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RESPONSES OF DIATOM COMMUNITIES TO HEAVY METALS IN STREAMS: THE INFLUENCE OF LONGITUDINAL VARIATION

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Abstract. We investigated longitudinal variation (e.g., from upstream to downstream) in diatom community composition in an unpolluted stream (the Cache la Poudre River, Colorado, USA) and demonstrated how natural variation in community structure and morphological growth forms influence responses of diatoms in metal-polluted streams. Upstream communities in the Cache la Poudre River were physiognomically simple and dominated by small, adnate species (*Achnanthes minutissima* and *Fragilaria vaucheriae*), which decreased in relative abundance downstream. Responses of diatom communities to Zn in six metal-polluted streams were influenced by elevation and other variables correlated with the stream's longitudinal gradient (pH, conductivity, alkalinity). Although clear community responses were observed at sites where Zn concentration exceeded 200 μg/L, effects of metals at moderately polluted sites were not significant.

These field results were compared to responses of periphyton communities in experimental streams dosed with heavy metals. We hypothesized that communities collected from high elevation sites, which were dominated by early successional species (*A. minutissima*), would be more tolerant to metals than downstream communities. To test this hypothesis, diatom communities collected from a high elevation site (2340 m) and a low elevation site (1536 m) on the Cache la Poudre River were exposed to a mixture of Cd, Cu, and Zn in experimental streams. Small, adnate species (*Achnanthes minutissima* and *Fragilaria vaucheriae*) were tolerant to metals and increased in abundance in treated streams. In contrast, late successional species, which were found only at the low elevation site (*Diatoma vulgare* and *Melosira varians*), were highly sensitive to metals and were eliminated from treated streams after 24 h. These results indicate that natural variation in diatom communities may complicate interpretation of routine biomonitoring studies. We suggest that it may be useful to study periphyton community responses to pollutants in relation to ecological characteristics and morphological growth forms. Conducting research within this framework may improve the predictive ability of periphyton models and provide a theoretical basis for understanding the relationships between community patterns and the evolutionary processes that determine responses to disturbance.

Key words: diatom communities; heavy metals; longitudinal variation; morphological growth forms; Rocky Mountain streams.

INTRODUCTION

Understanding the relative influence of natural spatial and temporal variation on the responses of aquatic organisms to pollutants is one of the most persistent problems in stream biomonitoring studies (Clements and Kiffney 1995). Although approaches such as the before-after-control-impact design (BACI) have been developed to address this problem (Stewart-Oaten et al. 1986, Underwood 1992), they are not applicable in field investigations where pre-impact data are unavailable or where appropriate reference sites cannot be found. Experimental approaches, such as the use of microcosms, mesocosms (Genter et al. 1987, Clements et al. 1992, Kiffney and Clements 1996), or natural field experiments (Feldman and Connor 1992, Clements and Kiffney 1995), support results of traditional stream surveys and allow researchers to test specific hypotheses generated by field studies (Lamberti and Steinman 1993).

Diatoms are routinely used to assess water quality, and the literature suggests that they are excellent biological indicators for many types of pollution in aquatic systems (Lowe 1974, Patrick and Palavage 1994, Kelly et al. 1995). However, diatom communities are extremely sensitive to natural variation in abiotic factors, including current (McIntire 1966, Horner and Welch 1981, Korte and Blinn 1983, Lamb and Lowe 1987, Peterson and Stevenson 1990, Poff et al. 1990), light (Shortreed and Stockner 1983, Steinman and McIntire 1987, Stevenson et al. 1991, DeNicola et al. 1992), and nutrients (Marcus 1980, Bothwell 1988). It is well established that longitudinal variation (e.g., from upstream to downstream) in these and other physicochemical characteristics significantly influence benthic communities (Vannote et al. 1980). However, unlike research on longitudinal variation in benthic mac-

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roinvertebrate communities (Vannote et al. 1980, Minshall et al. 1983, Minshall et al. 1985, Bruns and Minshall 1985, Perry and Schaeffer 1987), little information is available on the longitudinal distribution of diatoms in streams. The few studies published indicate that some algal species are restricted to upstream or downstream reaches, but it has been difficult to find clear longitudinal patterns based on algal species composition (Kawecka 1971, Ward 1986, Molloy 1992).

Rocky Mountain streams of Colorado provide an excellent opportunity to investigate longitudinal variation in diatom communities because they exhibit marked altitudinal gradients, often dropping several thousand meters across distinct vegetation and climatological zones (Ward 1986). Communities in high elevation streams are often exposed to extremely harsh winter conditions and intense scouring. Consequently, high-elevation streams possess simple two-dimensional periphyton communities dominated by small, early successional species (e.g., *Achnanthes minutissima* and *Fragilaria vaucheriae*), which are regulated by frequent disturbance (Oemke and Burton 1986). In contrast, low-elevation streams have warmer temperatures, higher nutrients, and rarely produce frazil ice in the winter. These downstream sites are less physically disturbed, have longer growing seasons, and are characterized by mature, physiognomically complex periphyton communities.

Many of the mountain streams in Colorado are also polluted by heavy metals from abandoned mines (Smith 1987, Clements 1994, Clements and Kiffney 1995), the effects of which may persist for many kilometers downstream (Moore et al. 1991, Clements 1994). Dilution by tributaries, binding of metals to sediments, and uptake by organisms create a gradient of metal concentrations from upstream to downstream, which is superimposed on the natural longitudinal gradient of the stream. Assessing effects of heavy metals on these high gradient streams is complicated by longitudinal changes in stream structure and function, which may occur over relatively short distances (Clements 1994, Clements and Kiffney 1995).

Species life history traits are influenced by natural disturbance regimes (Odum 1969, Pianka 1970), which may also vary from upstream to downstream. Headwater streams exhibit relatively small fluctuations in water chemistry and temperature, which are hypothesized to be important factors influencing benthic community structure (Ward and Stanford 1983). Species inhabiting headwater streams may be more susceptible to anthropogenic disturbance than species adapted to the fluctuating conditions in mid-order streams (Ward and Stanford 1983, Clements and Kiffney 1995). Alternatively, periphyton communities in high gradient, headwater streams experience greater turbulence and shear stress, especially during spring runoff. These conditions may have a dramatic influence on morphological growth forms and life history characteristics of diatoms (Oemke and Burton 1986, Peterson and Stevenson 1990, Molloy 1992). We hypothesize that species from these physically harsh environments possess traits that confer tolerance to both natural and anthropogenic disturbance.

In this study we used field biomonitoring and laboratory experiments to examine the responses of diatom communities to heavy metals in Colorado Rocky Mountain streams. The specific objectives of the study were: (1) to describe the natural longitudinal variation in diatom communities in an unpolluted stream; (2) to investigate the confounding effects of longitudinal variation in community structure and morphological growth forms on stream biomonitoring studies; and (3) to compare responses of diatoms observed at metal-polluted sites to those measured experimentally in stream microcosms.

