ELSEVIER

Ecological Indicators 5 (2005) 117–136

ECOLOGICAL INDICATORS

This article is also available online at:
www.elsevier.com/locate/ecolind

Comparative application of indices of biotic integrity based on periphyton, macroinvertebrates, and fish to southern Rocky Mountain streams

Michael B. Griffith[a,*], Brian H. Hill[b], Frank H. McCormick[c,1], Philip R. Kaufmann[d], Alan T. Herlihy[e], Anthony R. Selle[f]

[a]*US Environmental Protection Agency, National Center for Environmental Assessment, 26 W. Martin Luther King Drive, Cincinnati, OH 45268, USA*

[b]*US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Duluth, MN 55804, USA*

[c]*US Environmental Protection Agency, National Exposure Research Laboratory, Cincinnati, OH 45268, USA*

[d]*US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Corvallis, OR 97333, USA*

[e]*Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97333, USA*

[f]*US Environmental Protection Agency, Region 8, Denver, CO 80202, USA*

Accepted 11 November 2004

Abstract

To assess the relative sensitivity of assessments using community metrics for macroinvertebrates, periphyton, and fish assemblages, we compared the results of three parallel assessments using these assemblages at 86 stream reaches sampled in 1994 and 1995 by the Regional Environmental Monitoring and Assessment Program (R-EMAP) in the mineralized zone or historical mining region of the Southern Rockies Ecoregion in Colorado. We contrasted assessments using community metrics for each taxa group selected to be diagnostic of the two large-scale stressor gradients identified in this ecoregion: discharges from historical hardrock metal mines and agriculture, particularly pasturing of livestock. While principal components analysis (PCA) extracted axes from the metrics for all three assemblages correlated with increased metal concentrations, the axes differed in their sensitivity to different environmental gradients. Two axes extracted from the fish metrics were correlated with dissolved metals, suspended solids, and sediment embeddedness or with sediment metals. Two axes extracted from the macroinvertebrate metrics partially separated these two stressor gradients, while the single correlated axis extracted from the periphyton metrics did not. The second macroinvertebrate PCA axis was correlated with an environmental gradient correlated both with agricultural effects and with stream size, as were the second and third periphyton PCA axes. The third fish PCA axis was correlated with stream size and slope, but was not sensitive to agricultural effects. Fish, macroinvertebrates, and periphyton differ in their sensitivity to different stressors, and combining metrics for these assemblages into a mixed

* Corresponding author. Tel.: +1 513 569 7034; fax: +1 513 569 7916.
E-mail address: griffith.michael@epa.gov (M.B. Griffith).

[1] Present address: USDA Forest Service, Pacific Northwest Research Station, Olympia Forestry Sciences Laboratory, 3625 93rd Avenue SW, Olympia, WA 98512, USA.

1470-160X/$ – see front matter
doi:10.1016/j.ecolind.2004.11.001

assemblage index of biotic integrity may increase the utility of the multimetric approach to diagnose environmental stressors at impaired reaches.

Keywords: Community metrics; Biotic indices; Fish; Macroinvertebrates; Periphyton; Streams; Southern Rockies Ecoregion

1. Introduction

Characterization of stream condition requires assessment of physical and chemical characteristics as well as the composition and structure of biotic assemblages (Karr and Chu, 1999). Water quality monitoring programs have previously focused on comparison of water chemistry at reaches downstream from point-sources with ambient water quality criteria derived from bioassays (McCarron and Frydenborg, 1997). However, this approach generally ignores higher-level responses of in situ biological assemblages to chemicals, the in-stream transformations of chemicals, the temporal and longitudinal variation in chemical concentrations, and the interaction of these chemical effects with other environmental stressors, such as physical alterations of riparian zones and in-stream habitats (Karr and Chu, 1999). The structure and taxonomic composition of biological communities integrate these aspects of exposure and higher-level effects (Karr et al., 1986; Deshon, 1995; Rosen, 1995).

Three different taxonomic assemblages have been proposed for use in monitoring and assessment of water quality and stream biological integrity: fish, macroinvertebrates, and periphyton (Barbour et al., 1999). All are relatively easy to collect with established methods, respond predictably to changes in stream quality, and are distinct and generally taxonomically diverse subgroups within stream communities. However, besides differences in the physical and chemical tolerances among taxa, differences in life-history and biogeography of taxa among these assemblages may affect their responses to changes in stream quality (Townsend and Hildrew, 1994). The algae and cyanobacteria that compose periphyton assemblages have generation times of hours to days (Hill et al., 2000). Generation times for most macroinvertebrates, particularly aquatic insects, range from weeks to several years (Wallace and Anderson, 1996), while those of fish are longer, generally a year or more (Schlosser, 1990). Algae and cyanobacteria may be relatively ubiquitous across geographic regions, while fish and macroinvertebrate species may be more limited geographically (Barbour et al., 1999; Angermeier et al., 2000; Feminella, 2000). Also, recolonization mechanisms for these assemblages following disturbance differ. Colonization of substrates by periphyton is by passive dispersal with currents, followed by reproduction (Stevenson and Peterson, 1991). For macroinvertebrates, colonization occurs either by drift or active movement or, for insects, by oviposition by flying adults (Mackey, 1992). For fish, colonization occurs largely by active dispersal within the stream drainage (Niemi et al., 1990). As a result, these assemblages may differ in their responses to specific environmental stressor gradients, particularly if stressors have differing spatial or temporal scales (Barbour et al., 1999).

In 1994 and 1995, USEPA in cooperation with U.S. Fish and Wildlife Service conducted a Regional Environmental Monitoring and Assessment Program (R-EMAP) study that focused on wadeable streams in the mineralized region of the Southern Rockies Ecoregion in Colorado (Omernik, 1987). This region is characterized by historical and some active mining for precious and base metals that has occurred for more than a century (Lyon et al., 1993). It is estimated that acidic and heavy metal contaminated discharges from more than 21,000 abandoned mines affect at least 2000 km of streams in Colorado (Colorado Office of Active and Inactive Mines, 1996). Agriculture also occurs in the region, primarily as livestock grazing in the broader valleys. The 1997 agricultural census estimated that 26% of the acreage in the Colorado counties within this region was privately owned farmland (NASS, 1999), of which 77% was pasture or rangeland. Also, a substantial number of grazing permits exist for federal lands (NASS, 1999). Silvicultural activities are relatively low, as are human populations and urban–suburban development. Therefore, the major anthropogenic impacts on stream quality in this region are related to metal mining or pasturing for livestock.

Indices of biotic integrity (IBI), whether based on fish, macroinvertebrate, or periphyton assemblages, should be designed to be responsive to both specific sources of stress and to general, cumulative perturbations (Karr, 1993). We present the results of our efforts to develop ecoregion-specific indices of biotic integrity for the Southern Rockies Ecoregion of Colorado and compare the behavior of metrics and indices based on data for periphyton, macroinvertebrates, and fish. We wanted to answer the following questions. Do metrics and indices for each assemblage differ in their sensitivity to the major stressor gradients in these Rocky Mountain streams? Do the resulting indices for each assemblage differ in their relative ranking of reaches in terms of their biotic integrity?

2. Methods

2.1. Survey methods

2.1.1. Study area and survey design

The mineral belt of the Southern Rockies Ecoregion extends southwest from the vicinity of Denver to the Colorado–New Mexico state line and includes headwater drainages of the South Platte, Arkansas, Rio Grande, and Colorado Rivers (Fig. 1). As part of USEPA's R-EMAP surveys in this region, 73 sampling reaches were selected using a randomization method with a spatial systematic component (Herlihy et al., 2000). Sample probabilities were set so that roughly equal number of wadeable streams defined as second, third, and fourth-order (Strahler, 1957) on a digitized version of the 1:100,000 scale U.S. Geological Survey (USGS) topographic map appeared in the sample. Thirteen additional reaches were selected that were upstream or downstream of known mining sites. Random subsets of reaches were revisited either within each year or between years to assess variability between visits. Including revisits, 107 sampling events were conducted for which there are relatively complete macroinvertebrate, physical, and chemical data sets. However, 12 events lacked fish data, and two events lacked periphyton data. In total, data were collected from 86 different stream reaches representative of 6628 km of wadeable streams in the Southern Rockies Ecoregion.

Streams were sampled from late July to late September each year. Stable base flows occur during this period of the water year in these Rocky Mountain streams. A length of stream equal to 40 times the mean low-flow, wetted width, but with a minimum of 150 m and maximum of 500 m, was delineated around each randomly chosen sampling point.

2.1.2. Water and sediment chemistry

Five 4 L samples of stream water were collected in low-density polyethylene containers, as appropriately filtered and preserved in the field (Griffith et al., 2001), and sent to the analytical laboratory. Detailed information of the analytical procedures used for water chemistry can be found in USEPA (1987). In brief, base cations and metals were determined by atomic absorption, anions and nutrients by ion chromatography, DOC and total organic carbon (TOC) with a carbon analyzer, and hardness was calculated from dissolved Ca and Mg (APHA, 1995).

Sediments for metal analysis were placed in resealable plastic bags, placed on ice, and sent to the analytical laboratory. Samples were digested with HNO_3 and HCl (USEPA, 1994) and metals measured by atomic absorption (USEPA, 1987).

Some chemistry data were censored, because concentrations were less than the method detection limits. A value equal to one-half the method detection limit was assigned to these data in any further analyses (Heisel, 1990).

