EPA/600/R-06/078
February 2007

Relationships Among Exceedances of Chemical Criteria or Guidelines, the Results of Ambient Toxicity Tests, and Community Metrics in Aquatic Ecosystems

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NOTICE

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ABSTRACT

In order to use bioassessments to help to diagnose or identify the specific environmental stressors affecting aquatic or marine ecosystems, a better understanding is needed of the relationships among community metrics, ambient chemical criteria or guidelines and ambient toxicity tests. However, these relationships are not necessarily simple, because metrics generally assess measurement endpoints at the community level of biological organization, while ambient criteria or guidelines and ambient toxicity tests assess measurement endpoints at the organism level. Although a basic hierarchical relationship exists between the levels of biological organization used as measurement endpoints by these methods, quantification of this relationship may be further complicated by the influence of other differences among these methods that affect their sensitivity and specificity to the stressors present at individual sites.

Since 1990, the U.S. Environmental Protection Agency has conducted Environmental Monitoring and Assessment Program surveys of both wadeable stream and estuarine sites. These surveys have collected data on biotic assemblages, physical and chemical habitat characteristics and, in some cases, water and sediment chemistry and toxicity. Among these studies is a survey of wadeable streams in the Southern Rockies ecoregion of Colorado in 1994 and 1995 and a survey of estuaries in the Virginian Province of the eastern United States from 1990 to 1993. Streams in the Southern Rockies ecoregion are affected by contamination from hardrock metal mining, while the estuarine sites may be affected by sediment contamination by polyaromatic hydrocarbons and metals. We characterized streams as metals-affected based on exceedance of hardness-adjusted metals criteria for Cd, Cu, Pb and Zn in surface water; on water column toxicity tests (48-hour *Pimephales promelas* and *Ceriodaphnia dubia* survival); on exceedance of sediment threshold effect levels; or on sediment toxicity tests (7-day *Hyalella azteca* survival and growth). Estuarine sites were characterized as affected by sediment contamination based on exceedance of sediment guidelines or on sediment toxicity tests (i.e., 10-day *Ampelisca abdita* survival). The results of these classifications were contrasted by use of contingency tables and a measure of association, γ. Then, assemblage metrics were compared statistically among affected and unaffected sites to identify metrics sensitive to the contamination. In streams, a number of macroinvertebrate metrics, particularly richness metrics, were less in groups of sites identified as affected by metals with the criteria or

ambient toxicity tests, while other metrics were not. Fish metrics were less sensitive to the metal contamination, but this lack of sensitivity is likely because of the low diversity of fish assemblages in these Rocky Mountain streams. Similarly at the estuarine sites, a number of benthic metrics differed between the groups of sites segregated using the organism-level measure, while other metrics did not. These same metrics also exhibited relationships with contaminant concentrations in regression analyses. This variation among metrics depends on the sensitivity of the individual metrics to the stressor gradients of interest as many metrics may not measure the community responses characteristic of a specific stressor. The differences between groups for the more sensitive metrics imply that a relationship exists between the organism-level effects assessed by ambient chemistry or ambient toxicity tests and the community-level effects assessed by community metrics. However, the organism-level effects are only predictive to a limited extent of the community-level effects at individual sites.

Beyond the differences in the levels of biological organization represented by their measurement endpoints, these methods differ in their specificity and sensitivity to different stressors. Criteria or guidelines are specific to the contaminants being measured and assessed and cannot assess contaminants or stressors that are not measured or that lack guidelines for comparison. Ambient toxicity tests should detect effects of any toxicants present and bioavailable, but cannot assess other characteristics of a site that can affect the biotic community. Community metrics are the least specific of the three methods, because they measure directly community-level effects in the native assemblages. Metrics may be selected that are sensitive to a specific stressor, but they also will be sensitive to other stressors, such as alterations in physical habitat that are not addressed by the other methods.

Other factors also affect the relative sensitivity and predictiveness of these different methods. Toxicity tests and chemical criteria or benchmarks based on measurement endpoints that are chronic in duration would be more predictive of community-level effects. Toxicity tests often use one or two standard species, which can be more tolerant of specific contaminants than other indigenous species and would be less predictive of community-level effects than a chemical criterion or benchmark based on a species sensitivity distribution composed of many species.

Preferred citation:
U.S. EPA. 2006. Relationships Among Exceedances of Chemical Criteria or Guidelines, the Results of Ambient Toxicity Tests, and Community Metrics in Aquatic Ecosystems. U.S. Environmental Protection Agency, National Center for Environmental Assessment, Cincinnati, OH. EPA/600/R-06/078.

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LIST OF ABBREVIATIONS

ANCOVA	Analysis of Co-variance
ANOVA	Analysis of Variance
APHA	American Public Health Association
AVS	Acid Volitile Sulfide
AWQC	Ambient Water Quality Criteria
EMAP	Environmental Monitoring and Assessment Program
EPT	Ephemeroptera, Plecoptera, and Trichoptera
ER-M	Effects Range - Median
LCL	Lower Confidence Limit
PAHs	Polyaromatic hydrocarbons
PEL	Potential Effects Level
R-EMAP	Regional Environmental Monitoring and Assessment Program
SEM	Simultaneously-Extracted Metals
TEL	Threshold-effect Level
UCL	Upper Confidence Limit
USGS	United States Geological Survey

PREFACE

U.S. EPA's Office of Water, Regional Offices, and other program offices use three general approaches for the ecological assessment of contaminant exposure and effects in surface waters or sediments: (1) comparisons of chemical concentration data in water or sediments to chemical criteria or other guidelines, (2) ambient toxicity assessments of sediment or water, and (3) bioassessments of biotic assemblages, such as fish, invertebrates, or periphyton. In practice, these methods are used independently to assess the attainment of aquatic life use in various water bodies. Chemical criteria and ambient toxicity assessments are indirect approaches, because they evaluate the suitability of a water body to support a healthy biotic community, whereas bioassessments directly assess the existing biotic community. Moreover, these different methods measure effects using differing measurement endpoints that assess different levels of biological organization. Chemical criteria and ambient toxicity assessments are based on measures of the responses of organisms and are generally indicative of organism- or possibly population-level effects. Bioassessments, while usually working with selected biotic assemblages, are generally indicative of the community level effects. In addition, chemical criteria and ambient toxicity assessments differ, because chemical criteria or guidelines can be based on bioassay data from a broad range of taxa, whereas ambient toxicity assessments use a few standard bioassay species.

It is not clear whether these three approaches provide similar levels of protection to aquatic organisms, populations and communities. The two studies presented in this report begin to address that question. Results of the first study suggest that, for metals in Colorado streams, chemical criteria combined in a concentration additivity model approximate the threshold for effects on aquatic communities observed in bioassessments. Results of the second study are not as clear but suggest that biotic metrics can be more protective then chemical thresholds or ambient toxicity assessments.

This report is intended for ecological risk assessors and field biologists in the Office of Water, Regional Offices, other program offices, and the States interested in the application of these methods for evaluating the attainment of aquatic life use in streams and estuaries and for assessing the causes of impairment in affected systems. This report may also be of interest to research scientists interested in the further development of these methods.

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ACKNOWLEDGMENTS

For the Colorado R-EMAP study in Chapter 2, field sampling design and data collection were funded by U.S. EPA's Office of Research and Development as part of its Regional Environmental Monitoring and Assessment Programs. P. Johnson (U.S. EPA, Region VIII, Denver, Colorado) helped coordinate the field work and analysis of the chemistry and macroinvertebrate samples and, along with W. Schroeder (U.S. EPA, Region VIII, Denver, Colorado), provided details on the sampling and analyses for water and sediment chemistry. Comments by M. Kravitz, F. McCormick, G. Suter and two anonymous reviewers greatly improved the quality of the manuscript on which Chapter 2 is based. Also, preparation of that manuscript was supported in part by a U.S. EPA cooperative agreement (CR824682) with Oregon State University.

For the Virginian Estuarine Province EMAP study in Chapter 3, field sampling design and data collection were funded by U.S. EPA's Office of Research and Development as part of its Environmental Monitoring and Assessment Program - Estuaries and managed by D. Keith, C.J. Strobel, J. Martinson, J.B. Frithsen, K.J. Scott, J. Paul, A.F. Holland, R.W. Latimer and S.C. Schimmel. Comments by J. Paul improved the quality of Chapter 3.

1. INTRODUCTION

In general, the U.S. EPA has used three different methods for the ecological assessment of contaminant exposure and effects in surface waters or sediments. These methods are (1) comparisons of chemical concentration data in water or sediments to chemical criteria or other guidelines, (2) ambient toxicity assessments of sediment or water and (3) bioassessments of selected biotic assemblages, such as fish, invertebrates or periphyton.

Chemical criteria or other guidelines are generally concentrations of specific contaminants of interest that are associated with some threshold for biological effects. These guidelines are derived using numerical methods from compilations of laboratory bioassay or other effects data, such as species sensitivity distributions (Suter et al., 2001). The most commonly-used chemical criteria are the national ambient water quality criteria for the protection of aquatic life that have been derived from laboratory bioassay data following U.S. EPA guidelines (1985). Procedures have been proposed for deriving sediment guidelines for non-ionic organic chemicals or metals by applying the theory of equilibrium-partitioning to water quality criteria to estimate threshold concentrations of these contaminants in sediment pore water (U.S. EPA, 2003a; Hansen et al., 1996). This approach has been extended to assess mixtures of polyaromatic hydrocarbons (PAHs) and divalent metals (Swartz et al., 1995; U.S. EPA, 2003b,c). Other paired chemistry and effects data sets, usually for natural sediments containing mixtures of contaminants, have been used to derive sediment-effects concentrations such as Effects Range - Median (ER-M), and Potential Effects Level (PEL, MacDonald et al., 1996). An ER-M is defined as a sediment chemical concentration above which effects were frequently observed or predicted for most species (Long et al., 1995). A PEL is defined as a sediment chemical concentration above which adverse effects were frequently observed. Paired chemistry and sediment toxicity test data have been used to derive sediment effect concentrations (U.S. EPA, 1996) or logistic regressions that estimate the probability that a sediment is toxic (Field et al., 2002). Quantitative chemical data for water or sediments are compared with these chemical criteria, guidelines or sediment-effects concentrations to determine whether a contaminant of interest is at a concentration that may have adverse effects on aquatic organisms.

In ambient toxicity assessments, samples of sediments or water are tested directly in laboratory bioassays with standard organisms and protocols. These standard organisms include *Pimephales promelas* Rafinesque (fathead minnow) and *Ceriodaphnia dubia* (Jurine) (a cladoceran) for testing freshwater (U.S. EPA, 1993), *Hyalella azteca* Saussure (an amphipod) and *Chironomus tentans* Fabricius (a midge) for testing freshwater sediments (U.S. EPA, 2000a), *Mysidopsis bahia* (M.) (mysid shrimp) or *Cyprinodon variegatus* Lacepède (sheepshead minnow) for testing estuarine water (U.S. EPA, 1993) or *Ampelisca abdita* Mills (an amphiod) and *Rhepoxynius abronius* (J.L. Barnard) (an amphipod) for testing estuarine sediments (U.S. EPA, 1994a). Acute tests for water are conducted for 24 to 96 hours, while those for sediments are conducted for 7 to 10 days, and the measurement endpoints are survival and sometimes growth. Chronic tests may be conducted for 7 to 42 days, and the measurement endpoints are survival, growth, and usually some measure of reproductive success. A sample is identified as having adverse effects on aquatic organisms if a measurement endpoint is significantly reduced compared with concurrently-run controls.

In bioassessments, samples of a selected biotic assemblage, such as fish or benthic invertebrates, are collected, and the organisms are identified, counted, and sometimes weighed. These data are then used to calculate and score metrics that describe the assemblage. The metric scores are then summed to produce an index of biotic integrity (Barbour et al., 1999). A broad range of metrics can be calculated depending on the diversity of the selected biotic assemblage. General classes of metrics include richness metrics (i.e., counts of the number of specified taxa in the assemblage), evenness metrics, composition metrics, trophic or habitat guild metrics. Whether a metric is indicative of adverse effects at a site can be determined by comparison with its value at sites determined to represent reference conditions (Barbour et al., 1999). Variation in a metric relative to a known stressor gradient, particularly in relation to a threshold in a stressor gradient, can also show adverse effects (Karr and Chu, 1998). We use this second definition in this report.

These different methods assess effects using differing assessment and measurement endpoints at different levels of biological organization (U.S. EPA, 2003d). Moreover, assumptions exist about the relationships among the levels of protection associated with each of these assessment tools. Chemical criteria, guidelines, or effects-concentrations that are based on laboratory bioassay data and ambient toxicity assessments that use laboratory bioassays are based on measures of the responses of

organisms, such as survival, growth and fecundity, and, therefore, are show organism-level effects. Bioassessments, because they quantify characteristics of selected biotic assemblages, show community-level effects. In addition, chemical and ambient toxicity assessments differ, because chemical assessments can be based on laboratory bioassay or other data from a broad range of taxa, whereas ambient toxicity assessments use a few standard, bioassay species to test environmental samples.

A premise about the relationships among the measurement endpoints of each of these assessment tools and the protection for higher levels of biological organization is that these levels of biological organization are hierarchical (O'Neill et al., 1986). Laboratory bioassays measure survival, growth, and fecundity, but these organism-level effects may be extrapolated to population-level effects because rates of mortality and reproduction affect the number of individuals in a population (Kuhn et al., 2000). Chemical water quality criteria, as derived by U.S. EPA (1985), are assumed to be protective of at least 95% of the taxa in aquatic communities because the thresholds are set at the fifth percentile of the genera sensitivity distribution for a chemical. Other methods for deriving chemical guidelines may use different thresholds. The level of protection at the community level for ambient toxicity assessments may be variable because of variable sensitivity of the bioassay species to different chemicals compared with the indigenous taxa in communities.

Some of these premises have been previously addressed in studies intended to validate whole effluent and ambient toxicity tests (Mount et al., 1984, 1985, 1986a,b,c; Mount and Norberg-King, 1985, 1986; Norberg-King and Mount, 1986; Birge et al., 1989; Eagleson et al., 1990; Dickson et al., 1992; Clements and Kiffney, 1994; Diamond and Daley, 2000), but many of those studies predate the full development of standardized bioassessment protocols and the use of many community-level metrics. Moreover, these studies were mostly conducted at relatively few individual sites on single stream systems upstream and downstream of known point-sources.

Mount et al. (1984) and related studies compared the results of chronic 7-day tests with *Ceriodaphnia* spp. and *P. promelas* of serial dilutions of effluents and of ambient water and the results of community surveys of fish or macroinvertebrates. Their study reaches included from one to more than ten point sources, which included publically-owned treatment plants (POTWs), industrial plants, and chemical plants. Community measurements included the total number of taxa, total density, Shannon-Weaver species diversity, a community-loss index, and the density and percentage

composition of individual species and of major taxa, such as Ephemeroptera, Trichoptera, Chironomidae, and Mollusca.

Birge et al. (1989) compared the results of 8-day embryo-larval tests with *P. promelas* of ambient water and the results of community surveys of macroinvertebrates and fish. Their study reaches were upstream and downstream from a POTW, and community measurements included Shannon-Weaver species diversity, a coefficient of dominance, species richness, total density, the percent composition of macroinvertebrate functional groups, and the presence or absence of fish species.

Eagleson et al. (1990) compared the results of chronic, 7-day tests with *C. dubia* of effluents taking into account the site-specific dilution of the effluent in the receiving stream and the results of community surveys of macroinvertebrates conducted upstream and downstream of the effluent discharge. The sources of the effluents were classified as either municipal or industrial. Community measurements were total taxa richness and the taxa richness of major taxa groups, such as Ephemeroptera, Plecoptera, Trichoptera, Chironomidae, Oligochaeta, and Crustacea.

Dickson et al. (1992) reanalyzed data from several of the above studies along with data from the Trinity River collected upstream and downstream six major POTWs. The Trinity River study compared short-term, chronic tests with *C. dubia* and *P. promelas* of ambient water with the results of community surveys of macroinvertebrates and fish. Community measurements were fish or macroinvertebrate richness and evenness, and a fish index of biotic integrity.

Clements and Kiffney (1994) compared the results of chronic, 7-day tests with *C. dubia* of ambient water collected along a metals contamination gradient upstream and downstream of California Gulch, a point source of mine drainage to the Arkansas River, with the results of community surveys of macroinvertebrates. Community measurements were taxa richness, total abundance, and the percent abundance of Ephemeroptera and Orthocladiinae.