Methods

Longitudinal variation in the Cache la Poudre River

Natural longitudinal variation of epilithic diatom communities was investigated in the Cache la Poudre River, a relatively pristine stream that originates from Poudre Lake (elevation 3267 m) in Rocky Mountain National Park, Colorado (Fig. 1). The stream flows freely through the Cache la Poudre Canyon for ~100 km before becoming a plains river east of Fort Collins (elevation ~1500 m). The river has a similar topology to other western streams (Ward 1986) and shows typical changes in stream width (7–24 m), temperature (8.9–20.5°C), and water chemistry from upstream to downstream (Table 1).

Periphyton samples were collected from the Cache la Poudre River in August 1992. To reduce sampling variability, all samples were collected from large cobble substrate (128–256 mm) in shallow, unshaded riffle areas (~50 cm deep) with similar current velocity, substrate composition, and canopy cover. Individual stones were gently removed from the stream and periphyton samples were collected using a neoprene cuffed sampler (7.1 cm^2), which was pressed firmly on the smooth upper surface of the substrate. A small amount of water was placed in the sampler, periphyton was loosened from the rock surface with a battery-operated dental plaque remover (model 4726, Braun North America, Woburn, Massachusetts), and the resulting slurry was removed with a pipette. Periphyton removed from three rocks was combined, diluted to a standard volume of 100 mL, and preserved in 1% lugol's solution. Three samples were collected from each site.

In the laboratory, periphyton samples were homogenized for 60 s in a blender. Cell densities were enumerated by counting live diatom cells in 50 random fields, defined by a Whipple grid in the ocular lens, at 400× magnification using a Palmer-Maloney counting chamber. Data are reported as live cells per unit area of substrate sampled.

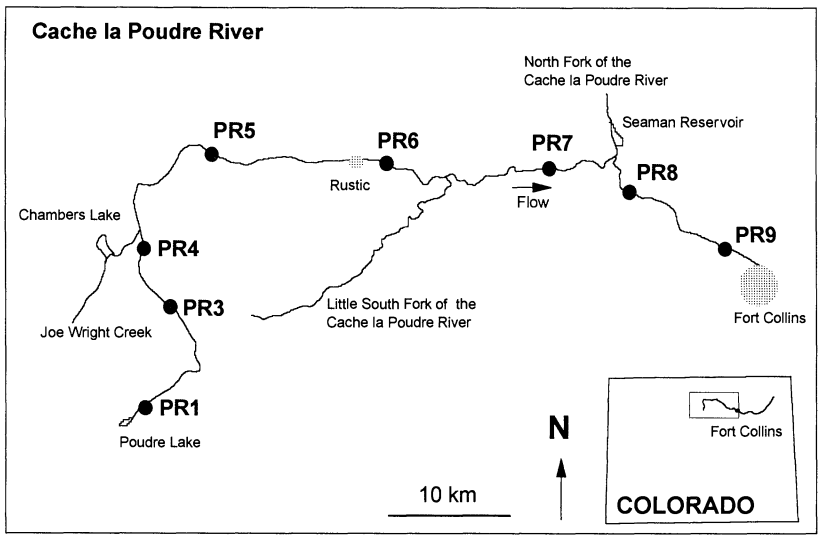

FIG. 1. Map of sampling locations on the Cache la Poudre River (Colorado) in August 1992.

Relative abundance of diatom species was determined by examination of permanent slides of cleaned diatom frustules using phase contrast microscopy at 1000× magnification. Diatom frustules were counted in lengthwise strips across the mount, defined by a Whipple grid, until at least 250 cells were counted. Studies have shown that this number is sufficient to characterize the dominant species in a community (Johnson and Lowe 1995).

Water temperature, dissolved oxygen (YSI, model 57), pH (Jenco, model no. 6009), and conductivity (YSI, model 33) were measured at all sites in the field. Water samples for alkalinity and hardness were placed on ice, returned to the laboratory, and analyzed by titration following standard methods (APHA 1989).

Community responses to metals in the field

To assess longitudinal variation in metal-polluted streams, diatom community structure was measured at reference, impacted, and recovery sites (n = 34) in six Rocky Mountain streams (Arkansas River, Blue River, Chalk Creek, Eagle River, Snake River, and the South Platte River) in north-central Colorado (Fig. 2). Between four and seven stations were located on each stream, upstream and downstream from known metal inputs. All streams were qualitatively similar and characteristic of streams in the southern Rocky Mountain ecoregion. Each stream originates from small headwaters above treeline, high on the Continental Divide, and is characterized as cold, clear, high-gradient, and oligotrophic. Additionally, each stream is polluted by heavy metals, usually from a discrete source in its upper reaches, and metal concentrations decrease downstream (Clements and Kiffney 1995).

Periphyton and water samples were collected in August 1992 from each station using the same procedures described previously. Water samples for determination of total recoverable metals (Cd, Cu, Zn) were preserved by acidification with nitric acid to a pH of <2. Samples for dissolved metals were filtered in the field through a 0.45 μm filter before acidification. Metals were analyzed using flame or furnace atomic adsorption spectrophotometry at the Colorado Division of Wildlife, Fort Collins, Colorado. Analytical limits of detection for Cd, Cu, and Zn were 0.1, 1.0, and 5.0 μg/L, respectively.

Community response to metals in experimental streams

To test the hypothesis that differences in periphyton community composition influenced responses to heavy metals, we conducted experiments in stream microcosms. Periphyton communities were collected from Cache la Poudre River stations PR5 and PR9 (Fig. 1) using unglazed ceramic tiles (5 × 5 cm) secured to a 0.6-m^2 section of concrete. Substrate composition, gradient, flow, and canopy cover were similar at the two sites; however, the lower elevation site (PR9) had greater temperature, hardness, alkalinity, and conductivity (see Table 1). Because of differences in abundance of dominant taxa between these sites, this approach allowed us to compare effects of metals on two distinct periphyton communities from the same stream.

Tiles (n = 108) were placed at each site in unshaded riffle areas, ~50 cm deep on 7 September 1993. Tiles were removed from the stream after 42 d colonization and transported on shelves in large coolers to the Stream Research Laboratory at Colorado State University. Descriptions of the experimental streams in this facility have been published previously (Clements et al. 1989, Kiffney and Clements 1994). The 18 oval, flow-through streams (76 × 46 × 14 cm) are located in a greenhouse and are supplied with untreated water from nearby Horsetooth Reservoir, a deep, cold-water

TABLE 1. Characteristics at sites sampled on the Cache la Poudre River (Colorado), August 1992.

Site	Elevation (m)	pH	Temperature (°C)	Conductivity (μS/cm)	Alkalinity (mg/L)	Hardness (mg/L)	Dissolved oxygen (mg/L)	Density (cells/mm²)	Richness	Diversity
PR1	3267	8.44	8.9	92	40	37	9.5	201	32	2.70
PR3	2938	7.66	9.4	47	13	12	8.0	81	27	1.74
PR4	2566	7.52	9.4	42	13	14	8.0	167	22	1.49
PR5	2340	7.91	12.8	48	19	16	9.0	29	34	2.28
PR6	2133	8.18	15.0	59	23	20	10.0	220	26	2.32
PR7	1780	8.07	15.5	54	20	18	10.0	14	28	2.03
PR8	1603	8.52	18.3	115	46	42	8.0	167	36	2.61
PR9	1536	8.67	20.5	227	73	95	9.5	11 190	34	2.87

Note: Locations of sampling stations are shown in Fig. 1.

impoundment. Water was delivered to each stream at a rate of 1.0 L/min, resulting in a turnover time of ~13 min. Current in each stream was maintained by motor-driven paddlewheels at 30 cm/s.