2.1.3. Physical habitat and riparian disturbance

Physical habitat data were collected from longitudinal profiles and from 11 cross-sectional transects spaced evenly along a stream reach. Thalweg depth was measured at 100 evenly spaced points along the length of the stream reach if the stream was greater than 2.5 m wide or at 150 points if the stream was less than 2.5 m wide. Channel habitat classes and location and amount of woody debris were recorded while measuring the thalweg. At each transect, additional measurements included channel dimensions, particle sizes at nine points across the transect, channel gradient, compass bearing for calculating sinuosity, riparian vegetation cover and structure on each side of the stream, and the occurrence and proximity of 11

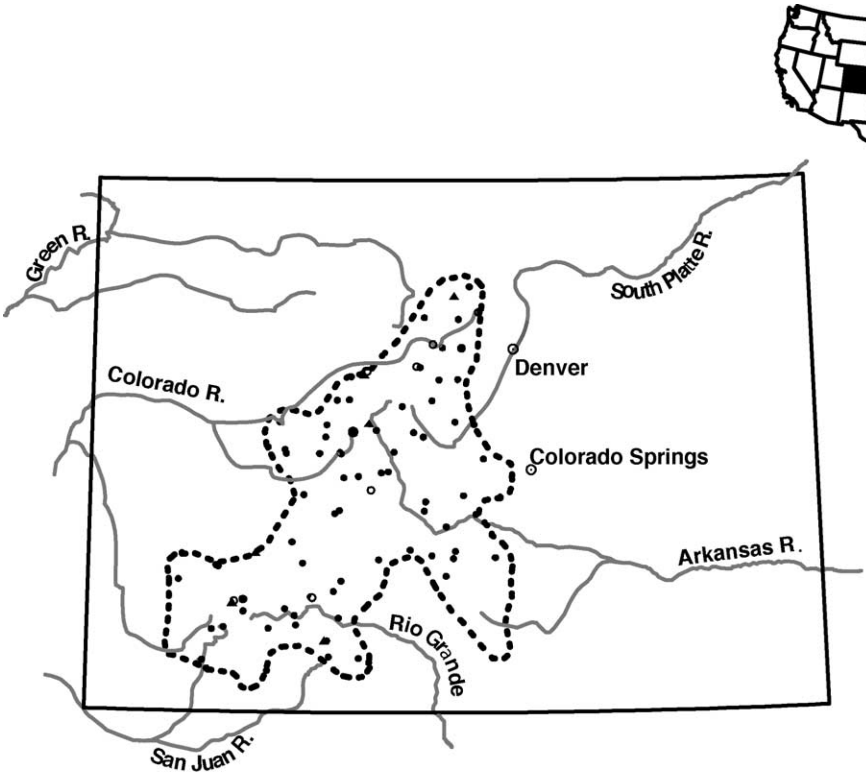


Fig. 1. Map of Colorado with the mineralized zone of the Southern Rockies Ecoregion and locations of the 1994–1995 R-EMAP reaches (- - -, mineralized region; ●, random-selection reaches, ▲, upstream reaches; ○, downstream reaches).

human disturbances in the riparian zone (i.e., buildings, channel revetment, pavement, roads, pipes, trash and landfill, parks and lawns, row crop agriculture, pasture and grass fields, logging, and mining). Lazorchak et al. (1998) describe the physical habitat field methods in greater detail.

Most physical habitat data were summarized as reach means or cover percentages. Riparian human disturbance indices were summarized as proximity-weighted means for each of the 11 disturbance types. Kaufmann et al. (1999) describe procedures for habitat data reduction and interpretation.

2.1.4. Landscape characterization

Catchment basins for each sample location were delineated using standard GIS methods. Metrics, such as slope, aspect, and shape/conformity were calculated for each catchment. Percentage of land-cover classes in each catchment was determined by overlaying Colorado Geographical Approach to Planning for Biological Diversity (GAP) land-cover data provided by the Colorado Division of Wildlife on each catchment. GAP land-cover was classified from various date, Landsat MSS images using unsupervised classification methods and extensive field verification. Similarly, miles of roads, miles of streams, and number of historic mines were calculated for each catchment by overlay processes with 1:200,000-scale USGS Digital Line Graph data, 1:100,0000-scale USGS National Hydrography data, and the Bureau of Mines 1992 MILS/MAS data. Landscape data were available only for random-selection reaches.

2.1.5. Macroinvertebrate collection and identification

Macroinvertebrates were collected from riffles or pools at each of the interior nine transects along a reach. At the first transect, the sampling position in the channel (i.e., right, middle, or left) was assigned randomly, and then varied systematically for the other transects. At each sample position, habitat type (i.e., riffle or pool) was identified; a semi-quantitative sample (0.5 m^2 quadrat) was collected with a modified kick net (Lazorchak et al., 1998). Net contents from each transect were combined into separate composite riffle and pool samples for each reach. Each sample was sieved (595 μm mesh) and preserved in 95% ethanol. Because of the preponderance of riffle habitats at all reaches (i.e., a pool composite sample was collected at only 12 of the 107 reaches), only data from composite riffle macroinvertebrate samples were used in these analyses.

If the riffle composite sample was composed of individual samples from less than four transects, then the entire composite sample was processed. Otherwise, the composite sample was split into three subsamples with a sample splitter; one subsample was randomly selected for processing (Lewis and Klemm, 1994). The resulting sample was placed in a white pan marked in a numbered grid pattern. A grid was selected randomly, and all organisms were removed under 2× magnification. This process was repeated until a minimum of 300 organisms or all organisms in the sample were removed. Specimens were generally identified genus but sometimes family or tribe. Abundances per m^2 were estimated based on the number of grids sorted, subsamples, and transects in a composite sample.

2.1.6. Periphyton collection and identification

Periphyton was collected adjacent to the macroinvertebrate quadrat at each of the interior nine transects (Lazorchak et al., 1998). At each transect, periphyton was collected from a 12 cm^2 area of stream bed delineated by a 1.5 cm long piece of 3.9 cm diameter PVC pipe. Periphyton was loosened from the substrate with a tooth brush and rinsed with stream water into a collection bottle. The composite periphyton sample for each reach was subsampled for identification, chlorophyll a content, and biomass. Total and subsample volumes were recorded.

Subsamples were fixed for periphyton taxa identification on-site with formalin (5%, v/v). Samples were homogenized in a high-speed blender and counted (400× magnification) in a Palmer-type chamber. Subsamples for permanent diatom slides were rinsed several times to remove the formalin, cleaned with concentrated HNO_3 and $K_2Cr_20_7$ to remove organic matter, then rinsed and dried (20–60 °C) on cover glasses and mounted in HYRAX® (Patrick and Reimer, 1966). At least 500 diatom valves in each sample were counted at 1000× magnification.

Subsamples for measurement of chlorophyll and periphyton biomass standing crop were filtered onto pre-leached, pre-ashed, pre-weighed glass fiber filters (1.0 μm average pore size). Filters were wrapped in foil, frozen, and analyzed within 30 days of collection. Periphyton biomass (ash-free dry mass, AFDM) was determined as weight loss following incineration (525 °C, 30 min) of the sample collected on glass fiber filters. Chlorophyll was extracted from each filter by leaching with cold (4 °C), 90% (v/v) acetone for 18 h; chlorophyll *a* content of the leachate was measured by light absorbance at 750 and 664 nm with a spectrophotometer, before and after addition of 1N HCl. Chlorophyll *a* concentrations were scaled for subsample area and AFDM (APHA, 1995).

2.1.7. Fish collection and identification

Fish were collected from the entire stream reach according to time and distance criteria using pulsed DC backpack electrofishing equipment supplemented by seining (Lazorchak et al., 1998). Sampling duration ranged from 45 to 180 min depending on stream size and complexity. The objective was to collect a representative sample of the fish assemblage by methods designed to collect all except very rare species, and provide a robust measure of proportional abundances of species. Sport fish and easily recognized species were identified and released. Voucher specimens (up to 25) of smaller individuals of each species and unidentified specimens were retained for museum verification. Collections are archived at the National Museum of Natural History, Smithsonian Institution.

2.2. Calculation and scoring of community metrics

We used the macroinvertebrate and periphyton data to calculate 10 macroinvertebrate metrics (Table 1) and 10 periphyton metrics (Table 2). These metrics were selected based on previous analyses of their relationships with the main environmental gradients and lack of redundancy in these streams (Griffith et al., 2001, 2002) and an understanding of their relationships with stressor gradients in other streams.

Table 1
Metrics included in the macroinvertebrate index of biotic integrity (MIBI) for the Southern Rockies Ecoregion, Colorado

Metric	Code	Calculation	Potential range	Actual score
Ephemeroptera and Plecoptera genera richness	RICH_EphPle	No. Plecoptera + Ephemeroptera genera/expected no. P + E genera	0–1	0–10
Trichoptera genera richness	RICH_Trichop	No. Trichoptera genera/expected no. Trichoptera genera	0–1	0–10
RA[a] Crustacea and Mollusca	RA_CrusMoll	1 − (no. crustacea and mollusca/total no. macroinvertebrates)	0–1	1.8–10
Chironomidae genera richness	RICH_Chiron	No. Chironomidae genera/expected no. Chironomidae genera	0–1	0–10
RA (five most dominant genera)	DOM_Taxa	1 − (no. five most dominant taxa/total no. macroinvertebrates)	0–1	0–6.9
Macroinvertebrate density	TOT_density	Total macroinvertebrate density/expected macroinvertebrate density	0–1	0–10
RA (EPT[b])	RA_EPT	No. EPT/total no. macroinvertebrates	0–1	0.8–10
Orthocladiinae/Chironomidae ratio	CRA_Orthocl	1 − (no. Orthocladiinae/total no. Chironomidae)	0–1	0–10
Tanytarsini/Chironomidae ratio	CRA_Tanytar	No. Tanytarsini/total no. Chironomidae)	0–1	0–9.7
Hilsenhoff's biotic index	HBI	1 − (HBI/10)	0–1	3.9–9.2
Range of realized MIBI scores				23.0–67.7

[a] Relative abundance.
[b] Ephemeroptera, Plecoptera, and Trichoptera.

Insufficient fish diversity was found in these streams to calculate a similar set of 10 community metrics for use in a fish IBI (McCormick et al., 1994). Therefore, a smaller set of five community metrics (Table 3) was selected for comparison with those of macroinvertebrates and periphyton.

