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HEAVY METALS STRUCTURE BENTHIC COMMUNITIES IN COLORADO MOUNTAIN STREAMS

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Abstract. The development of field sampling designs that employ multiple reference and polluted sites has been proposed as an alternative to the traditional upstream vs. downstream approach used in most biomonitoring studies. Spatially extensive monitoring programs can characterize ecological conditions within an ecoregion and provide the necessary background information to evaluate future changes in water quality. We measured physicochemical characteristics, heavy-metal concentrations, and benthic macroinvertebrate community structure at 95 sites in the Southern Rocky Mountain ecoregion in Colorado, USA. Most sites (82%) were selected using a systematic, randomized sampling design. Each site was placed into one of four metal categories (background, low, medium, and high metals), based on the cumulative criterion unit (CCU), which we defined as the ratio of the instream metal concentration to the U.S. Environmental Protection Agency criterion concentration, summed for all metals measured. A CCU of 1.0 represents a conservative estimate of the total metal concentration that, when exceeded, is likely to cause harm to aquatic organisms. Although the CCU was less than 2.0 at most (66.3%) of the sites, values exceeded 10.0 at 13 highly polluted stations. Differences among metal categories were highly significant for most measures of macroinvertebrate abundance and all measures of species richness. We observed the greatest effects on several species of heptageniid mayflies (Ephemeroptera: Heptageniidae), which were highly sensitive to heavy metals and were reduced by >75% at moderately polluted stations. The influence of taxonomic aggregation on responses to metals was also greatest for mayflies. In general, total abundance of mayflies and abundance of heptageniids were better indicators of metal pollution than abundance of dominant mayfly taxa. We used stepwise multiple-regression analyses to investigate the relationship between benthic community measures and physicochemical characteristics at the 78 randomly selected sites. Heavy-metal concentration was the most important predictor of benthic community structure at these sites. Because of the ubiquitous distribution of heavy-metal pollution in the Southern Rocky Mountain ecoregion, we conclude that potential effects of heavy metals should be considered when investigating large-scale spatial patterns of benthic macroinvertebrate communities in Colorado's mountain streams.

Key words: *benthic macroinvertebrate community structure; biomonitoring; heavy-metal concentrations; heavy metals and benthic communities; macroinvertebrates; metal pollution and Rocky Mountain streams; mining; mountain streams and mining pollution; Rocky Mountain streams (Colorado, USA); Southern Rocky Mountain ecoregion; spatial scale; taxonomic aggregation.*

INTRODUCTION

Experimental designs employed in stream biomonitoring often involve trade-offs between spatially and temporally extensive sampling (Resh et al. 1995, Wiley et al. 1997). Although long-term sampling of a single stream can provide important insights into seasonal and annual variation, this approach may overrepresent the significance of temporal variation relative to spatial variation (Wiley et al. 1997). In addition, the typical approach used in most assessments of water quality, in which upstream reference sites are compared to downstream polluted and recovery sites, is confounded by natural, longitudinal variation (Clements and Kiffney 1995). Finally, because most biomonitoring studies in streams do not employ true replicates (Hurlbert 1984), our ability to generalize to other systems is greatly limited.

The development of more sophisticated sampling designs that employ multiple reference and polluted sites has been proposed as an alternative to the traditional upstream-vs.-downstream approach used in most biomonitoring studies (Feldman and Connor 1992, Clements and Kiffney 1995, Humphrey et al. 1995, Resh et al. 1995). Hughes et al. (1986) recommended a watershed-classification approach to select reference streams with similar hydrological and geomorphological characteristics within an ecoregion. Although such spatially extensive assessments often lack estimates of temporal variation, they can be used to characterize ecological conditions at a regional scale when temporal variation is controlled. In addition, spatially extensive

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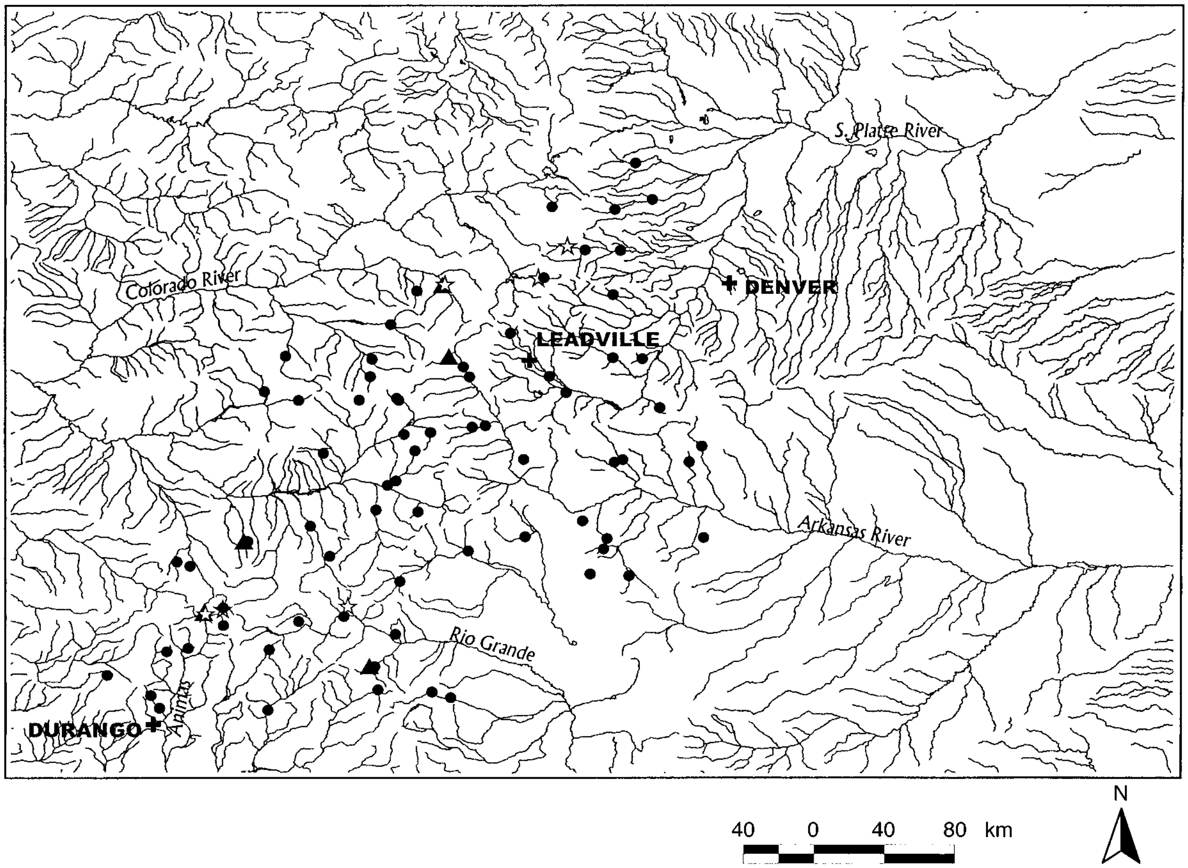

FIG. 1. Map of Colorado (USA) showing the major watersheds and sampling stations in the Southern Rocky Mountain ecoregion. Solid circles = randomly selected sites; open stars = test sites; solid triangles = reference sites. Note that some symbols are hidden, in cases where two sites were sampled from the same stream.

monitoring within an ecoregion provides the necessary background information to evaluate future changes in water quality (EPA 1995).

