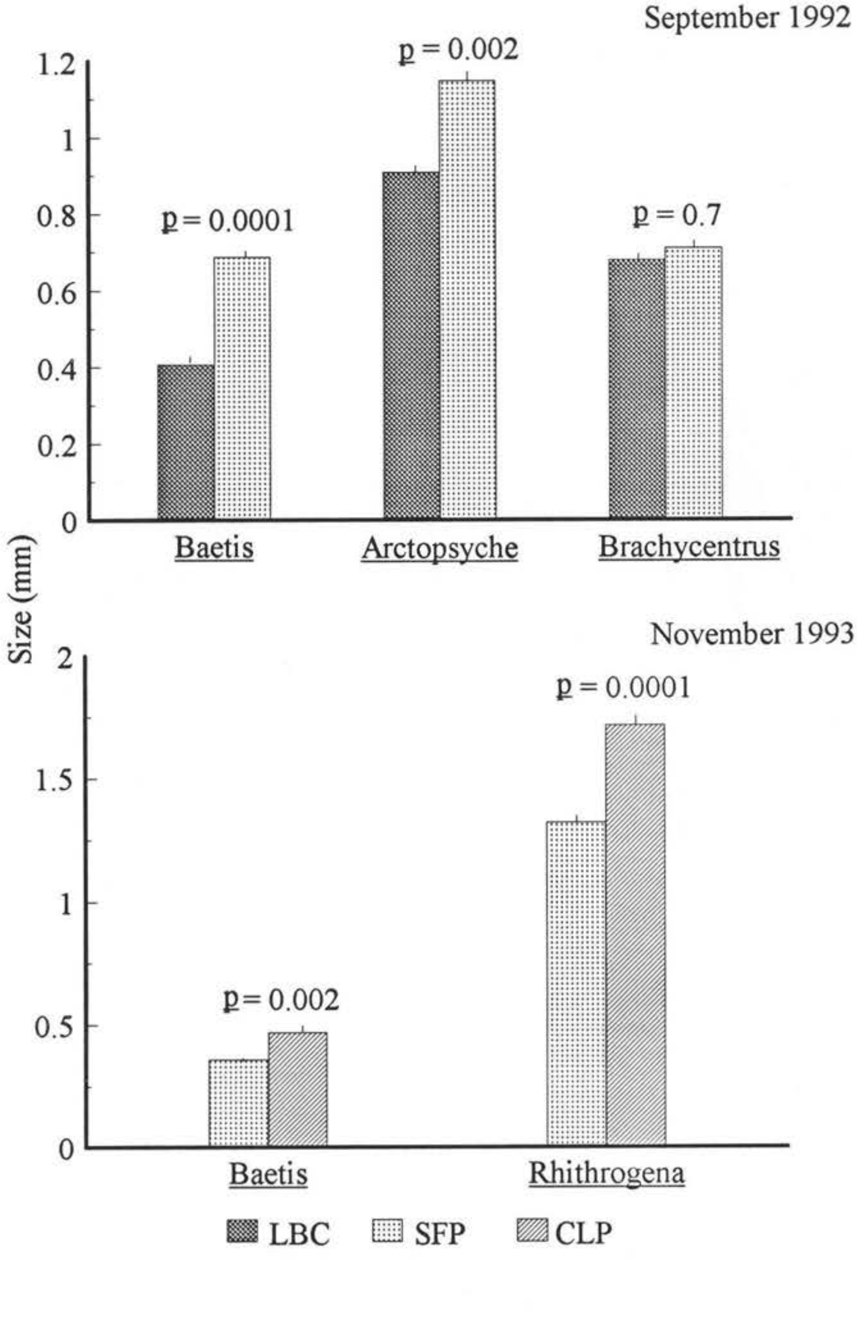
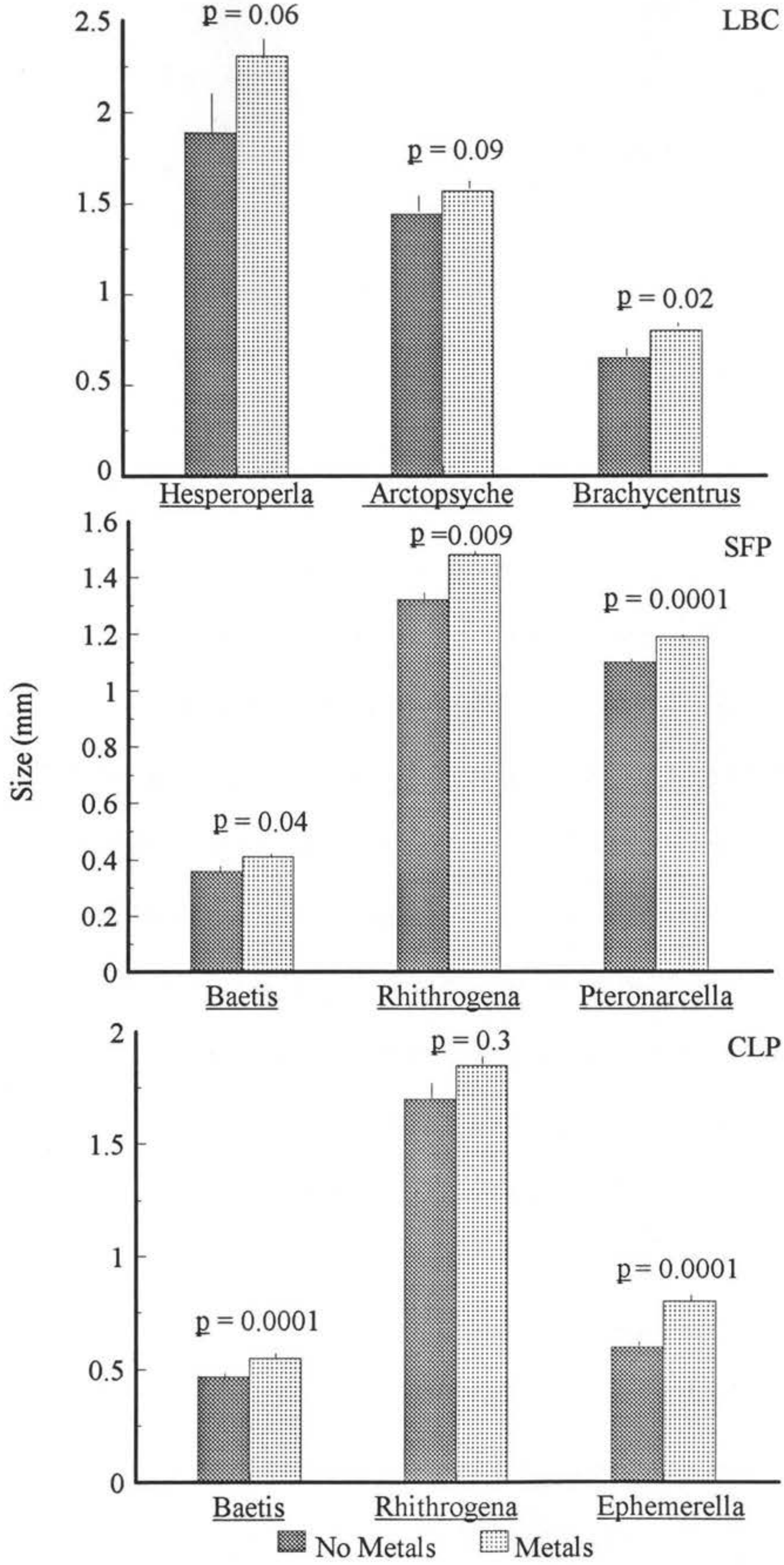

September 1992
p = 0.0001
p = 0.002
p = 0.7
1.2
1
0.8
0.6
0.4
0.2
0
Baetis
Arctopsyche
Brachycentrus
Size (mm)
November 1993
p = 0.0001
p = 0.002
2
1.5
1
0.5
0
Baetis
Rhithrogena
LBC
SFP
CLP

Figure 3.3. Mean ($\pm$ 1SE) head capsule width of invertebrates from LBC, SFP, and CLP in control (no metals) and metal-dosed microcosms after 10 days.


LBC
p = 0.06
p = 0.09
p = 0.02
Hesperoperla
Arctopsyche
Brachycentrus
SFP
p =0.009
p = 0.0001
p = 0.04
Baetis
Rhithrogena
Pteronarcella
CLP
p = 0.3
p = 0.0001
p = 0.0001
Baetis
Rhithrogena
Ephemerella
Size (mm)
No Metals
Metals

controls (November 1993; Figure 3.3).

Logistic regression showed there was a statistically significant relationship between percent survival and body size in seven out of the eight comparisons (Table 3.2). The negative slopes suggest that survival increased with increased body size, and that smaller size classes were more affected by metals than larger size classes within a population.

Discussion

Previously, Kiffney and Clements (1994b) reported that benthic macroinvertebrates from small, high-altitude streams were more sensitive to metals than macroinvertebrates from larger, lower-altitude streams. It was hypothesized that colder water temperatures influenced body size so that taxa from high-altitude streams were smaller, and therefore more sensitive to heavy metals. Moreover, evidence was presented that suggested sensitivity to metals decreased with increased body size (Kiffney and Clements 1994a). In this research, additional evidence is presented that supports these observations, as insects collected from high-altitude streams were smaller than the same species from low-altitude streams. In addition, due to the lower survival of small individuals, mean insect size was smaller in control microcosms than in metal-dosed microcosms.

Water temperature is a key variable influencing the distribution and abundance of stream macroinvertebrates, because of its influence on metabolism, growth, and reproduction (Sweeney and Vannote 1978, Vannote and Sweeney 1980, Ward 1982, Sweeney 1984, Rader and Ward 1990). The Thermal Equilibrium Hypothesis was

Table 3.2. Estimates of parameters (slope and intercept) and associated p-values for the relationship between survival (number per size class in control streams relative to metal-dosed streams) of aquatic invertebrates to metal exposure and size class using logistic regression.

Species	Site	Experiment	Parameter Estimates Intercept (p-value)	Slope (p-value)
Baetis tricaudatus	CLP	November 1993	3.03 (0.0001)	-4.45 (0.0001)
B. tricaudatus	SFP	September 1992	2.60 (0.16)	-8.71 (0.05)
Rhithrogena hageni	CLP	November 1993	5.75 (0.008)	-3.50 (0.007)
R. hageni	SFP	September 1992	2.79 (0.0001)	-2.03 (0.0001)
Brachycentrus americanus	LBC	September 1992	1.40 (0.009)	-2.92 (0.0003)
Arctopsyche grandis	LBC	September 1992	0.68 (0.25)	-0.78 (0.05)
Hesperoperla pacifica	LBC	September 1992	-0.33 (0.34)	0.14 (0.30)
Pteronarcella badia	SFP	September 1992	7.90 (0.0001)	-6.93 (0.0001)

developed to explain the causal relationship between water temperature and life history traits of stream insects (Sweeney and Vannote 1978, Vannote and Sweeney 1980). One of the predictions of this hypothesis is that aquatic insect body size is maximized in geographic areas where the thermal regime is optimal. Some researchers have observed that aquatic insects from high-altitude, colder sites were smaller (Anderson and Cummins 1979, Markarian 1980, Wise 1980, Rader and Ward 1990, Snyder et al. 1991), whereas others have noted insects from colder streams were larger (McCafferty and Pereira 1984, Hogue and Hawkins 1991). Rader and Ward (1990) ranked sites along an altitudinal gradient of a Colorado stream according to how closely they approximated the optimal temperature conditions for four mayfly taxa. Their highest altitude site, which was at an altitude similar to LBC and had a similar number of degree-days, was considered cooler than optimum with respect to maximizing body size (Rader and Ward 1990). I speculate that cold annual water temperatures at high-altitude Rocky Mountain streams may limit the amount of time an insect can grow, resulting in smaller body size.

Greater sensitivity of small individuals to contaminants compared to large individuals is well documented (Powlesland and George 1986, De Nicola Guidici et al. 1986, Green et al. 1986, Diamond et al. 1992, Stuhlbacher et al. 1993, Kiffney and Clements 1994b). Naylor et al. (1990) noted that small isopods (Asellus aquaticus) and amphipods (Gammarus pulex) were generally less tolerant to pH and Zn than large individuals. Further support for the relationship between size and effects of metals was provided by this study. Specifically, mean insect size was less in control microcosms than in metal-dosed microcosms, and logistic regression indicated survival increased with body size. The only case where body size was not significantly related to survival was observed in the stonefly H. pacifica, even though mean size was larger in metal-dosed microcosms ($p=0.06$). The small numbers of size classes generated by PROC CHART for this species may explain the non-significant slope. Thus, for natural populations of aquatic invertebrates, body size appears to be an important factor influencing metal-sensitivity.

Water quality criteria for metals are usually determined by evaluating the effects of contaminants using single-species toxicity tests. Although water hardness or alkalinity are considered when establishing these criteria (EPA 1980a, 1980b, 1980c), this approach ignores other factors, such as organism size and/or phenology that may influence response to contaminants. For example, I observed 100% mortality of the smallest size classes (0.5-1.0 mm head capsule width) of the mayfly Rithrogena hageni when exposed to a mixture of Cd, Cu, and Zn at chronic criterion levels (November 1993 experiment). The loss of these small, metal sensitive size classes may have important effects on recruitment, and population stability. If metal exposure occurs when metal sensitive lifestages are present, local population extinctions may occur (Luoma and Carter 1991, Kiffney and Clements 1994a). To protect biological integrity of aquatic systems (Karr 1991), I recommend using indigenous organisms in toxicity tests, and that a range of size-classes be exposed to the contaminant of interest. Moreover, the most sensitive size class, as determined by these experiments, should be used when formulating criteria for contaminants.