Nine tiles were randomly assigned to each of the 18 experimental streams. After a 24-h acclimation period, streams were randomly assigned to one of three metal treatments: 0.0x, 0.5x, and 5.0x, where x was a mixture of Cd, Cu, and Zn at 1.1 μg/L, 12 μg/L and 110 μg/L, respectively. Metal levels in the 0.5x treatment were comparable to the U.S. EPA chronic criterion values (at water hardness of 30 mg/L), whereas levels in the 5.0x treatment are within the range of concentrations measured at highly polluted sites in Colorado streams (Clements and Kiffney 1995). Metals were delivered to treated streams by peristaltic pumps at a rate of 10 mL/min from stock solutions prepared in 25 L carboys. The final experimental design was a completely randomized 2 × 3 factorial design, with location (two levels) and metal treatment (three levels) as the main effects. Each treatment combination had three replicates, with individual stream microcosms as the unit of replication.

After 10 d, periphyton was removed from three ran-

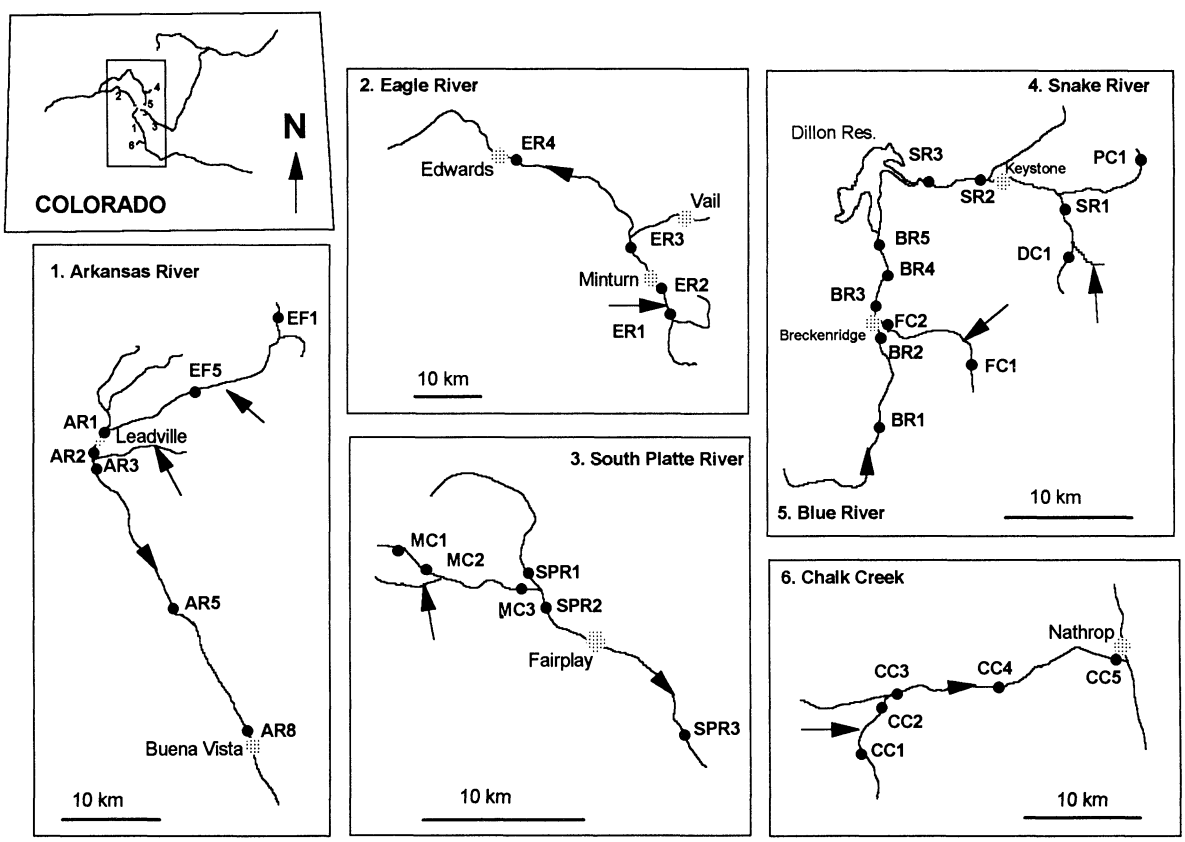

FIG. 2. Map of sampling stations in the metal-polluted streams (Colorado) in August 1992. Arrows indicate the locations of known metal inputs to each stream.

domly selected tiles with a sharp edged plastic scraper and a hard bristled toothbrush. Material from three tiles was combined and diluted to a standard volume of 100 mL. This procedure was repeated for the six remaining tiles and these subsamples were used for species enumeration, determination of ash-free dry mass (AFDM), and metal bioaccumulation (not reported here) for each stream.

Water samples were collected from both field sites at the beginning and end of the colonization period, and on days 0, 2, 6, and 10 from the experimental streams. Routine water quality parameters (temperature, dissolved oxygen, conductivity, hardness, alkalinity, and pH) and heavy metal concentrations were measured. Additional water samples were collected from the two field sites and the experimental streams at the end of the experiment for analysis of dissolved organic carbon.

Biomass was estimated by measuring ash-free dry mass (AFDM), using standard methods (APHA 1989) and the recommendations of Aloi (1990). Species were enumerated in a similar manner to the field study, with one important exception. Most field studies assume that all species within a community will have a similar ratio of live : dead cells, and that an accurate estimate of their relative abundance can be obtained from examination of the cleared diatoms. However, in short-term toxicity tests differential sensitivity of individual species may result in different ratios of live : dead cells. Accordingly, counts obtained from the permanent mounts were corrected at the generic level using live: dead ratios of dominant genera obtained by counting at least 250 cells in wet mounts at 1000× magnification. We distinguished live diatoms from dead diatoms by visual inspection (live diatoms appeared more lustrous and translucent, with the cytoplasm completely occupying the cell).

Statistical analyses

All statistical analyses were performed using a PC version of Statistical Analysis Systems (SAS 1988). In the study of the six metal-polluted streams, each of the 34 stations was assigned to one of four metal categories based on the concentration of Zn (total recoverable), the dominant metal sampled at all sites. Concentrations at background, low, medium, and high Zn stations were <20, 21–50, 51–200, and >200 μg Zn/L, respectively. These four categories were chosen because they bracketed the U.S. EPA chronic criterion value for Zn (50 μg/L) and because they resulted in an approximately equal number of stations within each category.