Selection of ecological indicators and the appropriate level of taxonomic resolution are also critical decisions when designing stream biomonitoring studies. Because of taxonomic difficulties with certain groups of macroinvertebrates, particularly chironomids and oligochaetes, large savings in sample processing cost may be realized by using relatively coarse (e.g., family level) taxonomic resolution (Lenat and Barbour 1994, Vanderklift et al. 1996). In addition, non-taxonomic categories, such as functional feeding groups and life-history traits, have been employed in multimetric indices (Wallace et al. 1996, Richards et al. 1997) and to characterize environmental conditions in streams (Kerans and Karr 1994, Barbour et al. 1996).

Separating natural spatiotemporal variability in ecological indicators from variation due to anthropogenic disturbance is greatly simplified when stressors are restricted to a single class of contaminants. Heavy-metal pollution from active and historic mining operations is ubiquitous in the U.S. west and is generally recognized as one of the most significant environmental problems in Rocky Mountain streams. Since the discovery of gold and other minerals in the mid-1800s, mining activities have had a major impact on watersheds in this region. Approximately one third of the U.S. Environmental Protection Agency (EPA) Superfund Sites in Colorado are mining sites, and it is estimated that heavy metals from over 5000 abandoned mines affect >2600 km of Colorado's streams (Colorado Department of Health 1992). This large number of streams polluted by the same class of contaminants within a single ecoregion provides a unique opportunity to employ sophisticated sampling designs to evaluate biological measures of integrity.

In 1993 the U.S. EPA initiated a Regional Environmental Monitoring and Assessment Program (R-EMAP) to assess the ecological status and condition of Colorado's mountain streams. Physicochemical characteristics and benthic macroinvertebrate data were collected from 73 streams in the Southern Rocky Mountain ecoregion (Fig. 1) in 1994 and 1995. The objectives of the present study were to (1) describe the extent of heavy-metal pollution in streams of the Southern Rocky Mountain ecoregion of Colorado; (2) determine the level of taxonomic resolution necessary to detect effects of heavy metals at a large spatial scale; and (3) characterize the role of heavy metals in structuring benthic macroinvertebrate communities in this region.

METHODS

Study sites

We analyzed physical, chemical, and biological data collected from 95 stations in the Southern Rocky Mountain (USA) ecoregion during late summer (August to September) of 1994 ($n = 46$ sites) and 1995 ($n = 49$ sites). The Southern Rocky Mountain ecoregion extends from southern Wyoming through Colorado and into northern New Mexico (Omernik 1987). The stations were located on 73 different streams within the Colorado mineral belt, an irregular-shaped area located in the central and southwestern portions of the state that runs approximately parallel to the continental divide and contains the major mineral deposits (Fig. 1). The mineral belt was delineated using current and historic mine-site locations obtained from the U.S. Bureau of Mines (U.S. Bureau of Mines 1992). Most (78) of the 95 study sites were randomly selected using a probabilistic sampling design developed by U.S. Environmental Protection Agency (EPA 1995). A systematic grid covering the Southern Rocky Mountain ecoregion in Colorado was used to select sites from the network of perennial streams on U.S. Geological Survey 1: 100 000 scale topographical maps. We restricted our sampling to shallow streams (2nd to 4th order) for logistical reasons and because variation in stream size confounds biological assessments using benthic macroinvertebrates (Clements 1994, Clements and Kiffney 1995). In addition to the randomly selected stations, we included eight metal-polluted ‘‘test’’ sites (streams with known inputs of heavy metals) and nine ‘‘reference’’ sites (streams with no known metal inputs) in our analysis. Except for seven streams that were sampled in 1994 and 1995, all sites were sampled only once during the study.

Details of the procedures used to collect and analyze water-quality data and benthic macroinvertebrates have been described previously (EPA 1989, 1995). Water temperature, stream depth, width, dissolved oxygen, and current velocity were measured in the field. Stream discharge was estimated by dividing the stream width into 15–20 equal intervals and was based on measurements of cross-sectional area and current velocity (measured at 60% of depth). Water samples for pH were collected in sealed 60-mL syringes to prevent equilibration with atmospheric CO_2 and measured in the laboratory. For other water-chemistry analyses, stream water was collected in a 4-L container and analyzed in the laboratory using the following standard U.S. EPA protocols (EPA 1983): water hardness (method number 200.7), alkalinity (310.1), total organic carbon (415.2), sulfate (300), phosphate (365.4), total organic nitrogen (353.2), and concentrations of metals (Al, Cd, Cu, Fe, Mn, Pb, and Zn) (200.7 and 213.2).

Benthic macroinvertebrates were collected from each station using a modified kick net (mesh size = 595 μm). At each station, nine subsamples located approximately four-stream-widths apart were sampled by disturbing a 0.5-m^2 area immediately upstream from the net for 20 s. In smaller streams ($<$4 m wetted width across the channel), these nine subsamples were collected from a 150-m stream reach. These subsamples were combined in a single container, rinsed through a 595-μm sieve, and preserved in 70% ethanol. The level of taxonomic resolution differed among the major groups of benthic macroinvertebrates. All aquatic insects were identified to either genus or species in the laboratory. Non-insect groups (molluscs, oligochaetes, crustaceans, water mites), which accounted for a very small portion of the benthic community at most sites, were generally identified to genus or family.

Characterization of heavy-metal concentrations

Because we expected that most metal-polluted streams in the study area were impacted by a mixture of metals, we used a cumulative measure of total metal concentration to examine the relationships between benthic community structure and heavy metals. This approach is a modification of the method described originally in the U.S. EPA water-quality criteria documents (National Research Council 1972). Water-quality criteria for individual chemicals represent concentrations that, when exceeded, may harm aquatic organisms. Because criterion values are only available for individual chemicals, alternative models are necessary to estimate toxic effects of metal mixtures. Although most research investigating toxicity of metal mixtures has focused on acute effects, previous studies have shown additive effects at chronic concentrations (Spehar and Fiandt 1986, Enserink et al. 1991). Therefore, we assumed that interactions among metals were additive and defined the ‘‘cumulative criterion unit’’ (CCU) as the ratio of the measured metal concentration to the U.S. EPA criterion value, summed for all metals at a station. The cumulative criterion unit is given as:

$$\mathrm{CCU} = \sum m_i/c_i$$

where m_i is the total recoverable metal concentration and c_i is the criterion value for the ith metal. For Al, Fe, and Mn we used chronic criterion values of 150, 1000, and 1000 μg/L, respectively. Because water hardness affects toxicity and bioavailability of some heavy metals, criterion values for Cd, Cu, Pb, and Zn were modified to account for variation in water hardness among streams (EPA 1986). For example, at a water hardness of 100 mg/L, criterion values for these four metals would be 1.1, 11.8, 3.2, and 106 μg/L, respectively. Metals that were below detection were not included in the CCU. Thus, a CCU value of 1.0 represents a conservative estimate of the chronic criterion value for all metals measured at a station.