These results may also have implications for biomonitoring studies using stream invertebrates as indicators of water quality (Rosenberg and Resh 1993). An important consideration, when collecting benthic organisms during biomonitoring studies, is mesh size of the sampler. Resh and McElravy (1993) report that 51% of stream studies used a mesh size >401 μm. Rapid bioassessement techniques frequently use even a coarser mesh due the taxonomic problems with identifying small instars (Resh and Jackson 1993). If smaller instars are generally more sensitive to contaminants, researchers using a coarser mesh may underestimate pollutant effects because smaller instars are under-represented. These results suggest that water quality standards for metals should consider stream altitude, as it is an important variable influencing invertebrate life history traits, which, in turn, can influence metal-sensitivity.

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CHAPTER IV

INFLUENCE OF DENSITY AND METALS ON PREDATOR-PREY INTERACTIONS IN STREAM INVERTEBRATE COMMUNITIES

Introduction

There is theoretical (Menge and Sutherland 1987, Menge and Olson 1990, Dunson and Travis 1991) and empirical (Walde 1986, Peckarsky et al. 1990, Dunson and Travis 1991, Warner et al. 1993) evidence to support the notion that the relative importance of species interactions in organizing communities shifts along environmental stress gradients. While plant (Tilman 1988) and marine ecologists (Connell 1975, Menge and Sutherland 1987) have devoted substantial effort to examining the interaction between abiotic and biotic forces in structuring communities, stream ecologists have only recently addressed these issues (Power et al. 1985, Walde 1986, Peckarsky et al. 1990, Hansen et al. 1991, Hart 1992). To better understand patterns of stream invertebrate communities, it is essential to explore how the relative importance of species interactions shifts along environmental gradients.

The influence of abiotic factors on species interactions has been examined in artificial ponds (Warner et al. 1993), natural lakes (Arnott and Vanni 1993) and streams (Walde 1986, Peckarsky et al. 1990). Low pH altered the outcome of competition between Hyla femoralis and H. gratiosa (Warner et al. 1993). Invertebrate predation and pH interacted to determine zooplankton community structure in fishless Wisconsin lakes (Arnott and Vanni 1993). Walde (1986) showed sedimentation either eliminated or enhanced predator effects, depending upon the relative susceptibility of the predator versus prey.

Metal pollution is an important source of environmental stress to stream organisms in western North America. For example, over 1,950 km of streams in Colorado receive metal-laden discharge from abandoned and active mines (Wilkinson 1992). It has been determined that metals reduce the abundance and species richness of macroinvertebrate communities in western streams (Chadwick et al. 1986, Leland et al. 1989, Clements 1994, Kiffney and Clements 1994a, 1994b, in press); however, very little information is available on whether metals interact with biotic processes to influence macroinvertebrate community structure. In the only study to date that tested the effects of metals on predator-prey interactions in stream invertebrate communities, Clements et al. (1989) observed that vulnerability of net-spinning caddisflies (Hydropsychidae) to predation was greater in copper-dosed experimental streams (6 μg/L Cu) than in controls.

It has been observed in some western streams that invertebrate densities reach their highest level during fall (Leland et al. 1989, Kiffney, unpublished data). Biotic interactions (i.e., predation and/or competition) may be the most intense during these periods of high densities. Furthermore, the outcome of biotic interactions may be altered by the presence of metals in water because invertebrate species vary in their sensitivity to the effects of metals (Kiffney and Clements 1994a, 1994b, in press). With this in mind, I designed an experiment to test the main and interactive effects of invertebrate density, metals, and predation on Rocky Mountain stream invertebrate communities in stream microcosms. I hypothesized that metals would affect prey species more than the predator, thus making the prey more vulnerable to predation.

Methods

Experimental System

General

To evaluate the effects of invertebrate density, metals, and predation on benthic invertebrate communities, artificial substrates were placed in the South Fork of the Poudre River (SFP), Colorado, a fourth-order stream in the Cache la Poudre catchment (Kiffney and Clements 1994a, 1994b). The artificial substrates consisted of 10 x 10 x 6 cm plastic trays (0.01 m^2 surface area) filled with air-dried pebbles and small cobbles (2-6 cm diameter). Previous studies have demonstrated that macroinvertebrate assemblages colonizing trays were similar to those collected from surrounding natural substrates (Clements et al. 1989, Kiffney and Clements 1994b); however, macroinvertebrate densities were generally higher on trays (Kiffney and Clements 1994b). Moreover, low densities of large predatory stoneflies (Plecoptera) have been observed on these trays when colonized at SFP.

Invertebrate Density Treatments

To manipulate invertebrate density, 36 trays were colonized for either 13 days (low density) or 42 days (high density) in riffle sections of SFP during late summer and early fall 1993. The trays were retrieved by placing a 100- μm net directly downstream to prevent loss of organisms. Trays collected from SFP were randomly assigned to 18 plastic coolers (four trays per cooler) filled with stream water. Each cooler was aerated by an airstone connected to a 12- volt air pump.

Trays were transferred to oval, fiberglass experimental streams (76 x 46 x 14- cm) in a greenhouse at Colorado State University, Fort Collins, CO. Trays from each density treatment were randomly assigned to a stream (n=18); therefore, there were streams with either low density or high density trays. Each flow-through stream received aerated, untreated reservoir water at rate of 0.5 L/min. Turnover time in the 13-L streams was approximately 26 min, and water depth was 10 cm. Current was provided by a paddlewheel at an average velocity of 30 cm/s. Streams were exposed to a natural photoperiod.

Predation Treatment

Hesperoperla pacifica (Plecoptera:Perlidae), a ubiquitous stonefly predator found in many streams in western North America (Stewart and Stark 1988), was collected from Little Beaver Creek, CO (LBC) a third-order stream also in the Cache la Poudre catchment. During previous studies, densities of H. pacifica ranging from 30-150 m^{-2} have been measured at LBC (Kiffney, unpublished results). H. pacifica has been observed to feed on a variety of benthic invertebrates including Baetis sp., Chironomidae, and Trichoptera in experimental streams (Kiffney, unpublished results), and in field studies (Richardson and Gaufin 1971, Fuller and Stewart 1977, Johnson 1983). Only stoneflies that were 15-20 mm long (tip of head to end of abdomen) were used. Stoneflies were transported to experimental streams in aerated coolers filled with natural stream water. Two densities of H. pacifica were added to experimental streams: one treatment received four stoneflies (100 m^2), while the other treatment received no stoneflies.

Metals Treatment

After a 48-h acclimation period, metal-treated microcosms were dosed with a mixture of Cd, Cu, and Zn at half the federal chronic levels for water of less than 50 mg/L $CaCO_3$ (EPA 1986) (i.e., 0.5, 6.0, and 55 μg/L Cd, Cu, and Zn, respectively). Peristaltic pumps delivered a stock solution of $CdCl_2$, $CuSO_4$, and $ZnSO_4$ from separate 20- L acid washed carboys into each dosed stream at a rate of 5 ml/min. Because only 18 experimental streams were available, some treatments were replicated twice, whereas others were replicated in triplicate (Table 4.1). Thus, this full factorial experiment consisted of a planned, unbalanced 3 x 2 treatment structure, and a completely randomized design.

Data Collection

After seven days, the four trays in each stream were pooled, washed into a 355-µm sieve, and retained organisms were preserved in 100% ethanol. Samples were sorted in the laboratory in white enamel pans and all organisms were identified to genus or species using available taxonomic keys, and a reference collection.

Water samples were collected from LBC and SFP ($\underline{n}$=1), and on days 0, 2, 4, and 7 in experimental streams ($\underline{n}$=18) for water chemistry and metals analysis. Alkalinity and hardness were determined in the laboratory by titration (APHA 1989). Conductivity was analyzed with a conductivity meter (VWR Scientific Model 1054) and pH with Chemcadet

Table 4.1. Treatment structure for the 3 x 2 factorial design.

Treatment	Number of Replicates
LD[1]; NM; NP	2
LD; NM; P	3
LD; M; NP	2
LD; M; P	2
HD; NM; NP	2
HD; NM; P	3
HD; M; NP	2
HD; M; P	2

[1] Treatments include: LD = low invertebrate density, HD=high invertebrate density, NM = no metals, M=metals, NP = no predators, P = predators.

meter. Dissolved oxygen and water temperature were measured in field and laboratory streams with an O_2 meter (Yellow Springs Instrument Company model 51B). All samples for metals analyses were acidified with reagent-grade HNO_3 to pH < 2. Cadmium and copper were analyzed on an IL 22 video dual channel atomic absorption spectrophotometer with a graphite furnace attachment, while zinc was measured on the same machine using a flame attachment. All metals were analyzed using standard methods (method #3111 A, APHA 1989). Metal levels in water are presented as total (suspended + dissolved) metals.

Statistical Analysis

PROC GLM and a three-way analysis of variance was used to test the main effects of invertebrate density, metals, and predation, and all interactions (SAS 1990). Treatment effects on species richness, total density, and abundance of dominant taxa (i.e., relative abundance > 0.1) were examined. Backward selection (criteria level < 0.05) was used to eliminate parameters that did not contribute significantly to the model. The only factor eliminated by this analysis was the three-way interaction. Estimates of means and standard errors were determined using SAS's LSMEANS (Milliken and Johnson 1992). If the F-statistic was significant, t-tests were used to compare means within the same effect. Since the experimental design was unbalanced, Type III sum of squares were used in all analyses. Because inspection of residual plots indicated non-homogeneity of variances, a square-root transformation was used to normalize variances. All values are presented as means $\pm$ 1 standard error.

Results

Water quality in experimental and natural streams was similar except for lower water temperatures in the field and elevated concentrations of metals in treated streams (Table 4.2). Concentrations of Cd (0.7 ± 0.07 µg/L), Cu (6.5 ± 0.4 µg/L), and Zn (50.3 ± 3.5 µg/L) were significantly higher in metal-dosed microcosms than in control microcosms. Concentrations of these metals in control streams and field sites were consistently below detection (detection limit of Cd, Cu, and Zn 0.1, 1.0, and 10.0 µg/L, respectively) of our analytical equipment.

Manipulating colonization time of artificial substrates significantly influenced invertebrate density and species richness (Table 4.3). For example, mean species richness was greater in high density treatments (18.0 taxa) than in low density treatments (14.0 taxa; t-test, $p=0.0006$). This difference was primarily due to the presence of three stonefly (Plecoptera) taxa, Suwallia sp. (Chloroperlidae), Capnia sp. (Capniidae), and Diura knowltni (Perlodidae), which were present in high density treatments but generally absent in low density treatments. Furthermore, densities of dominant taxa, such as Baetis sp. (Ephemeroptera:Baetidae), Ephemerella infrequens (Ephemeroptera:Ephemerella), Pteronarcella badia (Plecoptera:Pteronarcyidae), Prostoia besametsa (Plecoptera:Nemouridae), and Hydropsyche sp. (Trichoptera:Hydropsychidae) were significantly greater in high density than in low density microcosms. In contrast, density of the most abundant taxon, Rhithrogena hageni, was not affected by colonization time (Table 4.3).

Table 4.2. Water quality conditions of stream microcosms (n=18) during the experimental period, and Little Beaver Creek (LBC), and the South Fork of the Poudre River (SFP) when trays and Hesperoperla pacifica were collected.

	Experimental Streams	LBC	SFP
pH	7.5[1]	7.5	7.7
Water Temperature (C°)	11.1	6.0	8.0
Dissolved Oxygen (mg/L)	7.8	11.0	10.1
Conductivity	0.08	0.05	0.05
Hardness (mg/L)	30.1	10.0	20.0
Alkalinity (mg/L)	31.9	20.0	19.0
[Cd]µg/L[2]	0.7±0.07*	BD[3]	BD
[Cu]µg/L	6.0±0.4*	BD	BD
[Zn]µg/L	50.3±3.5*	BD	BD

[1] Water quality conditions in control streams and metal-dosed streams were the same except for metal concentrations in treatment streams; therefore, values for control streams are omitted.

[2] Metal values are mean ± 1 standard error. Means followed by an asterisk are statistically different from the control at a $p<0.05$.

[3] Below detection of analytical equipment. Detection limits for Cd, Cu, and Zn were 0.1, 1.0, and 10.0 µg/L, respectively.

Table 4.3. F-tests and statistical significance for overall ANOVA model, main effects, and two-way interactions for species richness, total abundance, and abundance of dominant taxa.