Use of these methods in ecological assessment and management of environmental contaminants can benefit from greater understanding of the relationships among these levels of biological organization and their protection by the measurement endpoints assessed by these methods. Although the Office of Water follows a policy of independent applicability (U.S. EPA, 1991), this policy has been questioned because of misunderstandings about the relationships among these methods and their relative limitations.

The following described research tested the assumptions about the relationships between the measurement endpoints at the organism level used by chemical criteria or guidelines and other bioassay-based regulatory tools with assemblage metrics, which are measurement endpoints at the community level of biological organization. The objectives of this project were to

(1) assess the availability of data sets from studies that have used two or all three of the methods to assess sediment or surface water quality at a number of sites,

(2) compare and contrast statistically the results produced by the different methods at different sites to determine the relationships among the measurement endpoints assessed by each method,

(3) assess the extent to which the methods that are based on measurement of organism-level effects are predictive and protective of effects at the assemblage or community level as measured by assemblage metrics.

1.1. DATA SETS USED

A limitation to this approach is the availability of data sets from studies that have used two or all three of the methods to assess sediment or surface water quality at a number of sites. Several regional data sets were identified from the U.S. EPA's Environmental Monitoring and Assessment Program (EMAP), and these data sets encompass studies of both wadeable streams and estuaries. However, these EMAP data sets have limitations. First, many EMAP studies have not analyzed potentially toxic contaminants in surface water, either in streams or estuaries. Because of the random-selection approach of EMAP, only a small proportion of sites are likely to have surface water concentrations of these contaminants above detectable limits, unless widespread sources for a contaminant exist across a region. In 1994 and 1995, a Regional EMAP (R-EMAP) survey of the Southern Rocky Mountains ecoregion (Omernik, 1987) of Colorado had widespread sources. These sources consisted of historical and active hard rock, metals mining sites (Lyon et al., 1993), and these streams were sampled for total and dissolved metals in surface water. For the same reasons, ambient toxicity tests of surface water have not been conducted in many EMAP studies, but ambient toxicity tests using *Pimephales promelas* and *Ceriodaphnia dubia* were conducted in this Colorado R-EMAP study. Also for these reasons, sampling of sediments for chemical analyses or ambient toxicity tests has been uncommon in EMAP wadeable stream studies. However, again this Colorado R-EMAP study collected sediment samples that were analyzed for metals and tested with

ambient toxicity tests using *Hyalella azteca*. EMAP - Estuaries has routinely collected sediment samples for chemical analyses and for ambient toxicity tests, often using *Ampelisca abdita*. These studies have been conducted in cooperation with the National Oceanographic and Atmospheric Administration's National Status and Trends Program, which has routinely collected sediments and bivalves for chemical analysis (O'Connor, 1994). An EMAP - Estuaries study of the Virginian Estuarine Province (Strobel et al., 1999) conducted from 1990 to 1993 was selected for analysis.

A common thread of most EMAP studies has been the sampling and analysis of biotic assemblages, particularly benthic invertebrates and fish. Both the Colorado R-EMAP study and the Virginian Province EMAP study collected benthic invertebrates and fish. However, because only sediment chemistry and ambient toxicity test data were available for the Virginian Province EMAP study, we used only the benthic invertebrate data from that study.

Several limitations are imposed on our assessment by use of these data sets and by technical aspects of the three methods used for the ecological assessment of contaminant exposure and effects. These data sets are secondary data, because they were collected for purposes that were different from those for which they are used in this report. As a result, some aspects of their study design are not optimal for our purposes. For example, the ambient toxicity tests conducted in both studies were acute in duration (U.S. EPA, 1993, 1994a,b), whereas the results of chronic toxicity tests would have been more comparable to the community metrics, which generally reflect longer-term effects (Karr and Chu, 1998). Moreover, EMAP generally uses a random-selection approach to identifying sampling sites (Strobel et al., 1999; Herlihy et al., 2000), although both studies included some sites where contamination was known or suspected to occur. While both studies were conducted in regions (i.e., the historical mining region of the Southern Rockies in Colorado and estuaries of the Virginian estuarine province of the eastern United States), where widespread contamination of surface water or sediments is known to occur, the number of sites classified into the unaffected or affected groups was unbalanced (i.e., the number of sites in the unaffected groups was larger than the number in the affected group). Many sites were also potentially affected by other stressors that may not be identifiable by comparisons of chemistry to available criteria or guidelines or by the ambient toxicity tests but may affect community metrics.

Also, technical differences among the three methods go beyond the methods' differences in the levels of biological organization used as their measurement

endpoints. For example, differences are related to laboratory testing versus field sampling and the selection of test species that are amenable to their use in a laboratory setting. The intent of this report is to address the relationships among the measurement endpoints used by the three methods. However, these aspects of study design and technical differences among the methods are discussed in the following chapters to clarify how they affect the observed relationships among the measurement endpoints.

The following chapters outline our comparisons of the results of the three methods for assessment of contaminant exposure and effects at sites sampled by

(1) the R-EMAP study conducted in 1994 and 1995 of wadeable streams in the Southern Rockies ecoregion of Colorado and

(2) the EMAP study conducted from 1990 to 1993 of poly-euhaline estuarine sites in the Virginian Province of the eastern United States.

The chapter on the R-EMAP study of wadeable streams in the Southern Rockies ecoregion of Colorado has already been published in a slightly different form in the journal, *Environmental Toxicology and Chemistry* (Griffith et al., 2004). Similarly, the chapter on the EMAP study of poly-euhaline estuarine sites in the Virginian Province was written to be published soon in a scientific journal. The final chapter summarizes our conclusions based on these two comparisons.

2. WADEABLE STREAMS IN THE SOUTHERN ROCKIES ECOREGION OF COLORADO

2.1. INTRODUCTION

In this chapter, we compare and contrast statistically the results of three different methods used by the U.S. EPA for the ecological assessment of contaminant exposure and effects in surface water and sediments of freshwater ecosystems: (1) chemical criteria for the protection of aquatic life such as ambient water quality criteria (AWQC) or sediment-effects concentrations, (2) ambient toxicity assessments of water or sediments, and (3) bioassessments of fish or macroinvertebrate assemblages to determine the relationships among the levels of biological organization assessed by each method. We also assess the extent to which organism-level effects predict effects at the community level. This approach is applied to the effects of metals contamination in streams associated with hard rock, metal mining in the mineralized belt of the Southern Rockies ecoregion of Colorado. This region is characterized by historical and active mining for base metals, and discharges from approximately 23,000 abandoned mines affect more than 2000 km of streams in Colorado (Lyon et al., 1993; Colorado Division of Minerals and Geology, 2003).

2.2. MATERIALS AND METHODS

2.2.1. Study Area and Survey Design. The mineralized belt of the Southern Rockies ecoregion includes headwater drainages of the South Platte, Arkansas, Rio Grande, and Colorado Rivers (Figure 1). We present data compiled from R-EMAP surveys conducted in 1994 and 1995. As part of these surveys, 73 sampling sites were selected using a randomization method with a spatial systematic component (Herlihy et al., 2000). The stream network on the digitized version of the 1:100,000 scale USGS topographic map was used as the sample frame. The surveys were restricted to 2nd, 3rd and 4th order (Strahler, 1957) on the 1:100,000 scale map. Sample probabilities were set so that roughly equal numbers of 2nd-, 3rd- and 4th-order streams appeared in the sample. Besides the 73 random sites, 13 other sites were selected that were variable distances either upstream (i.e., six sites) or downstream (i.e., seven sites) of known mining sites. Subsets of sites were revisited either within a year or during the second year to assess variability between visits, but data from only the first visit to a site were considered in these analyses. Nevertheless, some sites lacked data for one or more of the measurements, such as chemistry, toxicity tests, macroinvertebrates or fish.

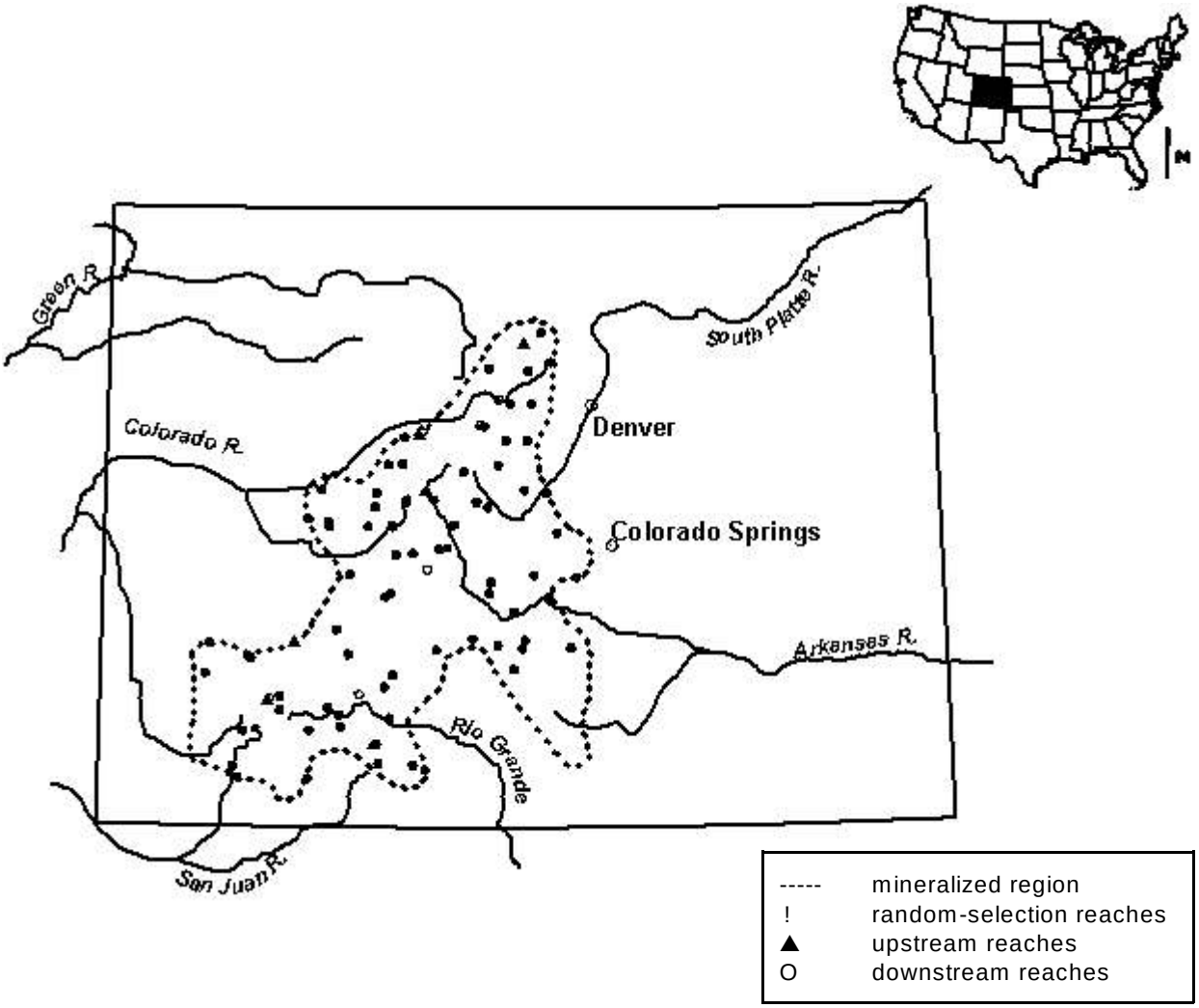


FIGURE 1

Map of Colorado, USA, with the Mineralized Region of the Southern Rockies Ecoregion and Locations of the 1994-1995 Regional Environmental Monitoring Assessment Program (R-EMAP) Reaches

Streams were sampled from late July to late September each year. This period of the water year is when stable base flows occur in these Rocky Mountain streams. Sampling was conducted to avoid episodic events when biological and chemical conditions were likely different from those during baseflow (Herlihy et al., 2000). A length of stream equal to 40 times the mean low-flow, wetted width (minimum of 150 m and maximum of 500 m) was delineated around each randomly chosen sampling point. The reach length was based on EMAP pilot studies that suggested this reach length was necessary to characterize the physical habitats in the stream (Herlihy et al., 2000). Eleven cross-section transects were established at equal intervals along the length of the reach.

2.2.2. Water and Sediment Chemistry. Stream water samples were collected in a flowing portion near the middle of each stream reach in low-density polyethylene containers (Lazorchak et al., 1998). Samples for dissolved cations and metals were filtered (0.45-µm filter) in the field, and samples for dissolved and total metals were preserved with 2 mL of concentrated HNO_3 (U.S. EPA, 1987). All samples were placed on ice and sent to the analytical laboratory (Lazorchak et al., 1998). Base cations and metals were determined by atomic absorption (U.S. EPA, 1987). Hardness was calculated from dissolved Ca and Mg (APHA, 1995). The detection limits achieved for Cd, Cu, Pb, and Zn were 0.3, 0.5, 2.0, and 2.0 µg/L, respectively.

Sediments for metal analysis were collected from depositional areas near each of the nine interior cross-section transects along a reach and placed in resealable plastic bags, placed on ice and sent to the analytical laboratory (Lazorchak et al., 1998). Samples were digested with HNO_3 and HCl, and metals were measured by atomic absorption (U.S. EPA, 1994b). The detection limits achieved for Cd and Pb were 0.025 and 1.08 mg/kg dry weight of sediment, respectively. Cu and Zn were detected in all tested samples.

2.2.3. Invertebrate and Fish Toxicity Tests. Subsamples of the water and sediments were also used in ambient toxicity tests. Water toxicity tests were conducted with <24-hour-old *Ceriodaphnia dubia* and 3- to 7-day-old *Pimephales promelas* using standard water column toxicity testing procedures (U.S. EPA, 1993). The bioassays were 48-hour, static-renewal tests, conducted at 20°C. Moderately-hard reconstituted water was used for the control water. Negative controls with moderately-hard reconstituted water were run with each set of field samples, and 90% survival in the negative control was required for a test to be valid. Also, tests with a reference toxicant, KCl, were used to evaluate the condition of the *C. dubia* and *P. promelas*. The measurement endpoint for

these bioassays was percent survival. Preliminary comparisons showed that survival in the test bioassays where survival was 80% or less was significantly less than survival in the control bioassays.

Sediment toxicity tests were conducted with 7-day-old *Hyalella azteca* using sediment toxicity testing procedures (U.S. EPA, 1994b). The tests were conducted in several sets, with 10 to 14 sediments tested in each set. The bioassays were 7-day, static-renewal tests, conducted at 25°C. Reformulated, moderately-hard, reconstituted water was used as the overlying water (Smith et al., 1997), and potting soil sediment was used as the control sediment. Animals were fed and the temperature of the overlying water was recorded daily. At the end of the test, the sediments were sieved through a U.S. standard #60 screen (250-µm mesh), and the live animals were collected and counted. Animals were euthanized with 70% ethanol, dried for 2 hours at 100°C, and placed in a desiccator until weighed. Negative controls with a potting soil sediment were run with each set of field samples, and 80% survival in the negative control was required for a test to be valid. Also, a water-only test with a reference toxicant, KCl, was used to evaluate the condition of the amphipods. The measurement endpoints for this bioassay were percent survival and percent growth. Preliminary comparisons indicated that survival and growth in the test bioassays where survival was 85% or less (Minimum significant difference [MSD] = 4.93%, Thursby et al., 1997) or growth was 90% or less (MSD = 8.93%), were significantly less than survival and growth in the control bioassays.

2.2.4. Macroinvertebrate Collection and Identification. Semi-quantitative macroinvertebrate samples were collected from riffles or pools at each of the nine interior cross-section transects along a reach with a kick net (Lazorchak et al., 1998). The samples from each transect were combined into separate composite riffle and pool samples for each reach. Because of the preponderance of riffle habitats at all sites (i.e., a pool composite sample was collected at only 11 of 86 sites), only data from composite riffle samples were used in these analyses. A 300-organism subsample was counted for each composite sample. Abundance per m^2 was estimated based on the number of grids sorted, subsamples and transects in a composite sample.