Fixed effects, one-way ANOVA was used to test for differences between Zn categories in the field study. To check the assumptions of normality and heterogeneity of variance, we examined scatter plots of residuals and performed F_{max} tests. Where necessary, densities and relative abundance of dominant taxa were transformed using the log and arcsine transformation, respectively. Canonical discriminant analysis (CDA) was used to examine overlap and separation of stations within each category, based on proportions of the 10 dominant species (e.g., those species that comprised >1% total abundance). Loading coefficients were examined to determine which species were responsible for the separation of Zn categories along each canonical axis. Stepwise multiple regression analysis was conducted to examine the relationship between diatom community measures and abiotic variables (Zn concentration, water hardness, conductivity, alkalinity, dissolved oxygen, pH, and elevation).

We used two-way ANOVA in the microcosm study to test the effects of site (PR5 and PR9), metal treatment (0.0x, 0.5x, and 5.0x), and the interaction between site and metal treatment for diatom community variables. If the overall F test was significant ($P < 0.05$), Ryan's Q multiple comparison test was used to test for differences between metal treatments. This test is recommended because of its strict control of the experimentwise error rate (Day and Quinn 1989).

RESULTS

Longitudinal variation in the Cache la Poudre River

Except for station PR1, hardness, alkalinity, conductivity, pH, and temperature were greater at the downstream stations compared to the upstream stations (Table 1). Water chemistry at stations PR8 and PR9 was distinct from other stations, as conductivity, alkalinity, and water hardness increased 4× between stations PR7 and PR9. Due to the influence of Poudre Lake, water chemistry at PR1 was anomalous relative to other upstream stations and was more similar to lower elevation sites.

Differences in water chemistry between upstream sites (PR3 to PR7) and downstream sites (PR8 and PR9) were reflected in changes in diatom community composition (Table 1; Fig. 3). Cell density was much greater at the lowest elevation site (PR9) compared to upstream sites. Most upstream sites were dominated by *Achnanthes minutissima, Fragilaria vaucheriae, Hannaea arcus,* and/or *Synedra rumpens.* Relative abundance of *A. minutissima* and *F. vaucheriae* decreased downstream, and stations PR8 and PR9 were characterized by higher species diversity and greater abundance of *Cymbella, Gomphonema, Navicula,* and *Melosira varians.* The diatom community at PR1 was more diverse and structurally different from other upstream sites, probably due to the influence of Poudre Lake.

Community responses to metals in the field

Routine water quality characteristics measured in the six metal-polluted streams are shown in Table 2. Other habitat characteristics of these sites (e.g., elevation, current velocity, depth, canopy cover) have been reported previously (Clements and Kiffney 1995). All streams were cold, oligotrophic, and characterized by

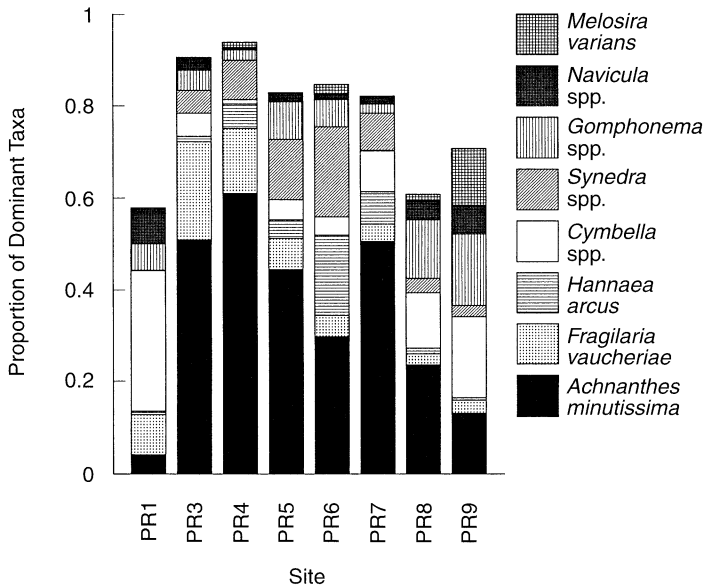

FIG. 3. Proportional abundance of dominant taxa at sites sampled on the Cache la Poudre River (Colorado) in August 1992.

low to moderate conductivity (range: 40–240 μS/cm), hardness (range: 10–126 mg/L), and alkalinity (range: 10–119 mg/L). Temperature, conductivity, alkalinity, and hardness increased from upstream to downstream in most streams and were inversely correlated with elevation (temperature, $r = -0.52$, $P = 0.0016$; conductivity, $r = -0.52$, $P = 0.0015$; alkalinity, $r = -0.36$, $P = 0.036$ and hardness, $r = -0.41$, $P = 0.015$). The pH was neutral or slightly alkaline at all sites, with the lowest values (6.7) measured in the Snake River.

Zn was the dominant metal measured at all polluted sites and ranged from below detection (<5 μg/L) to 1735 μg/L. Cu and Cd were present at some polluted sites, although usually in much lower concentrations. Because we observed little difference between total and dissolved Zn concentrations during the low flow conditions when this study was conducted, only total Zn concentration was used in these analyses.

Over 200 species of diatoms were collected from the study area. Ten dominant taxa (*Achnanthes minutissima, Fragilaria vaucheriae, F. pinnata, F. constreuens, Hannaea arcus, Cymbella minuta, Nitzschia palea, Synedra rumpens, S. ulna, Cocconeis placentula*) typically represented >80% of the diatom community at all stations. Although a few other species were found in high relative abundance, these species were restricted to a few sites or found only within one river basin.

Differences in Zn concentrations among background, low, medium, and high Zn sites were highly significant (Table 3). Species diversity was significantly lower and relative abundance of *Achnanthes minutissima* was significantly greater at the high Zn sites. Cell density, species richness, and abundance of other dominant taxa did not vary significantly among Zn categories ($P > 0.05$).

Canonical discriminant analysis, based on relative abundance of the 10 dominant species, clearly separated the high Zn sites from background, low, and medium Zn sites (Fig. 4). Wilk's lambda statistic indicated that differences between Zn categories were highly significant ($F_{3,30} = 2.32$; $P = 0.0037$). Canonical variables one and two explained 55 and 36% of the total variation, respectively. Inspection of loading coefficients indicated that separation along canonical variable one resulted primarily from greater abundance of *Achnanthes minutissima* at high Zn sites. Separation along canonical variable two resulted from greater abundance of *Fragilaria vaucheriae* and *Nitzschia palea* at medium Zn sites.

Stepwise multiple regression showed that Zn concentration, elevation, pH, and conductivity explained most of the variation in diatom community structure in the metal-polluted streams (Table 4). Zn concentration was the most important predictor variable for species richness and diversity, and explained 23 and 33% of the total variation in these measures, respectively. Zn was also included in the stepwise regression models for relative abundance of *Achnanthes minutissima* and *Fragilaria vaucheriae*; however, unlike the effects on species richness and diversity, Zn had a positive effect on relative abundance of these taxa. The positive relationship between Zn concentration and relative abundance of *A. minutissima* and *F. vaucheriae* resulted from the elimination of metal-sensitive diatoms at polluted stations. Zn was not included in the stepwise multiple regression model for any other dominant species.

Community response to metals in experimental streams

Periphyton communities on tiles collected from stations PR5 and PR9 for the microcosm study were mark-

TABLE 2. Physicochemical characteristics and diatom community variables from the six metal-polluted streams in August 1992.