Variable	Model	metals	predation	density	density x predation	density x metals	predation x metals
Species Richness	5.2***	0.9	6.9*	20.3***	0.1	3.8	0.01
Total Abundance	9.0***	0.5	20.0***	31.0***	3.8	0.2	0.1
Rhithrogena hageni	0.6	0.1	1.3	1.5	0.01	0.3	0.4
Baetis sp.	4.0*	1.1	14.5**	5.2*	1.3	0.4	0.3
Ephemerella infrequens	25.0***	1.0	11.2**	130.6***	9.3**	0.3	0.01
Pteronarcella badia	8.4**	0.04	16.0**	26.7***	3.0	0.4	4.1
Prostoia besametsa	8.4**	6.6*	8.5**	20.4***	3.3	0.1	15.7***
Hydropsyche sp.	9.7***	0.1	34.3***	9.1**	0.01	6.1*	6.1*

* $p < 0.05$

** $p \leq 0.01$

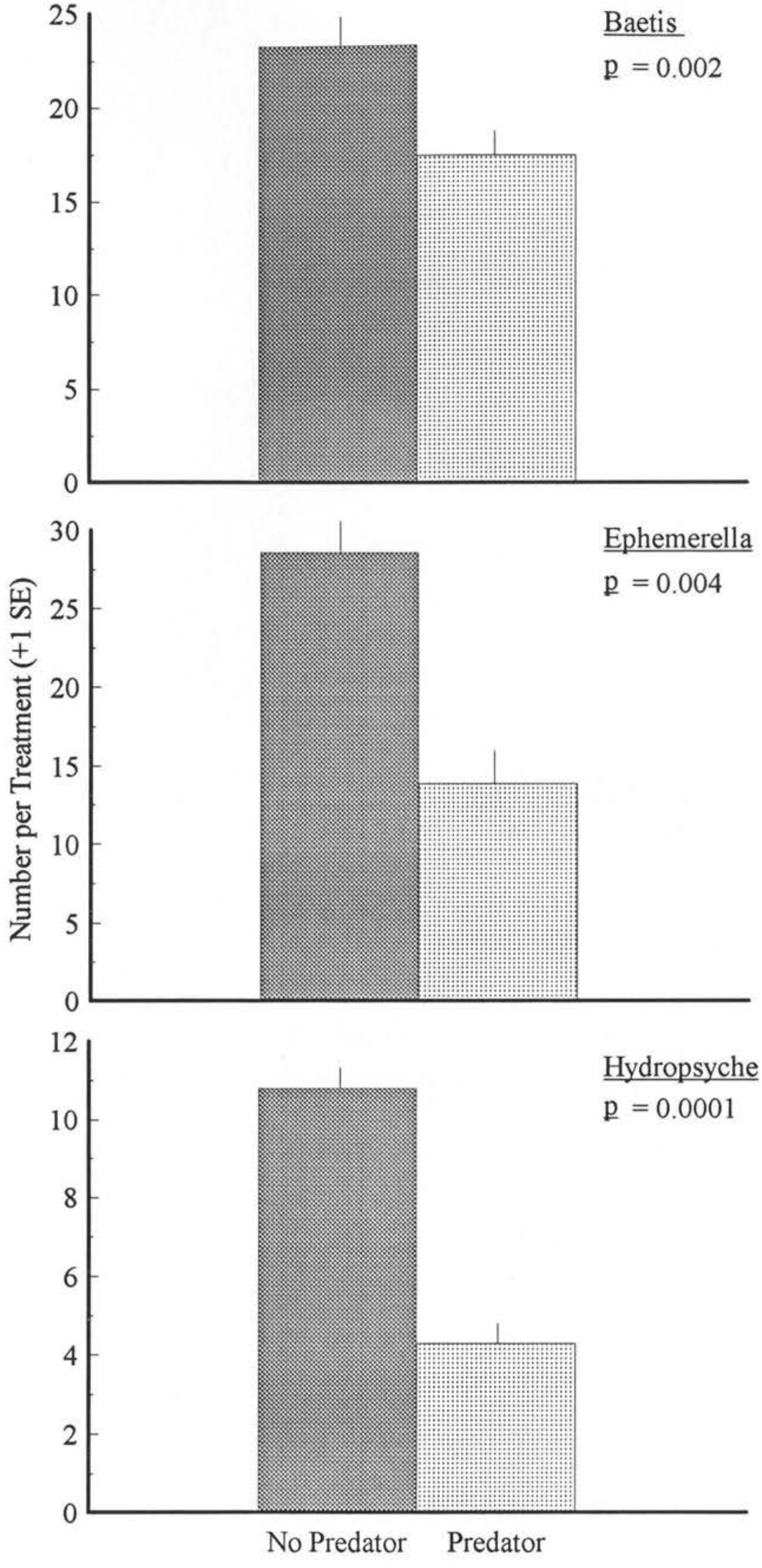
*** $p \leq 0.001$

The addition of metals to stream microcosms had little affect on benthic community structure. P. besametsa was the only species significantly reduced in metal-dosed microcosms (1.7 ± 0.84) compared to controls (4.8 ± 0.92; t-test, p=0.03) (Table 4.3).

Predation by H. pacifica significantly reduced the abundance of most dominant taxa, and this effect varied with invertebrate density for one species, E. infrequens (Table 4.3). Stonefly predation significantly reduced total density (t-test, p=0.0005), species richness (t-test, p=0.02), and abundance of P. badia (t-test, p=0.002) and P. besametsa (t-test, p=0.01). Taxa common in the diet of H. pacifica were also affected, as the abundance of Baetis sp., E. infrequens, and Hydropsyche sp. was significantly less in streams with predators versus streams without predators (Figure 4.1). Predation intensity on E. infrequens was greater in high density treatments than in low density treatments (p x d, p=0.01) (Table 4.3, Figure 4.2).

The effects of metals on the caddisfly Hydropsyche sp. varied depending on invertebrate density and predation. Survival of Hydropsyche sp. was lower in high density, metal-dosed streams than in low density, metal-dosed streams (density x metals, p=0.03) (Table 4.3, Figure 4.3). Furthermore, survival of Hydropsyche sp. was less in metal-dosed, predator-added streams than in no metal, predator-added streams (predation x metals p=0.03) (Table 4.3, Figure 4.4). Although predation on P. badia was 2X greater in metal-dosed microcosm than in controls, this difference was not significant (p=0.07) (Table 4.3, Figure 4.4). P. besametsa exhibited a different response than that of Hydropsyche sp. and P. badia. It was not affected by predators in metal-dosed

Figure 4.1. Effects of Hesperoperla pacifica on abundance of three dominant taxa in stream microcosms.


Baetis
p = 0.002
Ephemerella
p = 0.004
Hydropsyche
p = 0.0001
Number per Treatment (+1 SE)
25
20
15
10
5
0
30
25
20
15
10
5
0
12
10
8
6
4
2
0
No Predator
Predator

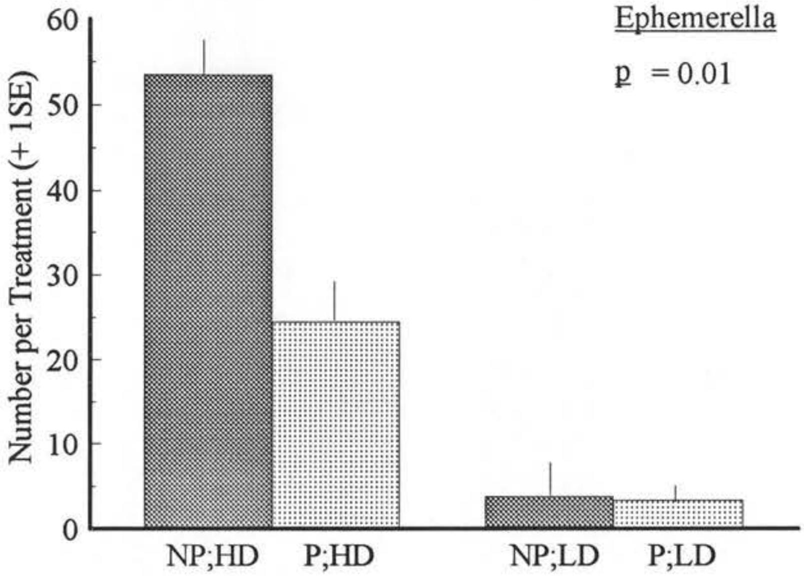


Figure 4.2. Effects of H. pacifica on abundance of Ephemerella infrequens in low density and high density microcosms. Treatments include: NP = no predators, P = predators, LD = low density, and HD = high density.

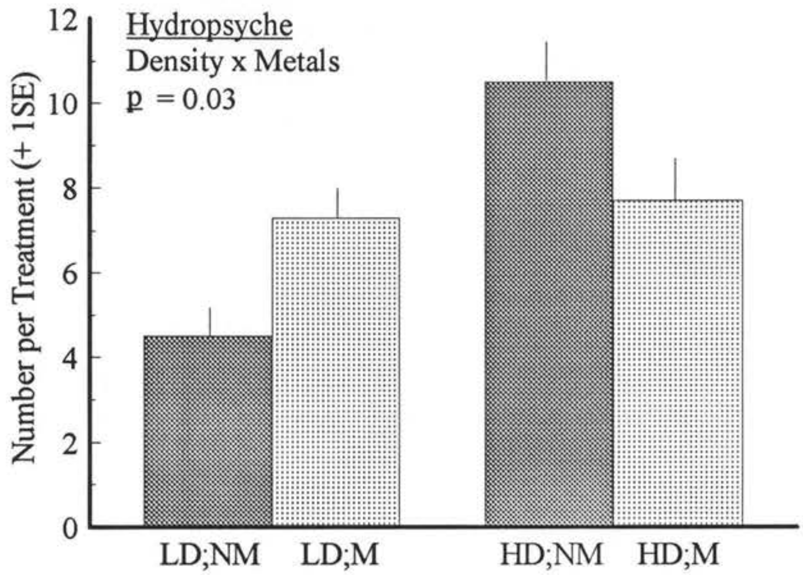
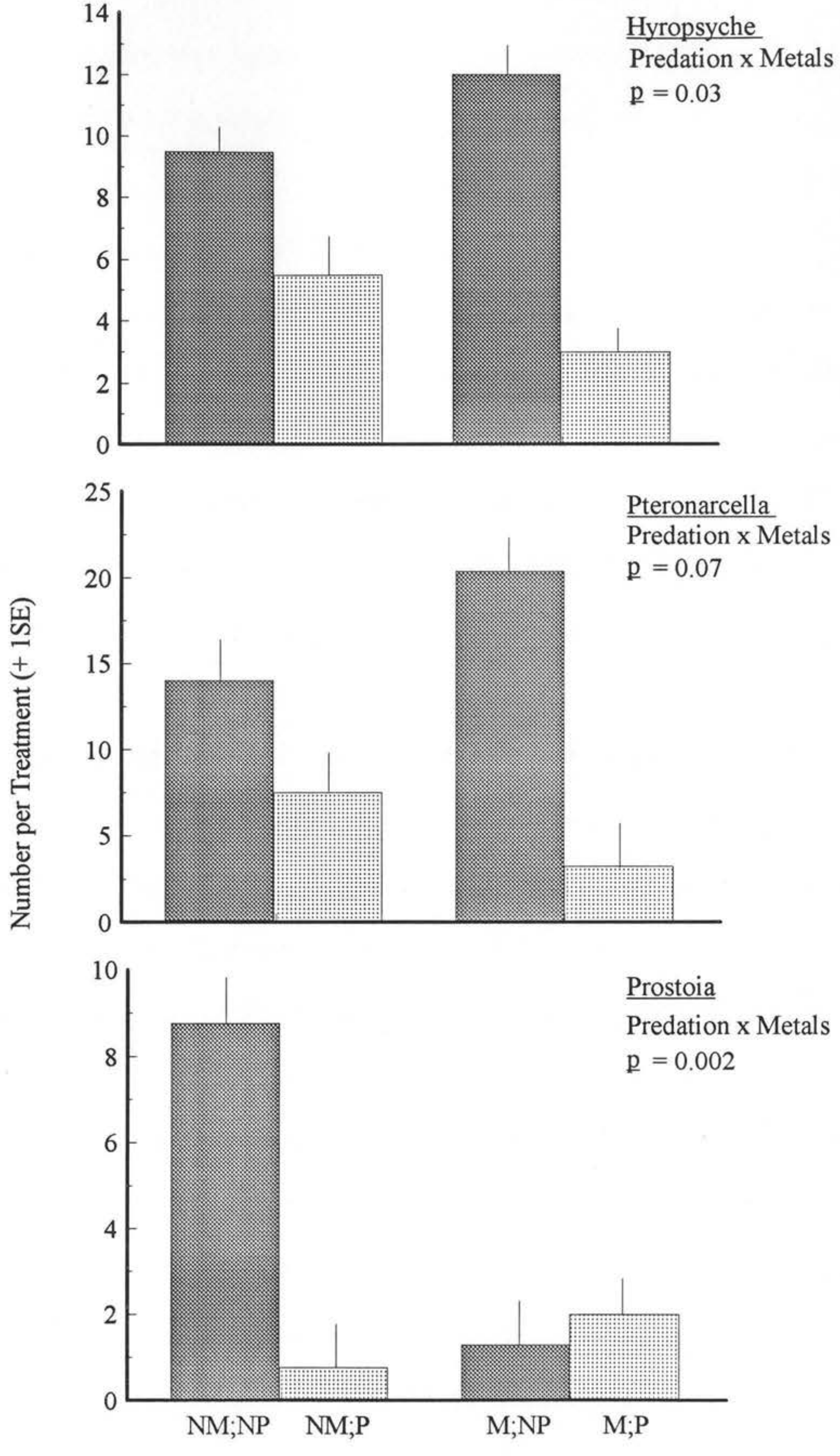


Figure 4.3. Effects of metals on abundance of Hydropsyche sp. in low density and high density microcosms. Treatments include: LD = low density, HD = high density, NM = no metals, M = metals.

Figure 4.4. Effects of H. pacifica on abundance of three dominant taxa in contol and metal-dosed microcosms. Treatments include: NP=no predators, P=predators, NM=no metals, M=metals.