2.2.5. Fish Collection and Identification. Fish were collected from the entire stream reach according to time and distance criteria using pulsed direct-current backpack electrofishing equipment supplemented by seining (Lazorchak et al., 1998). Total collection time was not less than 45 minutes and not longer than 3 hours within the defined sampling reach and was divided in proportion to the area of the stream reach

within each of the ten intervals between the eleven cross-section transects. Seining was used in conjunction with electrofishing to ensure sampling of species that may otherwise have been under-represented by an electrofishing survey alone or when a stream was too deep for electrofishing to be conducted safely. 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.

2.2.6. Calculation of Community Metrics. We used the macroinvertebrate data to calculate various community metrics (Tables 1 and 2) proposed in the literature (Barbour et al., 1999). Richness metrics are the number of taxa identified in a sample within the specified group (e.g., total taxa richness, Plecoptera taxa richness). Abundances metrics are the number of individuals found in a sample within the specified group (e.g., total abundance). Composition metrics are the abundance of individuals in the specified taxonomic group divided by total abundance or by the specified larger group (e.g., Chironomidae) and expressed as a percentage (% individuals that were Ephemeroptera, % Tanytarsini of Chironomidae). Evenness metrics are either total abundance divided by total taxa richness (e.g., abundance per taxon) or the abundance of the most common taxon or five most common taxa divided by total abundance and expressed as a percentage (e.g., % individuals that were the most common taxon) Trophic or habitat guild metrics can quantify taxa richness of a particular trophic or habitat guild (e.g., collector-gatherer taxa richness), or the abundance of individuals in the trophic or habitat guild divided by total abundance and expressed as a percentage (e.g., % individuals that were collector-gatherers). Pollution-indicator metrics can quantify taxa richness of a group of indicator taxa (e.g., intolerant taxa richness), or the abundance of individuals in the group of indicator taxa divided by total abundance and expressed as a percentage (e.g., % individuals that were tolerant taxa). Similarly, we calculated community metrics for fish (Tables 1 and 2), but these were limited by the low natural diversity of fish assemblages in these coldwater systems (McCormick et al., 1994). The maximum total fish species or subspecies richness observed was six, while maximum native fish species or subspecies richness observed was four. Of those sites with fish, the mean proportion of fish that were trout was 82.7%, and a mean 97.4% of the trout were not native.

TABLE 1

Macroinvertebrate and Fish Metrics that Exhibited Differences Between the Two Groups Segregated Using at Least One of the Measurement Endpoints. The values are *F* for the one-way analysis-of-variance (ANOVA) comparing the metric between the unaffected and affected groups segregated based on the measure endpoints: D, the hardness-adjusted dissolved chronic criteria for Cd, Cu, Pb, or Zn; WT, the results of 48-hour, water toxicity tests with *C. dubia* or *P. promelas*; S, sediment threshold-effects-levels for Cd, Cu, Pb, or Zn based on 28-day *H. azteca* tests; and ST, results of 7-day, sediment toxicity tests with *H. azteca*. The *p* associated with *F* is in parentheses

Community Metrics	D	WT	S	ST
Macroinvertebrate Metrics				
Total taxa richness	21.36 (<0.001)[a]	39.67 (<0.001)[a]	10.08 (0.002)[a]	11.42 (0.001)[a]
Total abundance	11.99 (<0.001)[a]	6.90 (0.010)	1.21 (0.27)	3.10 (0.082)
Abundance per taxon	9.11 (0.003)[a]	2.98 (0.088)	0.68 (0.41)	1.65 (0.20)
Intolerant taxa richness	10.81 (0.002)[a]	23.12 (<0.001)[a]	7.24 (0.009)[a]	11.71 (0.001)[a]
Ephemeroptera taxa richness	7.82 (0.006)[a]	15.55 (<0.001)[a]	8.48 (0.005)[a]	6.65 (0.012)
Plecoptera richness	5.04 (0.027)	10.55 (0.002)[a]	0.88 (0.35)	1.83 (0.18)
Trichoptera taxa richness	6.36 (0.014)	15.15 (<0.001)[a]	3.42 (0.068)	3.42 (0.068)
EPT taxa richness	10.74 (0.002)[a]	24.41 (<0.001)[a]	6.31 (0.014)	6.31 (0.014)
Chironomidae taxa richness	5.81 (0.018)	12.07 (<0.001)[a]	1.69 (0.20)	3.97 (0.050)
% Ind.[b], tolerant taxa	0.56 (0.46)	4.68 (0.033)	0.43 (0.51)	0.54 (0.47)
Orthocladinae taxa richness	3.84 (0.053)	11.23 (0.001)[a]	0.42 (0.52)	0.92 (0.34)
Tanytarsini taxa richness	6.14 (0.015)	13.02 (<0.001)[a]	5.57 (0.021)	10.77 (0.002)[a]
Coleoptera taxa richness	2.71 (0.10)	5.14 (0.026)	4.98 (0.028)	0.55 (0.46)
% Ind., Ephemeroptera	2.55 (0.11)	4.24 (0.043)	0.39 (0.54)	1.70 (0.20)
% Orthocladinae of Chironomidae	2.10 (0.16)	5.35 (0.023)	0.01 (0.94)	0.92 (0.34)
% Tanytarsini of Chironomidae	1.95 (0.17)	7.62 (0.007)	3.53 (0.064)	9.71 (0.003)[a]
% Ind., Coleoptera	3.20 (0.078)	3.88 (0.052)	7.27 (0.009)[a]	2.96 (0.089)
% Ind., Diptera and noninsects	0.01 (0.93)	2.77 (0.10)	4.54 (0.036)	0.04 (0.84)
% Ind., Most common taxon	6.90 (0.010)	4.21 (0.043)	0.21 (0.65)	0.55 (0.46)
% Ind., Five most common taxa	6.02 (0.016)	5.83 (0.018)	0.77 (0.38)	2.38 (0.13)
Collector-filterer taxa richness	2.94 (0.090)	4.30 (0.041)	2.70 (0.10)	0.51 (0.48)
Collector-gatherer taxa richness	11.94 (<0.001)[a]	19.46 (<0.001)[a]	5.10 (0.027)	8.49 (0.005)[a]

TABLE 1 cont.

Community Metrics	D	WT	S	ST
Predator taxa richness	4.30 (0.041)	5.01 (0.028)	1.98 (0.16)	2.84 (0.10)
Shredder taxa richness	6.87 (0.010)	16.41 (<0.001)[a]	7.43 (0.008)[a]	0.91 (0.34)
Scraper taxa richness	5.52 (0.021)	7.25 (0.009)	4.54 (0.036)	4.61 (0.035)
Fish Metrics				
Total species richness	4.61 (0.030)	8.36 (0.005)	5.85 (0.018)	0.93 (0.34)
Salmonidae species richness	5.40 (0.023)	7.08 (0.010)	3.69 (0.059)	0.51 (0.48)
Total abundance	3.21 (0.077)	4.36 (0.040)	3.93 (0.051)	1.88 (0.18)
Adult abundance	3.10 (0.082)	4.50 (0.037)	3.85 (0.054)	1.72 (0.19)
Salmonidae abundance	5.83 (0.018)	3.45 (0.067)	0.75 (0.39)	3.12 (0.081)
% Ind., native species	0.00 (0.98)	2.32 (0.13)	7.86 (0.006)[a]	0.20 (0.66)
% Ind., Salmonidae	3.99 (0.049)	12.18 (<0.001)[a]	0.06 (0.81)	1.31 (0.26)
% Ind., native Salmonidae	0.65 (0.42)	1.84 (0.18)	6.14 (0.015)	0.86 (0.36)
% *Oncorhynchus* of Salmonidae	0.42 (0.52)	3.35 (0.071)	5.60 (0.021)	0.04 (0.85)

[a] statistically significant when *p* was corrected with the sequential Bonferroni technique
[b] % Ind. = Percentage of individuals

TABLE 2

Metrics that Did Not Exhibit Differences among the Groups

Macroinvertebrate Metrics	Fish Metrics
% Ind.*, Plecoptera % Ind., Trichoptera % Ind., EPT taxa Ratio, EPT to EPT + Chironomidae % Ind., Chironomidae % Ind., Diptera Crustacea and Mollusca taxa richness % Ind., Oligochaeta and Hirundea Hilsenhoff's biotic index % Ind., Collector-filterers % Ind., Collector-gatherers % Ind., Predators % Ind., Shredders % Ind., Grazers	Native species richness Native species abundance Native, non-Salmonidae species richness Native, non-Salmonidae abundance % Ind., native, non-Salmonidae

* % Ind. = Percentage of individuals

2.2.7. Data Handling and Analysis. We classified sampling events into two groups: those sites potentially affected and those sites unaffected by metals in surface water or sediment. We repeated this segregation four times, each based on one of the four different organism-level measures (Table 3). We classified the sites based on the chemistry data using chronic AWQCs from U.S. EPA (1999, 2001) and the sediment threshold-effect levels (TELs) from U.S. EPA (1996). Because the water quality criteria for Cd, Cu, Pb and Zn are hardness-dependent, the exact values of these criteria varied among sites. The TELs are based on a compilation of data from 28-day *H. azteca* sediment toxicity tests and were total concentrations of 0.583, 28.0, 37.2 and 98.1 mg/kg dryweight of sediment for Cd, Cu, Pb and Zn, respectively (U.S. EPA, 1996). Because contamination associated with metal mining generally consists of a mixture of metals, a site was included in the potentially affected groups based on water or sediment chemistry if the concentration of at least one metal exceeded its criterion.

Classifications of sites to the two groups were compared between surface water and sediments and between the ambient criteria and ambient toxicity tests with contingency tables. We calculated the index γ (Goodman and Kruskal, 1972) to assess the association between the groups. The index γ is a measure of association in the assignment of sites to groups that ranges from -1, if there was no agreement in the assignment of sites to groups by the two methods, to +1, if there was complete agreement. We used PROC FREQ (SAS, 1999) in these analyses.

Selected macroinvertebrate and fish metrics were individually compared between each pair of groups using a one-way analysis of variance (ANOVA) to answer the question, “Was the mean value of the metric different between the groups identified as affected or unaffected by metals based on the organism-level measures?” Statistical significance was set at $\alpha = 0.05$, and the probabilities for simultaneous tests were corrected with the sequential Bonferroni technique (Rice, 1989). We used PROC GLM in this analysis.

These methods are often used concurrently to make decisions about adverse effects at individual sites. Therefore, we quantified the frequency of disagreement between an assessment of sites based on organism-level effects and that based on the significant community metric. If a community metric decreases as a stressor increases, an assessment based on that metric would differ if the metric was “greater than expected” at a site identified as affected based on organism-level effects or if the metric was “less than expected” at a site identified as unaffected based on organism-level effects. In this study, all the statistically significant metrics decreased in the affected

TABLE 3

Criteria Used to Divide Sites into the Impacted or Unimpacted Groups

Variable	Organism-level Measure
Dissolved concentrations of Cd, Cu, Pb, or Zn*	$\geq$ hardness-adjusted dissolved chronic criteria (U.S. EPA, 1999, 2001)
Survival of *C. dubia* or *P. promelas** in a 48-hour toxicity test	$\leq$ 80% survival
Sediment concentrations of Cd, Cu, Pb, or Zn*	$\geq$ TEL for the 28-day *H. azteca* sediment toxicity test (U.S. EPA, 1996)
Survival or growth* of *H. azteca* in 7-day toxicity test	$\leq$ 85% survival or $\leq$ 90% growth

* At least one of

group, and we defined community metrics as “greater than expected” when the metrics were greater than the 95% upper confidence limit (UCL) of an affected group and as “less than expected” when the metrics were less than the 95% lower confidence limit (LCL) of the unaffected group as calculated in the one-way ANOVA. We used PROC MEANS (SAS, 1999) to calculate the 95% UCL and LCL.

We used piecewise or segmented regression (Toms and Lesperance, 2003) further to explore the relationships between the significant metrics and the concentrations of Cd, Cu, Pb and Zn in surface water or sediments relative to the organism-level-based criteria. Piecewise regression is an approach to modeling data where the regression changes at one or more points, called join points, along the range of the independent variable (Bellman and Roth, 1969). If the criteria or effects-level values (i.e., the chronic AWQC for surface water or the TEL for sediments) represent threshold concentrations for effects at the community level as measured by the metrics, then α_1 or β_1 should be significantly less than 0 in the piecewise regression model,

$$y = \alpha_0 + \alpha_1 x_1 + \beta_0 \log_e x_2 + \beta_1 x_1 \log_e x_2 \qquad \text{(Eq. 1)}$$

where:

x_1 = a dummy variable with a value of 1 if at least one metal exceeded its criterion or sediment-effects concentration and a value of 0 otherwise

x_2 = the summation of the ratios of the concentration of each metal to its criterion or sediment-effects concentration

y = the metric value.

By designing the analysis in this way, the model is reduced to

$$y = \alpha_0 + \beta_0 \log_e x_2 \qquad \text{(Eq. 2)}$$

when no metals exceed their criteria or sediment-effects concentration because $\alpha_1 x_1 = 0$ and $\beta_1 x_1 \log_e x_2 = 0$. The coefficients, α_1 and β_1, then are the changes in the intercept and slope of the regression when at least one metal exceeds its criterion or sediment-effects concentration. We used PROC GLM (SAS, 1999) in these regression analyses.

This approach, using the summed ratios of the concentration of each metal to its criterion or sediment-effects concentration as the continuous independent variable, assumed that the effects of the four metals were concentration additive and that the criteria or sediment-effects concentrations represent their common mechanism and threshold level of effect. The criteria do not account for possible synergistic or antagonistic effects among these metals (U.S. EPA, 2000b).

2.3. RESULTS AND DISCUSSION

Because data were not complete for some sites (i.e., some sites lacked fish data, chemistry data or toxicity data), macroinvertebrate metrics could be compared for 83 to 85 sites depending on the organism-level measurement endpoint. Fish metrics could be compared for 76 to 78 sites.

2.3.1. Organism-level Measures. Using either metal concentrations or ambient toxicity tests, we identified more sites as affected by sediment contamination than by surface water contamination because there were more sites where metal concentrations or ambient toxicity tests indicated sediments were toxic whereas surface water was not than sites showing the reverse (Table 4). The association among groups, γ, was +0.89 between assessments based on water or sediment metal concentrations and +0.83 for those based on water or sediment toxicity tests.

As described in the literature on the hydrogeochemistry of the mine drainage that results in this metal contamination (Chapman et al., 1983; Filipek et al., 1987), metal concentrations in water are greatest closer to the mine source, but decrease as metal solubility changes in relation to pH and other factors. Metal concentrations in sediments increase downstream of the mine source within the zone where the metals are deposited. Although pH data for these sites were considered invalid, dissolved organic carbon ranged from less than a detection limit of 1.0 mg/L to 10.8 mg/L. Therefore, we would expect some sites to have elevated concentrations of these metals in sediment but not water. Also, the tests of sediment measure incrementally more sensitive endpoints than those for water (i.e., survival and growth versus just survival).