We placed all 95 stations (randomly selected, test, and reference) into one of four categories based on the measured CCU. We treated metal contamination as a categorical variable in these analyses because previous research suggested that some benthic community responses to metals were nonlinear (Clements and Kiffney 1995) and because we were interested in quantifying metal effects at specific levels of contamination (Wiens and Parker 1995). Background (unpolluted) sites ($n = 31$) were defined as stations where the CCU was less than 1.0. If metal effects are additive, a CCU of 1.0 represents the point at which we may expect to see adverse effects on aquatic organisms. We selected a value of 1.0 as a cutoff point because we were interested in determining if this conservative estimate of chronic metal effects would be protective of benthic communities. The low-metal category consisted of 32 sites with CCU values between 1.0 and 2.0. This category provided an opportunity to test the hypothesis that benthic communities would be protected when metal levels exceed the cumulative criterion value by a factor of 2 or less. The medium-metal category consisted of 19 sites with CCU values between 2.0 and 10.0. We selected this cutoff point because metal levels at 2–10 times the criterion are expected to cause significant mortality to sensitive species and alter benthic community structure in western streams (Roline 1988, Moore et al. 1991, Clements and Kiffney 1995). High-metal sites consisted of the 13 remaining stations where the CCU exceeded 10.0. We expected that all measures would show significant responses at these highly polluted stations. We recognize that the specific cutoff points for medium- and high-metal categories were somewhat arbitrary and that we may miss threshold responses that occurred within categories (Wiens and Parker 1995); however, our primary goal was to provide water-resource managers with specific guidelines (e.g., 1, 2, or 10 times the U.S. EPA criterion value) that could be employed to evaluate the severity of metal contamination in a stream. Furthermore, minor adjustments in these categories did not influence the final outcome of the analyses.

Data analysis

All statistical analyses were performed using a PC version of the Statistical Analysis System (SAS Institute 1994). We initially analyzed for differences between years using data collected from streams sampled in 1994 and 1995. Although relatively few of the randomly selected sites were sampled in both years ($n = 7$), we felt that it was important to determine if benthic communities from streams sampled in 1994 were similar to those sampled in 1995. Results of one-way ANOVA showed no significant differences between years for any of the benthic community variables that we examined. The only variables that were close to significant were abundance ($P = 0.1366$) and species richness ($P = 0.1389$) of Trichoptera. Therefore, we combined data from 1994 and 1995 and tested for differences among metal levels using one-way ANOVA (SAS Institute 1994: GLM procedure). We examined differences in physicochemical characteristics, abundance of the 16 dominant taxa (taxa that accounted for $>1.0\%$ of total abundance) and 16 community metrics. The community metrics that we examined included those used in other multimetric indices (e.g., the EPT index; Plafkin et al. 1989, Kerans and Karr 1994, Barbour et al. 1996), as well as metrics known to be sensitive to heavy metals in Rocky Mountain streams (e.g., abundance and species richness of mayflies; Clements 1994). The 16 community metrics used in these analyses were: total macroinvertebrate abundance, EPT abundance (number of Ephemeroptera, Plecoptera, and Trichoptera), total taxonomic richness, EPT richness (species richness of Ephemeroptera, Plecoptera, and Trichoptera), abundance and taxonomic richness of the four major aquatic insect groups (mayflies, stoneflies, caddisflies, chironomids), and the four dominant functional feeding groups (scrapers, shredders, collectors, predators).

We used Dunnett's test to determine which metal levels differed significantly from background stations (CCU < 1.0) for each variable. Although we ran separate one-way ANOVA for each of the 16 community-level metrics, we did not adjust P values for these multiple tests. As in most environmental assessments, we were particularly interested in detecting subtle effects of heavy metals and were concerned with protecting against Type II errors. However, because P values for most of these analyses were highly significant ($P < 0.0001$), these adjustments would not have affected our interpretation of these data. Assumptions of ANOVA were tested using F-max tests and by inspection of residuals plots. Where necessary, data were log-transformed to satisfy these assumptions.

We used stepwise multiple-regression analyses (SAS Institute 1994: REG procedure) with forward selection (significance level for entry = 0.05) to examine the relationship between benthic community variables and physicochemical characteristics (CCU, pH, hardness, alkalinity, conductivity, total organic carbon, nutrients, temperature, dissolved oxygen, current velocity, depth, width, and discharge). We limited these analyses to the 78 randomly selected sites because we wanted to generalize about the effects of heavy metals on benthic communities in all mountain streams in the region.

RESULTS

Physicochemical characteristics

Heavy-metal concentrations, expressed as cumulative criterion units (CCU), differed greatly among stations and ranged from 0.1 to 293.5. Although the CCU was less than 2.0 at the majority (66.3%) of stations

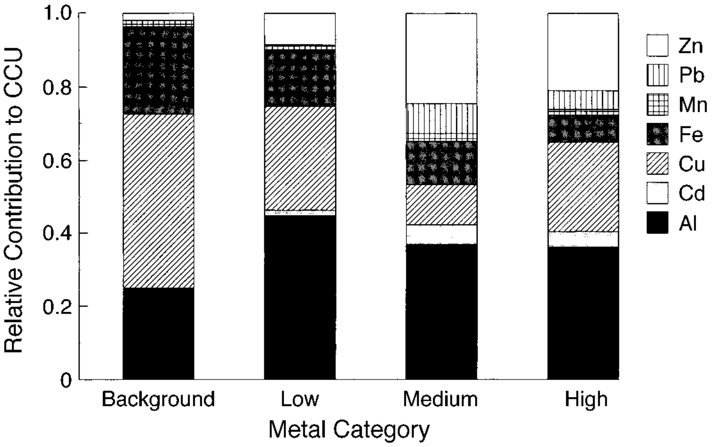

FIG. 2. Relative contributions of various metals to the cumulative criterion unit (CCU) at background ($n = 31$), low-metal ($n = 32$), medium-metal ($n = 19$), and high-metal ($n = 13$) sites. For definition of CCU, see *Methods: Characterization of heavy-metal concentrations.*

(median = 1.4), values exceeded 10.0 at 13 stations. Interestingly, only two of these stations were test sites with known metal inputs. The remaining 11 stations in this high-metals category were selected randomly. In other words, 14% of the randomly selected stations were highly contaminated by heavy metals.