Hyropsyche
Predation x Metals
p = 0.03
Pteronarcella
Predation x Metals
p = 0.07
Prostoia
Predation x Metals
p = 0.002
Number per Treatment (+ 1SE)
NM;NP
NM;P
M;NP
M;P

microcosms, but abundance of this species was lower in predator-added, controls (p x m, $p=0.005$) (Table 4.3, Figure 4.4).

Discussion

Results of this experiment suggest that abiotic and biotic factors interact to influence benthic invertebrate community structure. Specifically, predation intensity by the invertebrate predator H. pacifica was influenced by prey density and metal exposure. Moreover, similar to Clements et al. (1989), predatory interactions between stoneflies and caddisflies were more sensitive to metal exposure than caddisfly mortality alone.

Main Effects

Manipulating colonization time, metal concentrations, and predation had significant effects on invertebrate community structure. Variation in density and community composition between the two colonization periods may have been a result of changes in conditions of substrates, such as differences in epilithic texture and interstitial deposition (Mackay 1991). For the purposes of this experiment, these differences were important for evaluating how density interacted with predation and metals.

Experiments (Winner et al. 1980, Leland et al. 1986, Clements et al. 1988, Kiffney and Clements, 1994a, 1994b, in press) and field studies (Chadwick et al. 1986, Roline 1989, Clements 1994) have shown that metals can reduce species richness and abundance of stream invertebrate communities. Generally, mayflies and stoneflies are the most metal-sensitive groups, while caddisflies and chironomids are relatively tolerant. Because concentrations of Cd, Cu, and Zn used were approximately one-half the EPA chronic values (EPA 1986), and because only one species P. besametsa was significantly reduced by this treatment, it appears that these metal levels did not have a significant direct effect on most Rocky Mountain stream invertebrates.

There has been considerable controversy over the importance of predation as an organizing force of stream benthic communities (Allan 1975, Reice and Edwards 1986, Peckarsky and Dodson 1980, Walde 1986). The stonefly Megarcys signata significantly depressed prey colonization in the East River, Colorado (Peckarsky and Dodson 1980). Walde (1986) showed that another stonefly predator, Kogotus modestus, depressed densities of invertebrate prey, but this effect varied with abiotic factors (level of sedimentation). In my experiments, Hesperoperla pacifica significantly reduced the densities of most dominant taxa, especially those taxa (e.g., Baetis sp., Hydropsyche sp., and E. infrequens) that are common prey items of H. pacifica (Richardson and Gaufin 1971, Fuller and Stewart 1977, Johnson 1983). An unexpected result was the reduction in the abundance of P. badia, a relatively large (but smaller than H. pacifica), armored stonefly, in predation treatments. This negative effect may be a result of direct predation, however, P. badia was not found in the guts of H. pacifica. Alternatively, interspecific competition between H. pacifica and P. badia may have made P. badia more susceptible to the effects of metals. Other researchers have shown that interference competition can occur between stonefly nymphs, and this interaction is asymmetrical with the smaller species more affected than the larger species (Peckarsky 1991).

The large predator impacts measured may have been an artifact of the experimental system. The semi-closed microcosms used may have increased the probability of observing a significant predator impact (Cooper et al. 1990). Moreover, the small spatial and temporal scale of this experiment does not demonstrate that an invertebrate predator can significantly affect invertebrate community structure in natural streams. Nonetheless, these results do provide insights into processes, such as the interaction between abiotic and biotic factors, that may be occurring in stream invertebrate communities (Walde 1986).

Interactive Effects

Predation intensity on E. infrequens was greater at high invertebrate densities, and similar results have been reported by other researchers. Significant reductions in densities of various orthoclad chironomids by K. modestus only occurred at high prey densities (Walde and Davies 1984). Moreover, consumption rates of the predatory caddisfly Plectrocnemia conspersa were greater at high prey densities, which may reflect a functional response of predators to prey density (Lancaster et al. 1990). A predator's effectiveness may be dependent on background prey density, and invertebrate predators may exert a greater effect at high prey densities because prey refuges are less available.

Exposing invertebrates to metals had interesting and complex effects on biotic processes. Survival of Hydropsyche sp. was lower in high metal, high density treatments than in high metal, low density treatments suggesting that metals increased the intensity of intraspecific and/or interspecific competition. Behavioral experiments have shown that hydropsychid caddisflies will fight over retreats when an individual is placed in front of a occupied retreat (Jansson and Tunlikki 1979). If metals weaken the structural integrity of hydropsychid capture nets (Petersen and Petersen 1983) so that some individuals disperse and actively search for alternate retreats, an increase in intraspecific interactions may occur. Other researchers have observed similar interactive effects between density and abiotic environment. Travis and Trexler (1986) observed that the negative effects of higher densities of Bufo terrestris were more pronounced in physically stressful environments. Low pH increased the susceptibility of the anuran Hyla gratiosa tadpoles to the adverse effects of higher densities (Warner et al. 1991). These authors hypothesized that pH directly influenced the intensity of intraspecific competition; thus, abiotic factors not only limits where an organism can live, but can also affect intraspecific competition (Warner et al. 1991).

Similar to Clements et al. (1989), vulnerability of hydropsychid caddisflies to a stonefly predator (H. pacifica) was greater in experimental streams dosed with metals than in controls. As was mentioned previously, Petersen and Petersen (1983) provided evidence to suggest that metals disrupt silk-spinning in Hydropsychidae resulting in anomalies in the structure of the capture net. Clements et al. (1989) hypothesized that net-spinning caddisflies in metal-dosed streams are more vulnerable to predation because they spend more time outside retreats repairing and maintaining capture nets. These results suggest that hydropsychid caddisflies may be more at risk to the effects of predation in streams contaminated by metals.

Predation impacts on P. besametsa were in contrast to those observed for Hydropsyche sp. Because densities of P. besametsa were much less in metal-dosed streams compared with controls, H. pacifica may have not developed a search image for these organisms. Peckarsky and Penton (1989) speculated that stonefly attack probabilities may be frequency-dependent; therefore, there should be a greater attack rate with the most frequently encountered prey.

Management Implications

There is considerable variability in the tolerance of benthic macroinvertebrates to heavy metals (Winner et al. 1980, Leland et al. 1986, Clements et al. 1988, Kiffney and Clements 1994a, 1994b, in press), and this variation in metal-tolerance may influence the importance of competition and predation (Warner et al. 1991). The low concentrations (lower than EPA chronic criteria) of metals dosed into stream microcosms did not have substantial direct effects on benthic community structure; however, there were some unexpected indirect effects. If biotic interactions are more important in metal-contaminated streams than in pristine streams, the structure of benthic invertebrate communities may be indirectly affected by these metals. Since single-species toxicity studies would not have been able to predict the indirect effects observed in our experiment, it is important to conduct more complex multispecies toxicity experiments, using indigenous organisms, in conjunction with single-species tests when assessing risk of contaminants on ecological systems. Results from such experiments may provide greater insights into how contaminants influence community structure. In conclusion, I support the recent assertion by Dunson and Travis (1991) "that it is only through studying the interaction of biotic and abiotic factors that community structure can be fully understood."

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CHAPTER V

EFFECTS OF HEAVY METALS ON A MACROINVERTEBRATE ASSEMBLAGE FROM A ROCKY MOUNTAIN STREAM IN EXPERIMENTAL MICROCOSMS

Introduction

Biological monitoring is becoming increasingly important in assessing the biological integrity of aquatic systems (Karr 1991). The United States Environmental Protection Agency (US EPA) has proposed that biological criteria be included in its water-quality standards program (EPA 1988), and state agencies must eventually adopt narrative biological criteria into state water quality standards (EPA 1990). Failure of chemical criteria in protecting aquatic life has necessitated incorporating biological criteria into managing water resources (Karr 1991). Aquatic macroinvertebrates are particularly well-suited as indicators of biological integrity. These organisms are relatively sedentary and thus representative of local conditions, have long life cycles, perform a variety of ecological functions, and exhibit a range of sensitivities to contaminants (Rosenberg and Resh 1993).

Field biomonitoring is frequently used to assess effects of contaminants on lotic systems. Based on the assumption that contaminants affect benthic community structure, differences in community structure between upstream reference and downstream polluted sites may be attributed to contaminants (Cairns and Pratt 1993). Several investigators have noted that aquatic insect community structure differs between upstream reference sites and downstream metal-polluted sites in western streams (Chadwick et al. 1986, Roline 1988, Clements 1994). These studies have limitations, however, because of a number of statistical problems. A common problem with field biomonitoring studies is that they are frequently pseudoreplicated (sensu Hurlbert 1984), which limits statistical inference (Hurlbert 1984, Eberhardt and Thomas 1991). Furthermore, biological processes that occur at an upstream reference site, such as larval drift, can influence the distribution and abundance of organisms at a downstream polluted site. Hence the two locations (reference site and polluted site) are not spatially independent from one another at the time of sampling (Hurlbert 1984). Temporal independence is another factor to be considered in field biomonitoring (Hurlbert 1984), because an invertebrate sample collected from a site during fall may be correlated with a sample collected in winter. Moreover, in observational studies the variable of interest is not directly manipulated, therefore a direct cause and effect relationship cannot be established (Cooper and Barmuta 1993).

Single-species laboratory experiments can provide a strong link between a contaminant and a biological response because environmental factors are controlled (Norton et al. 1992). Although control of confounding factors is important in assessing the effects of contaminants on aquatic organisms, single-species tests do not always predict effects of toxicants on natural communities in the field (Cairns 1983, Kimball and Levins 1985). The introduction of contaminants into the environment has been used to assess the effects of anthropogenic disturbance on aquatic systems (Winner et al. 1980, Schindler 1987, Leland et al. 1989); however, numerous logistical, economic and legal considerations limit this approach. The use of stream microcosms and mesocosms containing indigenous stream organisms offers a practical alternative to the difficulties involved with experimental introduction of pollutants into the natural environment (Clements 1991), statistical problems associated with field studies (Hurlbert 1984, Eberhardt and Thomas 1991, Underwood 1994), and the lack of ecological realism of single-species tests (Cairns 1983, Kimball and Levins 1985).

The goals of this research were: (1) to determine the effects of a complex metal mixture on abundance, number of taxa, and species composition of an assemblage of stream macoinvertebrates collected using artificial substrates, (2) to evaluate the dose-response relationship of taxa common in southern Rocky Mountain streams and, (3) to compare experimental results with those observed in metal-polluted streams.

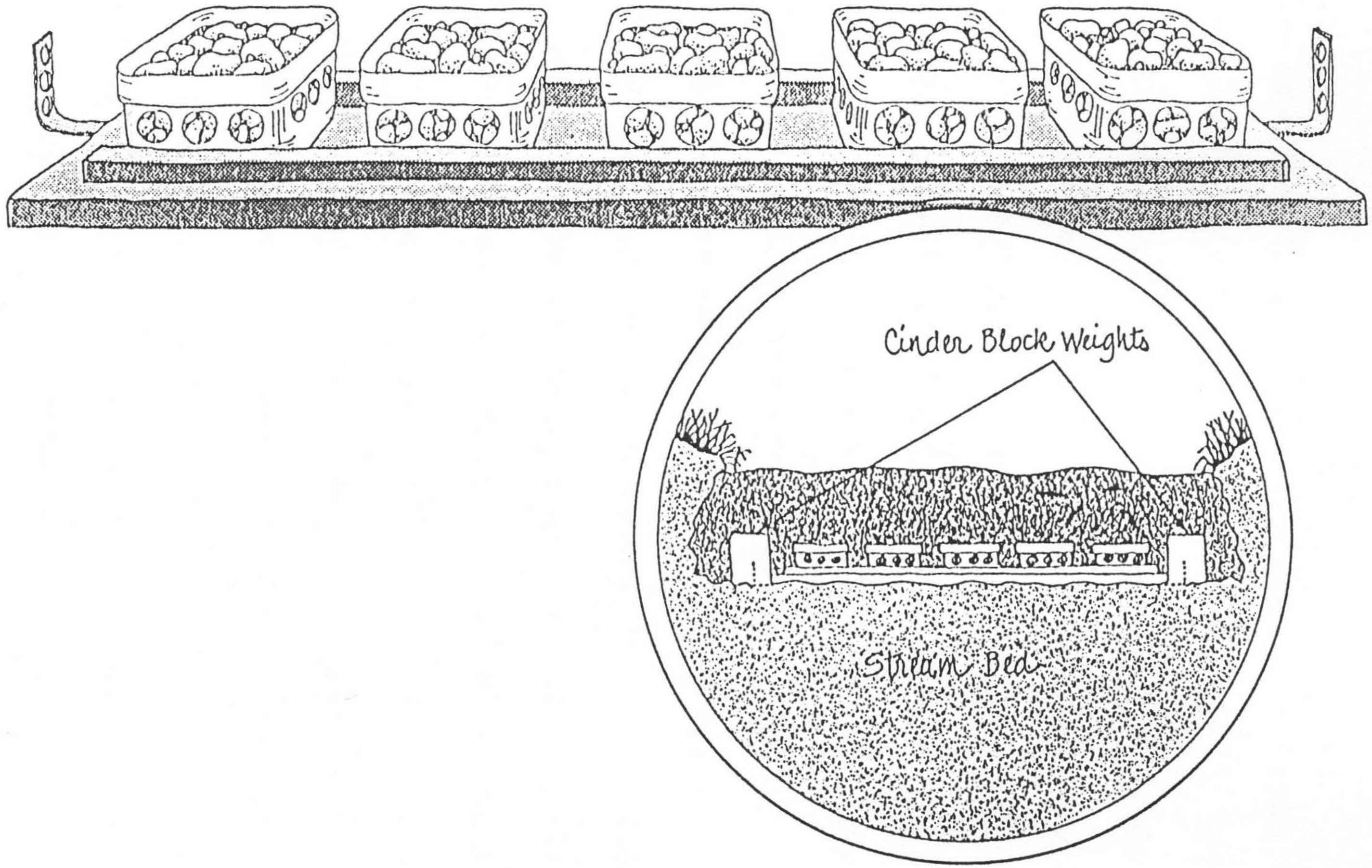
Methods

Experimental System

Benthic macroinvertebrates were collected from the Big South Fork of the Cache la Poudre, a 4th-order stream approximately 50 km west of Fort Collins, Colorado at an altitdude of 2320 m (above mean sea level). Riparian canopy consisted of willows (Salix sp.), ponderosa pine (Pinus ponderosa), and quaking aspen (Populus tremuloides). Substrate consisted of mainly pebbles, and small and large cobbles.