Comparing metal concentrations versus ambient toxicity tests, more sites were identified as affected based on metal concentrations than on ambient toxicity tests (Table 5), because metal concentrations indicated surface water or sediments were toxic whereas ambient toxicity tests did not indicate toxicity at more sites than in the reverse where ambient toxicity tests indicated toxicity although criteria did not. The association among groups, γ, was greater for the assessments based on water (γ = +0.98) than those based on sediment (γ = +0.73). The mean summed ratios of the dissolved concentrations of the four metals to their chronic AWQCs and the mean summed ratios of the sediment concentrations of the four metals to their TELs were greater at sites classified as affected by the ambient toxicity tests for water and sediment, respectively (Figure 2). However, these two measures agreed in their classification of a site at only 53% of the 19 sites identified as affected by at least one

TABLE 4

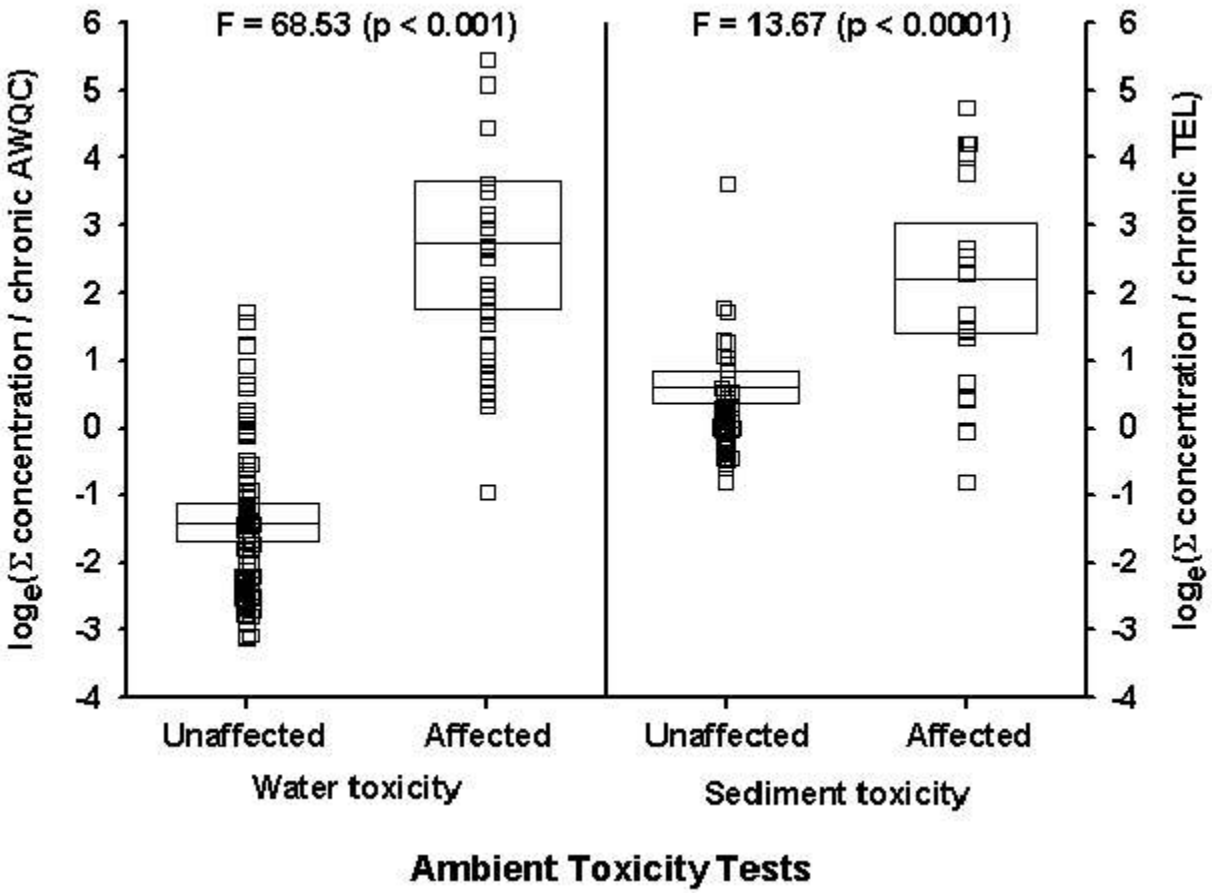
Correspondence of Conclusions of Assessments for Surface Water and Sediment for Sampling Events

Criteria (γ = +0.89)		Were water criteria exceeded?		
		No	Yes	Total
Were sediment TELs exceeded?	No	53	3	56
	Yes	15	15	30
	Total	68	18	n = 86
Ambient toxicity tests (γ = +0.83)		Did water ambient toxicity tests show effects?		
		No	Yes	Total
Did sediment ambient toxicity tests show effects?	No	63	4	67
	Yes	10	7	17
	Total	73	11	n = 84

TABLE 5

Correspondence of Conclusions of Assessments Based on Chemical Criteria and Ambient Toxicity Tests for Sampling Events

Water (γ = +0.98)		Were metal AWQC exceeded?		
		No	Yes	Total
Did water toxicity tests show effects?	No	65	8	73
	Yes	1	10	11
	Total	66	18	n = 84
Sediment (γ = +0.73)		**Were metal sediment TELs exceeded?**		
		No	Yes	Totals
Did sediment toxicity tests show effects?	No	49	18	67
	Yes	5	12	17
	Totals	54	30	n = 84



G = the raw data
The boxes show the mean and 95% confidence limits.

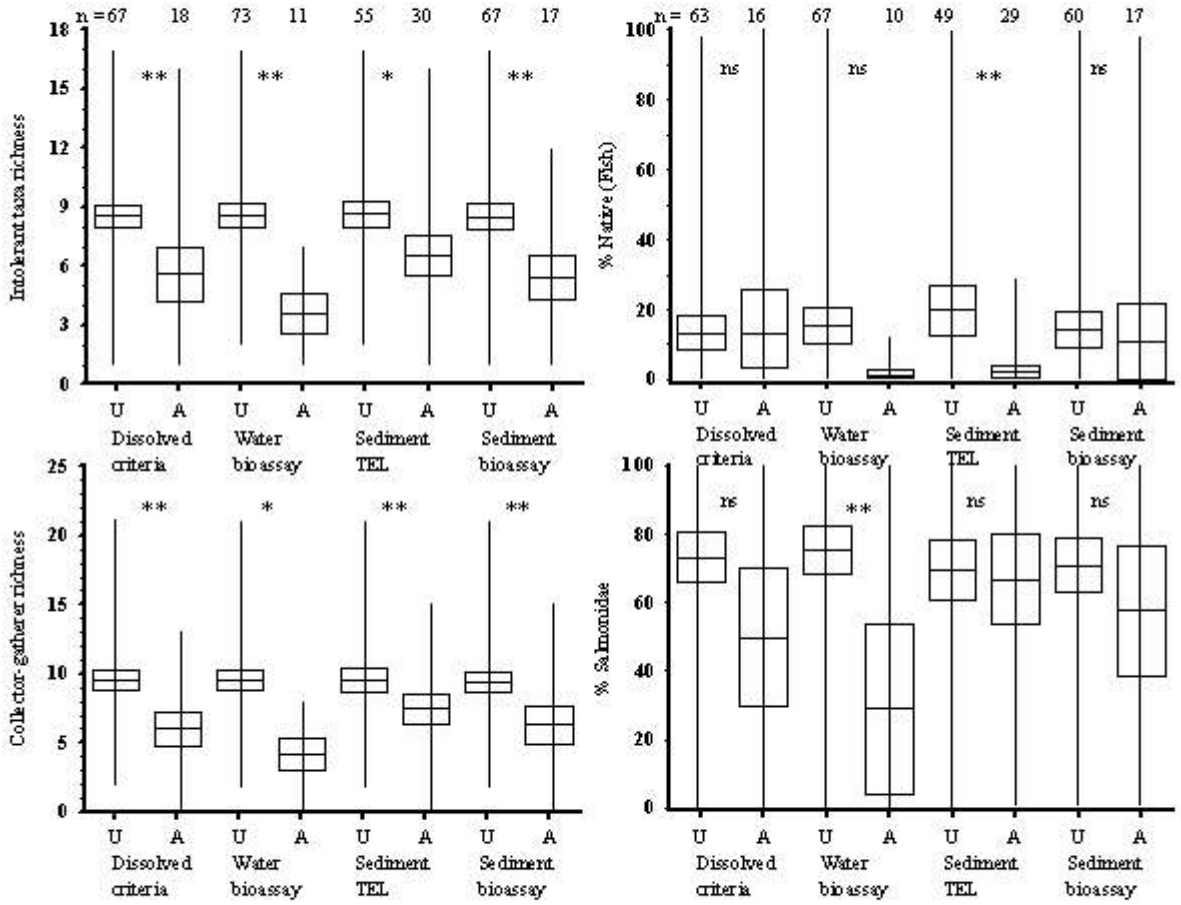
FIGURE 2

Comparison of Metals Concentrations in Water [$\log_e$(Σ Concentration / Chronic AWQC)] and in Sediment [$\log_e$(Σ Concentration / TEL)] Between Groups Identified as Potentially Affected or Unaffected by the Ambient Toxicity Tests of Water and Sediment, Respectively

measure for water and only 34% of the 35 sites identified as affected by at least one measure for sediment.

2.3.2. Organism-level Measures versus Community Metrics. When each metric was compared between pairs of groups segregated using the organism-level measures using a one-way ANOVA, a number of macroinvertebrate metrics exhibited significant differences between at least one pair of groups segregated using the organism-level measures (Table 1), whereas other metrics did not exhibit significant differences between any pairs of groups (Table 2). To be conservative, we will concentrate on those metrics for which F was statistically significant when p was corrected with the sequential Bonferroni technique. The metrics listed in Table 1 with the greatest *F* values from the one-way ANOVA are generally richness metrics: total taxa richness [AWQC - F = 21.36 ($p<0.001 <$ adjusted $p=0.050$), water toxicity test - F = 39.67 ($p<0.001 <$ adjusted $p=0.050$), sediment TEL - F = 10.08 ($p=0.002 <$ adjusted $p=0.050$), sediment toxicity test - F = 11.42 ($p=0.001 <$ adjusted $p=0.050$)], Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa richness [AWQC - F = 10.74 ($p=0.002 <$ adjusted $p=0.010$), water toxicity test - F = 24.41 ($p<0.001 <$ adjusted $p=0.025$)], Tanytarsini taxa richness [water toxicity tests - F = 13.02 ($p<0.001 <$ adjusted $p=0.006$), sediment toxicity tests - F = 10.77 ($p=0.002 <$ adjusted $p=0.017$)], intolerant taxa richness [AWQC - F = 10.81 ($p=0.002 <$ adjusted $p=0.013$), water toxicity test - F = 23.12 ($p<0.001 <$ adjusted $p=0.016$), sediment toxicity test - F = 11.71 ($p=0.001 <$ adjusted $p=0.050$)], and collector-gatherer richness [AWQC - F = 11.94 ($p<0.001 <$ adjusted $p=0.017$), water toxicity test - F = 19.46 ($p<0.001 <$ adjusted $p=0.013$), sediment toxicity test - F = 8.49 ($p=0.005 <$ adjusted $p=0.010$)], for macroinvertebrates (Figures 3 and 4). An exception is the total number of individuals [AWQC - F = 11.99 ($p=0.001 <$ adjusted $p = 0.025$)] for macroinvertebrates (Figure 4), which is an abundance metric. The metrics that exhibited significant differences between pairs of groups and are listed in Table 1 are relatively sensitive to the stressor gradient represented by metals contamination, whereas the metrics listed in Table 2 are insensitive to this gradient. Similar metrics were identified for being sensitive to this gradient by multivariate analyses in Griffith et al. (2001).

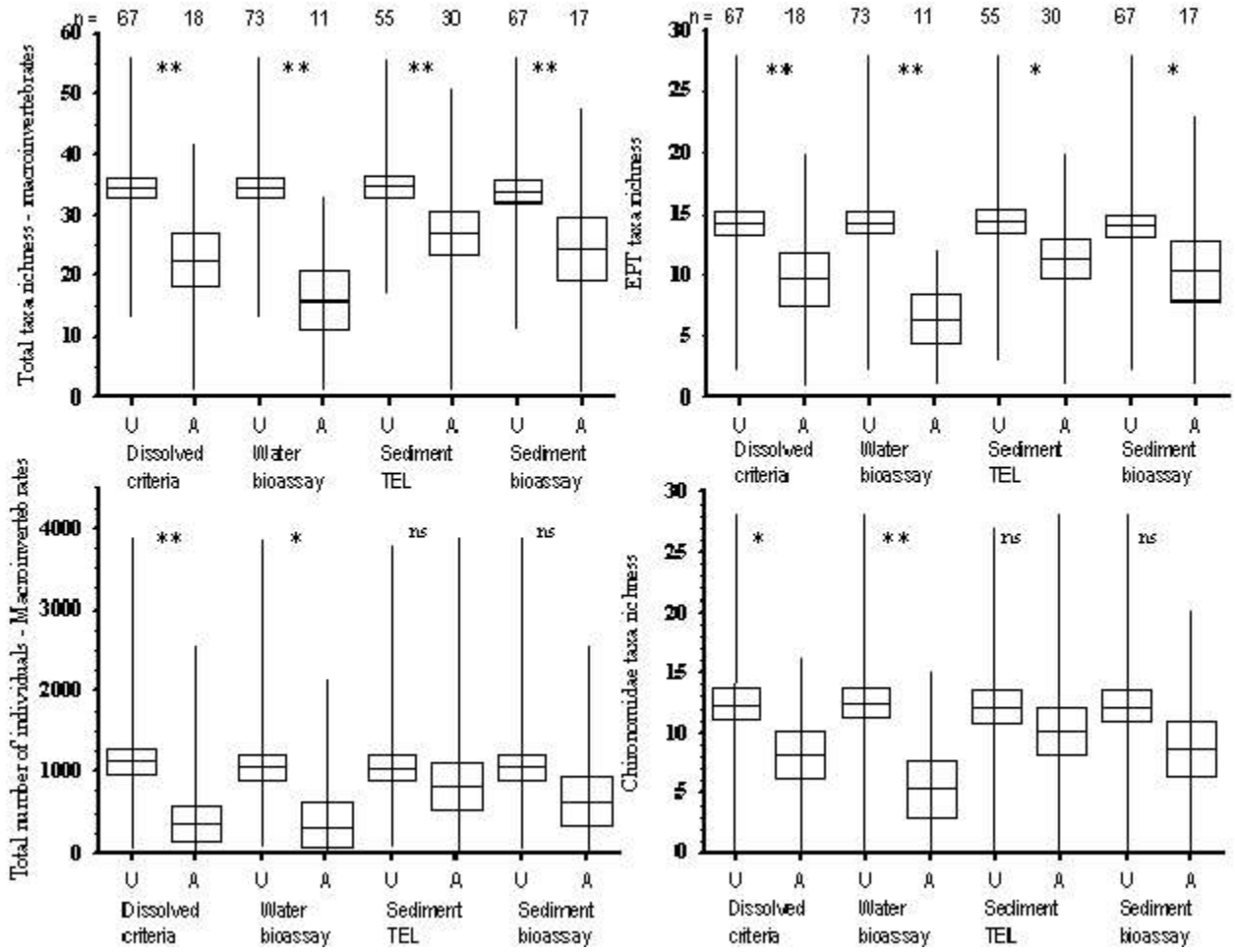
This sensitivity of richness metrics to metal contamination is consistent with an assumption that effects at the organism and population levels are the basis of effects observed at the community level. Persistent toxicants, such as metals, increase mortality and decrease growth and reproduction of individuals within an exposed population. These are organism-level effects that result in reduced abundances at the



n = number of sites classified in each group
U = unaffected group
A = affected group
ns = not significant
* = $p < 0.05$
** = significant when probabilities for simultaneous tests were corrected with a sequential Bonferroni technique

FIGURE 3

Comparison of Macroinvertebrate Metrics Between Groups Identified as Potentially Affected or Unaffected by Each of the Organism-level Endpoints. The boxes show the mean and 95% confidence limits of each metric for each group, while the whiskers show the range.



n = number of sites classified in each group
U = unaffected group
A = affected group
ns = not significant
* = $p < 0.05$
** = significant when probabilities for simultaneous tests were corrected with a sequential Bonferroni technique

FIGURE 4

Comparison of Macroinvertebrate and Fish Metrics Between Groups Identified as Potentially Affected or Unaffected by Each of the Organism-level Endpoints. The boxes show the mean and 95% confidence limits of each metric for each group, while the whiskers show the range.

population level (Kuhn et al., 2000). At some threshold, population recruitment fails, and more sensitive species will be eliminated from the community (Sheehan, 1984). Because the threshold concentrations at which different species are affected vary, more of the species in a community would be affected with increasing toxicant concentrations, and taxa richness would decrease (Barnthouse et al., 1986). The insensitivity of various composition metrics suggests no concomitant increase in more tolerant species, which could adapt or acclimatize themselves to these toxicants, occurred in compensation for the eliminated species (Vinebrooke et al., 2003). Such population effects would also be the basis of the observed decrease in the total number of individuals collected. We did not test other abundance metrics for macroinvertebrates because such metrics are not normally used in bioassessments. Abundance metrics require quantitative samples, and many states and other entities collect only qualitative samples as part of bioassessments (Barbour et al., 1999). However, this R-EMAP study collected semi-quantitative samples.

Fish metrics were less sensitive to the metal contamination. Only two composition metrics were significantly different between one pair of groups (Table 1, Figure 4): % individuals that were native species [sediment TEL - $F = 7.86$ ($p=0.006 <$ adjusted $p=0.017$) and % individuals that were Salmonidae [water toxicity test - $F = 12.18$ ($p<0.001 <$ adjusted $p=0.006$)]. However, this lack of sensitivity by the fish metrics might be a result of the low diversity of the fish assemblage in these cold-water streams. Maximum total fish species or subspecies richness in these streams was six, and maximum native fish species or subspecies richness was four. In streams with fish, a mean of 83% of the fish were Salmonidae, and a mean of 97% of the Salmonidae were not native species or subspecies.