To score the richness metrics, we assumed the expected number of taxa to be the maximum number of taxa observed in any sample from the data set. Therefore, the expected number of Trichoptera genera was 11, Plecoptera and Ephemeroptera genera was 18, Chironomidae genera was 28, algal divisions was 5,

Table 2
Metrics included in the periphyton index of biotic integrity (PIBI) for the Southern Rockies Ecoregion, Colorado

Metric	Code	Calculation	Potential range	Actual score
Algal division richness	RICH_ADiv	No. algal divisions/expected no. algal divisions	0–1	2–10
Non-diatom genera richness	RICH_SAlgae	No. non-diatom genera/expected no. non-diatom genera	0–1	0–10
RA (diatoms)	RA_Diatoms	No. diatom cells/total no. algal cells	0–1	0–10
Diatom tolerance value	Tolvalue	$\sum$Species tolerance value × no. cells diatom species/total no. diatom cells	0–1	3–10
RDA (*A. minutissima*)	RA_Achnm	No. *A. minutissima* cells/total no. diatom cells	0–1	0–9.9
RDA (nitrogen-heterotrophic diatoms)	RA_NHetD	1 − (no. nitrogen-heterotroph diatoms/total no. diatoms)	0–1	7.4–10
Diatom abundance	Abun_Diatom	$\log_{10}$(no. diatom cells + 1)/$\log_{10}$(expected no. diatom cells + 1)	0–1	0–10
Algal cell abundance	Abundance	$<2.77 \times 10^5$: total no algal cells/2.77×10^5; $\geq 2.77 \times 10^5$: 2.77×10^5/total no. algal cells	0–1	0–10
Chlorophyll	Chlorophyll	<4.18 mg/m^2: Chl *a* [mg/m^2]/4.18; ≥ 4.18 mg/m^2: 4.18/Chl *a* [mg/m^2]	0–1	0–10
Biomass (AFDM)	AFDM	<2.58 g/m^2: AFDM [g/m^2]/2.58; ≥ 2.58 g/m^2: 2.58/AFDM [g/m^2]	0–1	0–10
Range of PIBI realized scores				25.2–80.5

Table 3
Metrics calculated from fish data for the streams sampled in the Southern Rockies Ecoregion, Colorado

Metric	Code	Calculation	Potential range	Actual score
Fish richness	RICH_Fish	No. fish species/subspecies/expected no. fish species/subspecies	0–1	0–10
Fish abundance	Abundance	No. fish/expected no. fish	0–1	0–10
RA (Salmonidae)	RA_Trout	No. Salmonidae/total no. fish	0–1	0–10
RA (native fish)	RA_Natives	No. native fish/total no. fish	0–1	0–10
Oncorhynchus/Salmonidae ratio	TRA_Oncorhyn	No. Oncorhynchus/no. Salmonidae	0–1	0–10
Sum of realized fish metric scores				0–37.6

and non-diatom genera was 11. Similarly, we assumed that expected macroinvertebrate density, number of diatom cells, and number of fish collected were the maxima observed in any sample or 4360 macroinvertebrates/m^2, 1.88×10^6 diatom cells, and 261 fish, respectively. Preliminary analyses indicated that fish richness was related to stream width and the maximum number of fish species/subspecies increased with stream width. Therefore, we adjusted the expected number of fish species/subspecies as a function of stream width (Fausch et al., 1984). Maximum values were used as the expected values in order to avoid censoring the individual metric scores (Gilbert, 1987). However when used in a regulatory context, it may be appropriate to score these metrics using expected values that are the 95th percentile of the data (Hughes et al., 1998).

The Orthocladiinae/Chironomidae and Tanytarsini/Chironomidae ratios were assigned values of 0, if chironomid abundance was 0. For algal cell abundance, chlorophyll, and AFDM in the periphyton IBI, we expected either positive or negative responses to different environmental stressors, and median regional values of 2.77×10^5 cells/cm^2, 2.80 mg chlorophyll *a*/m^2, and 2.58 mg AFDM/m^2 were used as reference values (Table 2). For these metrics, positive or negative deviations from median values resulted in lower metric scores. To score each metric, we assumed the sign of the relationship between the raw values of the metric and the disturbance gradients. So that all metrics decreased with increasing disturbance, we subtracted metrics, whose raw values were expected to increase with increasing disturbance, from 1.

Potential raw values of each metric ranged from 0 to 1 (Tables 1–3). Raw values were multiplied by 10 to create metric scores that ranged from 0 to 10, with decreasing metric scores corresponding to increasing anthropogenic disturbance. With 10 metrics for macroinvertebrates and for periphyton, potential values of the biotic indices range from 0 to 100 (Tables 1 and 2). Because we used only five metrics for fish, the potential sum of the fish metric scores ranges from 0 to 50 (Table 3).

2.3. Description of macroinvertebrate metrics

2.3.1. Ephemeroptera and Plecoptera genera richness

EPT (Ephemeroptera, Plectopera, and Trichoptera) taxa have been widely used as indicators of environmental disturbances, including sediment, organic enrichment, and exposure to toxic chemicals, because of their general sensitivity to anthropogenic stressors (Wallace et al., 1996). However, analyses of Rocky Mountain streams indicate there are differences between the sensitivity of Ephemeroptera and Plecoptera and that of Trichoptera to specific stressors (Clements, 1991; Kiffney and Clements, 1994; Griffith et al., 2001). We expected Ephemeroptera and Plecoptera genera richness to decrease with increasing environmental stress, particularly those associated with effects from metal mining or grazing by livestock.

2.3.2. Trichoptera genera richness

While Trichoptera are generally sensitive to anthropogenic stressors, analyses of Rocky Mountain streams indicate that Trichoptera may be less sensitive to environmental disturbances, such as sedimentation and organic enrichment associated with grazing by livestock, then Ephemeroptera and Plecoptera (Griffith et al., 2001). However, Trichoptera are similar to these orders in their sensitivity to exposure to metals associated with mining. We expected Trichoptera genera richness to decrease with increasing environmental stress associated with effects from metal mining, but to be less affected by effects associated with livestock grazing.

2.3.3. Relative abundance (RA), Crustacea and Mollusca

Crustacea and Mollusca are characteristic of streams with lower gradients and greater water hardness, nutrients, and suspended solids associated with sedimentation and organic enrichment in streams affected by livestock grazing (Griffith et al., 2001). We expected the relative abundance of these taxa to increase with increasing perturbation by these stressors.

2.3.4. Chironomidae genera richness

The Chironomidae are a very diverse family with many genera in Rocky Mountain streams. Although some genera are tolerant of toxic substances and may be dominant under these conditions (Deshon, 1995; Barbour et al., 1996), total genera richness of Chironomidae generally decreases. We expected Chironomidae genera richness to decrease with increasing environmental stress.

2.3.5. Relative abundance (RA), five most dominant genera

The dominance of the most abundant macroinvertebrate genera is a measure of the evenness of taxonomic assemblages (Plafkin et al., 1989). Dominance, usually by tolerant species that are better adapted to unfavorable conditions, results in reduced diversity and an uneven distribution of individuals among taxa (Barbour et al., 1996). Relative abundance of the five most abundant genera is a measure of this redundancy (Plafkin et al., 1989), and we expected this metric to increase with increasing environmental stress.

2.3.6. Macroinvertebrate density

Although at least semiquantitative samples are needed to estimate this metric, population abundances generally reflect in-stream habitat quality and the effects of toxic substances on assemblages (Karr et al., 1986). As a result, this metric was expected to decrease with increased contaminant loading or with decreased habitat quality in these streams.

2.3.7. Relative abundance (RA), EPT taxa

Because of their sensitivity to various environmental stressors, the relative abundance of EPT taxa has also been used as a composition measure for macroinvertebrate assemblages (Barbour et al., 1999). We expected the relative abundance of EPT taxa to decrease with increasing environmental stress.

2.3.8. Orthocladiinae/Chironomidae ratio

Orthocladinae is a subfamily of the midge family Chironomidae in which many species are tolerant of toxic substances, particularly metals (Barbour et al., 1996). At high metal concentrations, genera, such as *Cricotopus*, may dominate (Lenat, 1983; Plafkin et al., 1989). We expected the ratio of Orthocladiinae to all Chironomidae to increase with increasing environmental stress.

2.3.9. Tanytarsini/Chironomidae ratio

Tanytarsini is a tribe of the subfamily Chironominae of the family Chironomidae. This group is generally intermediate in tolerance to toxic substances, and many genera decline in abundance under moderate pollution stress (Deshon, 1995). We expected the ratio of Tanytarsini to Chironomidae to decrease with increasing environmental stress.

2.3.10. Hilsenhoff's biotic index (HBI)

The HBI is a tolerance index intended to measure the average individual sensitivity of the macroinvertebrate assemblage to pollution (Hilsenhoff, 1987). Different taxa are assigned tolerance values from 0 for the most intolerant to 10 for the most tolerant. Although originally designed to be specific to organic pollution, the HBI often works well for other perturbations. Therefore, we expected the HBI to increase with increasing environmental stress.

2.4. Description of periphyton metrics

2.4.1. Algal division richness

The number of algal divisions represented by all taxa in the assemblage should be the highest at reaches with good water quality (Stevenson and Bahls, 1999). We expected algal division richness to decrease with increasing nutrient enrichment or toxic conditions.

2.4.2. Non-diatom genera richness

Although organic enrichment or toxic conditions may cause shift in algal dominance from diatoms to non-diatom taxa, such as chlorophytes or cyanobacteria (Palmer, 1969; Patrick, 1977; Bott and Rogenmuser, 1978; Steinman et al., 1991), this dominance is generally by a few tolerant taxa and we expected the richness of non-diatom taxa to decrease with increasing environmental stressors.

2.4.3. Relative abundance (RA), diatoms

Organic enrichment or toxic conditions can cause shifts in domination of the periphytic community from diatoms to non-diatom taxa, particularly to Chlorophyta or cyanobacteria (Palmer, 1969; Patrick, 1977; Bott and Rogenmuser, 1978; Steinman et al., 1991). Therefore, we expected the relative abundance of diatoms to decline with increases in these types of environmental stressors.

2.4.4. Diatom tolerance value

As suggested by Bahls (1993), the diatom tolerance value is a weighted pollution tolerance index that assigns a pollution tolerance value of 3 to eutraphentic to hypereutraphentic or polysaprobous diatom species, 2 to mesotraphentic or mesosaprobous diatom species, and 1 to oligotraphentic or oligosaprobous diatom species as defined by van Dam et al. (1994). This classification system for diatoms has been used to identify and assess reaches impacted by nutrients (Palmer, 1969; Lange-Berlatot, 1979; Hall and Smol, 1992; Christie and Smol, 1993; Pan et al., 1996). We expected the tolerance value to decrease with increasing nutrient and organic matter enrichment.

2.4.5. Relative diatom abundance (RDA): *Achnanthes minutissima*

A. minutissima is a cosmopolitan diatom that is tolerant of a broad range of abiotic conditions (Stevenson and Bahls, 1999). Although it may be tolerant of certain toxic conditions, it is also very tolerant of the low nutrient conditions characteristic of more pristine Rocky Mountain streams (Griffith et al., 2002). We expected the relative abundance of *A. minutissima* to decrease with increasing nutrient enrichment.

2.4.6. Relative diatom abundance (RDA): nitogen-heterotrophic diatoms

Nitrogen-heterotrophic diatoms are facultatively or obligately nitrogen-heterotrophic diatoms as classified by van Dam et al. (1994). These diatoms can use or require organic nitrogen compounds for their metabolism (van Dam et al., 1994) and increase with increasing nutrient or organic enrichment.

2.4.7. Diatom abundance

Diatom abundance often decreases in response to toxic effects or nutrient or organic enrichment. Diatom abundance decreases as a direct response to increasing toxic effects (Patrick, 1977), while it decreases as an indirect response to increasing nutrient or organic enrichment, because of increases in non-diatom algae (Steinman et al., 1991). Therefore, we expected diatom abundance to decrease in response to increasing toxic effects or enrichment.