Artificial substrates were used to collect invertebrates and transfer them to experimental streams. The artificial substrates consisted of 10 x 10 x 6-cm plastic trays (0.01 m^2 surface area) filled with air-dried pebbles and small cobbles (2-6 cm diameter) (Figure 5.1). Details of this experimental approach have been published previously (Clements et al. 1988a, 1988b, Clements 1989). Fifty-five trays were placed in riffle sections of the Big South Fork on 1 April 1992 and retrieved on 5 July 1992. The trays were retrieved by placing a 100-μm mesh net directly downstream to prevent loss of

Figure 5.1. Schematic of artificial substata used to collect invertebrates.

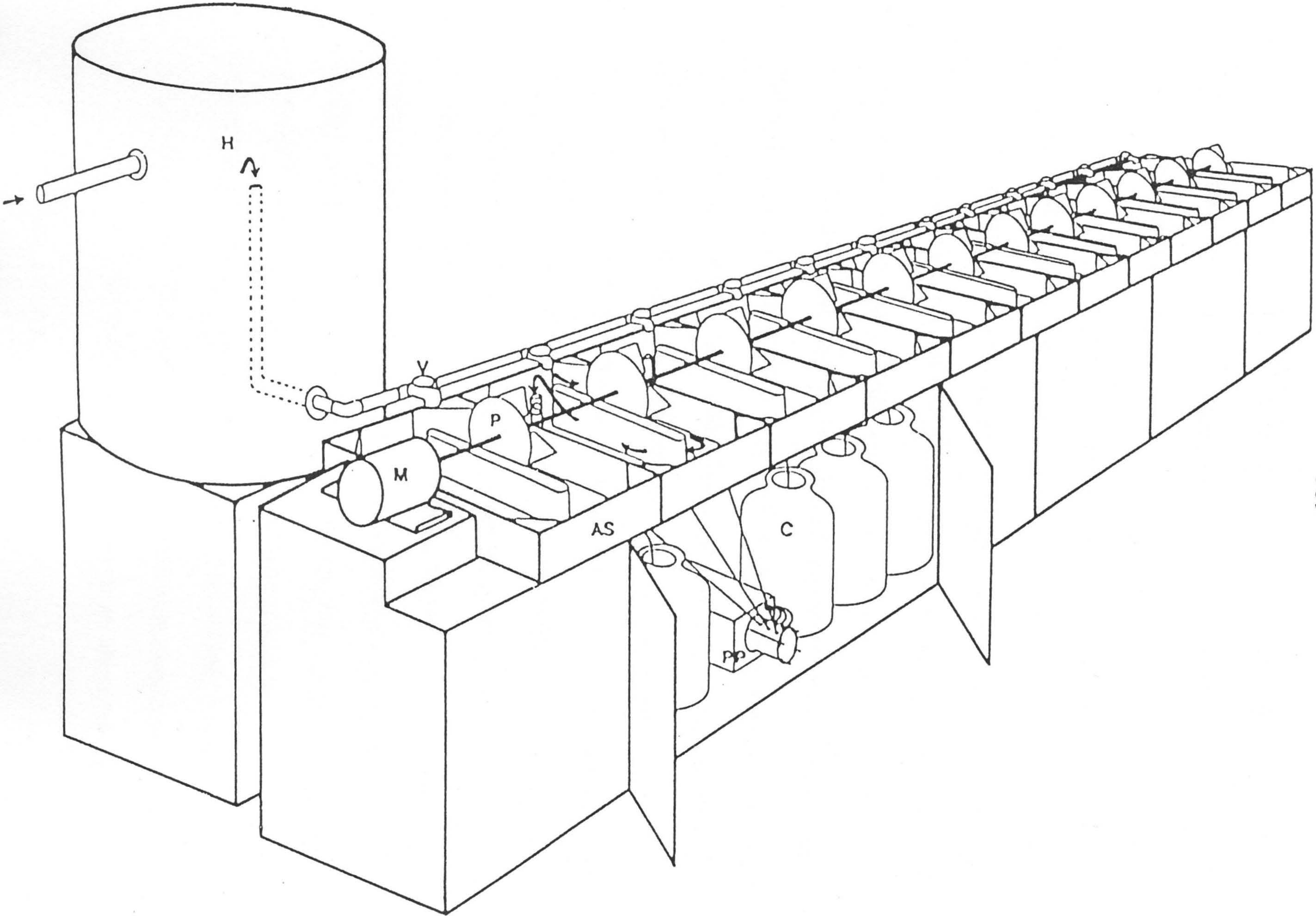

Cinder Block Weights
Stream Bed

organisms. Forty-eight trays were randomly assigned to 12 plastic coolers (four trays per cooler) filled with stream water. Coolers were aerated by an airstone connected to a 12-volt air pump.

Trays were transferred to fiberglass experimental streams (76 x 46 x 14-cm) at Colorado State University, Fort Collins (Figure 5.2). The four trays ($\underline{n}$=4) from each cooler were randomly assigned to a stream ($\underline{n}$=12 streams). Each oval, flow-through stream received chilled, dechlorinated tap water at a rate of 0.5 L/min. Turnover time in the 13-L streams was approximately 26 min, and water depth in each stream was 10 cm. Current was provided by a paddlewheel at an average velocity of 25 cm/s. A 14 light:10 dark photoperiod was maintained with cool-white fluorescent lights. Previous studies using a similar experimental system have shown that control mortality is minimal (Clements et al. 1988b, Kiffney and Clements 1994, Kiffney, unpublished results).

After a 48-h acclimation period, each experimental stream was randomly assigned to one of four target treatments (three replicates per treatment): 0, 1X (X=1.1, 12, and 110 μg/L Cd, Cu, and Zn, respectively), 5X or 10X. The 1X treatment was similar to US EPA recommended chronic criteria for these metals (EPA 1986). Cadmium, Cu, and Zn levels in the 1X, 5X, 10X metal mixture were also similar to total metal concentrations measured in water at metal-polluted stations at the Arkansas River (Kiffney and Clements 1993, Clements 1994). Peristaltic pumps delivered a stock solution of $CdCl_2$, $ZnSO_4$ and $CuSO_4$ from separate 20-L acid-washed carboys into each treated stream at a rate of 5 mL/min. After 10 d, the four trays in each stream were pooled, washed into a 355-μm sieve, and organisms preserved in 100% ethanol. Samples were sorted in the laboratory

Figure 5.2. Schematic of experimental streams. AS=artificial stream; H=headbox; M=motor; P=paddlewheel; V=valve; C=carboy; PP=peristaltic pump.


H
V
P
M
AS
C
PP

and all organisms, except for chironomids, were identified to genus or species. Chironomids were identified to subfamily or tribe (Merritt and Cummins 1984).

To examine selectivity of trays, benthic invertebrates were also collected from the Big South Fork using a 0.1 m^2 Hess sampler ($\underline{n}$=5). Samples were collected adjacent to colonization trays on the same day that trays were retrieved from the field. For each sample collected, substrate was disturbed to a depth of approximately 10 cm. Samples were washed through a 355 μm sieve and organisms retained were preserved in 70% ethanol. Insects were sorted and identified following procedures described previously.

Unfiltered water samples were collected from SFP ($\underline{n}$=1) when trays were collected and on days 0, 2, 4, 8, and 10 in experimental streams ($\underline{n}$=12) for water chemistry and metals analysis. Alkalinity and hardness were determined in the laboratory by titration (APHA 1989). Conductivity was measured with an electrical conductivity meter (VWR Scientific Model 1054) and pH with a Chemcadet meter. Dissolved oxygen and temperature were measured in the field and the laboratory with an O_2 meter (Yellow Springs Instrument Company model 51B). Current velocity was measured with a digital flow meter (General Oceanics 2030R). All water samples for metals analysis were acidified with reagent-grade HNO_3 to pH < 2. Total (dissolved+suspended) Zn and Cu levels in water were analyzed on an dual channel atomic-absorption spectrophotometer (video IL 22, Instrumentation Laboratory, Franklin, Massachusetts) using standard method #3111 A (APHA 1989). Cadmium (total metals) was measured using a graphite furnace attachment to the same AA spectrophotometer used to measure Zn and Cu.

Field Study

Benthic macroinvertebrate samples (n=5) were collected from reference and metal-polluted sites at the Arkansas River and Eagle River, Colorado using a 0.1/m^2 Hess sampler (355 μm mesh net) during August of 1992. For a comparison of field and experimental results, three sites from each river were chosen (e. g., one reference and two metal-polluted sites), because total concentrations of Zn in water at these sites during August 1992 were similar to levels measured in 0X, 1X, and 5X experimental treatments (Clements, unpublished results). Cadmium and copper were also measured in the water, but for this comparison we present only Zn levels. For each sample collected, substrate was disturbed to a depth of approximately 10 cm. Samples were washed through a 355 μm sieve and organisms retained were preserved in 70% ethanol. Insects were sorted and identified following procedures described previously.

Water samples were collected from each site for water chemistry. Chemical parameters were measured as described above.

Statistical Analysis

All statistical analyses were conducted using a personal-computer-version of Statistical Analysis Systems' PROC GLM (SAS 1988). A fixed effects, one-way analysis-of-variance was used to test the effects of metal treatment on number of taxa, number of individuals, abundance of major orders, and dominant genera in laboratory streams. A taxon was defined as dominant if its relative abundance was >1% in control streams. Larval densities of Epeorus longimanus and Rhithrogena hageni

(Ephemeroptera:Heptageniidae) were low, which made detecting statistical differences difficult; therefore, these two taxa were combined for all statistical analysis. If the F-test for the model was statistically significant ($p<0.05$), the Ryan-Einot-Gabriel-Welsch-Q (REGWQ) multiple range test (SAS 1988) was employed to test for differences between treatments. The REGWQ test controls for maximum experiment-wise error rate in a manner similar to Tukey's HSD multiple comparison test, but is more powerful (Day and Quinn 1989). Least-squares, linear regression using natural logarithms of Zn metal concentrations in water and larval densities was used to develop response curves for dominant taxa and major groups. If response surface analysis indicated that a linear function described the dose-response relationship, estimates for the regression equation were derived. Relative sensitivities were determined by comparing densities of dominant taxa and major insect groups in the 1X treatment with densities in the control treatment. Plots of residuals indicated heterogeneity of variances among treatments; therefore, all statistical analyses were performed on $\ln(n+1)$ transformed data. Data are presented as untransformed means ($\pm$ 1 SE).

Results

Experimental results

Water temperature, dissolved oxygen, and pH in experimental streams and at the field collection site were similar (Table 5.1). Alkalinity, hardness, and conductivity in the field were approximately three times lower than in experimental streams. Measured levels of Cd and Zn (e.g., total metals) in water from experimental streams approximated target

Table 5.1. Chemical and physical characteristics of experimental streams and the Big South Fork of the Poudre River, Colorado. The 1X treatment consisted of a mixture of Cd, Cu, and Zn (target levels were 1.1 μg/L Cd, 12 μg/L Cu, and 110 μg/L Zn). Mean (and standard error) measured concentrations of metals in the water followed by the same letter were not statistically different ($p<0.05$).

	Experimental streams				
	Control	1X	5X	10X	Big South Fork, Poudre River
Water temperature (C°)	13	13	13	13	12
Dissolved oxygen (% saturation)	>90	>90	>90	>90	>90
pH	7.3	7.3	7.2	7.2	7.3
Alkalinity (mg/L)	39	39	39	39	14
Hardness (mg/L)	38.3	38.5	38.5	39	12
Conductivity (m℧)	0.13	0.13	0.13	0.13	0.04
Width (m)	0.18	0.18	0.18	0.18	15
Depth (m)	0.10	0.10	0.10	0.10	0.40
Current (m/s)	0.25	0.25	0.25	0.25	0.40
Cd μg/L	BD[1]^{a}	1.00^{b} (0.05)	5.12^{c} (0.3)	8.90^{d} (0.9)	BD
Cu μg/L	BD^{a}	10.0^{b} (0.8)	33.3^{c} (1.4)	48.0^{c} (3.6)	BD
Zn μg/L	BD^{a}	108.0^{b} (2.9)	534.0^{c} (22.7)	992.0^{d} (37.6)	BD

[1] Below detection limits of analytical equipment (e.g., 10 μg/L Zn, 10 μg/L Cu, and 0.1 μg/L Cd).

concentrations, while the 5X and 10X Cu treatments were 40-50% lower than target levels. Statistical differences were detected between all metal concentrations except for the 5X and 10X Cu treatments.