When classification of sites to the affected and unaffected groups based on organism-level effects is compared with individual metric values, the methods differ in their assessment of adverse effects at some sites (Table 6). For example, the total taxa richness metric for macroinvertebrates was greater than the 95% upper confidence limit of the mean of the affected group for 6 of the 18 sites classified as affected based on exceedance of the dissolved metals criteria and was less than the 95% lower confidence limit of the mean of the unaffected group for 28 of the 67 sites classified as unaffected.

Sites in the unaffected group where metrics are less than the expected range may be affected by other stressors. Previous analyses also identified increased nutrients and fine sediments and decreased canopy cover associated with livestock

TABLE 6

Enumeration of Sampling Events in Wadeable Streams in the Southern Rockies Ecoregion of Colorado Where Classification Based on the Organism-level Measures and that Based on the Community Metric Disagree

Metric	Number of Sampling Events*			
	Classified as Unaffected	Metric <95% LCL for Unaffected Group	Classified as Affected	Metric >95% UCL for Affected Group
Dissolved Chronic Criteria				
Total taxa richness (macroinvertebrates)	67	28	18	6
Total number of individuals	67	36	18	1
Number, Individuals per taxon	67	32	18	3
Intolerant taxa richness	67	23	18	5
Ephemeroptera taxa richness	67	22	18	7
EPT taxa richness	67	20	18	4
Collector-gatherer taxa richness	67	30	18	6
Water Toxicity Tests				
Total taxa richness (macroinvertebrates)	73	29	11	3
Intolerant taxa richness	73	25	11	2
Ephemeroptera taxa richness	73	24	11	2
Plecoptera taxa richness	73	28	11	3
Trichoptera taxa richness	73	29	11	4
EPT taxa richness	73	25	11	4
Chironomidae taxa richness	73	32	11	3
Orthocladinae taxa richness	73	31	11	3

TABLE 6 cont.

Metric	Number of Sampling Events			
	Classified as Unaffected	Metric <95% LCL for Unaffected Group	Classified as Affected	Metric >95% UCL for Affected Group
Tanytarsini taxa richness	73	27	11	3
Collector-gatherer taxa richness	73	33	11	4
Shredder taxa richness	73	40	11	1
% Individuals, Salmonidae	67	25	11	3
Sediment Threshold Effects Levels				
Total taxa richness (macroinvertebrates)	55	21	30	13
Ephemeroptera taxa richness	55	25	30	9
% Coleoptera	55	28	30	9
Shredder taxa richness	55	30	30	8
% Individuals, native species	49	39	29	0
Sediment Toxicity Tests				
Total taxa richness (macroinvertebrates)	67	26	17	7
Intolerant taxa richness	67	22	17	6
Tanytarsini taxa richness	67	23	17	4
% Tanytarsini of Chironomidae	67	33	17	2
Collector-gatherer taxa richness	67	33	17	5

* The total number sampling events is the sum of the columns labeled “Classified as unaffected” and “Classified as affected.”

grazing in riparian zones as another stressor gradient in these Rocky Mountain streams (Griffith et al., 2001). Also, because most sites were only sampled once, we do not know the temporal variability of metal concentrations in these streams, and these single measurements may underestimate exposure of fish or macroinvertebrates to metals in some streams.

At sites in the affected group where metrics were greater than the expected range, exposure to metals in surface water and sediments may differ from that measured, in part because of unaccounted for effects on metal bioavailability. In surface water, factors, such as dissolved organic carbon, pH, or other cations besides water hardness, may also affect metal bioavailability (Di Toro et al., 2001), but U.S. EPA water quality criteria are currently only adjusted for water hardness. The TELs were derived from analyses of laboratory bioassay data (U.S. EPA, 1996) that did not consider possible factors affecting metal bioavailability in sediments (Chapman et al., 1999). Acid volatile sulfide (AVS) can affect the bioavailability of metals in sediments (Liber et al., 1997). However, AVS was not measured in this study, and significant concentrations of AVS are unlikely to occur in the coarse, well-aerated sediments of these shallow, high-gradient streams. Including these additional factors that affect metal bioavailability in models used to adjust the criteria or other guidelines may be appropriate.

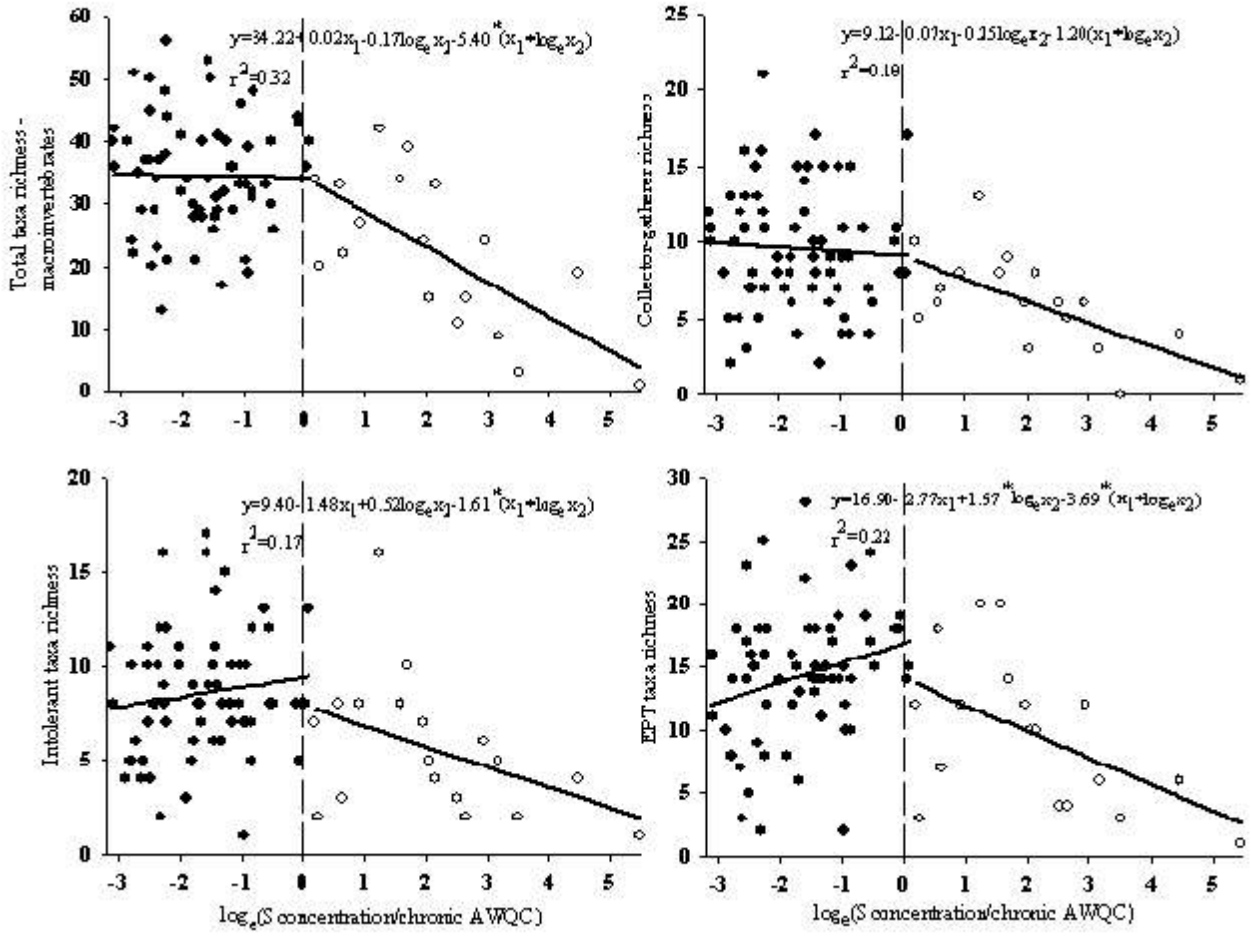
The differences in assignment of sites to affected and unaffected groups based on criteria or sediment-effects concentrations versus ambient toxicity tests likely also result from the direct assessment of bioavailability by the ambient toxicity tests. However, there is also a difference in duration between the organism-level endpoints for the chemical criteria and ambient toxicity tests. The criteria we used for surface water are chronic criteria, whereas the ambient toxicity tests would be considered acute in duration. Chronic effects are expected at lower concentrations of toxicants than acute effects, and chronic effects would be reflected by the community metrics.

2.3.3. Piecewise Regression Analyses. Metal contamination associated with hard-rock metal mining is a complex impact on streams. In the mineralized zone of the Southern Rockies Ecoregion, the contamination is a mixture of primarily four metals, Cd, Cu, Pb and Zn, that changes as surface water chemistry changes downstream from the mine source (Chapman et al., 1983). To simplify our analyses, we assumed a potential impact if one or more of the concentrations of these four metals in surface water exceeded their hardness-adjusted criteria or in sediments exceeded their TEL. Therefore, the affected group includes a continuum of sites from those in which one

metal minimally exceeded its criterion to those in which all four metals greatly exceeded their criteria. Moreover, the criteria may not necessarily represent actual threshold concentrations for adverse effects at the community level. For surface water, the slope of the piecewise regression of the four macroinvertebrate metrics; total taxa richness, intolerant taxa richness, collector-gatherer richness and EPT taxa richness; on the summed ratios of the dissolved concentrations of the four metals to their chronic AWQCs was positive or not significantly different from 0 when the metal concentrations were all less than the AWQCs (Figure 5). When at least one metal exceeded its AWQC, the piecewise regressions for the summed ratios were negative and significantly different from 0. This suggests that the chronic criteria for water approximate threshold levels for adverse effects the for macroinvertebrate assemblages in these streams. Conversely, for sediments, the slope of the piecewise regression of these same four metrics on the summed ratios of the sediment concentrations of the four metals to their TELs was negative and significantly different from 0 when the metal concentrations were all less than the TELs (Figure 6). When at least one metal exceeded its TEL, the slope was less negative, but this change in slope was significant only for EPT taxa richness. This suggests that the TELs do not approximate threshold levels for adverse effects for macroinvertebrate assemblages in these streams, because taxa richness decreased with increasing metals although sediment concentrations of the four metals were less than the TELs.

Besides assessing measurement endpoints at different levels of biological organization, chemical criteria, ambient toxicity tests and community metrics differ in their specificity to different stressor gradients (Karr and Chu, 1998). Ambient criteria are very specific to whatever contaminants are being measured and assessed and ignore any unmeasured contaminants or stressors that lack criteria. Ambient toxicity tests detect toxicity associated with any bioavailable contaminant in the tested surface water or sediments but do not assess other characteristics of the stream. Community metrics are not generally designed to be stressor specific. Therefore, while community metrics may be sensitive to specific stressors (Norton et al., 2000; Griffith et al., 2001; Ofenbock et al., 2004), those metrics also will be sensitive to other concurrent alterations of the stream that affect the structure of the biotic assemblages. This includes alterations of physical habitat that are not addressed by chemical criteria.

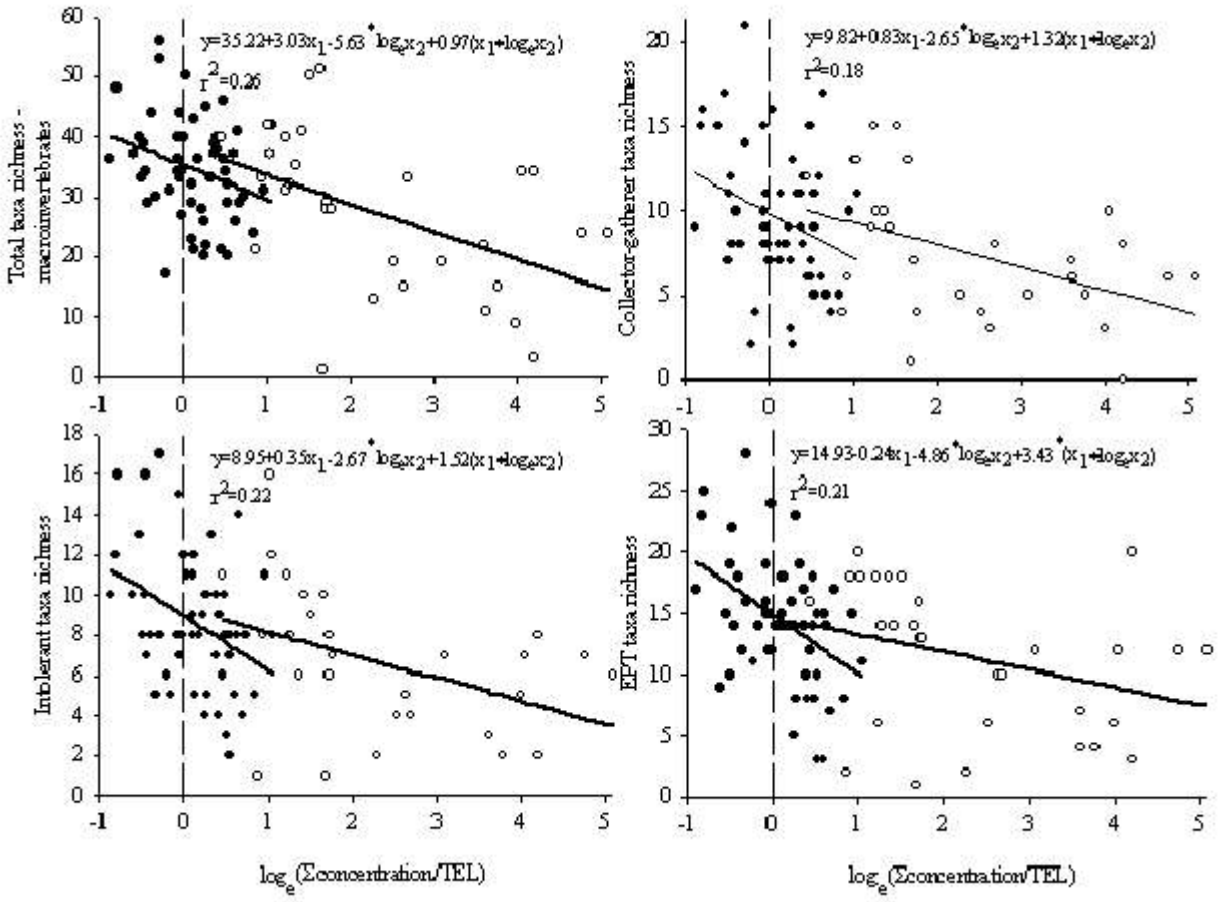
We used a simple approach in classifying the sites into unaffected and affected groups. This was done, recognizing that only recently have models been constructed to extrapolate accurately between the organism- and population-level effects (Kuhn et al.,



y = the metric value
x_1 (dummy variable) = 1 if at least one metal exceeds its chronic AWQC (open circles), or x_1 = 0 otherwise (solid circles)
x_2 = $\sum$ (ratios of the dissolved concentrations of Cd, Cu, Pb, and Zn to their chronic AWQC)
* = coefficient significantly different from 0 at $p < 0.05$
The solid lines are the predicted regression lines for each segment.

FIGURE 5

Piecewise Regressions of Taxa Richness Metrics on the Summed Ratios of the Dissolved Concentrations of Cd, Cu, Pb and Zn to their Chronic AWQC



y = the metric value
x_1 (dummy variable) = 1 if at least one metal exceeds its TEL (open circles), or $x_1 = 0$ otherwise (solid circles)
$x_2 = \sum$ (ratios of the sediment concentrations of Cd, Cu, Pb, and Zn to their TELs)
* = coefficient significantly different from 0 at $p < 0.05$
The solid lines are the predicted regression lines for each segment.

FIGURE 6

Piecewise Regressions of Taxa Richness Metrics on the Summed Ratios of the Sediment Concentrations of Cd, Cu, Pb and Zn to their TELs

2000), and we still cannot accurately model or extrapolate between population and community effects because of the difficulties of incorporating variation in exposure and response across the hierarchical levels of time, space and organization (de Kruijf, 1991; Karr and Chu, 1998). Considering this simple classification, one might expect few, if any, of the metrics would have exhibited differences in their means between the two groups. However, a number of metrics, particularly richness metrics, exhibited differences between the groups although the conclusions based on the organism-level measures and on community metrics disagreed at some sites. This would suggest that a relationship exists between the organism-level effects assessed by ambient criteria or guidelines or ambient toxicity tests and the community-level effects assessed by community metrics. However, the organism-level effects are only predictive to a limited extent of the community-level effects at individual sites, because this predictability is affected by differences among the methods that go beyond the hierarchical levels of biological organization used as their measurement endpoints. We need to assess the generality of these relationships for other contaminants besides metals.