2.4.8. Algal cell abundance

The relationship between total algal cell abundance and water quality can be complex and not readily interpreted. Increased cell abundances have been reported in response to nutrient loading and increased insolation from opening of the canopy (Steinman et al., 1991), whereas other chemical perturbations may result in reduced cell abundances (Kosinski, 1984; Amblard et al., 1990). We expected algal cell abundance, which decreases for both positive and negative deviations from the regional median cell abundance, to decrease with increasing nutrients, organic matter, and toxicants.

2.4.9. Chlorophyll

Chlorophyll *a* concentration has been used to assess nutrient environmental of streams, even in regional-scale studies (Leland, 1995; Pan et al., 1999). We expected the chlorophyll metric, which deceases for both positive and negative deviations from the regional median chlorophyll *a* concentration (Hill et al., 2000), to decrease with increasing nutrient, organic matter, and contaminant loadings to the stream.

2.4.10. Biomass (AFDM)

As with chlorophyll *a*, the relationship between standing crop and water quality is not easily interpreted. Periphyton biomass will increase in response to nutrient loading and increased light associated with agricultural effects (Leland, 1995), but periphyton biomass may also decrease in response to chemical perturbations (Sigmon et al., 1977; Clark et al., 1979; Boston et al., 1991). We expected the biomass metric, which decreases for both positive and negative deviations from the regional median AFDM concentrations (Hill et al., 2000), to decrease with increases in nutrients, light, or toxicants.

2.5. Description of fish metrics

2.5.1. Fish richness

The number of fish species or subspecies has been widely used as an indicator of environmental degradation in streams (Hughes and Gammon, 1986; Hughes et al., 1998). If stream size and other features are similar, the species richness will decrease along various environmental stressor gradients (Karr et al., 1986).

2.5.2. Fish abundance

As a measure of population abundance, the total number of individuals generally reflects in-stream habitat quality and the effects of toxic substances on fish assemblages (Karr et al., 1986). As a result, this metric was expected to decrease with increased contaminant loading or with decreased habitat quality in these wadeable streams.

2.5.3. Relative abundance (RA), Salmonidae

Salmonids are lithophilic spawners whose eggs develop in the interstices of sand, gravel, and cobble substrates (Barbour et al., 1999). The proportion of individuals that are Salmonidae is a measurement of stream habitat suitability for reproduction and is affected by environmental disturbances influence the embeddedness and chemical quality of stream sediments (Angermier and Karr, 1986; Berkman and Rabeni, 1987). Therefore, this metric is expected to decrease with increased embeddedness and sediment contamination.

2.5.4. Relative abundance (RA), native fish

The proportion of individuals that are native is an estimate of the reproductive isolation of fish assemblages and measures the loss of species segregation between midwestern and western fishes caused by the introduction of midwestern species to the Rockies (Hughes and Gammon, 1986; Miller et al., 1988; Barbour et al., 1999). Many of the introduced species are more tolerant of habitat degradation and chemical stressors. Therefore, this metric is expected to decrease along the environmental stressor gradients.

2.5.5. Oncorhynchus/Salmonidae ratio

In the southern Rockies, cutthroat (*O. clarki*) and rainbow (*O. mykiss*) trout are generally considered more intolerant of habitat degradation and chemical stressors than non-*Oncorhynchus* trout, such as brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) (Barbour et al., 1999). Therefore, the proportion of Salmonidae that are *Oncorhynchus* is expected to decrease along the environmental stressor gradients.

2.6. Data analysis

Principal components analysis (PCA) was used to identify the primary gradients in the macroinvertebrate, fish, or periphyton metrics, independent of the environmental data. The two tails of metrics whose raw values were expected either to increase or decrease relative to a regional median value in response to environmental stressors are likely responding to different stressor gradients. Therefore, separate variables were created for the increasing and decreasing tails prior to analysis in PCA. Analyses were conducted with the program CANOCO Version 3.12 (ter Braak, 1987). PCA axis scores from this analysis quantify correlations of individual community metrics to the axes.

Because we conducted an indirect gradient analysis (ter Braak, 1995), correlations of reach scores for each PCA axis with selected environmental variables (Table 4) were examined with Pearson product–moment correlation (SAS, 1996) to determine those environmental variables significantly correlated with the PCA axes (ter Braak, 1995). Statistical significance was set at $\alpha = 0.05$, and probabilities for simultaneous tests were corrected with the sequential Bonferroni technique (Rice, 1989). However, many environmental variables have low signal to noise variance ratios, and correlation coefficients will be low-biased, conservative estimates of correlations

Table 4
Summary of the reduced chemical and physical habitat environmental data set for the stream reaches sampled in the Southern Rockies Ecoregion, Colorado

Code	Variable	*N*	Minimum	Maximum	Mean	S.D.
K	*K* (μeq/L)	107	0.00	220	32.0	49.5
CD	Dissolved Cd (μg/L)	107	0.250[a]	3.30	0.468	0.640
CU	Dissolved Cu (μg/L)	107	0.500[a]	2460	42.0	269
ZN	Dissolved Zn (μg/L)	107	2.00[a]	902	63.3	166
FE	Total Fe (μg/L)	107	2.50[a]	11200	633	1520
ALK	Alkalinity (μeq/L)	106	0.00	9110	1510	1620
HARDN	Hardness (mg $CaCO_3$/L)	107	6.78	481	79.9	69.7
CL	Cl (μeq/L)	105	31.0	4400	150	573
F	*F* (μeq/L)	107	0.00	133	17.4	25.5
SO_4	SO_4 (μeq/L)	103	22.9	6480	671	1130
PO_4	Total P (mg/L)	107	0.005[a]	0.29	0.043	0.052
DOC	Dissolved organic carbon (mg/L)	107	1.00[a]	10.8	1.70	1.66
TOC	Total organic carbon (mg/L)	107	1.00[a]	10.0	2.23	1.82
TSS	Total suspended solids (mg/L)	107	2.00[a]	72.0	6.85	12.8
SED_CU	Sediment Cu (mg/kg)	107	1.29	597	45.7	102
SED_ZN	Sediment Zn (mg/kg)	107	17.4	2940	256	578
TEMP	Water temperature (°C)	106	4.0	22.5	11.9	3.9
SLOPE	Mean water surface gradient (%)	105	0.1	22.9	2.5	3.3
DEPTH	Mean thalweg depth (cm)	105	7.7	99.5	39.7	18.0
WIDTH	Mean wetted width (m)	105	0.5	22.4	6.7	4.1
EMBED	Mean substrate embeddedness (%)	105	3.3	100	38.8	27.1
LRBS	log(relative substrate stability)	105	−6.78	−3.01	−4.22	0.62
LSUB_DM	log[geometric mean substrate diameter (mm)]	105	−2.39	2.98	1.54	0.86
LWOOD	LWD[b] volume in or above active channel	105	0.0	100.0	6.7	13.6
CASCADE	% Cascades	105	0	82	5	13
RAPIDS	% Rapids	105	0	90	32	27
CDENBK	Mean % canopy density at banks	105	0.0	98.4	54.7	34.3
CAN_CON	PR[c], coniferous riparian canopy present	105	0.00	1.00	0.23	0.33
CAN_MIX	PR, mixed riparian canopy present	105	0.00	0.86	0.12	0.19
MID_MIX	PR, mixed riparian midlayer vegetation present	105	0.00	0.95	0.27	0.31
HERBS	PC[d], Riparian ground-layer herbaceous	105	0.06	0.88	0.43	0.22
WOODY	PC, Riparian ground-layer woody	105	0.00	0.83	0.29	0.18
RPASTURE	RHDI[e]-pasture and hayfields (weighted sum)	105	0.00	1.50	0.37	0.60
RPAVEM	RHDI-pavement (weighted sum)	105	0.00	0.67	0.12	0.17
DECIDFOR	% Watershed in deciduous forests	88	0.0	81.7	11.2	18.8
ROWCROP	% Watershed in row crops	88	0.0	22.5	0.8	3.1
BARREN	% Watershed barren	88	0.0	12.3	0.3	1.4

[a] Value is one-half the method detection limit, because some samples had concentrations less then the detection limit.
[b] Large woody debris.
[c] Proportion of reach.
[d] Proportional cover.
[e] Riparian human disturbance index.

between the environmental variables and PCA axes (Kaufmann et al., 1999). In addition, correlations among reach scores for each axis and the biotic indices were compared among assemblages to examine similarities among the environmental gradients measured by the three assemblages.

3. Results

3.1. Macroinvertebrate IBI (MIBI)

Principal component analysis extracted three axes with eigenvalues of 0.344, 0.225, and 0.147 and explained 72% of the variance in macroinvertebrate metrics data. Orthocladiinae/Chironomidae ratio ($r = -0.803$, $P < 0.001$), Tanytarsini/Chironomidae ratio ($r = -0.836$, $P < 0.001$), Trichoptera genera richness ($r = -0.597$, $P < 0.001$), and macroinvertebrate density ($r = -0.352$, $P < 0.001$) were most correlated with the first axis (Fig. 2A). We interpret the first axis as a disturbance gradient associated with mining impacts and was correlated with sediment and dissolved water column concentrations of metals, including Cd, Cu, and Zn, and with SO_4, TSS, and hardness (Fig. 2A). The RA (EPT) ($r = -0.865$, $P < 0.001$), Plecoptera and Ephemeroptera genera richness ($r = -0.798$, $P < 0.001$), and RA (Crustacea and Mollusca) ($r = -0.309$, $P = 0.001$) were most correlated with the second axis, which we interpret as a gradient associated with agricultural effects and stream size. Variables correlated with the second axis describe a gradient from larger streams with more dense riparian canopies composed of conifers and mixed midlayer vegetation and with more coarse substrates and woody debris to smaller streams lacking canopy and midlayer vegetation and with greater substrate embeddedness, $P0_4$, hardness, TSS, TOC, and riparian disturbance by pasture or hayfields (Fig. 2). Chironomidae genera richness ($r = 0.604$, $P < 0.001$) and RA (five most dominant taxa) ($r = 0.489$, $P < 0.001$) were most correlated with the third axis (Fig. 2B), which we interpret as a second gradient associated with mining effects and was correlated with dissolved concentrations of metals, including Cu, Cd, and Zn (Fig. 2B).

The MIBI ranged from 23.0 to 67.7 and was most correlated with the first axis ($r = -0.909$, $P < 0.001$), which was associated with mining impacts. While correlations of the MIBI with the second ($r = -0.314$, $P = 0.001$) and third axes ($r = 0.199$, $P = 0.04$) were less, MIBI was inversely correlated with increasing gradients of environmental disturbance associated with each axis.