The relative contribution of each metal to the CCU differed among the four metal categories (Fig. 2). Cd and Pb were below detection and the contribution of Zn to the CCU was <2.0% at background sites. Cu, Fe, and Al were the most important metals at background and low-metal sites (CCU < 2.0) and accounted for 88–96% of the CCU. The relative importance of Cu and Fe decreased and the contribution of Cd, Pb, and Zn to the CCU increased at medium-metal sites. Zn, Cu, and Al were the most important metals measured at high-metal sites.

Results of one-way ANOVA showed significant differences in some physicochemical characteristics among the four metal categories (Table 1). Conductivity, water hardness, total organic carbon (TOC), SO_4, and PO_4, were generally greater in metal-polluted streams, particularly those in the high-metals category. Increased conductivity and higher levels of SO_4 at high-metal sites were most likely a direct result of metal pollution. Other physicochemical characteristics (pH, water temperature, depth, width, stream discharge, dissolved oxygen, alkalinity, and nitrogen) did not differ significantly among metal categories.

Community-level responses to metals

Differences among metal categories for most measures of abundance and all measures of species richness were highly significant (Figs. 3 and 4). However, none of these variables were significantly lower at low-metals sites. The EPT index (species richness of mayflies,

TABLE 1. Physicochemical characteristics (means with 1 SD in parentheses) measured at 95 stations on second- to fourth-order streams in the Southern Rocky Mountain ecoregion (Colorado, USA), and ANOVA results.

	Sampling stations				ANOVA results	
Variable	Background $n = 31$	Low metal $n = 32$	Medium metal $n = 19$	High metal $n = 13$	*F*†	*P*
Conductivity (μmho/cm)	94.5 (66.5)	188.8 (254.0)	131.8 (91.6)	286.9 (241.9)*	$F_{3,79} = 3.56$	0.0032
pH	7.16 (1.01)	7.37 (1.16)	7.73 (1.00)	7.08 (0.90)	$F_{3,76} = 1.25$	0.2976
Hardness (mg/L)	59.4 (40.9)	70.9 (66.5)	73.7 (51.6)	144.7 (114.8)*	$F_{3,91} = 5.44$	0.0121
Alkalinity (mg/L)	51.3 (25.9)	85.1 (95.2)	77.2 (95.6)	76.9 (102.3)	$F_{3,90} = 1.01$	0.6067
Total organic carbon (mg/L)	2.2 (0.6)	2.6 (0.9)	3.2 (1.6)	3.5 (2.5)*	$F_{3,91} = 3.92$	0.0111
Chloride (mg/L)	1.3 (0.3)	11.5 (35.0)	2.9 (3.7)	4.7 (6.5)	$F_{3,91} = 1.37$	0.2570
SO_4 (mg/L)	13.3 (20.6)	21.1 (40.0)	26.6 (49.8)	111.0 (86.6)*	$F_{3,91} = 14.83$	0.0001
$NO_3 + NO_2$ (mg/L)	107.8 (334.8)	190.9 (454.4)	82.2 (357.9)	0.4 (0.9)	$F_{3,91} = 0.95$	0.2232
PO_4 (mg/L)	0.02 (0.02)	0.03 (0.03)	0.06 (0.06)*	0.08 (0.08)*	$F_{3,91} = 6.35$	0.0006
Temperature (°C)	10.8 (3.2)	12.0 (3.9)	12.8 (3.8)	12.8 (5.1)	$F_{3,91} = 1.32$	0.2615
Dissolved oxygen (mg/L)	7.9 (0.8)	8.5 (1.7)	8.4 (1.3)	8.1 (1.1)	$F_{3,88} = 1.17$	0.5993
Depth (m)	0.83 (0.58)	0.91 (0.46)	0.95 (0.63)	0.89 (0.57)	$F_{3,83} = 0.21$	0.8891
Width (m)	6.3 (3.9)	5.7 (3.5)	5.9 (4.3)	7.0 (3.7)	$F_{3,83} = 0.37$	0.7731
Current velocity (cm/s)	46.6 (27.4)	44.5 (24.4)	47.8 (23.8)	48.1 (27.1)	$F_{3,83} = 0.08$	0.9702
Discharge (m^3/s)	1.76 (2.51)	2.16 (2.35)	2.54 (2.68)	3.13 (2.64)	$F_{3,86} = 0.97$	0.4099

Notes: Stations were placed into four categories based on the level of metal pollution. *F* values and *P* values are from one-way ANOVA testing for differences among the four metal categories.

* $P < 0.05$; sites significantly different from background based on Dunnett's multiple-comparisons test.

† Degrees of freedom differ among these physicochemical characteristics because of missing values for some variables.