Overall, the invertebrate assemblages in artificial substrates were similar to those in Hess samples; however, the percent abundance of some taxa was lower in artificial substrates (Table 5.2). At a coarser level of taxonomic resolution, the percent abundance of stoneflies and caddisflies was greater in trays compared to Hess samples, while there was a greater percentage of mayflies and chironomids in Hess samples. The number of taxa collected in trays and in Hess samples was similar (number of taxa=23 and 22 in trays and Hess, respectively), whereas total densities were twice as high in trays (9800 individuals/m^2) than in Hess samples (3600 individuals/m^2).

Community composition in the control treatment was represented by approximately equal proportions of mayflies, stoneflies, caddisflies, and chironomids (Figure 5.3). A loss of mayflies and stoneflies at the higher metal treatments resulted in an increase in the proportion of caddisflies and chironomids. Exposure to the metal mixture significantly reduced the number of individuals and taxa compared with control streams (Figure 5.4, Table 5.3). These parameters also exhibited statistically significant log-linear, dose-response relationships.

Mayflies and stoneflies were the insects most sensitive to metal treatment (Figure 5.5, Table 5.3). Total mayfly densities were reduced by 68% in the 1X treatment and by 95% in the 10X treatment compared with the control (Figure 5.5). Densities of stoneflies declined 44% in the 1X and 77% in the 10X treatments compared with control streams.

Table 5.2. Percent abundance (mean and standard error) of dominant taxa and major insect groups in artificial substrates ($\underline{n}$=5) and in Hess samples ($\underline{n}$=5) from the Big South Fork, Poudre River, CO.

Taxa	Hess	Tray
Ephemeroptera	32.0(5.0)	22.0(4.0)
Heptageniidae	6.7(1.0)	1.7(0.5)
Baetis tricaudatus	2.8(0.5)	2.4(0.5)
Drunella grandis	3.4(0.8)	7.9(3.0)
D. doddsi	3.0(1.0)	1.4(0.8)
Plecoptera	7.8(0.8)	14.5(1.0)
Pteronarcella badia	2.0(1.0)	3.0(1.0)
Sweltsa coloradensis	1.1(0.5)	0.3(0.1)
Suwallia pallidula	4.9(1.0)	0.7(0.3)
Trichoptera	3.5(1.0)	11.1(1.0)
Lepidostoma ormeum	3.3(1.0)	5.2(3.0)
Chironomidae	50.6(5.0)	32.0(5.0)
Tanypodinae	3.2(0.8)	1.9(0.3)
Orthocladiinae	21.0(3.0)	8.1(1.0)
Chironomini	3.4(1.0)	3.6(1.0)
Tantytarsini	20.1(5.0)	14.0(3.0)

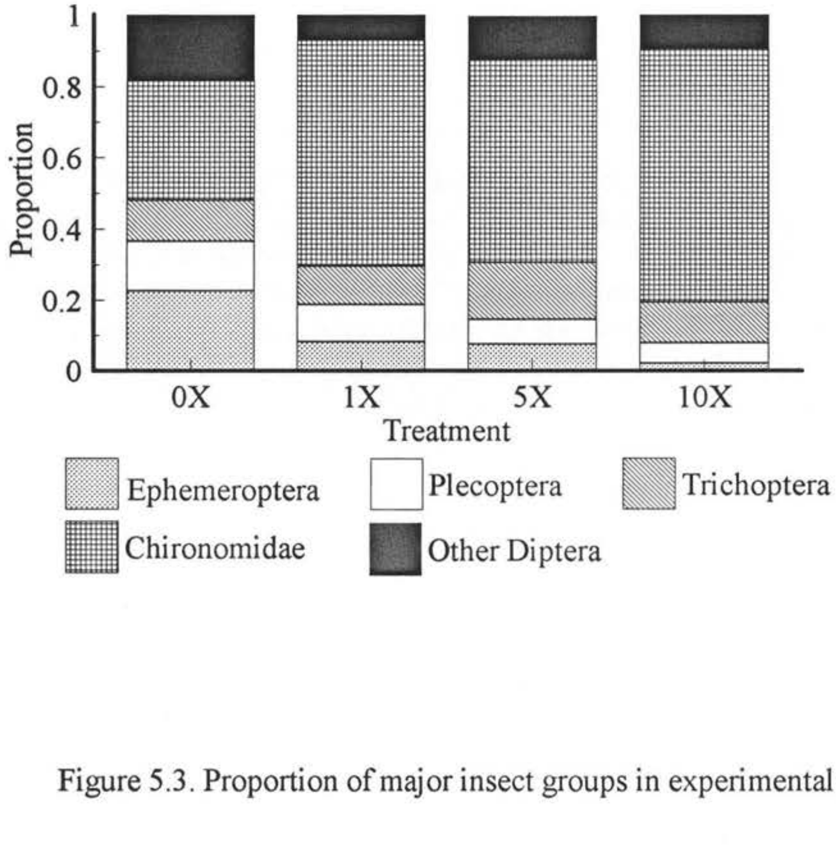


Figure 5.3. Proportion of major insect groups in experimental streams.

Figure 5.4. Effects of a mixture of Cd, Cu, and Zn on total density and species richness in experimental streams. Target concentrations for the 1x treatment were 1.1 µg/L Cd, 12 µg/L Cu, and 110 µg/L Zn. Values represent mean (± 1SE) density observed at each experimental treatment (n=3). Mean values with the same letter are not statistically different ($p<0.05$) based on the REGWQ multiple comparison test. Regression equations and correlation coefficients are presented for only those taxa and indices that exhibited a statistically significant natural log-linear relationship between Zn concentrations and density.

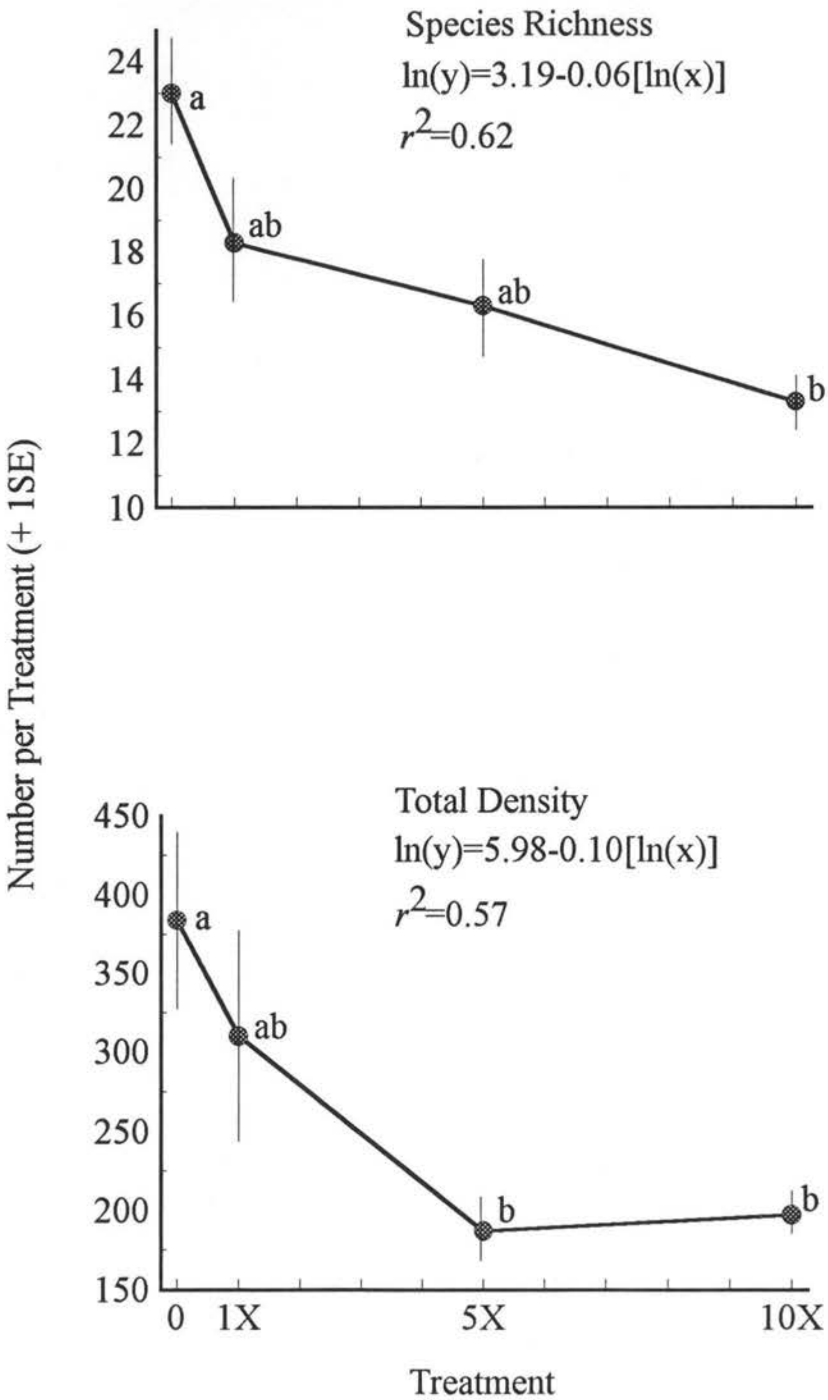
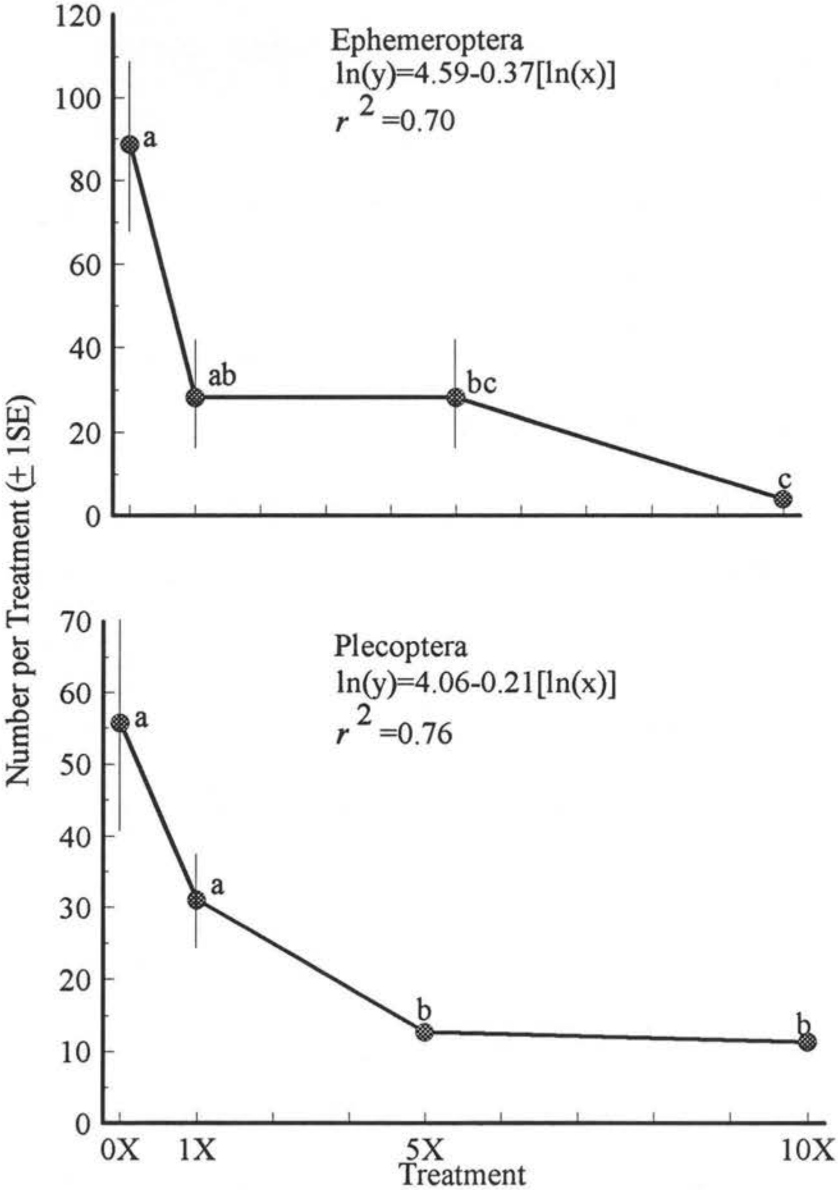

Species Richness
ln(y)=3.19-0.06[ln(x)]
r^2=0.62
24
22
20
18
16
14
12
10
a
ab
ab
b
Number per Treatment (+ 1SE)
Total Density
ln(y)=5.98-0.10[ln(x)]
r^2=0.57
450
400
350
300
250
200
150
a
ab
b
b
0
1X
5X
10X
Treatment

Table 5.3. Relative change in abundance of dominant taxa, major insect groups, and community-level indices in 1X treatment streams (n=3) compared to control streams (n=3).

Taxa	% Change
Ephemeroptera	(-)68
Heptageniidae	(-)90
Baetis tricaudatus	(-)76
Drunella grandis	(-)65
D. doddsi	(-)66
Plecoptera	(-)44
Pteronarcella badia	(-)60
Suwallia pallidula	(-)36
Sweltsa coloradensis	0
Trichoptera	(-)12
Lepidostoma ormeum	(-17)
Chironomidae	(+)56
Tanypodinae	(+)94
Orthocladiinae	(+)5
Chironomini	(+)8
Tanytarsini	(+84)
Number of Individuals	(-)20
Number of Taxa	(-)22

Figure 5.5. Effects of a metal mixture of Cd, Cu, and Zn on densities of Ephemeroptera and Plecoptera in experimental streams (see Figure 5.4 for further details).