Stream/ Station	pH	Dissolved oxygen (mg/L)	Conductivity (μS/cm)	Temperature (°C)	Alkalinity (mg/L)	Hardness (mg/L)	[Zn] (μg/L)	Species richness	Diversity H'	Proportion *Achnanthes*
Eagle River										
ER1	8.5	8.4	180	11	119	122	2	35.3	2.27	0.37
ER2	8.0	8.0	145	14	52	84	407	21.7	1.25	0.48
ER3	8.0	8.0	150	15	54	86	336	20.7	1.15	0.35
ER4	8.8	7.8	240	18	77	126	104	16.7	1.62	0.02
Blue River										
FC1	7.8	8.6	55	9	30	42	7	19.0	1.70	0.17
FC2	7.4	8.8	130	8	41	84	1735	5.7	0.63	0.76
BR1	7.5	8.2	75	11	43	50	3	26.0	2.05	0.44
BR2	8.1	8.1	85	12	52	60	9	27.3	1.98	0.43
BR3	7.9	8.3	100	12	42	66	691	14.3	1.04	0.60
BR4	7.3	8.3	105	11	52	64	72	22.0	2.19	0.22
BR5	7.8	8.4	100	9	51	64	56	21.3	2.10	0.17
Snake River										
DC1	7.5	7.6	65	10	10	10	13	34.0	2.20	0.43
PC1	7.3	8.1	70	8	17	48	72	23.7	2.06	0.36
SR1	6.7	7.5	100	11	11	52	364	28.7	1.90	0.44
SR2	6.8	7.6	90	14	16	48	399	29.3	1.88	0.53
SR3	6.8	7.3	90	16	18	48	276	10.7	0.85	0.61
Arkansas River										
EF1	7.8	7.4	90	12	32	48	39	14.0	1.40	0.36
EF5	8.4	8.8	170	8	70	108	28	26.3	2.18	0.35
AR1	8.2	7.6	155	13	64	94	24	23.0	1.83	0.51
AR2	8.0	7.6	150	15	67	92	26	26.3	1.88	0.50
AR3	7.9	7.6	170	15	69	100	181	27.0	2.02	0.46
AR5	8.8	8.2	170	17	65	98	79	22.7	1.94	0.13
AR8	7.8	7.3	100	18	34	44	27	27.0	2.10	0.33
Chalk Creek										
CC1	8.2	8.0	55	8	23	32	31	38.0	2.38	0.31
CC2	7.3	7.8	75	10	23	40	452	22.0	1.43	0.64
CC3	7.3	7.6	75	11	28	36	224	23.3	1.37	0.68
CC4	7.4	7.6	80	15	31	42	76	25.0	1.75	0.44
CC5	7.9	6.8	140	21	50	52	26	42.0	2.83	0.21
South Platte River										
MC1	7.8	7.6	40	11	17	18	2	20.7	1.53	0.57
MC2	7.9	7.3	75	14	35	40	12	7.0	0.76	0.41
MC3	8.3	7.2	155	16	69	96	168	7.7	0.80	0.12
SPR1	8.5	7.5	130	15	64	80	24	18.7	1.66	0.12
SPR2	8.7	7.3	150	16	62	86	52	11.3	0.98	0.15
SPR3	8.2	6.8	210	20	89	110	10	24.0	1.89	0.10

Note: Locations of sampling stations are shown in Fig. 2.

TABLE 3. Zinc concentration and diatom community variables at a range of Zn sites in the six metal-polluted streams (August 1992).

Variable	Background	Zn class: Low	Zn class: Medium	Zn class: High	F value†
Number of stations	8	8	9	9	
Zn concentration	7.3[a] (0.6)	28.1[b] (0.8)	95.5[c] (5.7)	542.6[d] (51.8)	94.90***
Cell density (cells/mm^2)	2905 (1471)	4095 (3597)	6263 (4789)	1539 (1293)	0.73 ns
Species richness	24.1 (3.2)	26.9 (3.3)	19.7 (2.2)	19.6 (2.7)	1.61 ns
Species diversity	1.80[a] (0.17)	2.03[a] (0.16)	1.72[ab] (0.17)	1.28[b] (0.14)	3.94*
Relative abundance of *Achnanthes minutissima*	0.37[a] (0.05)	0.34[a] (0.05)	0.23[a] (0.05)	0.56[b] (0.04)	7.97**

Notes: Results of one-way ANOVA are also shown. Means with the same letter were not significantly different. Standard errors are shown in parentheses. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant.

† df = 3,30.

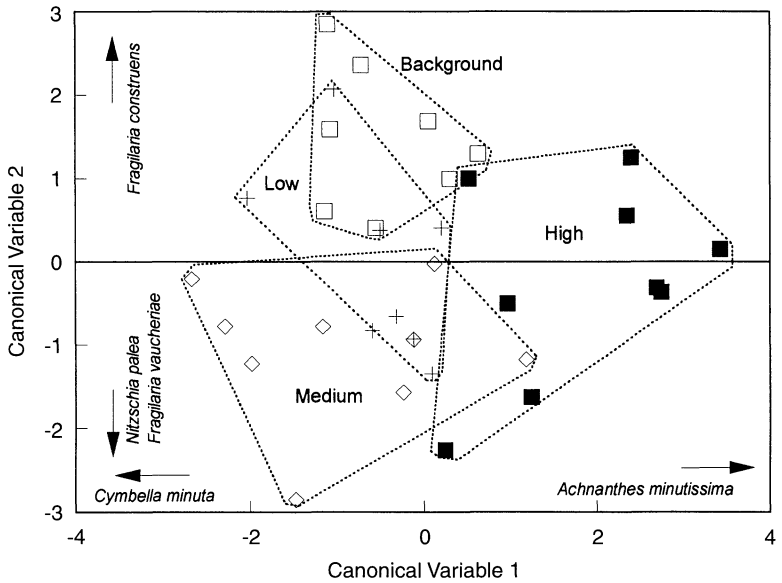

FIG. 4. Canonical discriminant analysis showing separation and overlap of background, low, medium, and high Zn stations in metal-polluted streams. The analysis was based on the relative abundance of the 10 dominant species, e.g., those species that comprised >1% total abundance. The primary taxa responsible for separation along each canonical axis is also shown.

edly different (Table 5). Cell density, species richness, and species diversity were lower at the upstream site, which was dominated by *Achnanthes minutissima, Fragilaria vaucheriae,* and *Gomphonema* spp. In contrast, the downstream community was physiognomically more complex and characterized by colonies of *Diatoma vulgare,* long filaments of *Melosira,* and several species of *Nitzschia, Navicula, Gomphonema,* and *Cymbella.*

Physicochemical characteristics in the experimental streams were within the range of those observed at stations PR5 and PR9 in the Cache la Poudre River, with the exception of temperature that was higher in the experimental streams (Table 6). Hardness, alkalinity, conductivity, pH, temperature, and dissolved organic carbon (DOC) were greater at PR9 compared to PR5. Mean concentrations of Zn, Cu, and Cd at the two field sites and in control experimental streams were low or below detection. Mean Zn and Cd concentrations approximated target concentrations in treated streams. Mean Cu concentrations approximated the target levels in 0.5x streams, but were only about half of the target value in the 5.0x streams. Total recoverable and dissolved metal concentrations were similar on all sampling occasions.