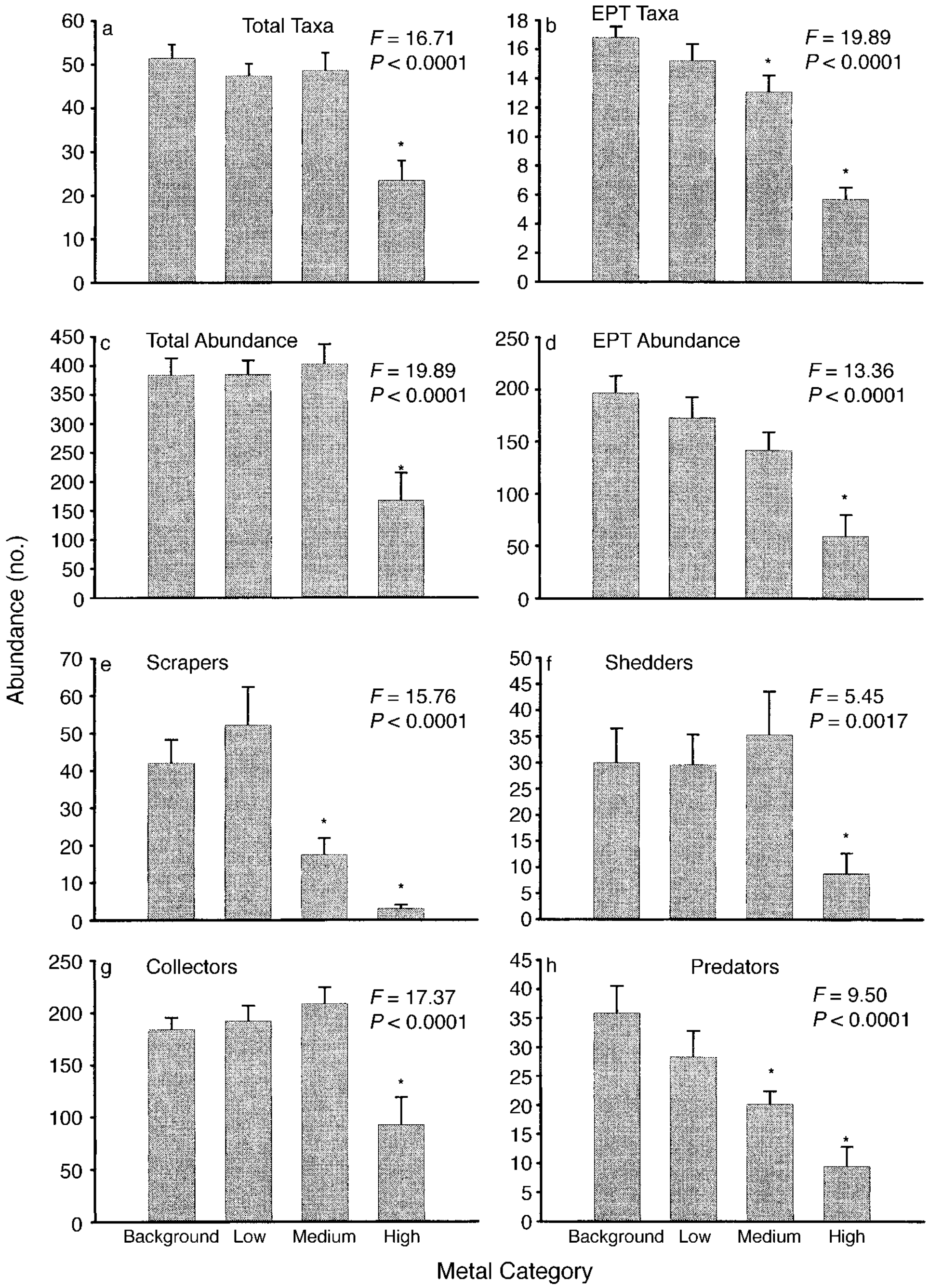

FIG. 3. Community-level responses (mean and 1 SE) to metal pollution of streams. (a) Number of taxa, (b) EPT taxa (mayflies, stoneflies, and caddisflies), (c) macroinvertebrate abundance, (d) EPT abundance, and (e–h) abundance of the four major functional feeding groups at background, low-, medium-, and high-metal sites. Results of one-way ANOVA for each variable are also shown (df = 3, 91). Asterisks indicate sites that were significantly different from background ($P < 0.05$), based on Dunnett's multiple-comparisons test.

stoneflies, and caddisflies) was significantly lower at medium- and high-metal stations compared to background stations (Fig. 3b). In contrast, the total number of taxa, total abundance, and EPT abundance (number of mayflies, stoneflies, and caddisflies) were significantly lower only at high-metal stations (Fig. 3a, 3c, 3d).

Effects of heavy metals on abundance of the four major functional feeding groups (scrapers, shredders, collectors, and predators) were highly significant (Fig. 3). The greatest response was observed for scrapers (Fig. 3e) and predators (Fig. 3h), which were significantly lower at medium- and high-metal stations. Shredders (Fig. 3f) and collectors (Fig. 3g) were rel-

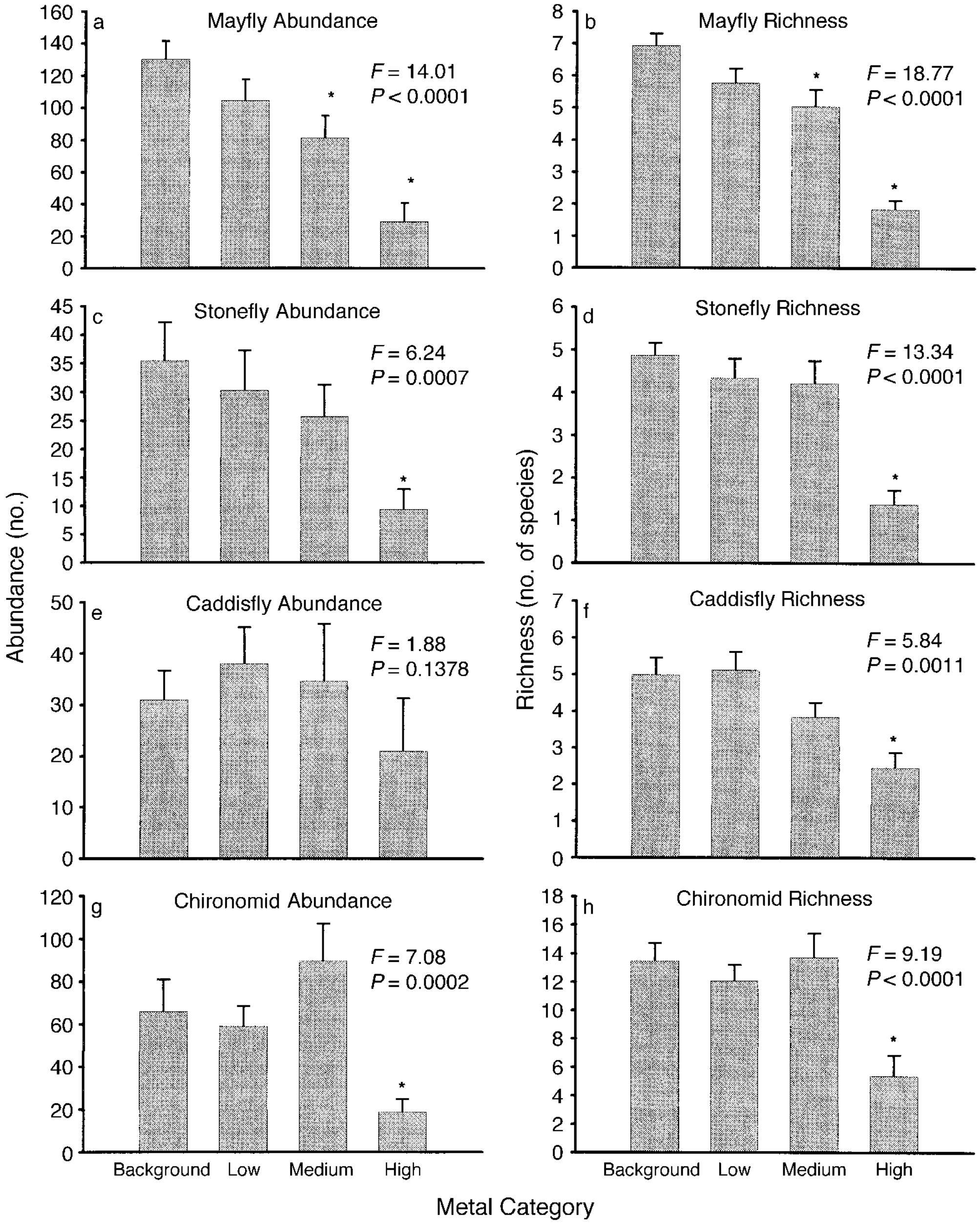

FIG. 4. Abundance and species richness of mayflies, stoneflies, caddisflies, and chironomids at background, low-, medium-, and high-metal sites. Data are means and 1 SE. See Fig. 3 for details of statistical analyses.

atively tolerant of metals and were significantly lower only at high-metal sites.