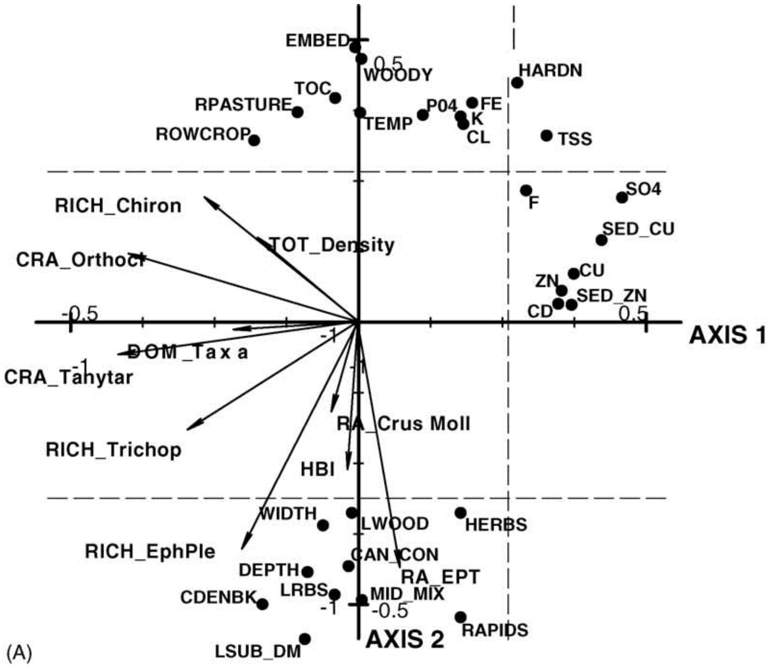
3.2. Periphyton IBI (PIBI)

Principle component analysis extracted three axes with eigenvalues of 0.356, 0.191, and 0.121 and that explained 67% of the variance in Periphyton metrics. Diatom abundance ($r = -0.437$, $P < 0.001$) and non-diatom genera richness ($r = -0.417$, $P < 0.001$) was most correlated with the first axis (Fig. 3A), which we interpret as a gradient associated with mining effects and was positively correlated with sediment Cu and dissolved Cd concentrations. RA (diatoms) ($r = -0.843$, $P < 0.001$) and AFDM ($r = -0.399$, $P = 0.003$) were most correlated with the second axis (Fig. 3B), which we interpret as a gradient primarily associated with sediment coarseness. Percent embeddedness and percent fines were positively correlated with this axis, while substrate diameter was negatively correlated (Fig. 3B). Alkalinity and TOC were also positively correlated with the second axis. Algal cell abundance was most correlated with the first axis when it was less than the median ($r = -0.654$, $P < 0.001$) but was most correlated with the second axis when it was greater than the median ($r = -0.360$, $P = 0.009$). RDA (*A. minutissima*) ($r = -0.694$, $P < 0.001$), diatom tolerance value ($r = -0.462$, $P < 0.001$), RDA (nitrogen-heterotrophic diatoms) ($r = -0.411$, $P < 0.001$), and chlorophyll (≤mean: $r = -0.546$, $P < 0.001$; >mean: $r = -0.370$, $P < 0.007$) were most correlated with the third axis (Fig. 3). We interpreted this axis as a disturbance gradient related to agricultural impacts. Environmental variables correlated with the third axis describe a gradient from streams characterized by greater canopy shading by a mixed canopy and midlayer, by a herbaceous ground-layer, and by coarser substrates to streams lacking canopy or midlayer vegetation, with a woody ground-layer, with finer substrates and greater embeddedness, and with greater conductivity and concentrations of PO_4, K and Cl (Fig. 3).

The PIBI ranged from 25.2 to 80.5 (Table 2) and was most correlated with the third axis ($r = -0.636$, $P < 0.001$), which we interpreted as associated with agricultural impacts (Fig. 3). As with the MIBI, the PIBI was less correlated with the other axes (first axis: $r = -0.383$, $P < 0.001$; second axis: $r = -0.195$, $P = 0.05$), but was inversely correlated with increasing disturbance gradients. However, the PIBI required greater effort in designing scoring for the metrics.

3.3. Fish metrics

Principle component analysis extracted two axes with eigenvalues of 0.421 and 0.311 that explained 73% of the variance in fish metrics. RA (Salmonidae)



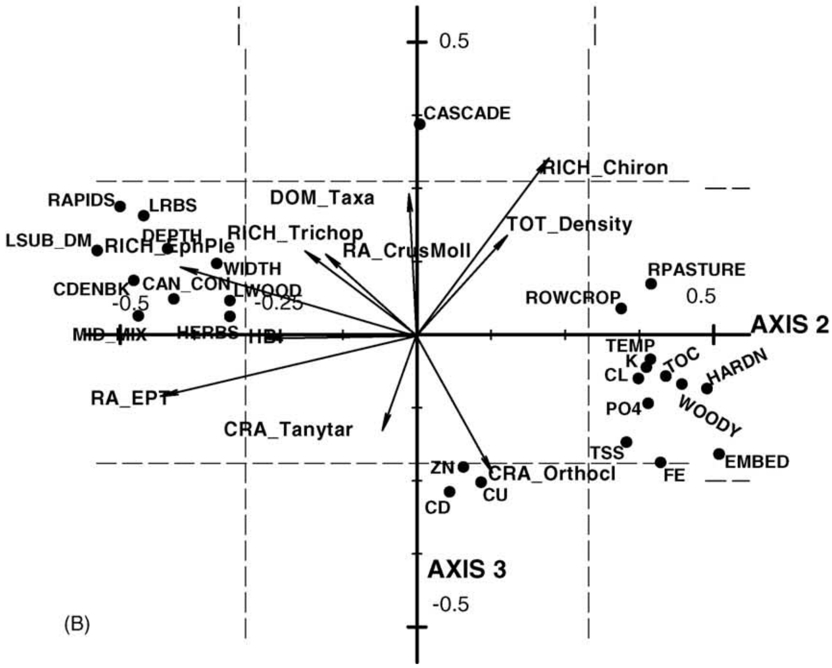
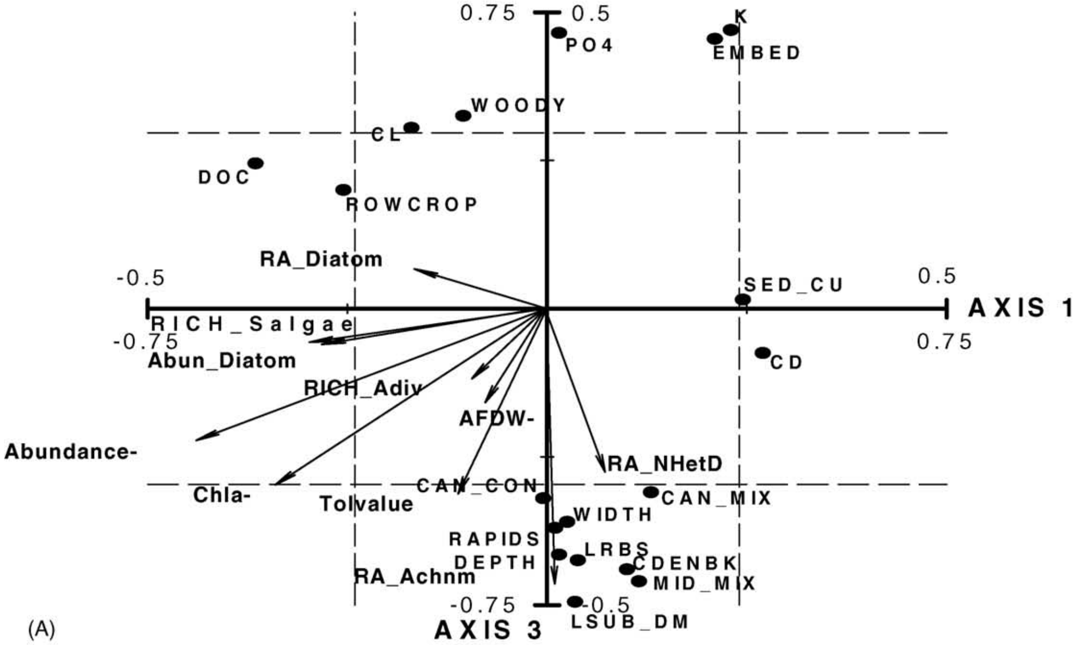


Fig. 2. Correlation biplots for (A) the first and second axes and (B) the second and third axes of the principal component analysis based on the macroinvertebrate metrics. The scale is −1.0 to 1.0 for correlations of the macroinvertebrate metrics (arrows) with the axes and −0.5 to 0.5 for correlations of the environmental variables (solid circles) the axes. For environmental variables whose correlation was more positive or negative than the dashed lines drawn perpendicular to an axis, P was statistically significant when corrected with the sequential Bonferroni technique. The abbreviation codes for the environmental variables are in Table 4. Those for the metrics are in Table 1.



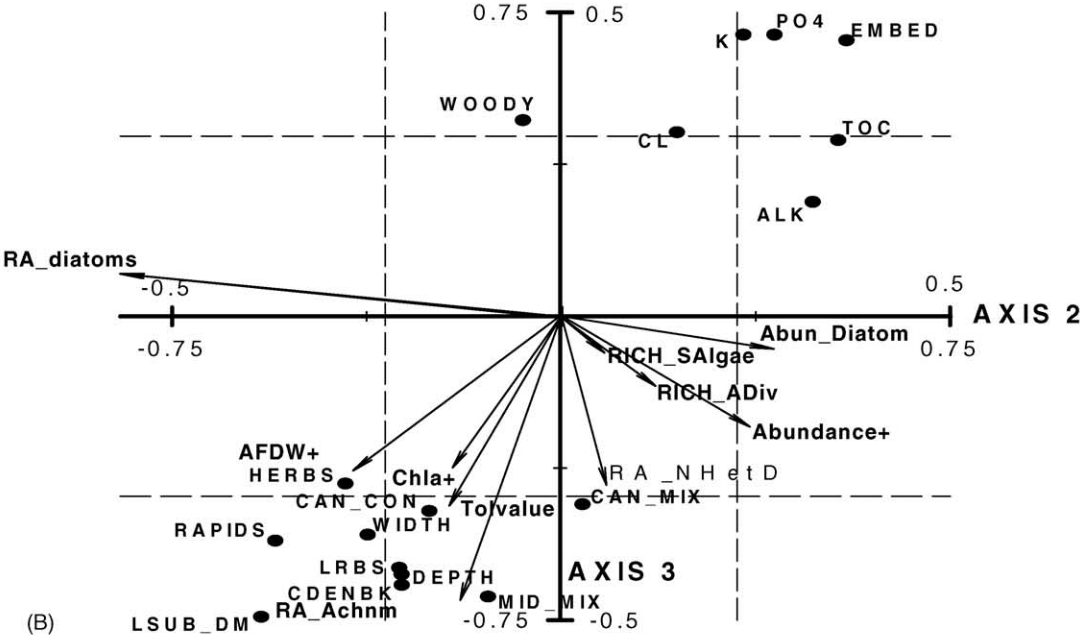


Fig. 3. Correlation biplots for (A) the first and third axes and (B) the second and third axes of the principal component analysis based on the periphyton metrics. The scale is −0.75 to 0.75 for correlations of the periphyton metrics (arrows) with the axes and −0.5 to 0.5 for correlations of the environmental variables (solid circles) with the axes. For environmental variables whose correlation was more positive or negative than the dashed lines drawn perpendicular to an axis, *P* was statistically significant when corrected with the sequential Bonferroni technique. The abbreviation codes for the environmental variables are in Table 4. Those for the metrics are Table 2. For the total cell abundance, chlorophyll, and biomass metrics, (+) = metric scores ≥ median and (−) = metric scores < median.