3. ESTUARINE SYSTEMS IN THE VIRGINIAN PROVINCE OF THE ATLANTIC COAST

3.1. INTRODUCTION

In this chapter, we compare and contrast statistically the results of three different methods used by the U.S. EPA for the ecological assessment of contaminant exposure and effects in sediments in estuarine ecosystems: (1) chemical guidelines, (2) ambient toxicity assessments, and (3) bioassessments of benthic invertebrates to determine the relationships among the levels of biological organization assessed by each method. We also assess the extent to which organism-level effects predict effects at the community level. Through these comparisons, we expected to assess the relationships among the levels of biological organization protected by the different methods and assess the extent to which organism-level effects are predictive of effects at the community level. In this paper, this approach is applied to the effects of sediment contamination in estuaries of the Virginian Province of the Atlantic coast of the United States. Contaminants in these sediments were expected to be metals, polyaromatic hydrocarbons (PAHs), some pesticides and polychlorinated biphenyl (PCB) congeners.

3.2. MATERIALS AND METHODS

3.2.1. Study Area and Survey Design. The Virginian Province of the United States includes estuarine habitats along the Atlantic coast extending from Cape Henry, Virginia to Cape Cod, Massachusetts. In the following tables, we present data compiled from U.S. EPA's EMAP surveys conducted from 1990 to 1993. As part of these surveys, sampling sites were selected in a stratified, random manner within each of three classes of estuaries based on size: large estuaries, large tidal rivers and small estuaries or tidal rivers (Strobel et al., 1999). In the Virginian Province, this sampling approach identified 12 large estuaries, five large tidal rivers and 144 small estuaries or tidal rivers. Additional sites were selected non-randomly in areas for which there was prior knowledge of ambient environmental conditions that represent areas with likely anthropogenic disturbance. Some sites were revisited during a subsequent year to assess variability among years, but data from only one visit to a site were considered in these analyses. Nevertheless, some sites lacked data for one or more of the measurements, such as chemistry, toxicity tests or benthic invertebrates.

Sites were sampled from July to September each year. This index period was selected as the period of the year when biotic responses to potential anthropogenic and natural stressors were anticipated to be most pronounced (Strobel et al., 1995).

3.2.2. Field and Laboratory Methods. Field methods for the Virginian Province surveys are fully documented in Reifsteck et al. (1993), and laboratory methods are documented in U.S. EPA (1995). These methods are summarized briefly below.

At each station, salinity (‰), temperature (°C), and dissolved oxygen (DO, mg/L) were recorded with a model SBE-25 Sealogger conductivity-temperature-depth profiler (Sea-Bird Electronics, Inc., Bellevue, WA).

At each station, generally three replicate grab samples were collected with a 0.044-m^2 Young-modified Van Veen grab (Theodore E. Young, Sandwich, MA) and processed for benthos (i.e., at two sites, only two replicate grab samples were collected and processed). Samples were sieved in the field with a 0.5-mm mesh screen. Material retained on the screen was preserved in 10% buffered formalin with rose bengal. In the laboratory, samples were sorted. Organisms were counted, weighed, and identified to the lowest possible taxonomic level, usually species (Strobel et al., 1995).

Additional grab samples were collected at each site, and the top two cm of sediment was composited for analysis of percent silt-clay, contaminant concentrations and sediment toxicity (Strobel et al., 1995). Percent silt-clay was the portion of sediment passing through a 63-µm screen.

3.2.3. Sediment Contaminant Concentrations. Subsamples of the composited sediments were analyzed for organic and metal contaminants. Analysis of organics involved Soxhlet extraction and extract drying with $NaSO_4$, concentration with a Kuderna-Danish apparatus and cleanup with activated Cu for elemental S and gel permeation chromatograph or alumina for organic interferents (Paul et al., 1999). PAHs were analyzed with gas chromatography/mass spectrometry. Pesticides and PCB congeners were analyzed with gas chromatography/electron capture detection confirmed by a second column. For Ag, Al, Cr, Cu, Fe, Mn, Ni, Pb and Zn, sediments were digested with HF and HNO_3 on a hot plate followed by analysis with inductively-coupled plasma, atomic emission spectrometry. For As, Cd, Sb, Se and Sn, sediments were digested with HNO_3 and HCl in a microwave oven followed by analysis with a Zeeman-corrected, stabilized-temperature graphite furnace atomic absorption spectrometry (Paul et al., 1999). Hg was analyzed by cold-vapor atomic absorption spectrometry.

3.2.4. Ambient Toxicity Tests. Other subsamples of the composited sediments were used in ambient toxicity tests. Standard, acute, 10-day static tests (U.S. EPA, 1995; Strobel et al., 1999) were conducted with juvenile *Ampelisca abdita*. Prior to testing,

the amphipods were acclimated at 20°C for at least 48 hours. During testing, the amphipods were not fed. For each sediment tested, five glass test chambers were filled with 200 mL of sediment and 600 mL of seawater with salinity of 30 ‰. The chambers were illuminated constantly to inhibit amphipod emergence from the sediment and maximize exposure. The water was aerated to maintain dissolved oxygen concentrations >90% saturation. Temperature of the overlying water was maintained at 20$\pm$1°C. Dead animals were counted and removed daily, and at the end of the test, the sediments were sieved through a 0.5-mm screen and live amphipods were collected and counted. Any amphipods, which were not accounted for, were presumed to have died during the test. Negative controls with an uncontaminated sediment were run with each set of field samples, and 85% survival in the negative control was required for a test to be valid. Also, a water-only test with a reference toxicant, $CdCl_2$ or $C_{12}H_{25}SO_4Na$ (sodium dodecyl sulfate), was used to evaluate the condition of the amphipods. The measurement endpoint for these bioassays was percent survival. These test bioassays indicated toxicity if survival was statistically different from ($\alpha = 0.05$) and $\leq$80% of survival the corresponding negative control bioassays (Thursby et al., 1997; Strobel et al., 1999).

3.2.5. Calculation of Community Metrics. We used the benthos data to calculate various community metrics (Tables 7 and 8), identified as indicative of community integrity in the literature (Fauchald and Jumars, 1979; Engle et al., 1994; Weisberg et al., 1997; van Dolah et al., 1999; Olsgard et al., 2003). Richness metrics are the number of taxa identified in a sample within the specified group (e.g., total taxa richness, Polychaeta species richness). Abundance metrics are the number of individuals found in a sample within the specified group (e.g., total abundance, Spionida abundance), while total biomass in the dry weight of organisms in a sample. Composition metrics are the abundance of individuals in the specified taxonomic group divided by total abundance or by the specified larger group (e.g., Polychaeta) and expressed as a percentage (e.g., % individuals that were Mollusca, % Polychaeta that were Spionida). Evenness metrics are either total abundance, the abundance of the specified group, or biomass divided by total taxa richness (e.g., abundance per taxon, biomass per taxon) or the abundance of the two most common taxa divided by total abundance and expressed as a percentage (e.g., % individuals in the two most common taxa). Trophic or habitat guild metrics can quantify taxa richness of a particular trophic or habitat guild (e.g., Polychaeta omnivore species richness, Infaunal taxa richness)or the abundance of individuals in the trophic or habitat guild divided by

TABLE 7

Benthic Metrics that Exhibited Differences Between the Two Groups Segregated Using at Least One of the Measurement Endpoints.[a] The values from the analysis of covariance (ANCOVA) are a, the intercept; b_1, the slope of the regression of the metric on percent silt and clay; b_2, the slope of the regression of the metric on percent total organic carbon, and F (b_3), the F value for comparison of the regression of the metric between the unaffected and affected groups segregated based on the measurement endpoint. For b_1 and b_2, NS indicates p>0.05, * indicates $p < 0.05$ and ** indicates p<0.01 for a t test that the slope was significantly different from 0. The *p* associated with *F* (b_3) is in parentheses and * indicates that the regression slopes were statistically significant different between the two groups when p was corrected with the sequential Bonferroni technique.

Community Metrics	Sediment Chemistry				Sediment Toxicity Test			
	a	b_1	b_2	F (b_3)	a	b_1	b_2	F (b_3)
Total taxa richness	34	NS	-4.2**	3.97 (0.048)	34	NS	-4.8**	0.53 (0.47)
Phyllodocida species richness	5.7	NS	-0.64**	5.24 (0.023)	5.8	NS	-0.78**	1.00 (0.32)
Capitellida species richness	3.0	NS	NS	11.10 (0.001)*	3.3	NS	-0.28**	1.05 (0.31)
Polychaeta omnivore richness	2.3	NS	NS	6.11 (0.014)	2.2	NS	NS	0.61(0.44)[b]
Crustacea species richness	13	-0.073**	NS	7.67 (0.006)	13	-0.082**	NS	0.30 (0.58)
Pollution-indicative taxa richness	1.6	NS	NS	5.06 (0.026)	1.7	NS	NS	0.00 (0.98)[b]
Number of individuals per taxon	7.3	NS	3.1**	0.09 (0.77)	10	NS	NS	5.15 (0.024)
Number of infaunal individuals per taxon	13	NS	5.9**	0.05 (0.83)	19	NS	NS	6.17 (0.014)
Biomass per taxon	013	NS	NS	2.91 (0.090)	0.012	NS	NS	9.48 (0.002)*
% Individuals[c,d], Polychaeta	0.71	NS	NS	6.15 (0.014)	0.72	NS	NS	0.15 (0.70)[b]
% Polychaeta[e], Phyllodocida	0.20	0.0010**	-0.31**	6.68 (0.011)	0.21	0.00087*	-0.033**	9.538 (0.003)*
% Polychaeta, Spionida	0.25	NS	NS	33.04 (<0.001)*	0.22	NS	0.043**	2.47 (0.12)
% Polychaeta, predators	0.40	NS	-0.030*	10.62 (0.001)*	0.40	NS	-0.042**	0.59 (0.44)

TABLE 7 cont.

Community Metrics	Sediment Chemistry				Sediment Bioassay			
	a	b_1	b_2	F (b_3)	a	b_1	b_2	F (b_3)
% Individuals, Gastropoda	0.25	NS	NS	9.60 (0.002)*	0.24	NS	NS	2.08 (0.15)[b]
% Individuals, Crustacea	0.44	-0.0047**	0.13**	4.93 (0.028)	0.45	-0.0051**	0.12**	0.04 (0.84)
% Individuals, Pollution-indicative taxa	0.085	0.0028**	NS	29.98 (<0.001)*	0.066	0.0021*	0.054*	10.87 (0.001)*
% individuals, Pollution-sensitive taxa	0.43	NS	NS	5.89 (0.016)	0.43	NS	NS	4.38 (0.038)
% Individuals, *Streblospio benedicti*	0.017	NS	0.047**	32.95 (<0.001)*	0.012	NS	0.075**	3.37 (0.068)
% Individuals, *Mulinia lateralis*	0.011	0.0014**	NS	1.91 (0.17)	0.0051	0.0015**	NS	6.54 (0.011)
% Individuals, *Paraprionospio pinnata*	0.042	0.0017**	-0.027*	7.02 (0.009)*	0.044	0.0018**	-0.035**	0.90 (0.34)
% Individuals, *Acteocina canaliculata*	0.081	0.00086**	NS	7.99 (0.005)	011	NS	NS	0.51 (0.48)[b]
Phyllodocida abundance[f]	3.4	0.011**	-0.42**	14.33 (<0.001)*	3.5	0.011**	-0.50**	7.21 (0.008)*
Spionida abundance	3.6	NS	NS	5.30 (0.022)	3.7	NS	NS	0.36 (0.55)[b]
Gastropoda abundance	3.5	0.012*	-0.46**	5.99 (0.015)	3.4	NS	NS	3.13 (0.08)[b]
Decapoda abundance	1.6	NS	NS	9.95 (0.002)*	1.7	NS	-0.18**	0.50 (0.48)
Survival	94	NS	NS	19.42 (<0.001)	))	))	))	))

[a] The measurement endpoints were: Sediment Chemistry = maximum *p* from logistic regressions of Field et al. (2002) and Sediment Bioassay = results of acute, 10-day, sediment toxicity tests with juvenile *Ampelisca abdita*.

[b] The *p* for F value for the overall equation was >0.05.

[c] % Individuals = Percentage of total individuals that were the specified subgroup.

[d] Percent metrics were transformed as arcsin $\sqrt{(y/100)}$.

[e] % Polychaeta = Percentage of Polychaeta individuals that were the specified subgroup.

[f] Abundance metrics were transformed by $\log_e(y+1)$.

TABLE 8

Benthic Metrics that Did Not Exhibit Differences among the Two Groups Segregated Using at Least One of the Measurement Endpoints[a]

Invertebrate Metrics	
Infaunal taxa richness	% Individuals[b], Mollusca
Polychaeta species richness	% Individuals, Bivalvia
Spionida species richness	% Bivalvia[c], Tellinidae
Terebellida species richness	% Bivalvia, Lucinidae
Polychaeta sessile richness	% Individuals, Crustacea[d]
Pollution-sensitive taxa richness	% Amphipoda[e], Ampeliscidae + Haustoriidae
% Individuals, two most common taxa	% Individuals, Pollution-sensitive taxa
Pielou's evenness index	% Individuals, pollution-sensitive Group A[f]
% Polychaeta[g], Terebellida	% Individuals, *Mediomastus* spp.
% Polychaeta, Hesionidae	Total abundance
% Polychaeta, Capitellidae	Infaunal abundance
% Polychaeta, Orbiniidae	Polychaeta abundance
% Polychaeta, Cirratulidae	Capitellidae abundance
% Polychaeta, Nereididae	Terebellida abundance
% Polychaeta, sessile or discretely motile individuals	Mollusca abundance
% Polychaeta, surface deposit feeders	Amphipoda abundance
%Polychaeta, subsurface deposit feeders	Total biomass
% Individuals, Decapoda	

[a] Sediment Chemistry = maximum *p* from logistic regressions of Field et al. (2002); Sediment Bioassay = results of acute, 10-day, sediment toxicity tests with juvenile *Ampelisca abdita*
[b] Percentage of individuals that were the specified subgroup
[c] Percentage of Bivalvia that were the specified family
[d] excluding Pycnogonida and Thoracica
[e] Percentage of Amphipoda that were the specified families
[f] pollution sensitive Group A = Ampeliscidae, Tellinidae, Hesionidae, Cirratulidae, *C. polita*, and *C. burbancki* (van Dolah et al., 1999)
[g] Percentage of Polychaeta that were the specified subgroup

total abundance or abundance of the specified larger group and expressed as a percentage (% Polychaeta that were predators). Pollution-indicator metrics are the abundance of one or more pollution-indicator taxa divided by total abundance or by the abundance of a larger taxonomic group (e.g., % individuals that were pollution-indicative taxa, % individuals that *Streblospio benedicti*).

3.2.6. Data Handling and Analysis. The sites ranged in salinity from freshwater tidal (<0.5 ‰) to poly-euhaline (>18 ‰), and many community metrics were correlated with this gradient, particularly because some metrics were often 0 either at the freshwater tidal or poly-euhaline sites. Therefore, to reduce this source of variation, we only used data from the poly-euhaline sites. To focus on effects associated with contaminants in sediments, we also excluded sites where the measured concentration of dissolved oxygen was less than 2.0 mg/L. As a result, data from 201 sites were used in these analyses.

We classified sampling events into two groups, those sites potentially affected and those sites unaffected by contaminants in sediment. This segregation was performed twice using the two different organism-level measures (Table 9). We used the logistic regression models from Field et al. (2002) to classify the chemistry data. The logistic regression models are for 10 metals, 22 PAHs, total PCBs and 4 organochlorine pesticides and are based on a compilation of matching data for sediment chemistry and 10-day sediment toxicity tests with the amphipods, *R. abronius* or *A. abdita* from a wide-range of estuarine habitats on the Atlantic, Gulf and Pacific coasts of North America. It should be noted that a subset of the data used by Field et al. (2002) was taken from these Virginian Province surveys. The logistic regression models estimate the probability that sediments from a site will exhibit toxicity based on individual chemical concentrations, though sediments may be contaminated with a mixture of chemicals. Field et al. (2002) warn that these logistic regression models are not dose-response relationships but can be considered indicators of toxicity. A site was included in the potentially affected group based on sediment chemistry if the predicted probability that the sediment was toxic exceeded 0.5 for at least one chemical (Field et al., 2002).