Effects of heavy metals were generally greater on mayflies compared to other macroinvertebrate groups (Fig. 4). Abundance and species richness of mayflies were significantly lower at both medium- and high-metal stations (Fig. 4a, b). Although species richness of stoneflies (Fig. 4d), caddisflies (Fig. 4f), and chironomids (Fig. 4h) differed among metal levels, only high-metal sites were significantly different from background. Caddisflies were the only macroinvertebrate group whose abundance did not vary significantly among metal levels (Fig. 4e).

Responses of dominant taxa to metals

Although abundance of most dominant benthic macroinvertebrate taxa differed significantly among metal categories, the effects of metals were generally greatest on mayflies and stoneflies (Fig. 5). In particular, abundance of the mayflies *Rhithrogena robusta* (Fig. 5b), *Cinygmula* sp. (Fig. 5c), and *Drunella doddsi* (Fig. 5d), and the stonefly *Sweltsa* sp. (Fig. 5e) was

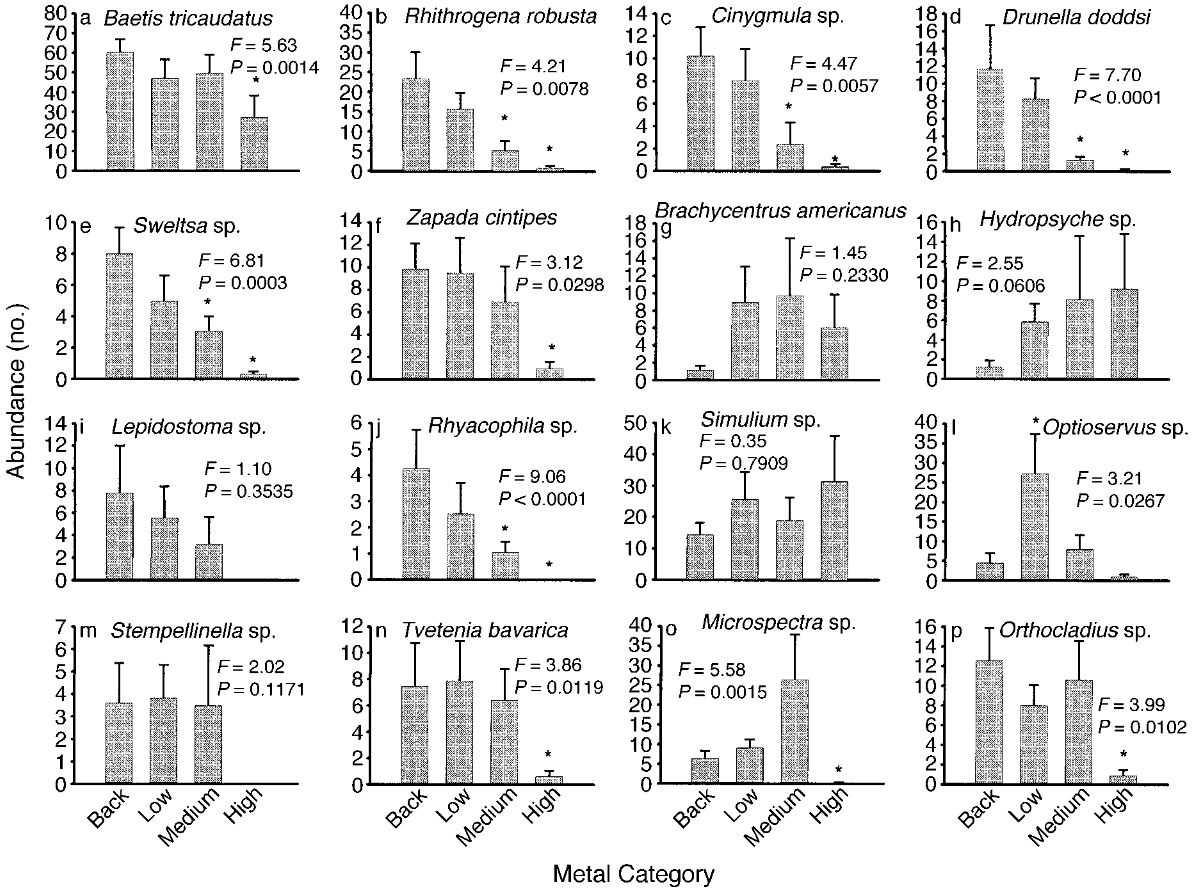

FIG. 5. Abundance of the 16 dominant taxa at background, low-, medium-, and high-metal sites. Data are means and 1 SE. See Fig. 3 for details of statistical analyses.

significantly lower at medium- and high-metal stations. *Rhyacophila* sp. (Fig. 5j) was the only caddisfly that showed a significant response to metal level and was lower at medium-metal sites. Differences among metal categories in abundance of the three other dominant caddisflies, (*Brachycentrus americanus, Hydropsyche* sp., and *Lepidostoma* sp.) and the blackfly *Simulium* sp. were not significant (Fig. 5g, h, i, k). Abundance of the beetle *Optioservus* sp. (Elmidae) (Fig. 5l) differed significantly among metal levels, but this difference resulted from increased abundance at low-metal sites compared to background. Three of the four dominant chironomid taxa (*Tvetenia bavarica, Microspectra* sp., and *Orthocladius* sp.) differed significantly among metal categories (Fig. 5n, o, p); however, effects were observed only at the highest metal levels.

Taxonomic aggregation and effects of heavy metals

To investigate the influence of taxonomic aggregation on responses of benthic macroinvertebrate to heavy metals, we selected the dominant genus within each of the four major macroinvertebrate groups (mayflies, stoneflies, caddisflies, and dipterans) that showed the most consistent response to heavy metals. Because our goal was to determine if coarser levels of taxonomic resolution improved our ability to assess effects of metals at large spatial scales, we chose a genus within each group that was sensitive to metals. The effects of taxonomic aggregation on responses of these four groups to heavy metals were most pronounced for mayflies (Fig. 6). The amount of variation explained (r^2 = 0.14 to 0.32) from one-way ANOVA increased with the level of taxonomic aggregation for this group. In contrast, total abundance of caddisflies was not influenced by heavy metals, but effects on *Rhyacophila* sp. (the only genus in the family Rhyacophilidae in Colorado) were highly significant. Taxonomic aggregation had relatively little influence on the responses of stoneflies and dipterans to heavy metals. The amount of variation explained (r^2 = 0.12 to 0.19) from one-way ANOVA for these groups was generally similar at different levels of taxonomic aggregation.