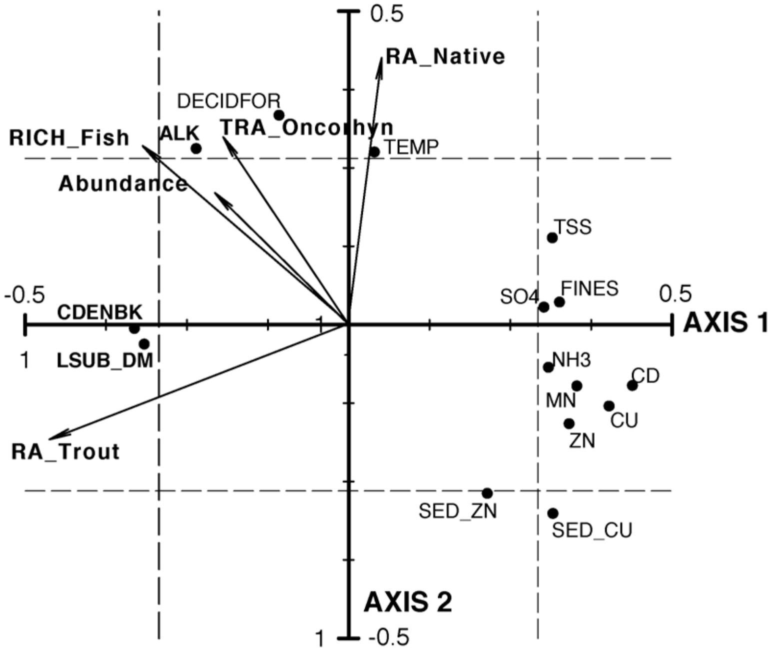


Fig. 4. Correlation biplots for the first and second axes of the principal component analysis based on the fish metrics. The scale is −1.0 to 1.0 for correlations of the fish metrics (arrows) with the axes and −0.5 to 0.5 for correlations of the environmental variables (solid circles) with the axes. For environmental variables whose correlation was more positive or negative than the dashed lines drawn perpendicular to an axis, P was statistically significant when corrected with the sequential Bonferroni technique. The abbreviation codes for the environmental variables are in Table 4. Those for the metrics are in Table 3.

($r = -0.921$, $P < 0.001$) and fish richness ($r = -0.641$, $P < 0.001$) were most correlated with the first axis (Fig. 4A), and environmental variables correlated with this axis described a gradient from streams with coarser substrates and greater riparian shading to streams characterized by fine substrates with greater suspended solids, SO_4 and dissolved concentrations of metals, such as Cd, Cu, and Zn (Fig. 4). We interpreted this axis as a mining effect gradient associated with increased dissolved metals, suspended solids, SO_4 and fine substrates and decreased riparian shading. RA (native fish) ($r = 0.854$, $P < 0.001$), *Oncorhynchus*/Salmonidae ratio ($r = 0.593$, $P < 0.001$), and fish abundance ($r = 0.417$, $P < 0.001$) were most correlated with the second axis (Fig. 4). We interpreted this axis as a second gradient also associated with mining effects, but primarily correlated with sediment metal concentrations (Fig. 4).

The sum of the fish metric scores was correlated with the first ($r = -0.810$, $P < 0.001$) and second axes ($r = 0.577$, $P < 0.001$); the sum was inversely correlated with the increasing stressor gradient associated with these axes (Fig. 4).

3.4. Comparison of indices of biotic integrity

Correlation between the PIBI and MIBI was 0.325, and their correlations with the summed fish metric scores were 0.338 and 0.419, respectively (Fig. 5). Eighteen reaches had summed fish metric scores that were zero, because no fish were found. When we excluded these 18 reaches, the correlations between the summed fish metric scores and the PIBI or MIBI were reduced to 0.006 ($P = 0.96$) and 0.091 ($P = 0.43$), respectively. PIBIs were generally greater than the MIBIs for the same reaches (see 1:1 line in Fig. 5A). Recognizing the potential summed fish metric score was half the potential PIBI or MIBI, the summed fish metric scores were less than half the PIBIs or MIBIs for the same reaches (see 2:1 line in Fig. 5B and C). However, when we segregated the reaches into quartiles based on their PIBI, MIBI, or

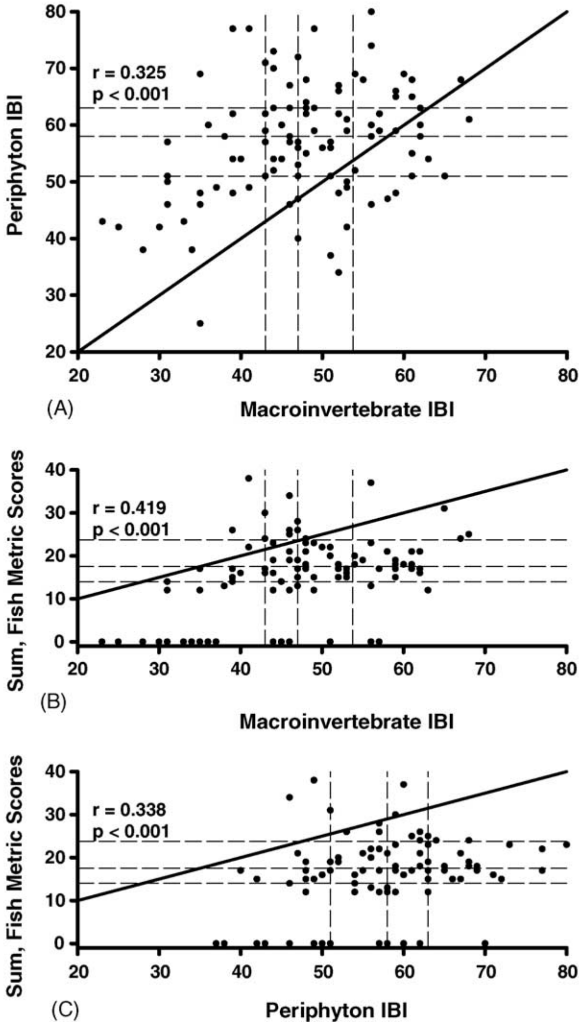


Fig. 5. Correlations for the periphyton and macroinvertebrate IBIs (A) and for the summed fish metric scores and MIBI (B) and PIBI (C), respectively (r = Pearson correlation between each pair of indices, P = the probability of the correlation). In (A), the solid, diagonal line is the 1:1 line, while in (B and C), the solid, diagonal line is the 1:2 line, because the potential maximum sum of fish metric scores was 50 compared with a potential maximum of 100 for the IBIs. The horizontal or vertical dashed lines separate the four quartiles of each index.

summed fish metric scores, from 72 to 75% of the reaches were assigned to the same or adjacent quartiles (Fig. 5).

When corrected with the sequential Bonferroni technique (Rice, 1989), the first macroinvertebrate and periphyton PCA axes were correlated ($r = 0.305$, $P = 0.002$), as were the third axes ($r = 0.444$, $P < 0.001$). The first fish PCA axis was weakly correlated with the first macroinvertebrate ($r = 0.288$, $P = 0.005$) and first periphyton ($r = 0.269$, $P = 0.009$) axes. Also, the third macroinvertebrate PCA axis was weakly correlated with the first ($r = -0.244$, $P = 0.01$) and second ($r = 0.257$, $P = 0.008$) periphyton axes.

4. Discussion

A question, which has faced the US Environmental Protection Agency and state agencies using the multimetric biotic index approach, is whether there is a need to assess all three assemblages? Are fish, macroinvertebrate, or periphyton assemblages individually sufficiently representative of the effects of environmental stressors on stream communities alone? Or do these assemblages differ in their sensitivity to various types of stressors sufficiently that more than one assemblage should be assessed? However, few studies have compared these assemblages collected concurrently at multiple reaches. Some streams, such as in coldwater systems like these in the Rocky Mountains, lack sufficient assemblage diversity to produce enough metrics for incorporation into an IBI (Lyons et al., 1996). As a result, many agencies assess at least two assemblages as part of their monitoring programs (Davis et al., 1996).

Our Rocky Mountain streams data suggest the selected metrics for fish, macroinvertebrates, and periphyton differ in their sensitivity to the major environmental gradients. Metrics for all three assemblages were sensitive to increased metal concentrations associated with mining impacts on these streams. However, fish metrics identified separate PCA axes correlated with dissolved metals concentrations, total suspended solids, and fine sediments or with sediment metals concentrations (Fig. 4A). As lithophilic spawners whose eggs develop in the interstices of stream sediments, we expected salmonids to be sensitive to embeddedness and sediment contamination (Berkman and Rabeni, 1987; McCormick et al., 1994; Hughes et al., 1998). In contrast to our expectation, the RA (Salmonidae) was most correlated with the axis associated dissolved metals and not sediment metals. The RA (native fish), *Oncorhynchus*/Salmonidae ratio, and fish abundance metrics were more correlated with the axis associated with sediment metals. Macroinvertebrate metrics partially separated these two metals gradients, which were not separated by the periphyton metrics. The second macroinvertebrate axis separates a complex environmental gradient correlated with sediment coarseness and embeddedness, PO_4, riparian shading, and the presence of woody debris. This gradient is associated with agricultural effects but cannot be separated from stream size. Similarly, the second and third periphyton axes are correlated with this gradient but do not separate the agricultural effects from stream size. In both cases, mean depth and width were inversely correlated with the agricultural gradient (Figs. 2 and 3A), and we had originally expected more agricultural effects lower in the drainage systems. Additional PCA axes were not considered, because they were uncorrelated with the IBIs.