Classifications of sites to groups was compared between sediment chemistry and ambient toxicity tests with contingency tables, and the index γ (Goodman and Kruskal, 1972) was calculated to assess the association between the groups. The index γ is a measure of association in the assignment of sites to groups that ranges from -1, if there was no agreement in the assignment of sites to groups by the two

TABLE 9

Criteria Used to Divide Sites into the Impacted or Unimpacted Groups

Variable	Organism-level Measure
Sediment concentrations of measured metals, polyaromatic hydrocarbons, total polychlorinated biphenyls, or pesticides	Maximum *p* from logistic regression models (Field et al., 2002) $\geq$0.50
Survival of *A. abdita* in a 10-day toxicity test	<80% of and significantly different from survival in controls

methods, to +1, if there was complete agreement. We used PROC FREQ (SAS, 1999) in these analyses. As the focus of this research is the relationships between classifications of sites with these two methods, sediment chemistry and ambient toxicity tests, and community metrics, this analysis was done to contrast how these two methods classify the sites.

Because many benthic metrics also varied with the silt and clay content or the organic carbon content of the sediment, we compared each benthic invertebrate metric between each pair of groups using analysis of covariance (ANCOVA). The question answered was, "Was the regression of the metric on percent silt and clay and percent organic carbon in the sediments different between the groups identified as affected or unaffected by contaminants based on the organism-level measures?" The data were fitted to the model:

$$y = a + b_1 x_1 + b_2 x_2 + b_3 x_3 \qquad \text{(Eq. 3)}$$

where:

x_3 = a dummy variable with a value of 1 if at least one metal exceeded its criterion or sediment-effects concentration and a value of 0 otherwise

x_1 = % silt and clay content of the sediment

x_2 = % organic carbon content of the sediment

y = the metric value.

By designing the analysis in this way, the model reduces to a two-way ANCOVA, if either b_1 or b_2 is not significantly different from 0, and reduces to a one-way ANOVA, if both b_1 and b_2 are not significantly different from 0. To homogenize the variance, abundance metrics were transformed by $\log_e(y+1)$ and percentage metrics were transformed by $\sqrt{(y/100)}$ arcsine. Statistical significance was set at $\alpha = 0.05$, and the probabilities for simultaneous tests were corrected with the sequential Bonferroni technique (Rice, 1989). We used PROC GLM (SAS, 1999) in this ANCOVA.

To explore further the relationships between the significant metrics and the organism-level measures, we examined the residual of each metric,

$$Residual = Observed - (a + b_1 X_1 + b_2 X_2) \qquad \text{(Eq. 4)}$$

where a, b_1, and b_2 are the estimated intercept and significant slopes from the regression in Equation 3 (Draper and Smith, 1981).

This approach removes the variation in the metric variables resulting from the silt and clay content and the organic carbon content of the sediments. Then, we regressed the residuals of the significant metrics either against maximum *p* from the logistic regressions or percent survival of *A. abdita* in the ambient toxicity tests.

These methods may be used concurrently to make decisions about whether adverse effects are occurring or are likely at individual sites. Therefore, we quantified the frequency of disagreement between assessments of sites based on organism-level effects and those based on the significant community metrics. An assessment based on a community metric would differ if the metric was "different from expected" at sites identified as affected or unaffected based on organism-level effects. However, whether a metric was "different from expected" changed depending on whether a metric increased or decreased at affected sites. We defined community metrics as "different from expected" using the 95% confidence limits as outlined in Table 10. We used PROC REG, PROC UNIVARIATE, and PROC GLM to calculate the parameters necessary to estimate the 95% confidence limits.

3.3. RESULTS AND DISCUSSION

Because data were not complete for some sites (i.e., some sites lacked invertebrate data, chemistry data particularly for PCBs or pesticides, or ambient toxicity test data), comparisons were made for 152 to 201 sites depending on the variables being compared.

3.3.1. Organism-level Measures. A few more sites were identified as affected based on chemistry than on ambient toxicity tests (Table 11) because chemistry indicated sediments were toxic whereas the ambient toxicity tests did not at more sites than did the reverse where ambient toxicity tests indicated toxicity whereas chemistry did not. The association between groups, γ, was +0.724 for the assessments using ambient toxicity tests versus chemistry, and mean percent survival of *A. abdita* in the toxicity tests was less among the sites where maximum *p* was greater than 0.5 (Figure 7). However, these two measures agreed in their classification of a site at only 25% of the 43 sites where sediments were identified as affected by least one measure.

This inconsistency in classification of sediments as affected between ambient toxicity tests and chemistry has been identified previously (O'Connnor and Paul, 2000; O'Connor et al., 1998) for other benchmarks. Although *A. abdita* has been a standard species for testing of estuarine sediments (U.S. EPA, 1994a), it may be more tolerant of many contaminants compared with other indigenous estuarine species (Hyland et al., 1996). The logistic equations we used were based on an analysis of compiled data on

TABLE 10

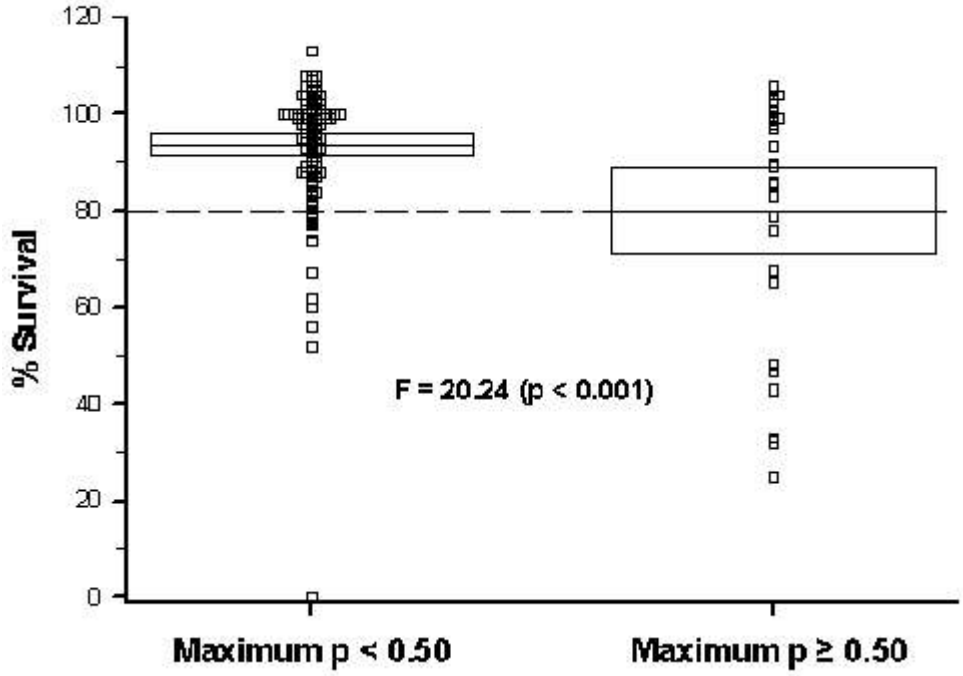
Criteria Used to Classify Metrics as Different than Expected

Metric	Unaffected sites	Affected sites
Increases at affected sites	Metric residual for individual site > Upper 95% confidence limit of mean metric residual for unaffected sites	Metric residual for individual site < Lower 95% confidence limit of mean metric residual for affected sites
Decreases at affected sites	Metric residual for individual site < Lower 95% confidence limit of mean metric residual for unaffected sites	Metric residual for individual site > Upper 95% confidence limit of mean metric residual for affected sites

TABLE 11

Correspondence of Conclusions of Assessments Based on Chemcial Criteria and Ambient Toxicity Tests for Sampling Events

γ = +0.724		Maximum *p* from logistic regression models ≥0.50?		
		No	Yes	Totals
Sediment toxicity tests show effects?	No	143	18	161
	Yes	14	11	25
	Totals	157	29	n = 186



G = the raw data
The boxes show the mean and 95% confidence limits.
The dashed line is 80%, the percent survival used to classify sites based on the ambient toxicity tests.

FIGURE 7

Comparison of Percent Survival of *A. abdita* Between Sites where Maximum $p < 0.50$ from the Logistic Regressions and those where Maximum $p \geq 0.50$

sediment chemistry and 10-day sediment toxicity tests with the amphipod *R. abronius* in addition to *A. abdita* (Field et al., 2002). Moreover, only mortality was used as the measurement endpoint in these data, instead of the multiple endpoints used by Long et al. (1995) to derive ER-Ms. Also, Field et al. (2002) used 90% survival in the test bioassay to classify sediments as toxic, whereas we used 80% survival relative to the negative control to classify sediments as toxic in the ambient toxicity tests.

A *p* from the logistic regression models for a least one measured constituent exceeded 0.5 at 32 of 211 sites. For each of these sites, a *p* exceeded 0.5 for one or more metals, one or more PAHs or both (Table 12). The *p* from the logistic regression for total PCBs exceeded 0.5 at only 1 site and p for pesticides exceeded 0.5 at 8 of the 152 sites where PCBs or pesticides data were available. However, all these sites also were contaminated by metals or PAHs.

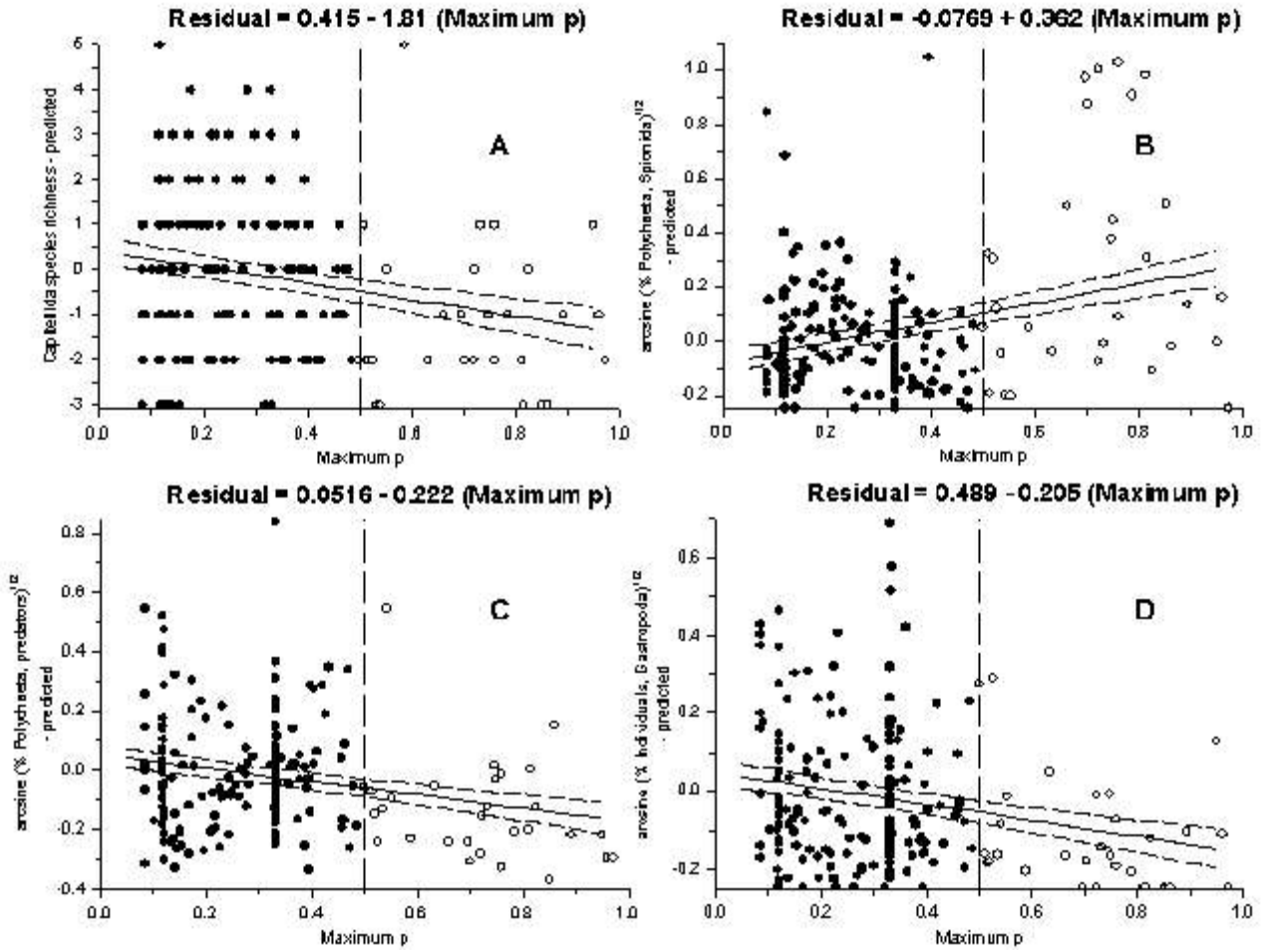
3.3.2. Organism-level Measures versus Community Metrics. A number of benthic metrics exhibited significant differences between at least one pair of groups segregated using the organism-level measures (Table 7). Other metrics did not exhibit significant differences between any pairs of groups (Table 8). However, these differences among metrics appear to depend on the sensitivity of the benthic metrics to the stressor gradient being examined (Griffith et al., 2001). The metrics with the greatest *F* statistics for the comparison between the two groups identified based on sediment chemistry in Table 7 included a richness metric, Capitellida species richness (Figure 8); composition metrics, percent Polychaeta that were Spionida and percent individuals that were Gastropoda (Figure 8); a trophic metric, percent Polychaeta that were predators; pollution-indicator metrics, percent individuals that were pollution-indicative taxa and percent individuals that were *Streblospio benedicti* (Figure 9); and abundance metrics, Phyllodocida abundance and Decapoda abundance (Figure 9). However, the comparisons of metrics between the groups identified based on the ambient toxicity tests showed fewer significant differences (Table 7), and the statistically significant metrics were percent Polychaeta that were Phyllodocida, percent individuals that were pollution-indicative taxa and Phyllodocida abundance and the evenness metric, biomass per taxon (Figure 10).