The role of metals in structuring benthic communities

Results of stepwise multiple regression using the 78 randomly selected stations indicated that heavy-metal

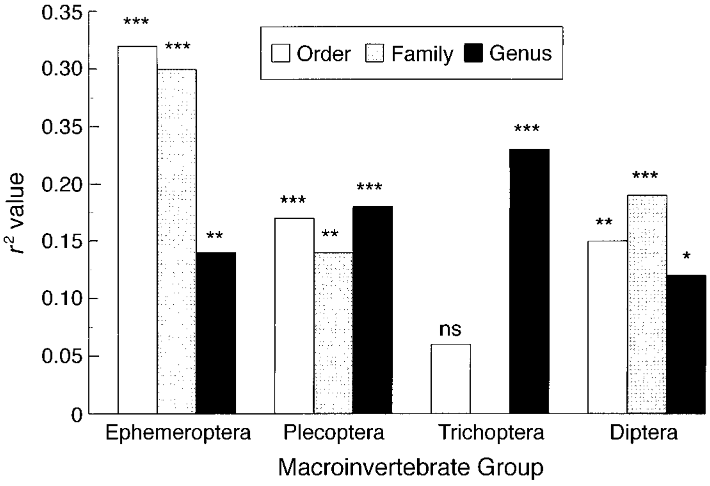

FIG. 6. The influence of taxonomic aggregation on responses of the four major macroinvertebrate groups to heavy metals in Rocky Mountain streams. The figure compares r^2 values (df = 3, 91) and levels of significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS = not significant) from one-way ANOVA testing for differences among metal categories for Ephemeroptera (Heptageniidae: *Rhithrogena* sp.), Plecoptera (Chloroperlidae: *Sweltsa* sp.), Trichoptera (Rhyacophilidae: *Rhyacophila* sp.), and Diptera (Chironomidae: *Ortholcadius* sp.). Note that *Rhyacophila* is the only genus in the family Rhyacophilidae in Colorado.

concentration was the most important predictor variable for 14 of the 16 community variables that we examined (Table 2). The CCU was included in all multiple-regression models except for caddisfly and shredder abundance. The amount of variation explained by the CCU in these models ranged from 16% (caddisfly richness) to 52% (EPT [Ephemeroptera, Plecoptera, and Trichoptera]). Although other physicochemical characteristics, such as water temperature, SO_4, PO_4, and conductivity were included in some models, they generally explained much less variation in community structure than did metal concentration.

DISCUSSION

Of the 32 biological variables examined in this study (16 community measures and 16 dominant taxa), 26 differed significantly among the four metal levels. Although none of these variables were significantly lower at low-metal sites (where CCU [cumulative criterion unit] values were between 1.0–2.0), abundance of predators, mayfly abundance, and mayfly richness appeared to respond to these low-metal levels (Fig. 3h and 4a, b). Benthic community composition was significantly altered at medium-metal sites, where the mean CCU was 4.7. Based on these results, we suggest that the cumulative criterion value for metals is protective of benthic communities in Colorado's mountain streams; however, alterations in benthic macroinvertebrate community structure are likely to occur when heavy-metal concentrations exceed 2 times the U.S. Environmental Protection Agency's chronic criterion value. These findings are consistent with results reported in a previous study of six Colorado streams polluted by Zn (Clements and Kiffney 1995).

Measures of species richness were generally less variable than abundance of major taxonomic groups or dominant taxa, a finding that has been reported previously for other stressors (Barbour et al. 1996, Fore et al. 1996, Johnson 1998). Benthic community measures that showed the strongest response in the present study are also known to be useful indicators of metal pollution (Clements et al. 1988, Clements 1994, Clements and Kiffney 1995, Kiffney and Clements 1996). For example, mayflies are highly sensitive to heavy metals in western streams and are often the first group eliminated downstream from metal inputs (Leland et al. 1989, Nelson and Roline 1993, Clements 1994, Clements and Kiffney 1995). In the present study, abundance and species richness of mayflies and abundance of most dominant mayfly taxa were significantly lower at medium-metal stations.

Previous studies have reported that the EPT index is a reliable indicator of water quality (Plafkin et al 1989, Lenat and Barbour 1994) and is closely related to functional measures (decomposition, secondary productivity) of biological integrity (Wallace et al. 1996). However, research on the Arkansas River, a Colorado stream degraded by heavy metals, showed that the EPT index was relatively insensitive to moderate levels of metal contamination because mayflies were replaced by metal-tolerant caddisflies at polluted sites (Clements and Kiffney 1994). In the present study the EPT index was lower at medium-metal sites, and heavy-metal concentration was the most important predictor variable for this metric in the multiple-regression model (partial $r^2 = 0.52$). These results suggest that the EPT index may be a more useful indicator of heavy-metal pollution at a large regional scale than in individual streams.

One of the most consistent responses to heavy metals in our study was lower abundance of heptageniid mayflies. In particular, abundance of the mayflies *Rhithrogena robusta* and *Cinygmula* sp. was reduced by >75% at medium-metal sites. Results of microcosm experiments (Kiffney and Clements 1994) and field studies in Colorado (Nelson and Roline 1993, Clements 1994, Clements and Kiffney 1995) indicate that heptageniid mayflies are highly sensitive to heavy metals. Increased abundance of *Rhithrogena hageni* has also

TABLE 2. Results of stepwise multiple regression (forward selection) showing the relationship between benthic macroinvertebrate community variables and physicochemical characteristics at 78 randomly selected stations on streams in the Southern Rocky Mountain ecoregion. The directional influence (+, −) of each predictor included in the regression model is also shown.

Dependent variable	Predictors in model	Partial r^2	Model r^2	Model F	Model P
Total abundance	CCU (−) PO_4 (+) SO_4 (−)	0.51 0.09 0.05	0.65	$F_{3,53} = 32.1$	0.0001
Total species richness	CCU (−) PO_4 (+)	0.39 0.06	0.45	$F_{2,54} = 22.3$	0.0001
EPT richness	CCU (−) hardness (−) NO_2 (+) PO_4 (+)	0.52 0.07 0.04 0.03	0.66	$F_{4,52} = 25.0$	0.0001
EPT abundance	CCU (−)	0.51	0.51	$F_{1,55} = 57.1$	0.0001
Scrapers	CCU (−) PO_4 (+)	0.35 0.05	0.40	$F_{2,54} = 17.8$	0.0001
Shredders	SO_4 (−) temperature (−)	0.18 0.10	0.28	$F_{2,54} = 10.6$	0.0001
Collectors	CCU (−) SO_4(−) alkalinity (+)	0.48 0.06 0.04	0.58	$F_{3,53} = 24.1$	0.0001
Predators	CCU (−) pH (+)	0.37 0.06	0.43	$\mathrm{F}_{2,54} = 20.8$	0.0001
Mayfly abundance	CCU (−)	0.46	0.46	$F_{1,54} = 47.5$	0.0001
Stonefly abundance	CCU (−) temperature (−)	0.27 0.20	0.47	$F_{2,54} = 24.2$	0.0001
Caddisfly abundance	SO_4 (−)	0.11	0.11	$F_{1,55} = 7.0$	0.0106
Chironomid abundance	CCU (−)	0.19	0.19	$F_{1,55} = 12.8$	0.0007
Mayfly richness	CCU (−) hardness (−)	0.43 0.07	0.50	$F_{2,54} = 41.1$	0.0001
Stonefly richness	CCU (−) temperature (−) NO_2 (+) conductivity (−) pH (+)	0.47 0.15 0.06 0.03 0.03	0.74	$F_{5,51} = 29.0$	0.0001
Caddisfly richness	CCU (−) PO_4 (+) conductivity (−)	0.16 0.08 0.07	0.31	$F_{3,53} = 8.0$	0.0002
Chironomid richness	CCU (−)	0.25	0.25	$F_{1,55} = 18.4$	0.0001