Community level responses of diatoms to metals in experimental streams varied between sites and among metal treatments (Fig. 5). Significant metal and site effects were observed for all variables except cell density. Metal × site interaction terms were significant for all variables except species richness, indicating that effects of heavy metals on periphyton communities differed between stations. For example, cell density was similar between sites in control streams, but the re-

TABLE 4. Results of stepwise multiple regression analyis showing the relationships between periphyton community variables, Zn concentration, and other abiotic variables in the six metal-polluted streams (August 1992).

Dependent variable	df	Overall F value	Overall r^2	Overall P value	Model variable	Partial r^2	P value
Species richness	3,30	5.43	0.35	0.0042	(−)Zn	0.23	0.0036
					(−)Elevation	0.06	0.1230
					(−)pH	0.06	0.1100
Shannon-Weiner diversity	1,32	15.49	0.33	0.0004	(−)Zn	0.33	0.0004
Relative abundance of *Achnanthes minutissima*	3,30	11.56	0.53	0.0001	(−)pH	0.36	0.0002
					(+)Zn	0.13	0.0072
					(−)Conductivity	0.04	0.1200
Relative abundance of *Fragilaria vaucheriae*	3,30	3.4	0.25	0.0300	(+)Zn	0.13	0.0350
					(+)pH	0.07	0.1230
					(+)Elevation	0.05	0.1460

Note: The table shows the F values and r^2 for the complete model, r^2 for each independent variable, and the directional effect (+, −) of the independent variable.

TABLE 5. Diatom community variables and relative abundance of dominant taxa on tile substrate at stations PR5 and PR9 in October 1993.

Variable	PR5	PR9
Cell density (cells/mm^2)	13 380	62 500
Species richness	23	38
Shannon diversity	2.01	2.81
Achnanthes minutissima	45.4	25.6
Achnanthes deflexa	–	4.3
Cocconeis placentula	–	3.9
Cymbella minuta	1.4	8.3
Cymbella sinuata	–	4.3
Cymbella turgidula	1.1	3.1
Diatoma vulgare	–	8.7
Fragilaria vaucheriae	10.8	+
Gomphonema parvulum	12.8	3.5
Gomphonema subclavatum	8.0	5.6
Hannaea arcus	2.8	5.6
Melosira sp.	2.0	8.1
Navicula cryptocephela	–	3.3
Nitzschia sp.	1.1	5.8
Synedra sp.	2.5	3.1

Notes: Data were collected on the same day that tiles were collected for the experimental stream study. Minus signs (–) denote absent; plus signs (+) denote <1.0%.

duction in cell density was greater for communities from the downstream site (PR9) in the 0.5x streams.

Exposure to heavy metals significantly altered densities of dominant taxa at both sites, and the 5.0x treatment was acutely toxic to all diatom species (Fig. 6). Abundance of *Achnanthes minutissima* increased by ~60% in the 0.5x treatments compared to controls for both sites; however, the responses of other taxa varied between sites. *Fragilaria vaucheriae* and *Cymbella minuta* were relatively tolerant to metals, and populations from the downstream site (PR9) increased in the 0.5x streams. *Nitzschia palea* and *Synedra rumpens* from both sites exhibited intermediate sensitivity to metals. The two most sensitive species, *Diatoma vulgare* and *Melosira varians,* were found only at the downstream site. Large colonies of these species detached from the substrate soon after dosing was initiated, and both species were eliminated from treated streams within 24 h.

DISCUSSION

Longitudinal variation in the Cache la Poudre River

Changes in community composition observed in the Cache la Poudre River from upstream to downstream were similar to the successional sequence observed in western streams following physical disturbance (Fisher et al. 1982). *Achnanthes minutissima,* which was most abundant at upstream sites, is often dominant immediately after scour events (Peterson and Stevenson 1990, Stevenson 1990). *Fragilaria vaucheriae* and *Hannaea arcus* showed similar trends and often dominate in high altitude streams or in early successional communities (Horner and Welch 1981, Poff et al. 1990). Conversely, species with greater vertical orientation that are more common in later successional communities (e.g., *Navicula, Gomphonema, Cymbella, Melosira,* and *Nitzschia*) increased in abundance downstream.

Although some researchers have proposed the general applicability of this periphyton successional sequence on a temporal scale (Hoagland et al. 1982, Korte and Blinn 1983), few studies have found clear spatial patterns and none have related succession to longitudinal patterns in physicochemical characteristics (Jones and Barrington 1985, Moss and Bryant 1985). Molloy (1992) found a relationship between morphological growth forms and stream size in three rivers in Kentucky. Small adnate species (*Achnanthes* spp.) were more abundant in headwater streams, whereas centric and filamentous species occurred with greater frequency downstream. Ward (1986) found similar trends in Saint Vrain Creek, Colorado, where later successional species were found in higher abundance at downstream sites.

The complex role of abiotic variables in determining periphyton community structure is clearly illustrated at PR1. We expected this high elevation site would have the greatest proportion of early colonizing species and be dominated by *Achnanthes minutissima.* However, species composition at PR1 was similar to that found at PR9, the furthest downstream site. The hydrologic

TABLE 6. Physicochemical characteristics of field sites (PR5 and PR9) and in experimental streams in October 1993.

	Field sites		Experimental streams		
Variable	PR5	PR9	0.0x	0.5x	5.0x
Temperature (°C)	2	7	12	12	12
Dissolved oxygen (mg/L)	12.4	10.1	8.1	7.8	7.3
Conductivity (μS/cm)	38	115	81	81	81
Hardness (mg/L $CaCO_3$)	18	70	31	31	31
Alkalinity (mg/L $CaCO_3$)	20	57	32	32	32
pH	7.6	8.8	7.6	7.5	7.3
Dissolved organic carbon (mg/L)	2	272	2	2	2
Zinc (μg/L)	7.0	6.0	5.6 (1.4)	54.0 (12.5)	547.0 (218.0)
Copper (μg/L)	bd†	bd	1.0 (0.4)	6.7 (1.5)	36.0 (17.0)
Cadmium (μg/L)	0.18	bd	bd	0.8 (0.2)	6.7 (2.9)

Notes: Field data were collected on the same day that tiles were collected for the experimental stream study. Standard errors are shown in parentheses.

† bd = below detection.