The periphyton and macroinvertebrate IBIs for these Southern Rockies streams were correlated, as were some of their respective PCA axes. The correlations between the sum of the fish metric scores and the periphyton or macroinvertebrate IBIs were also significant. However, the correlations were all weak and disappeared when 0 values were excluded, though the axes identify similar environmental gradients. This suggests that, at least in the ecoregion we examined, indices based on these metrics are not as redundant as suggested by Karr and Chu (1999). Moreover, the presence or absence of fish do contribute information for the diagnosis of environmental stressors, despite the low diversity of the assemblage in these streams.

Our data do not clearly support the suggestion that these assemblages might differ in their correlations to certain environmental gradients because of differences in their taxa's life-histories and biogeography. The PCA axes extracted from fish and macroinvertebrate metrics were correlated with some larger-scale environmental variables, such as the riparian habitat disturbance index for pasture and hayfields and percent watershed in row crops, but most environmental variables measured reach-scale (Frissell et al., 1986) variability among streams. Sampling was confined to a single ecoregion (Omernik, 1995) and limited the potential biogeographic variation in the assemblages. In addition, the two major stressor gradients represent press-type disturbances (Resh et al., 1988; Detenbeck et al., 1992), which have had longer durations than the generation times of any taxa in these assemblages and limits any potential effect of life-histories in causing variations in the recovery of the assemblages from these disturbances. Moreover, the sampling design of our study, where most reaches were visited once, may have limited our ability to distinguish the effects of shorter-term, pulse-type disturbances. Our chemical sampling may have missed such disturbances, as would typical monitoring programs (Karr and Chu, 1999). Therefore, this data set may be inadequate to observe effects of such life-history and biogeographic differences among assemblages.

Although Lyons et al. (1996) proposed a coldwater fish IBI composed of five metrics for Wisconsin, we do not propose that the fish metrics used here be combined as a fish IBI for the Southern Rockies Ecoregion without being further evaluated as to their variance ratios, responsiveness, and redundancy (Hughes et al., 1998). However, these five metrics appear to capture much of the variation in this low diversity assemblage and were able to separate reaches with high concentrations of dissolved metals and sulfate that generally occur closer to mine sources from reaches with high concentrations of sediment metals that may occur at greater distances from mine sources (Moore et al., 1991). This is consistent with the results of McCormick et al. (1994) and has potential to assist in designing regional programs to address the widespread effects of historical metal mining on Rocky Mountain streams (Lyon et al., 1993).

In conclusion, the three assemblages differed in their sensitivities to the different stressor gradients observed in these streams, and it would be warranted to use more than one assemblage to assess the biotic integrity of these streams. Because of these differences in the assemblages' sensitivities and the lack of diversity in the fish assemblages, another approach to designing an IBI for this ecoregion might be to select subsets of the metrics for each assemblage for incorporation into a multi-assemblage, multimetric index of biotic integrity. Such an index would further simplify interpretation of the biological data by managers and the public by providing a single index value, instead of several possibly conflicting values. Such a value may also be more indicative of the integrity of the entire stream community. Furthermore, examination of the differing responses of the component metrics in such an index to specific stressors could maximize the potential to diagnose the environmental stressors at individual reaches.

A single, 12-metric multi-assemblage index for the Southern Rockies Ecoregion might include the metrics, RA (native fish), fish richness, fish abundance, and RA (Salmonidae) for fish; Plecoptera and Ephemeroptera genera richness, Chironomidae genera richness, Tanytarsini/Chironomidae ratio, and RA (five most dominant genera) for macroinvertebrates; and non-diatom genera richness, diatom abundance, RA (diatoms), and RDA (*A. minutissima*) for periphyton. We feel these 12 metrics hold the greatest promise because of their correlations with the stressor gradients identified in our analyses. However, such an index need not necessarily consist of equal number of metrics from each assemblage, and the metrics selected should be based on their correlations with specific environmental stressors. Further testing with additional data is needed to assess the utility of combining these mixed metrics into a biotic index.

Acknowledgements

Field sampling design and data collection were funded by USEPA's Office of Research and Development as part of its Regional Environmental Monitoring and Assessment Programs. J. Lazorchak (USEPA, NERL, Cincinnati, OH) and P. Johnson (USEPA, Region 8, Denver, CO) coordinated the field work and analysis of the chemistry and macroinvertebrate samples and, along with W. Schroeder (USEPA, Region 8, Denver, CO), provided details on the sampling and analyses for water and sediment chemistry. C. Seeliger (USEPA, NHEERL, Corvallis, OR) helped with management of the physical environmental characteristics database. Comments by L. Yuan (USEPA, NCEA, Washington, DC), J. Lazorchak, S. Golladay (J.W. Jones Ecological Research Center, Newton, GA), I. Waite (USGS, WRD, Portland, OR), and an anonymous reviewer greatly improved the quality of the manuscript. Data analysis and preparation of this manuscript were supported in part by an appointment of the first author to the Postgraduate Research Program at the National Exposure Research Laboratory administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Environmental Protection Agency and by an EPA cooperative agreement (CR824682) with Oregon State University. The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

References

Amblard, C., Couture, P., Bourdier, G., 1990. Effect of a pulp and paper mill effluent on the structure and metabolism of periphytic algae in experimental streams. Aquat. Toxicol. 18, 137–162.

APHA (American Public Health Association), 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association, Washington, DC.

Angermier, P.L., Karr, J.R., 1986. Applying an index of biotic integrity based on stream fish communities: considerations in sampling and interpretation. N. Am. J. Fish. Manage. 6, 418–429.

Angermeier, P.L., Smogor, R.A., Stauffer, J.R., 2000. Regional frameworks and candidate metrics for assessing biotic integrity in mid-Atlantic highland streams. Trans. Am. Fish. Soc. 129, 962–981.

Bahls, L., 1993. Periphyton bioassessment methods for Montana streams. Water Quality Bureau, Montana Department of Health and Environmental Sciences, Helena, MT.

Barbour, M.T., Gerritsen, J., Griffith, G.E., Frydenborg, R., Mccarron, E., White, J.S., Bastian, M.L., 1996. A framework for biological criteria for Florida streams using benthic macroinvertebrates. J. N. Am. Benthol. Soc. 15, 185–211.

Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B., 1999. Rapid Bioassessment Protocols for Use in Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, 2nd ed. EPA 841/B-99/002. US Environmental Protection Agency, Washington, DC.

Berkman, H.E., Rabeni, C.F., 1987. Effect of siltation on stream fish communities. Environ. Biol. Fish. 18, 285–294.

Boston, H.L., Hill, W.R., Stewart, A.J., 1991. Evaluating direct toxicity and food-chain effects in aquatic systems using natural periphyton communities. In: Gorsuch, J.W., Lower, W.R., Wang, W., Lewis, M.A. (Eds.), Plants for toxicity assessments, Vol. 2. ASTM STP 1115. American Society for Testing and Materials, Philadelphia, PA, pp. 126–145.

Bott, T.L., Rogenmuser, K., 1978. Effects of no. 2 fuel oil, Nigerian crude oil, and used crankcase oil on attached algal communities: acute and chronic toxicity of water-soluble constituents. Appl. Environ. Microb. 36, 673–682.

Christie, C.E., Smol, J.P., 1993. Diatom assemblages as indicators of lake trophic status in southeastern Ontario lakes. J. Phycol. 29, 575–586.

Clark, J.R., Dickson, K.L., Cairns, J., 1979. Estimating aufwuchs biomass. In: Weitzel, R.L. (Ed.), Methods and measurement of periphyton communities: A review. ASTM STP 690. American Society for Testing and Materials, Philadelphia, PA, pp. 116–141.

Clements, W.H., 1991. Community responses of stream organisms to heavy metals: a review of observational and experimental approaches. In: Newman, M.C., McIntosh, A.W. (Eds.), Metal Ecotoxicology: Concepts and Applications. Lewis Publishers, Chelsea, MI, pp. 363–391.

Colorado Office of Active and Inactive Mines, 1996. Inactive mine reclamation program. Colorado Office of Active and Inactive Mines, Denver, CO.

Davis, W.S., Snyder, B.D., Stribling, J.B., Stoughton, C., 1996. Summary of state biological assessment programs for streams and wadeable rivers. EPA 230/R-96/007. U.S. Environmental Protection Agency, Office of Policy, Planning, and Evaluation, Washington, DC.

Deshon, J.E., 1995. Development and application of the invertebrate community index (ICI). In: Davis, W.S., Simon, T.P. (Eds.), Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making. Lewis Publishers, Boca Raton, FL, pp. 217–243.

Detenbeck, N.E., Devore, P.W., Niemi, G.J., Lima, A., 1992. Recovery of temperate-stream fish communities from disturbance: a review of case studies and synthesis of theory. Environ. Manage. 16, 33–53.

Fausch, K.D., Karr, J.R., Yant, P.R., 1984. Regional application of an index of biotic integrity based on stream fish communities. Trans. Am. Fish. Soc. 113, 39–55.

Feminella, J.W., 2000. Correspondence between stream macroinvertebrate assemblages and eoregions of the southeastern USA. J. N. Am. Benthol. Soc. 19, 442–461.

Frissell, C.A., Liss, W.J., Warren, C.E., Hurley, M.D., 1986. A hierarchical framework for stream habitat classification: viewing streams in a watershed context. Environ. Manage. 10, 199–214.

Gilbert, R.O., 1987. Statistical Methods for Environmental Pollution Monitoring. Van Nostrand Reinhold, New York, NY.

Griffith, M.B., Kaufmann, P.R., Herlihy, A.T., Hill, B.H., 2001. Analysis of macroinvertebrate assemblages in relation to environmental gradients in Rocky Mountain streams. Ecol. Appl. 11, 489–505.

Griffith, M.B., Hill, B.H., Herlihy, A.T., Kaufmann, P.R., 2002. Multivariate analysis of periphyton assemblages in relation to environmental gradients in Colorado Rocky Mountain streams. J. Phycol. 38, 1–13.

Hall, R.I., Smol, J.P., 1992. A weighted-averaging regression and calibration model for inferring total phosphorous concentration from diatoms in British Columbia (Canada) lakes. Freshw. Biol. 27, 417–434.

Heisel, D.R., 1990. Less than obvious: statistical treatment of data below the detection limit. Environ. Sci. Technol. 24, 1774–1787.