Ephemeroptera
ln(y)=4.59-0.37[ln(x)]
r 2 =0.70
a
ab
bc
c
120
100
80
60
40
20
0
Number per Treatment (± 1SE)
Plecoptera
ln(y)=4.06-0.21[ln(x)]
r 2 =0.76
a
a
b
b
70
60
50
40
30
20
10
0
0X
1X
5X
10X
Treatment

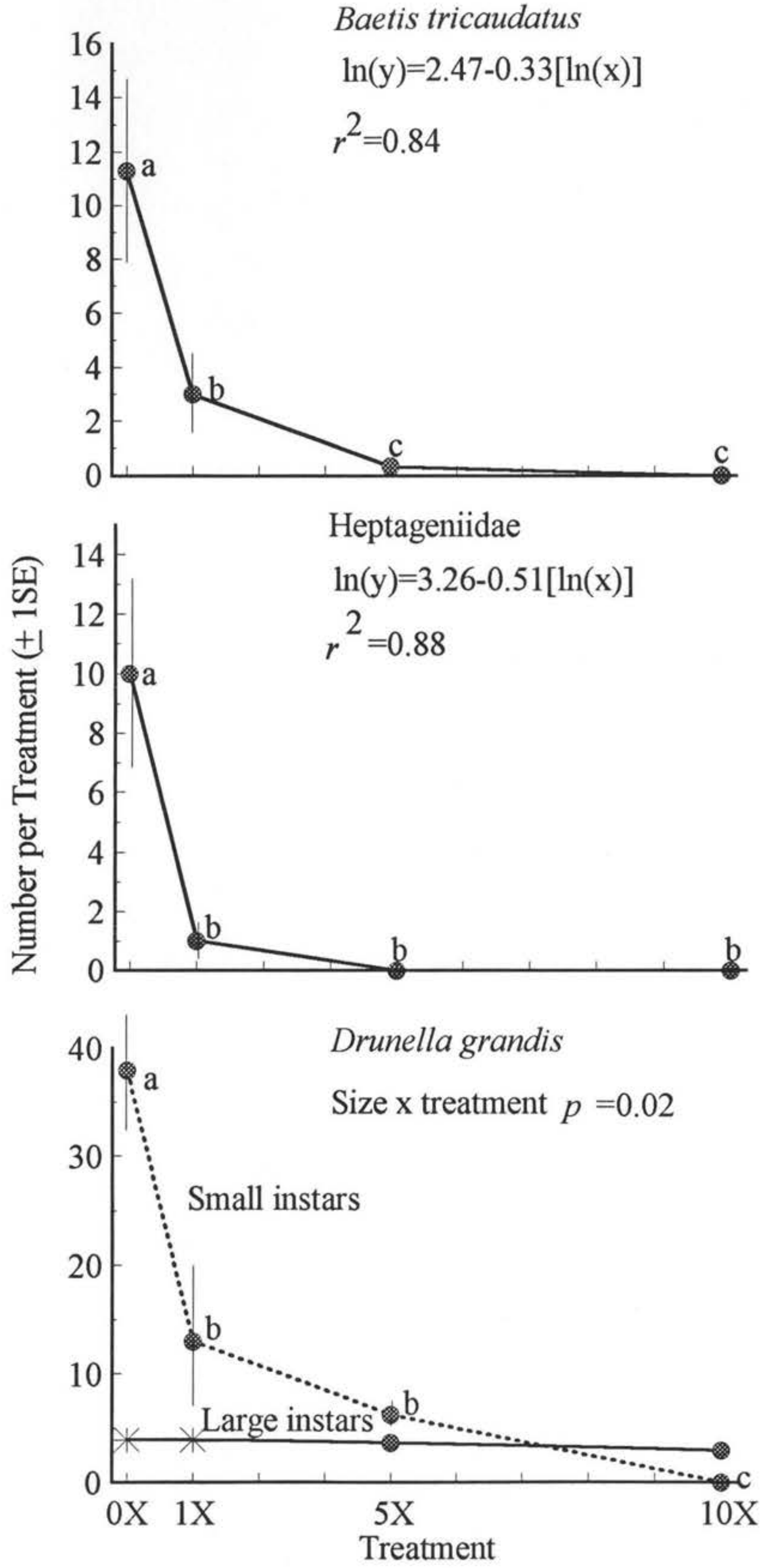
There was also a statistically significant log-linear relationship between Zn concentration in water and density of mayflies and stoneflies. The abundance of caddisflies, primarily Lepidostoma ormeum (Trichoptera:Lepidostomatidae), declined by 54% in the 10X treatment compared with the control. Reduced abundance of caddisflies was not statistically significant between treatments (p=0.54), nor was there a significant dose-response relationship.

Baetis tricaudatus and Heptageniidae (Epeorus longimanus and Rhithrogena hageni) were the most sensitive mayflies to metal treatment (Figure 5.6, Table 5.3). In the 1X treatment, densities of B. tricaudatus were reduced by 76%, while Heptageniidae was reduced by 90%. Both taxa were virtually eliminated in the 5X treatment. Furthermore, these taxa exhibited the strongest relationship between Zn concentration and density (r^2=0.84 for B. tricaudatus and r^2=0.88 for Heptageniidae). The density of Drunella doddsi in the 1X treatment was less than half the control density, but this difference was not statistically significant (p = 0.08) due to high control variability. However, there was a significant log-linear relationship between Zn concentration in water and density of D. doddsi (r^2=0.5, p=0.009)

The response of D. grandis was size-dependent, as indicated by the statistically significant size x treatment interaction (p=0.02) (Figure 5.6). Metal treatment had no effect on the number of large D. grandis; however, densities of small larvae were reduced by 70% in the 1X treatment.

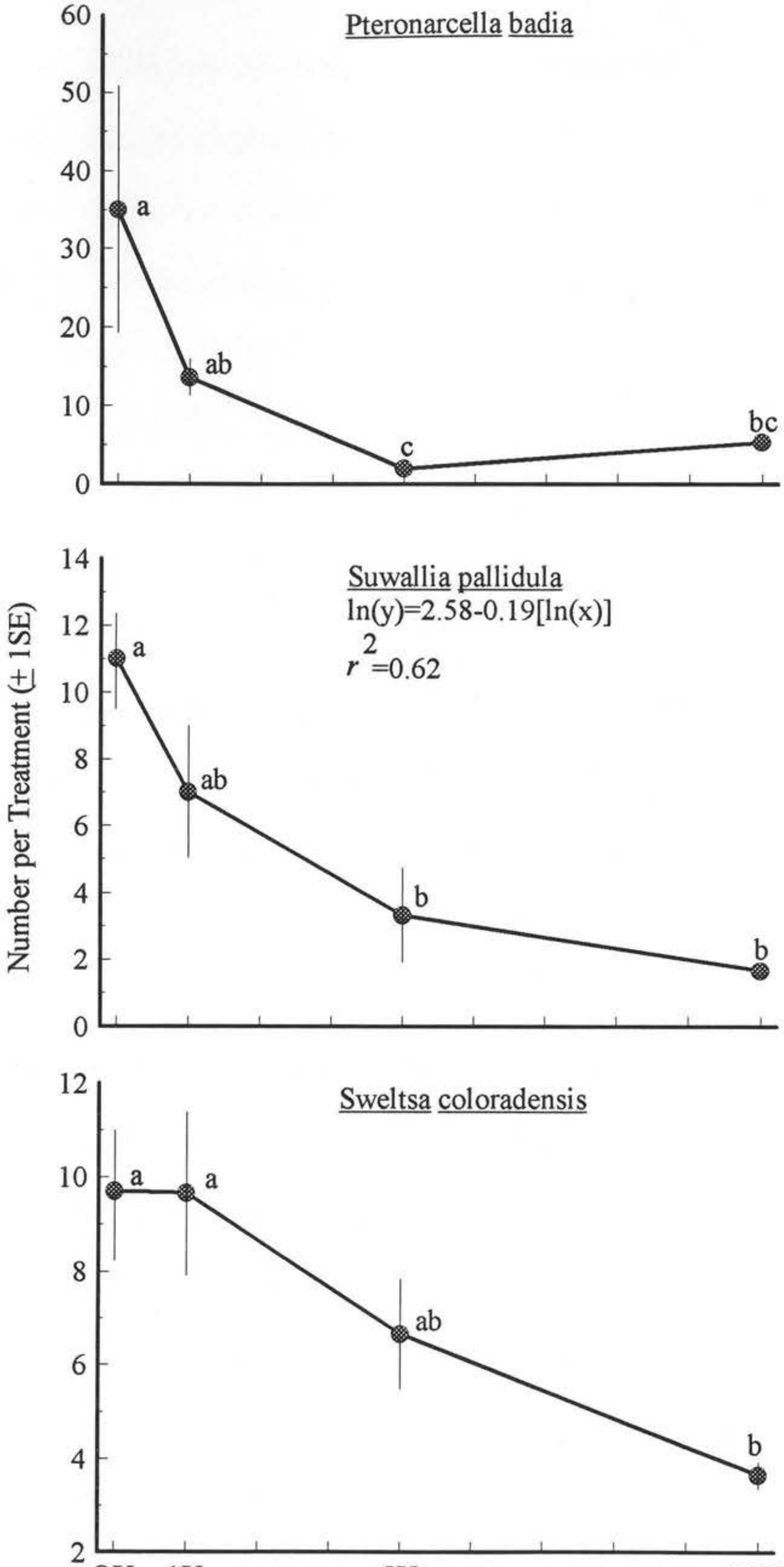
Although total abundance of stoneflies was reduced by metals, responses differed among species. Pteronarcella badia (Plecoptera:Pteronarcyidae) was the most sensitive

Figure 5.6. Effects of a mixture of Cd, Cu, and Zn on densities of dominant mayfly taxa (see Figure 5.4 for further details).



Baetis tricaudatus
ln(y)=2.47-0.33[ln(x)]
r2=0.84
16
14
12
10
8
6
4
2
0
a
b
c
c
Heptageniidae
ln(y)=3.26-0.51[ln(x)]
r2=0.88
14
12
10
8
6
4
2
0
a
b
b
b
Number per Treatment (± 1SE)
Drunella grandis
Size x treatment p =0.02
40
30
20
10
0
a
Small instars
b
b
Large instars
c
0X
1X
5X
10X
Treatment

Figure 5.7. Effects of a mixture of Cd, Cu, and Zn on densities of dominant stonefly taxa (see Figure 5.4 for further details).


Pteronarcella badia
Suwallia pallidula
ln(y)=2.58-0.19[ln(x)]
r^2=0.62
Sweltsa coloradensis
Number per Treatment (± 1SE)
OX
1X
5X
10X
Treatment
a
ab
c
bc
b

species, with a 60% reduction in density in the 1X treatment (Figure 5.7, Table 5.3). Suwallia pallidula and Sweltsa coloradensis, two species in the family Chloroperlidae, differed in their response to metal treatment, as the abundance of S. pallidula was reduced by 36% in the 1X treatment, while there was no change in the number of S. coloradensis in the same treatment. Densities of S. coloradensis were reduced by 31 in the 5X and 63% in the 10X treatment. S. pallidula was also the only stonefly to exhibit a statistically significant dose-response relationship between Zn levels in water and abundance.

The response of Orthocladiinae (p=0.8), Tanypodinae (p=0.1), Chironomini (p=0.9), and Tanytarsini (p=0.004) chironomids was highly variable, but they did not appear to be affected by metal treatment. For example, the abundance of Tanytarsini was greater in the 1X treatment compared with the control (Table 5.3), but abundance in the 5X and 10X treatments were similar to controls.

Field Studies

Heptageniid mayflies were consistently less abundant below sources of metal contamination, whereas the response of B. tricaudatus was more variable. The number of heptageniids was reduced by 60 to 100% at metal-polluted field sites compared with reference sites (Table 5.4). Densities of Baetis were also lower at Eagle River metal-polluted field sites compared with reference sites. In contrast, Baetis was more abundant at AR 5 (79 μg/L Zn) compared with AR 2 (26 μg/L Zn). Abundance of this species was lower at the most metal-polluted Arkansas River site (AR 3) compared with the reference site (AR 2).

Table 5.4. Mean (and standard error) density (no./m^2) of common taxa and species richness (and standard error) per sample at the Arkansas River (AR) and the Eagle River (ER).

				Taxa			
Site	[Zn] μg/L	Baetis tricaudatus	Heptageniidae	Sweltsa coloradensis	Orthocladiinae	Total Density	Species Richness
AR 2	26	758(379)	74(14)	46(14)	70(10)	3910(700)	21.2(0.5)
AR 5	79	922(190)	0	38(20)	1870(607)	6750(1150)	24.2(2.0)
AR 3	181	372(83)	30(15)	94(31)	892(275)	3800(437)	20.4(1.9)
ER 1	2	1214(191)	124(22)	56(25)	222(71)	4780(811)	30.8(2.1)
ER 4	104	192(42)	4.0(4.0)	20(8.0)	4130(1890)	6370(2010)	20.4(1.7)
ER 3	336	350(109)	10(4.0)	28(9.0)	358(84)	1890(196)	19.4(1.3)

Similar to Baetis, the response of S. coloradensis to metals was variable. Densities of Sweltsa were higher at the Arkansas River site with the highest Zn levels (AR 3) compared with the reference site. In contrast, this species was less abundant at Eagle River metal-polluted sites compared with the reference site.