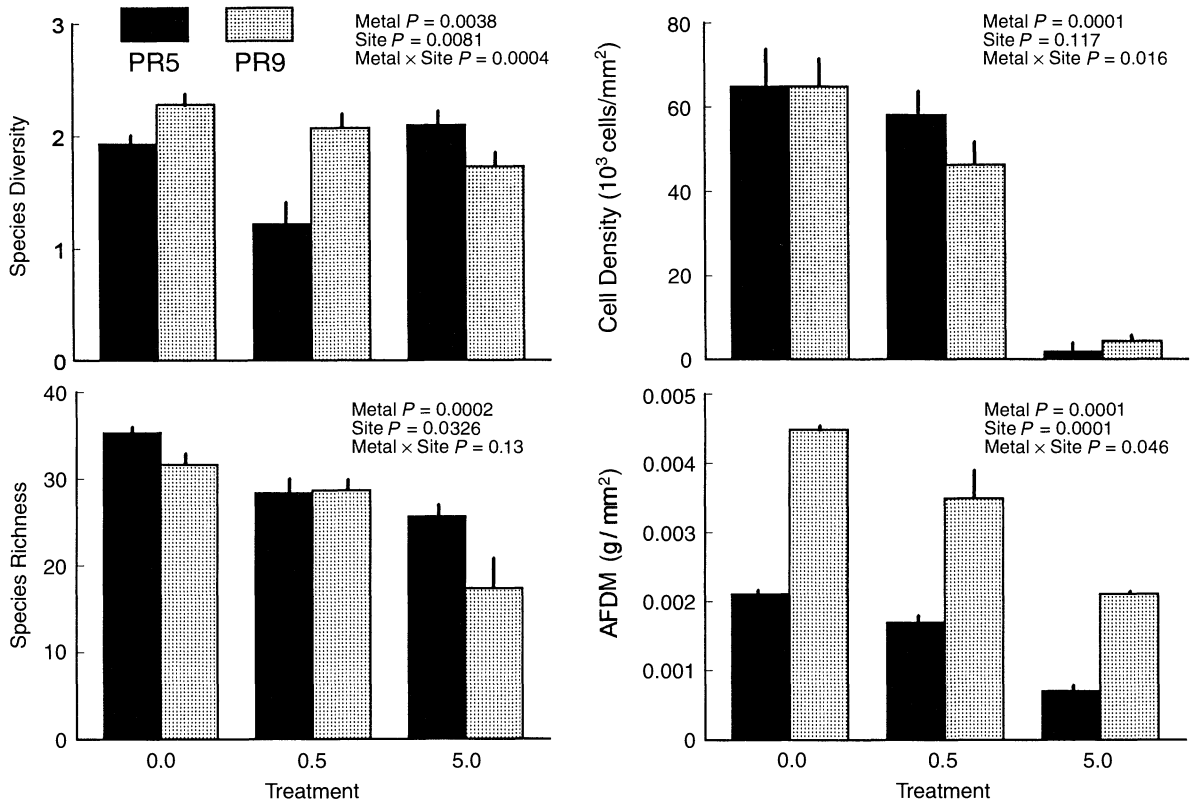

FIG. 5. Mean (+1 SE) Shannon-Wiener diversity, species richness, cell density, and ash-free dry mass (AFDM) of periphyton communities in 0.0x, 0.5x, and 5.0x treatments from the experimental stream study. Results of two-way ANOVA (site × metals treatment) are shown in each panel.

regime and water quality at PR1 were influenced by Poudre Lake, 0.3 km upstream from the sampling site. We hypothesize that the stable water quality conditions and flow regime at PR1 created an environment that was favorable to development of a midsuccessional community.

In summary, diatom communities are sensitive to a host of environmental variables that change along a stream's longitudinal gradient. We hypothesize that these physicochemical conditions constitute a general productivity gradient from upstream to downstream, which influences ecological succession and produces later successional communities at downstream sites. We suggest that this natural variation in periphyton community structure may complicate biomonitoring studies in Colorado mountain streams where physicochemical changes occur over relatively short distances.

Community responses to metals in the field

Most field studies that investigate the response of aquatic communities to contaminants are confined to a single stream, where upstream reference sites are compared to downstream polluted and recovery sites. Conclusions drawn from these studies are applicable only to the specific stream under study (Hurlbert 1984, Feldman and Connor 1992, Clements and Kiffney 1995). Although "metal treatments" in the six metal-polluted streams in our study were not assigned randomly, we feel that our study design is an improvement over single stream studies and that our results have more general applicability.

Periphyton communities in Colorado mountain streams showed little response to metal levels at or below the U.S. EPA water quality criterion for Zn (50 μg/L), a finding that was also reported for macroinvertebrate communities (Clements and Kiffney 1995). One-way ANOVA and canonical discriminant analysis (CDA) indicated that differences in community composition were apparent at sites where Zn levels exceeded 200 μg/L. Communities in these streams were dominated by *Achnanthes minutissima* and *Fragilaria vaucheriae,* a pattern found in other streams polluted by heavy metals (Leland and Carter 1984, Deniseger et al. 1986, Takamura et al. 1990, Kelly et al. 1995). We suggest that our results represent a general response of periphyton communities to mine drainage, which are applicable to other Colorado streams polluted by heavy metals.

Results of our study of metal-polluted streams are limited because sampling was restricted to late summer, low-flow conditions. Diatom community composition in streams often shows high temporal variation (Ste-