Herlihy, A.T., Larsen, D.P., Paulsen, S.G., Urquhart, N.S., Rosenbaum, B.J., 2000. Designing a spatially balanced, randomized site selection process for regional stream surveys: the EMAP Mid-Atlantic pilot study. Environ. Monit. Assess. 63, 95–113.

Hill, B.H., Herlihy, A.T., Kaufmann, P.R., Stevenson, J.R., Mccormick, F.H., Johnson, C.B., 2000. Use of periphyton assemblage data as an index of biotic integrity. J. N. Am. Benthol. Soc. 19, 50–67.

Hilsenhoff, W.L., 1987. An improved biotic index of organic stream pollution. Great Lakes Entomol. 20, 31–39.

Hughes, R.M., Gammon, J.R., 1986. Longitudinal changes in fish assemblages and water quality in the Willamette River, Oregon. Trans. Am. Fish. Soc. 116, 196–209.

Hughes, R.M., Kaufman, P.R., Herlihy, A.T., Kincaid, T.M., Reynolds, L., Larsen, D.P., 1998. A process for developing and evaluating indices of fish assemblage integrity. Can. J. Fish. Aquat. Sci. 55, 1618–1631.

Karr, J.R., 1993. Defining and assessing ecological integrity beyond water quality. Environ. Toxicol. Chem. 12, 1521–1531.

Karr, J.R., Chu, E.W., 1999. Restoring Life in Running Waters: Better Biological Monitoring. Island Press, Washington, DC.

Karr, J.R., Fausch, K.D., Angermeier, P.L., Yant, P.R., Schlosser, I.J., 1986. Assessing biological integrity in running waters: a method and its rationale. Special Publication 5. Illinois Natural History Survey, Champain, IL.

Kaufmann, P.R., Levine, P., Robison, E.P., Seeliger, C., Peck, D.V., 1999. Quantifying physical habitat in wadeable streams. EPA 620/R99/003. U.S. Environmental Protection Agency, Corvallis, OR.

Kiffney, P.M., Clements, W.H., 1994. Structural responses of benthic macroinvertebrate communities from different stream orders to zinc. Environ. Toxicol. Chem. 13, 389–395.

Kosinski, R.J., 1984. The effect of terrestrial herbicides on the community structure of stream periphyton. Environ. Pollut. A 36, 165–189.

Lange-Berlatot, H., 1979. Pollution tolerance of diatoms as a criterion for water quality estimation. Nova Hedwigia 64, 285–304.

Lazorchak, J.M., Klemm, D.J., Peck, D.V., 1998. Environmental Monitoring and Assessment Program—Surface Waters: Field Operations and Methods Manual for Measuring the Ecological Condition of Wadeable Streams. EPA 620/R-94/004F. U.S. Environmental Protection Agency, Washington, DC.

Leland, H.V., 1995. Distribution of phytobenthos in the Yakima River basin, Washington, in relation to geology, land use, and other environmental factors. Can. J. Fish. Aquat. Sci. 52, 1108–1129.

Lenat, D.R., 1983. Chironomid taxa richness: natural variation and use in pollution assessment. Freshw. Invert. Biol. 2, 192–198.

Lewis, P.A., Klemm, D.J., 1994. Laboratory methods for processing of benthic invertebrate samples. In: Klemm, D.J., Lazorchak, J.M. (Eds), Environmental Monitoring and Assessment Program—Surface Waters and Region 3 Regional Environmental Monitoring and Assessment Program: 1994 Pilot Laboratory Methods Manual for Streams. EPA 620/R-94/003. U.S. Environmental Protection Agency, Washington, DC, pp. 5.1–5.16.

Lyon, J.S., Hilliard, T.J., Bethel, T.N., 1993. Burden of Gilt. Mineral Policy Center, Washington, DC.

Lyons, J., Wang, L., Simonson, T.D., 1996. Development and validation of an index of biotic integrity for coldwater streams in Wisconsin. N. Am. J. Fish. Manage. 16, 241–256.

Mackey, R.J., 1992. Colonization by lotic macroinvertebrates: a review of processes and patterns. Can. J. Fish. Aquat. Sci. 49, 617–628.

McCarron, E., Frydenborg, R., 1997. The Florida bioassessment program: an agent for change. Hum. Ecol. Risk Assess. 3, 967–977.

McCormick, F.H., Hill, B.H., Parrish, L.P., Willingham, W.T., 1994. Mining impacts on fish assemblages in the Eagle and Arkansas Rivers, Colorado. J. Freshw. Ecol. 9, 145–179.

Miller, D.L., Leonard, P.M., Hughes, R.M., Karr, J.R., Moyle, P.B., Schrader, L.H., Thompson, B.A., Daniels, R.A., Fausch, K.D., Fitzhugh, G.A., Gammon, J.R., Halliwell, D.B., Angermeier, P.L., Orth, D.J., 1988. Regional applications of an index of biotic integrity for use in water resource management. Fisheries 13, 12–20.

Moore, J.M., Luoma, S.N., Peters, D., 1991. Downstream effects of mine effluent on an intermontain riparian system. Can. J. Fish. Aquat. Sci. 48, 222–232.

NASS (National Agricultural Statistics Service), 1999. 1997 Census of Agriculture. vol. 1. Geographic Area Series, Part 6. Colorado State and County Data. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, DC.

Niemi, G.J., Devore, P., Detenbeck, N., Taylor, D., Lima, A., Pastor, J., Yount, I.D., Naiman, R.J., 1990. Overview of case studies on recovery of aquatic systems from disturbance. Environ. Manage. 14, 471–587.

Omernik, J.M., 1987. Ecoregions of the conterminous United States. Ann. Assoc. Am. Geogr. 77, 118–125.

Omernik, J.M., 1995. Ecoregions: a spatial framework for environmental management.. In: Davis, W.S., Simon, T.P. (Eds.), Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making. Lewis Publishers, Boca Raton, FL, pp. 49–62.

Palmer, C.M., 1969. A composite rating of algae tolerating organic pollution. J. Phycol. 5, 78–82.

Pan, Y., Stevenson, R.J., Hill, A.H., Herlihy, A.T., Collins, G.B., 1996. Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment. J. N. Am. Benthol. Soc. 15, 481–495.

Pan, Y., Stevenson, R.J., Hill, B.H., Kaufmann, P.R., Herlihy, A.T., 1999. Spatial patterns and ecological determinants of benthic algal assemblages in mid-Atlantic streams. J. Phycol. 35, 460–468.

Patrick, R., 1977. Effects of trace metals in the aquatic ecosystem. Am. Sci. 66, 185–191.

Patrick, R., Reimer, C.W. 1966. The diatoms of the United States, exclusive of Alaska and Hawaii, vol. 1. Monogr. Acad. Nat. Sci. Phil., No. 13.

Plafkin, J.L., Barbour, M.T., Porter, K.D., Gross, S.K., Hughes, R.M., 1989. Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates, and fish. EPA 440/4-89/001. US Environmental Protection Agency, Washington, DC.

Resh, V.H., Brown, A.V., Covich, A.P., Gurtz, M.E., Li, H.W., Minshall, G.W., Reice, S.R., Sheldon, A.L., Wallace, J.B., Wissmar, R., 1988. The role of disturbance in stream ecology. J. N. Am. Benthol. Soc. 7, 433–455.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.

Rosen, B.H., 1995. Use of periphyton in the development of biocriteria. In: Davis, W.S., Simon, T.P. (Eds.), Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making. Lewis Publishers, Boca Raton, FL, pp. 209–215.

SAS (SAS Institute), 1996. SAS/STAT Users Guide. SAS Institute, Cary, NC.

Schlosser, I.J., 1990. Environmental variation, life history attributes, and community structure in stream fishes: implications for environmental management and assessment. Environ. Manage. 5, 621–628.

Sigmon, C.F., Kania, H.J., Beyers, R.J., 1977. Reductions in biomass and diversity resulting from exposure to mercury in artificial streams. J. Fish. Res. Bd. Can. 34, 493–500.

Steinman, A.D., Mulholland, P.J., Kirschtel, D.B., 1991. Interactive effects of nutrient reduction and herbivory on biomass, taxonomic structure, and P uptake in lotic periphyton communities. Can. J. Fish. Aquat. Sci. 48, 1951–1959.

Stevenson, R.J., Bahls, L.L., 1999. Peiphyton protocols. In: Barbour, M.T., Gerritsen, J., Synder, B.D., Stribling, J.B. (Eds.), Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Microinvertebrates, and Fish. 2nd ed. EPA 841/B-99/002. US Environmental Protection Agency, Washington DC, pp. 6.1–6.22.

Stevenson, R.J., Peterson, C.G., 1991. Emigration and immigration can be important determinants of benthic diatoms assemblages in streams. Freshw. Biol. 26, 279–294.

Strahler, A.N., 1957. Quantitative analysis of watershed geomorphology. Trans. Am. Geophys. Union 38, 913–920.

ter Braak, C.J.F., 1987. CANOCO—a FORTRAN program for canonical community ordination by [partial], [detrended], [canonical] correspondence analysis, principal components analysis, and redundance analysis. Agricultural Mathematics Group, Wageningen, The Netherlands.

ter Braak, C.J.F., 1995. Ordination. In: Jongman, R.H.G., ter Braak, C.J.F., van Tongeren, O.F.R. (Eds.), Data Analysis in Community and Landscape Ecology. New Edition Cambridge University Press, Cambridge, pp. 91–173.

Townsend, C.R., Hildrew, A.G., 1994. Species traits in relation to a habitat templet for river systems. Freshw. Biol. 31, 265–275.

USEPA (US Environmental Protection Agency), 1987. Handbook of Methods for Acid Deposition Studies: Laboratory Analyses for Surface Water Chemistry. EPA 600/4-87/026. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC.

USEPA (US Environmental Protection Agency), 1994. Test Methods for Evaluating Solid Waste, vol. 1A. EPA 600/54-87/032. U.S. Environmental Protection Agency, Washington, DC.

van Dam, H., Mertens, A., Sinkeldam, J., 1994. A coded checklist and ecological indicator values of freshwater diatoms from The Netherlands. Neth. J. Aquat. Ecol. 28, 117–133.

Wallace, J.B., Anderson, N.H., 1996. Habitat, life history, and behavioral adaptations of aquatic insects. In: Merritt, R.W., Cummins, K.W. (Eds.), An Introduction to the Aquatic Insects of North America. 3rd ed. Kendall/Hunt Publishing Co., Dubuque, IO, pp. 41–73.

Wallace, J.B., Grubaugh, J.W., Whiles, M.R., 1996. Biotic indices and stream ecosystem processes: results from an experimental study. Ecol. Appl. 6, 140–151.