The group that exhibited the most tolerance to metals was Orthocladiinae. Densities of orthoclad chironomids were higher at all metal-polluted field sites compared with reference sites. For example, the density of orthoclads was 1.6 to 27 times greater at metal-polluted field sites compared with reference sites.

Detecting patterns in community-level parameters, such as species richness and total density, was difficult. There was no apparent relationship between species richness or total density and metals at the Arkansas River. Total density at ER 4 (104 μg/L Zn) was greater than at ER 1 (2 μg/L Zn); however, total density was reduced by 60% at ER 3 (336 μg/L Zn) compared with ER 1. Species richness at Eagle River metal-polluted sites was lower than at the Eagle River reference site.

Discussion

Results from this study were similar to other experiments (Clements et al. 1988a, 1988b, Leland et al. 1989, Kiffney and Clements 1994) and field studies (Chadwick et al. 1986, Clements 1994, Clements, unpublished results) that have examined the effects of metals on stream macroinvertebrates. Specifically, mayflies and some stoneflies were sensitive, and caddisflies and chironomids were tolerant to metal exposure. However, the sensitivity to metals differed within families, genera, and across lifestages.

The effects of a metal mixture was most apparent in the mayflies, such as heptageniid mayflies (e.g., Rhithrogena hageni and Epeorus longimanus). Other taxa in this family have been reported to be reduced in other lotic systems in the western United States contaminated by metals (Chadwick et al. 1986, Leland et al. 1989, Nelson and Roline 1993, Clements 1994). As with other aquatic insects, little is know regarding the mechanism causing this sensitivity to metals, but it may be attributed to damage of respiratory membranes (Leland 1984).

Other researchers have reported that Baetis sp. is either sensitive (Clements et al. 1988b, Leland et al. 1989, Kiffney and Clements 1994) or tolerant to metals (Norris et al. 1982, Roline 1988, Hoiland and Rabe 1992). Baetis species are abundant in post-dusk drift (Leland 1985) and are known to rapidly colonize recently disturbed substrates (Mackay 1992). These behavioral attributes may explain the presence of Baetis sp. at metal-polluted stream sites (Clements 1994). Furthermore, the family Baetidae is composed of many species, and each will likely vary in its sensitivity to contaminants. I suggest experiments are the most rigorous method to estimate an organism's sensitivity to contaminants.

Although this research showed that mayflies as a group were sensitive to metals, I observed variation among species and across lifestage (Drunella grandis). An inverse relationship between sensitivity and size was also observed for the mayfly Stenonema modestum (Ephemeroptera: Heptageniidae) (Diamond et al. 1992). Organism size may also influence the results of single-species toxicity tests which have shown that certain mayflies (Baetis rhodani, Ephemerella ignita, and D. grandis) are tolerant to metals

(Warnick and Bell 1969, Williams et al. 1985). These differences may be due to using large, more resistant life stages, a common practice when collecting in the field organisms for laboratory toxicity tests. Higher sensitivity of small instars may result from larger surface area:volume ratios, higher initial lipid content, or a greater weight specific metabolism, which would facilitate uptake of toxicants and increased frequency of molting during early development (Powlesland and George 1986). Higher sensitivity of early instars could have a profound influence on persistence of populations of aquatic organisms (Luoma and Carter 1991). In the Rocky Mountain ecoregion, metal concentrations in water may increase greatly during spring snowmelt (Kiffney and Clements 1993, Clements 1994). Macroinvertebrate taxa with early lifestages present during these episodic events may have an greater risk of local extinction. As an example, Leland et al. (1989) observed that the effects of Cu on stream macroinvertebrates were more severe when dosing was initiated in the summer compared with fall. It was hypothesized the summer metal exposure occurred near the time of egg hatch for most species, which may be a period when individuals are especially sensitive to stress (Anderson 1980). Results from my study showed that densities of mayflies were significantly reduced by the metal mixture, but these data also suggests other factors, such as lifestage contributed to an organism's sensitivity to metals.

The varied responses of stoneflies to metals that were observed has also been seen by other researchers some noting declines (Chadwick et al. 1986, Leland et al. 1989), whereas others found stoneflies unaffected (Selby et al. 1985, Clements et al. 1988b, Roline 1988). Larval densities of <u>Pteronarcys</u> <u>princeps</u> (Plecoptera: Pteronarcyidae) eclined in a Sierra Nevada stream dosed with low concentrations of Cu (e.g., 5 μg/L) (Leland et al. 1989). In contrast, a single-species test determined the 96-h median tolerance limit for Pteronarcella badia was 18.0 mg/L Cd (Clubb et al. 1975), which was three orders of magnitude higher than Cd concentrations in our 10X treatment. It is difficult to make strict comparisons among these studies because differences in environmental conditions (e.g., hardness, pH, and alkalinity) can influence metal bioavailibility (Sprague 1984), and tests exposing multispecies benthic assemblages to metals can be more sensitive than single species tests (Clements et al. 1988b). Indirect effects (e.g., predation, parasitism, and competition) can exert additional stress on organisms and may be responsible for the greater sensitivity of taxa in multispecies experiments compared to single-species tests.

Orthocladiinae are known to be tolerant to metals (Winner et al. 1980, Clements et al. 1988a, Clements 1994) and the tribe Tanytarsini sensitive to Cu (Clements et al. 1988a, 1989a). In my experiment, at copper concentrations comparable to those used by Clements et al. (1989a), densities of Tanytarsini in the 1X treatment were greater than in control streams. Thus, I caution against classifying the family Chironomidae as tolerant or sensitive because of differences among taxa (Yasuno et al. 1985, Clements et al. 1988a). Because the family Chironomidae is extremely diverse in Rocky Mountain streams (Ward 1986) and their taxonomy is relatively difficult, it was decided a priori to identify this group to subfamily or tribe. The level of taxonomic resolution used may explain the difficulty in discerning negative effects of metals on chironomids; however, I was able to detect statistically significant effects of metals on other, less taxonomically difficult taxa.

It has reported elsewhere that some caddisflies dominate communities at metal-polluted sites (Norris et al. 1982, Clements et al. 1988a, Clements 1994). In an earlier experiment it was shown that caddisflies from two pristine Rocky Mountain streams were unaffected by 130 μg/L Zn (Kiffney and Clements 1994). A common index used to detect anthropogenic disturbance in lotic systems is the number of mayfly, stonefly, and caddisfly (e.g. EPT index) taxa (Resh and Jackson 1993). In my study, species richness of caddisflies ($p=0.2$) and stoneflies ($p=0.9$) was unaffected by metals. Previous studies have also demonstrated that some stonefly species are tolerant to metals (Clements et al. 1988b, Kiffney and Clements 1994, Clements 1994). I suggest that it may be difficult to discern differences in the EPT index at metal-contaminated sites, especially sites with low to moderate concentrations of metals, from reference sites in western streams.

Differences in metal effects observed in this study and in single-species experiments may be attributed to the route of metal exposure. In general, herbivores (Baetis, heptageniid mayflies) and detritivores (P. badia) were the most negatively affected by the metal mixture. Leland et al. (1989) observed that herbivores and detritivores were more sensitive to Cu exposure than predators. They hypothesized the primary route of Cu uptake was ingestion of particles rather than solute transport. Clements (1994) noted that heptageniid mayflies (e.g., scrapers) were the most sensitive group to heavy metals at the Arkansas River. I did not measure the concentrations of metals in other biotic or abiotic compartments (e.g., sediment, detritus, algae, or animal tissue); however, in a previous study aquatic insects that fed mostly on aufwuchs (Baetis) or detritus (P. badia) accumulated the highest concentration of metals compared to predators (Kiffney and

Clements 1993). These results suggest that feeding habits of aquatic insects may prove to be useful in assessing or predicting response to metal pollution.

The experimental results presented here were similar to findings from a biomonitoring study conducted on the Arkansas River and the Eagle River, Colorado, streams polluted by a mixture of metals (Cd, Cu, and Zn) (Clements 1994, Clements, unpublished results). Mayfly abundance (B. tricaudatus and heptageniids) was generally lower below sources of metals at field streams, and in experimental streams dosed with a metal mixture. Alternatively, stoneflies and orthoclad chironomids were not as affected by metals. Other researchers have noted similar biological responses in streams contaminated by metals (Peckarsky and Cook 1981, Chadwick et al. 1986, Clements et al. 1988a, Leland et al. 1989, Clements 1991, Clements 1994). Leland et al. (1989) reported that densities of Baetis sp. and two species of heptageniid mayflies (Epeorus dulciana and E. longimanus) were reduced in field streams dosed with 10 μg/L Cu. At Coal Creek, Colorado, there was an increase in the mortality of Baetis bicaudatus, Cinygmula sp. (Ephemeroptera:Heptageniidae), and E. longimanus below sources of acid mine drainage (Peckarsky and Cook 1981). Chadwick et al. (1986) noted that mayflies were virtually eliminated, whereas caddisflies and dipterans were more abundant at sites contaminated by metals. The general agreement between experimental and field results supports the hypothesis that metals can restrict the distribution and abundance of some aquatic insects. However, there were differences, such as greater total density at metal-polluted field sites compared to reference sites, indicating that a multitude of ecological factors influence aquatic insect community structure in metal-contaminated streams.

Heptageniids showed a stronger negative correlation between density and Zn concentration than all other taxa in field surveys of the Arkansas River (Clements 1994). Taxa within this family are found in streams throughout the United States and have been observed to be sensitive to both metals and other forms of pollution (Leland et al. 1989, Feldman and Conner 1992, Clements 1994). I advocate the use of heptageniid mayflies as "sentinel organisms" (Johnson et al. 1993, Nelson and Roline 1993, Clements 1994) to detect metal-pollution of lotic systems. Specifically, based on field surveys and microcosm experiments, I propose monitoring changes in density and species richness of heptageniid mayflies as a potential indicator of the biological effects of metals on Rocky Mountain stream macroinvertebrates.

The US EPA has mandated the use of biological criteria in restoring, maintaining, and protecting aquatic communities. Hughes et al. (1990) advocate the use of a regional framework for stratifying natural variation and for determining biological criteria, based on biological conditions of pristine streams within ecoregions. Although such an approach would facilitate the establishment of biological criteria, biological communities of lotic systems exhibit considerable spatial and temporal variability (Vannote et al. 1980, Minshall et al. 1985, Resh and Rosenberg 1993), which would make interpretation of results difficult. Kiffney and Clements (1994) observed that Baetis spp. and Rhithrogena hageni collected from two pristine streams located at different elevations differed in their response to Zn in experimental streams. This variation highlights the importance of evaluating the effects of metals on indigenous stream organisms experimentally, because results from biomonitoring studies may be confounded by other factors. I do not suggest that the experimental approach described in this research is a remedy for distinguishing between natural and anthropogenic variability in lotic systems; however, manipulative experiments in combination with a well-designed field study provide a rigorous approach to evaluating impacts of pollutants on streams.

Any experimental approach is simple in relation to the natural world, but the one used in this research is a much more complex system than standardized, single-species toxicity tests and provides an opportunity to examine some of the indirect effects of contaminants (Clements et al. 1989b). Artificial substrates were colonized by a diverse array of taxa and functional feeding groups, such as predators (Chloroperlidae), detritivores (P. badia), collector-filterers (Brachycentrus spp.), collector-gatherers (Drunella), and grazers (Baetis, Heptageniidae). In addition, detritus and periphyton accumulated on trays (Kiffney, personal observation), providing natural food sources. The composition of the macroinvertebrate assemblage collected on these substrates was similar to samples collected using a Hess, a device routinely used in biomonitoring studies (Merritt and Cummins 1984). In conclusion, I agree with other researchers that combining field observations with controlled experimentation is the most efficient method of gaining knowledge in ecological and environmental research (Eberhardt and Thomas 1991)

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CONCLUSIONS

This research has shown that abiotic and biotic factors can influence the response of stream macroinvertebrates to metals. For example, stream benthic invertebrate communities from high-altitude streams were more sensitive to metals than those from low-altitude streams. Differences in insect body size between high- and low-altitude streams may explain this variation in metal sensitivity. I suggest that water resource managers need to consider ecological factors on a regional basis when assessing the ecological risk of contaminants on lotic systems. Specifically, my results suggest that metal standards for high-altitude streams need to be lower than for low-altitude streams because of differences in sensitivity of aquatic invertebrates between these streams.

Testing the effects of contaminants on biotic processes may provide more sensitive indicators of environmental stress than single species toxicity experiments. For instance, predation intensity on Hydropsyche sp. was greater in microcosms dosed with half the chronic levels of Cd, Cu, and Zn than in controls. Therefore, to maintain ecological integrity of lotic ecosystems, it is important to understand how contaminants indirectly affect community structure.

Biological monitoring of lotic systems is an important aspect in determining effects of contaminants; however, to rigorously define sensitive bioindicators, results from these field surveys should be combined with controlled experiments using indigenous organisms. My research suggests that a reliable indicator of metal-contamination in western streams is the abundance and species richness of heptageniid mayflies.

In summary, my research has shown that the response of stream macroinvertebrates to metals was influenced by abiotic and biotic factors. Thus the response of natural communities to contaminants can be affected by a myriad of ecological factors. Furthermore, my results indicate that single species laboratory toxicity tests may not be adequate predictors of the response of indigenous organisms to toxicants, and that more ecologically realistic experiments are necessary to adequately assess the response of natural ecosystems to anthropogenic disturbance.