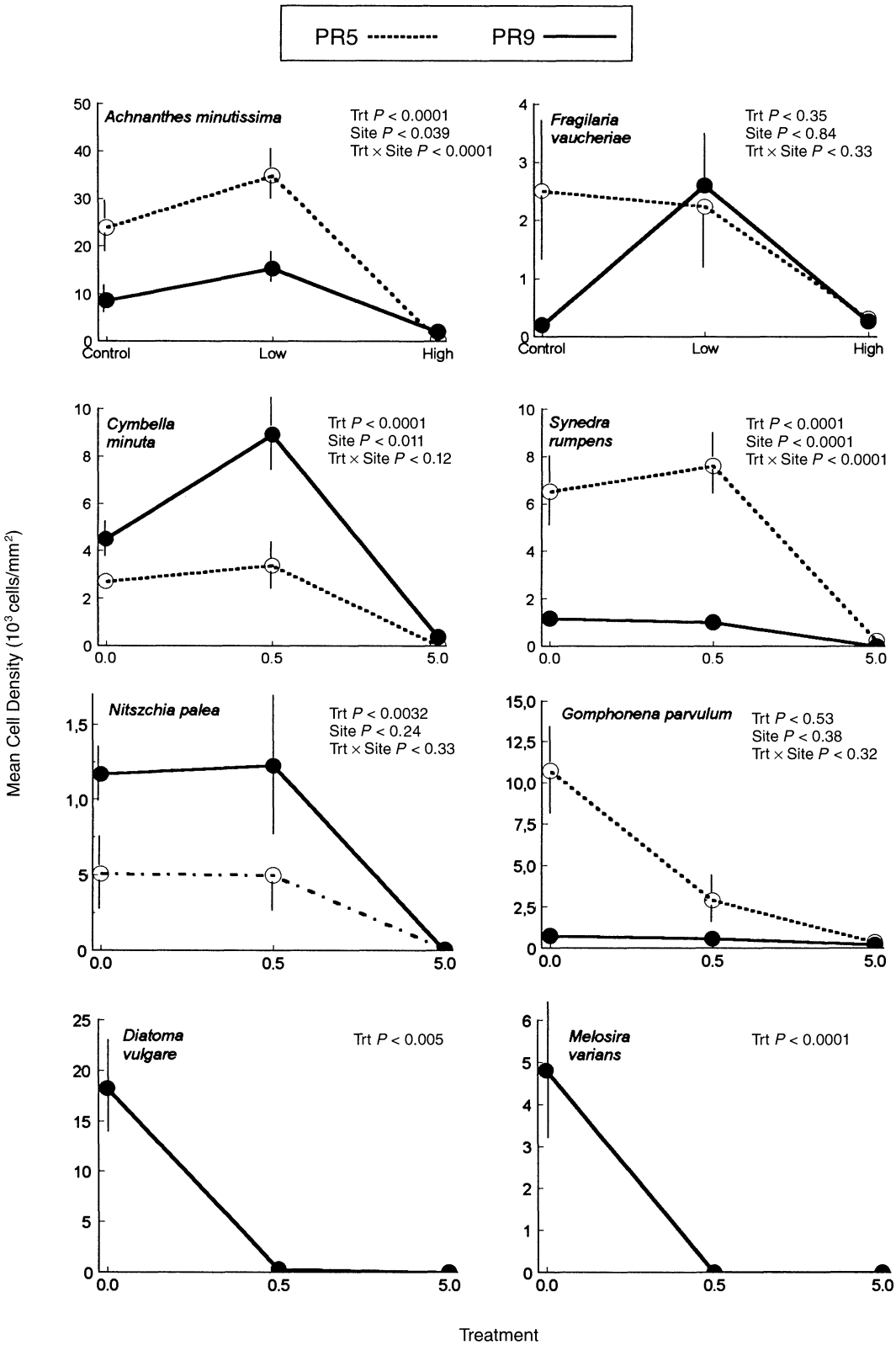

FIG. 6. Mean (±1 SE) abundance of dominant species in 0.0x, 0.5x, and 5.0x treatments from the experimental stream study. Results of two-way ANOVA (site × metals treatment) are shown in each panel.

venson 1990), which may complicate the use of diatoms as indicators of water quality (Pan et al. 1996). It is likely that seasonal variation in periphyton community composition and physicochemical characteristics will influence responses to heavy metals in Colorado streams. For example, we would expect that the early successional communities present in spring would be more tolerant of heavy metals than the late successional communities of summer. Seasonal variation in heavy metal concentrations could also influence periphyton community composition. Previous studies of Colorado's metal-polluted streams have reported the highest metal levels during spring runoff (Clements 1994). The influence of seasonal variation in diatom community composition and physicochemical characteristics could be examined by conducting microcosm experiments with spring and summer communities.

Results of our field study may have been influenced by the indirect effects of invertebrate grazers, particularly heptageniid mayflies. Our previous work has shown that heptageniids are highly sensitive to heavy metals and are usually absent in metal-polluted streams (Clements 1994, Clements and Kiffney 1995). It is possible that elimination of these metal-sensitive grazers may have altered diatom community composition in our study. In a review of grazer–periphyton interactions in streams, Feminella and Hawkins (1995) reported that the most consistent response of algal assemblages to grazers was reduced abundance of numerically dominant species, including *A. minutissima.* Therefore, the greater abundance of *A. minutissima* observed at metal-polluted sites in our study may have been influenced by reduced abundance of grazing mayflies. We suggest that additional experimental studies are necessary to understand the complex interactions between invertebrate grazers and heavy metal pollution in streams.

Community responses to metals in experimental streams

Results of the microcosm study support the hypothesis that effects of heavy metals were greater on communities collected from the downstream site. We suggest that differences in metal effects were a result of natural differences in community composition between sites. Communities from the upstream site were dominated by early successional species (*Achnanthes minutissima* and *Fragilaria vaucheriae*), which were relatively tolerant to metals. In contrast, downstream communities were dominated by colonial and filamentous species (*Melosira varians* and *Diatoma vulgare*), which were highly sensitive to metals. Although some diatom species collected from the downstream site were relatively tolerant to metals, *M. varians* and *D. vulgare* were eliminated in the 0.5x treatments. Differences in sensitivity to contaminants between locations, which have also been reported for benthic macroinvertebrate communities (Kiffney and Clements 1996), have important practical implications. If communities from low-elevation streams are naturally dominated by late successional species, then effects of metals on these communities will be greater. Consequently, it may be necessary to account for differences in sensitivity between locations when establishing water quality criteria to protect aquatic organisms (Kiffney and Clements 1996).

Some attempts have been made to relate morphological growth forms and reproductive strategies of algae to their ability to dominate disturbed habitats or during early stages of succession (Fisher et al. 1982, McCormick and Stevenson 1991, Stevenson et al. 1991, Molloy 1992, Peterson and Stevenson 1992). To our knowledge no attempts have been made to relate life history characteristics and morphological growth forms of algae to their tolerance of contaminants. We suggest that the same morphological and ecological characteristics that allow a species to dominate during early stages of primary succession also influence tolerance to heavy metals. In our field studies, communities from both reference sites and metal-polluted sites were similar and dominated by *Achnanthes minutissima* or *Fragilaria vaucheriae.* The natural dominance of these metal-tolerant species at reference sites may also explain why we did not observe large shifts in community composition at moderately polluted sites (e.g., where Zn levels were between 51 and 200 μg/L) in our study of metal-polluted streams. Our hypothesis that morphological and life history characteristics of diatoms influence tolerance to contaminants could be tested by exposing communities collected at different stages of succession to heavy metals. We predict that communities collected during the early stages of succession would be dominated by small, adnate species, which would be more tolerant to metals than those collected during later stages.

It may be possible to correct for the influence of natural longitudinal changes in community structure by developing predictive relationships between community composition and elevation from regional reference sites (sensu Fausch et al. 1984); however, because of the sensitivity of diatom communities to many abiotic variables, locating reference sites within adjacent river drainages may be difficult. Adjacent streams within the same watershed may have very different diatom assemblages because of differences in geology, water chemistry, and land use (Kutka and Richards 1996). Because routine biomonitoring studies cannot establish direct cause-and-effect relationships between community patterns and anthropogenic stressors, it is important to validate biological indicators experimentally. We suggest that integrating experimental studies with routine field biomonitoring will support the establishment of causal relationships between anthropogenic disturbances and community responses.

Conclusions

Despite the common use of diatoms as indicators of water quality, many of the relationships between spe-

cies abundance and specific water quality variables remain weak and untested. Cattaneo et al. (1993) concluded that the predictive power of diatom community models was limited because environmental variables included in analyses are inappropriate for the sampling scale and taxonomic level chosen. The use of diatoms as indicators of water quality is also hampered by a lack of information on how diatom assemblages respond across larger spatial scales (Kutka and Richards 1996). In our study, multiple regression analysis indicated that Zn was an important predictor for periphyton community measures; however, elevation and other variables significantly correlated with elevation (pH, conductivity, and alkalinity) were also important in explaining community composition. Differences in life history characteristics and morphological growth forms of dominant taxa between locations will influence responses to contaminants. We suggest that it may be useful to study periphyton community responses to pollutants in relation to life history traits, ecological strategies, and morphological growth forms. Conducting research within this framework may improve the predictive ability of periphyton models and provide a theoretical basis for understanding the relationships between observed community patterns and the evolutionary processes that determine a species response to disturbance.

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