Notes: Total degrees of freedom differ among response variables because of missing values for some predictors. CCU = cumulative criterion unit, the ratio of the measured metal concentration to the U.S. Environmental Protection Agency's criterion value, summed for all metals at a station (see *Methods: Characterization of heavy-metal concentrations*). EPT = Ephemeroptera, Plecoptera, and Trichoptera.

been proposed as a measure of recovery in metal-polluted streams (Nelson and Roline 1993, 1996). Our results support the hypothesis that lower abundance of heptageniid mayflies is one of the most useful indicators of metal pollution in Rocky Mountain streams.

The level of taxonomic aggregation in biomonitoring studies

Because of the problems and high cost associated with identifying certain groups of benthic macroinvertebrates to genus or species (e.g., chironomids), the level of taxonomic resolution in biomonitoring studies is an important consideration. Although it is necessary to identify organisms to a level that provides a reliable measure of response to perturbation, aggregating species or genera into higher taxonomic categories may result in more useful indicators (Cottingham and Carpenter 1998). Not surprisingly, chironomids were the most diverse group in our study and accounted for 141 of the 350 taxa identified. In retrospect, species- or even genus-level identification of chironomids was probably not necessary to characterize effects of heavy metals in this study. Abundance of the three dominant chironomid taxa was highly variable and was significantly lower only at severely polluted sites (Fig. 5n, o, p). In fact, F values for total taxonomic richness increased when chironomids were excluded from the analysis ($F_{3,91} = 16.7$ with chironomids; $F_{3,91} = 18.3$ without chironomids), indicating that species- or genus-level identification of this group did not improve our ability to distinguish between background and metal-polluted streams.

Several researchers have reported that relatively coarse levels of taxonomic resolution are sufficient to detect effects of pollution on benthic communities (Warwick 1993, Ferraro and Cole 1995, Vanderklift et al. 1996). For example, Bowman and Bailey (1997) concluded that patterns of community structure were similar when analyses were based on genus- or family-level identifications. Aggregate measures of phytoplankton community composition were more reliable indicators of eutrophication than were species populations in a whole-lake enrichment experiment (Cottingham and Carpenter 1998). The most likely explanation for these results is that closely related species often have similar sensitivity to the same contaminants and therefore aggregating species into higher taxonomic units reduces sampling variability (Warwick 1988).

When samples are collected over relatively large geographic areas, as in the present study, higher taxonomic aggregates (e.g., families, orders) will be represented at more sites than any individual species. If species in these aggregates show similar responses to disturbance, then measures at coarse levels of taxonomic resolution will most likely be better indicators. In our study, taxonomic aggregation had the greatest effects on responses of mayflies to heavy metals. Although the mayfly *Rhithrogena* sp. was sensitive to heavy metals, total abundance of heptageniids and Ephemeroptera were better indicators (Fig. 6). Unlike these aggregate measures, abundance of *Rhithrogena sp.* was highly variable, particularly at background and low-metal sites. Because most mayflies and almost all heptageniids are sensitive to metals, these aggregate measures were better indicators of pollution at the large spatial scale of our study. In contrast to these results, total caddisfly abundance was a poor indicator of metal pollution. Unlike mayflies, the order Trichoptera includes families that are both highly tolerant (Brachycentridae and Hydropsychidae) and relatively sensitive (Rhyacophilidae) to heavy-metal pollution.

In summary, the appropriate level of taxonomic resolution in biomonitoring studies represents a tradeoff between natural background variability, sensitivity to the stressor, and, in the case of problematic groups such as chironomids, practical considerations. Ultimately, the level of taxonomic resolution may also depend on the spatial scale of the investigation. Family-level or higher identification may be appropriate over a relatively large spatial scale, such as in the present investigation. However, this coarse taxonomic resolution may not be sufficient to detect effects of disturbance within a single stream (Marchant et al. 1995).

The structuring role of heavy metals in Colorado's mountain streams

The influence of heavy metals on the distribution and abundance of benthic macroinvertebrates in western streams has received considerable attention (Roline 1988, Nelson and Roline 1993, Clements 1994). However, because most of this research has been restricted to individual streams, where upstream reference sites were compared to downstream polluted sites, it is difficult to make generalizations from these studies (Hurlbert 1984, Feldman and Connor 1992, Clements and Kiffney 1995). Our study compared 31 relatively unpolluted sites (CCU < 1.0) to 64 metal-polluted sites in the Southern Rocky Mountain ecoregion of Colorado (USA). Because most sites were selected randomly, our results have broad implications for all streams in this region. Stepwise multiple-regression analyses using only randomly selected sites showed that heavy-metal concentration was the most important predictor for 14 of 16 community variables. These findings demonstrate that heavy metals play an important role in structuring benthic communities in streams of the Southern Rocky Mountain ecoregion. Because of the ubiquitous distribution of heavy-metal pollution in this region, we conclude that effects of heavy metals should be considered when investigating large-scale spatial patterns of benthic macroinvertebrate communities in Colorado's mountain streams.

Although our data are spatially extensive, we currently lack information on seasonal or long term responses to heavy metals in these streams. Previous research in Colorado has shown a strong seasonal response of benthic communities to heavy metals, with greatest effects observed during spring runoff when metal levels are highest (Clements 1994). Consequently, the late-summer sampling employed in the present study may have underestimated effects of metals. Because many of the remote, high-elevation streams in Colorado are inaccessible until early summer, estimating seasonal variation in community responses to metals will be difficult. In addition to accounting for seasonal variation, a complete analysis of heavy-metal effects should examine long-term changes in benthic communities. Although our study was limited to two consecutive years, these data provide the necessary baseline information to evaluate future changes in water quality in Colorado's mountain streams.

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