Percent silt and clay content of the sediments ranged from 0.1% to 99.4%, while the % organic carbon content of the sediments ranged from 0.01% to 7.0% and was correlated with the % silt/clay content (i.e., r = 0.77). Of the metrics that also showed significant differences between the groups classified as affected and unaffected based on sediment chemistry, % Polychaeta that were predators exhibited a negative

TABLE 12

Comparison of Sites where Maximum *p* from the Logistic Regression ≥0.50 for Metals versus for PAHs

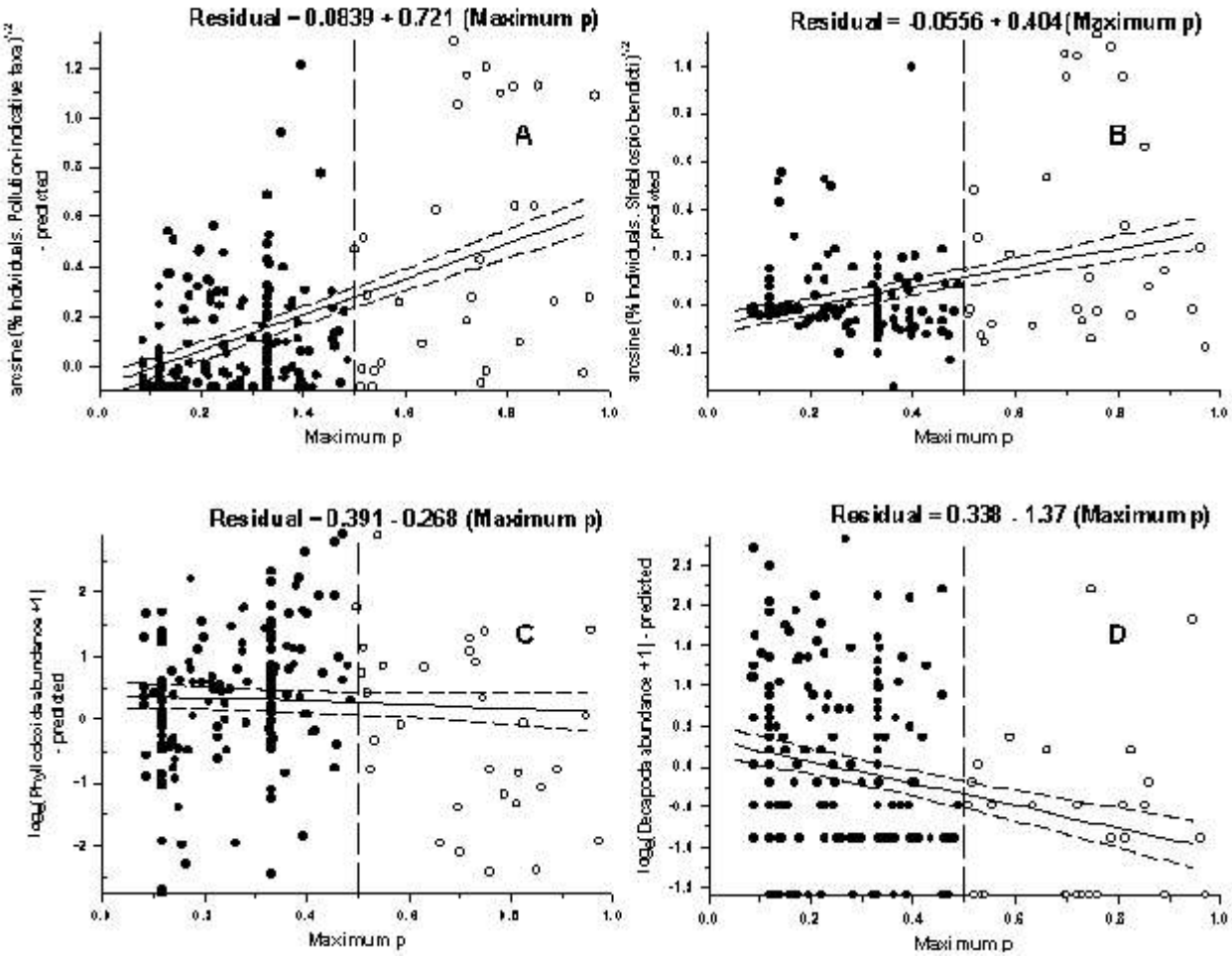
		Maximum *p* from logistic regression models for metals ≥0.50?		
		No	Yes	Totals
Maximum *p* from logistic regression models for PAHs ≥0.50?	No	179	9	188
	Yes	6	17	23
	Totals	185	26	n = 211



The solid lines are the predicted regression lines.
The dashed lines are the 95% confidence limits.
The vertical dashed line is the maximum *p* of 0.5 used to classify the two groups,
! = sites classified as unaffected
" = sites classified as affected.

FIGURE 8

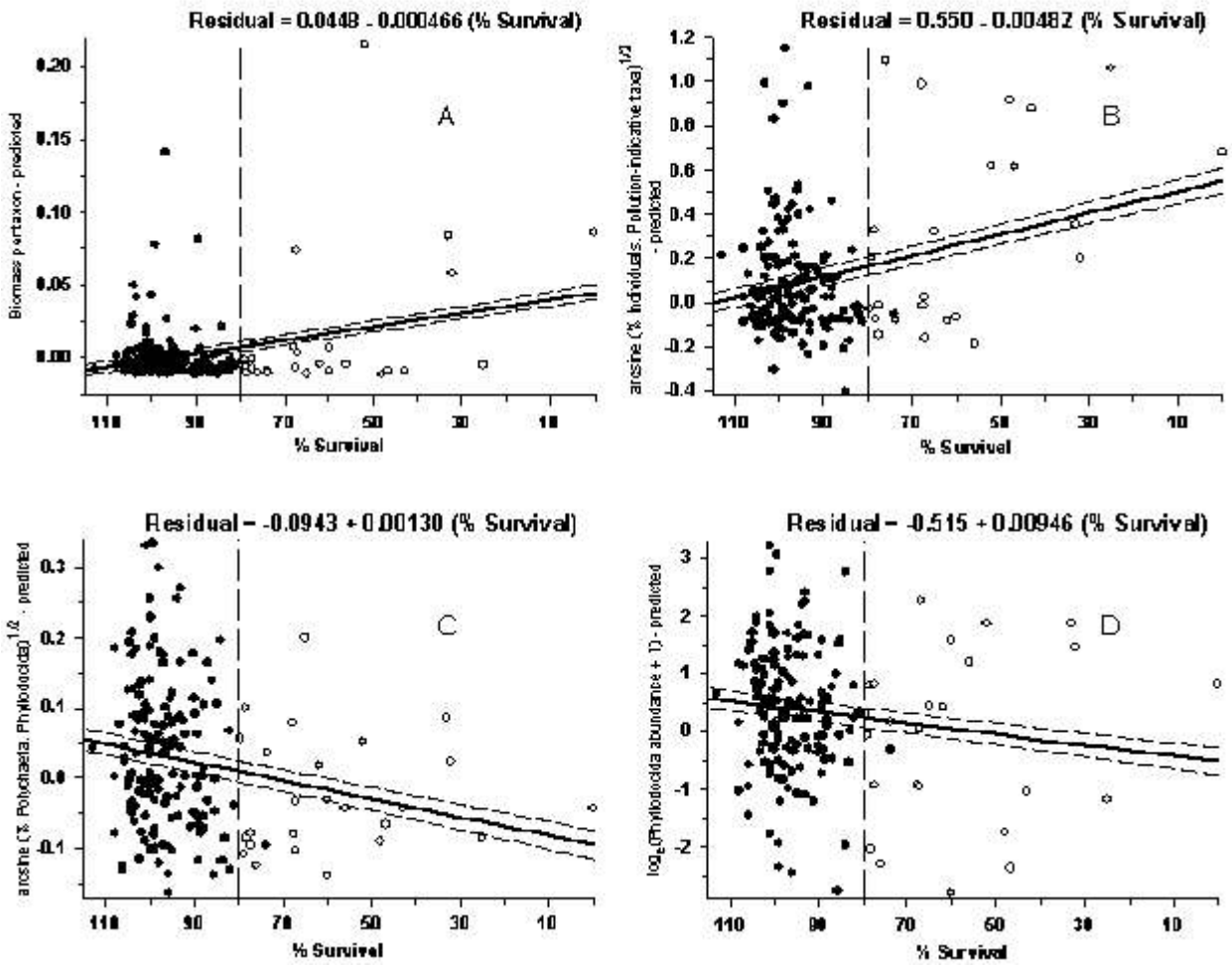
Regressions of Residuals (i.e., after variation due to the percent silt & clay content and the percent organic carbon content of the sediment were removed) of Benthic Metrics (Richness and Composition) on Maximum *p* from the Logistic Regressions: A. Capitellida species richness, B. percent Polychaeta that were Spionida, C. percent Polychaeta that were predators, and D. percent individuals that were Gastropoda.



The solid lines are the predicted regression lines.
The dashed lines are the 95% confidence limits.
The vertical dashed line is the maximum *p* of 0.5 used to classify the two groups,
! = sites classified as unaffected
" = sites classified as affected.

FIGURE 9

Regressions of Residuals (i.e., after variation due to the percent silt & clay content and the percent organic carbon content of the sediment were removed) of Benthic Metrics (Pollution-Indicator and Abundance) on Maximum *p* from the Logistic Regressions: A. percent individuals that were pollution-indicative taxa, and B. percent individuals that were *Streblospio benedicti*, C. Phyllodocida abundance, and D. Decapoda abundance.



The solid lines are the predicted regression lines.
The dashed lines are the 95% confidence limits.
The vertical line is the percent survival of 80% used to classify the two groups,
! = sites classified as unaffected
" = sites classified as affected

FIGURE 10

Regressions of Residuals (i.e., after variation due to the percent silt & clay content and the percent organic carbon content of the sediment were removed) of Benthic Metrics on Percent Survival for the Sediment Toxicity tests with *A. ampelisca:* A. Biomass per taxon, B. percent individuals that were pollution-indicative taxa, C. percent Polychaeta that were Phyllodocida, and D. Phyllodocida abundance. Note that % survival decreases along the X axis. Therefore, the slope of the regression equation estimates the change in the residual from right to left on the graph.

relationship with % organic carbon, % individuals that were *Streblospio bendicti* exhibited a positive relationship with % organic carbon, % individuals that were pollution-indicative taxa exhibited a positive relation with % silt and clay, and Phyllodocida abundance exhibited a positive relationship with % silt and clay and a negative relationship with % organic carbon (Table 7). Of the metrics that also showed significant differences between the groups classified as affected and unaffected based on the sediment toxicity tests, % individuals that were pollution-indicative taxa showed positive relationships with both % silt and clay and % organic carbon, and both % Polychaeta that were Phyllodocida and Phyllodocida abundance showed a positive relationship with % silt and clay and a negative relationship with % organic carbon.

The sensitivity of these richness, composition, trophic guild, pollution-indicator and abundance metrics to the identified sediment contamination is consistent with an assumption that effects at the organism and population levels are the basis of effects observed at the community level. Toxicants, such as PAHs and metals, may increase mortality and decrease reproduction of organisms within exposed populations that are less tolerant to the toxicants. In turn, more tolerant organisms in exposed populations may experience less of an increase in mortality and less of a decrease in reproduction, and these populations may increase, in part, because of reduced species interactions (Vinebrooke et al., 2003). These are organism-level effects that result in altered relative abundances at the population level (Kuhn et al., 2000). Such population effects would also be the basis of the observed changes in the absolute abundances of different taxa. If at some threshold population recruitment fails, less tolerant species will be eliminated from the community (Sheehan, 1984). Because the threshold concentrations at which different species are affected vary, more of the species in a community would be affected with increasing toxicant concentrations, and taxa richness would decrease (Barnthouse et al., 1986). However, single species toxicity tests may not be a very sensitive indicator for such community changes if the test organism is more tolerant than other indigenous taxa. This sensitivity may be further reduced because of the acute duration of the toxicity tests. The metrics measure chronic effects, which occur at lower concentrations of toxicants than acute effects. This may explain the fewer metrics that distinguished between sites classified based on the ambient toxicity tests.

While the assessments using toxicity tests and biotic metrics may have been more comparable if the duration of the toxicity tests were chronic, this is a limitation of our use of secondary data, which was collected for another purpose. We used EMAP data, and because of decisions made by the EMAP researchers, only data from toxicity

tests of acute duration were available. Moreover, the random site-selection approach of EMAP results in sampling of uncontaminated and contaminated sites in proportion to their occurance across a region. This resulted in the unbalanced distribution of sites between the unaffected and affected groups as identified by sediment chemistry or the ambient bioassays. The advantage of this data set is includes data from a large number of sites that do not exhibit spacial correlations.

When classification of sites to the affected and unaffected groups based on organism-level effects is compared with individual metric values, the methods differ in their assessment of adverse effects at some sites (Table 13). For example, Phyllodocida species richness was less than the 95% lower confidence limit of the mean of the unaffected group for 88 (51.5%) of the 171 the sites classified as unaffected. Moreover, Phyllodocida species richness was greater than the 95% upper confidence limit of the mean of the affected group for 8 (26.7%) of the 30 sites classified as affected by metals or PAHs based on the logistic equations. Sites in the unaffected group where metrics are different from expected are probably affected by other stressors. Previous analyses have identified other contaminants in sediments, such as some pesticides, butyltins, or selenium (Kiddon et al., 2003), that could not be assessed with the logistic equations (Field et al., 2002). Other stressors may include excess nutrients, with their effect on light penetration, on dissolved oxygen in the water column and on total organic carbon in the sediments; the presence of marine debris or other habitat alterations (Strobel et al., 1999; Kiddon et al., 2003). We only excluded sites where low dissolved oxygen was an obvious additional stressor.

At sites in the affected group where metrics were different from expected, exposure to contaminants in sediments may differ from that measured, in part because of unaccounted for effects on bioavailability. The logistic regressions were derived from analyses of bioassay data that did not consider possible site-specific factors affecting the bioavailability of the contaminants in sediments (Field et al., 2002). Alternate approaches to assessing sediment chemistry have measured AVS or the fraction of organic carbon, which may affect the bioavailability of metals and organics such as PAHs, respectively (Liber et al., 1997; U.S. EPA, 2003b). While these methods attempt to assess the bioavailability of these contaminants, there are limitations to these approaches, particularly with the assumption that equilibrium conditions exist within the sediments for metals and AVS or PAHs and organic carbon (O'Connor and Paul, 2000). In preliminary analyses, sediments from only 9 of 201 sites could be classified as potentially toxic based on the equilibrium partitioning model for chronic PAH effects (U.S. EPA, 2003b). Five of those sites had a maximum $p > 0.5$ and five sites exhibited

TABLE 13

Enumeration of Sampling Events in Estuarine Systems of the Virginian Province of the Atlantic Coast where Classification Based on the Organism-level Effects Measures and that Based on the Community Metric Disagree

Metric	Number of Sampling Events*			
	Classified as Unaffected	Metric Different from Expected for Unaffected Group	Classified as Affected	Metric Different from Expected for Affected Group
Classification Based on Sediment Chemical Concentrations				
Capitellida species richness	171	70	30	8
% Polychaeta, Spionida	171	54	30	16
% Polychaeta, predators	171	86	30	6
% Individuals, Gastropoda	171	92	30	7
% Individuals, Pollution-indicative taxa	171	54	30	15
% Individuals, *Streblospio benedicti*	171	27	30	17
Phyllodocida abundance	171	75	30	11
Decapoda abundance	171	81	30	9

TABLE 13 cont.

Metric	Number of Sites			
	Classified as Unaffected	Metric Different from Expected for Unaffected Group	Classified as Affected	Metric Different from Expected for Affected Group
Classification Based on Sediment Toxicity Tests				
Biomass per taxon	159	33	27	21
% Individuals, Pollution-indicative taxa	159	46	27	15
% Polychaeta, Phyllodocida	159	83	27	10
Phyllodocida abundance	159	69	27	11

* For each metric, the total number of sampling events is the sum of the columns labeled “Classified as unaffected” and “Classified as affected.”

toxicity in the ambient toxicity tests. The Simultaneously Extracted Metals/Acid Volatile Sulfide (SEM/AVS) ratio exceeded one for sediments from 27 of the 133 sites where AVS data were available. However, this means only that the metals may be bioavailable and not that their bioavailable concentrations are sufficient to cause toxicity (Hansen et al., 1996). This may be why only four of those sites exhibited toxicity in the ambient toxicity tests, and only three sites had a maximum $p > 0.5$.

Besides assessing measurement endpoints at different levels of biological organization, chemical guidelines, ambient toxicity tests and community metrics differ in their specificity to different stressor gradients (Karr and Chu, 1998). Chemical guidelines are very specific to the contaminants being measured and assessed and ignore any unmeasured contaminants or stressors that lack guidelines for comparison. Ambient toxicity tests detect toxicity associated with the entire milieu of bioavailable contaminants in the tested sediments but do not assess other characteristics of the estuarine site. Community metrics are not generally stressor specific. Therefore, while community metrics may be sensitive to specific stressors (Griffith et al., 2001), they also will be sensitive to other concurrent alterations of the ecosystem that affect the structure of the biotic assemblages, including alterations of physical habitat that are not addressed by chemical benchmarks.

We used a simple approach in classifying the sites into the unaffected and affected groups. This was done, recognizing that only recently have models been constructed to extrapolate accurately between the organism- and population-level effects (Kuhn et al., 2000), and we still cannot accurately model or extrapolate between population and community effects because of the difficulties of incorporating variation in exposure and response across the hierarchical levels of time, space and organization (de Kruijf, 1991; Landis, 2002). Considering this simple classification, one might expect few, if any, of the metrics would have exhibited differences in their means between the two groups. However, a number of metrics exhibited differences between the groups although the conclusions based on the organism-level measures and on community metrics disagreed at some sites. This would suggest that a relationship exists between the organism-level effects assessed by chemistry or ambient toxicity tests and the community-level effects assessed by community metrics. However, organism-level effects are only predictive to a limited extent of the community-level effects at individual sites. This also suggests benthic metrics may be used to confirm adverse effects at sites identified for further analysis based on chemical data as has been done with ambient toxicity tests (O'Connor et al., 1998). However, care is needed in the selection of appropriate metrics because metrics differ in their sensitivity to different stressors.

4. CONCLUSIONS

At least for the stressors identified, metals in stream water and sediments or metals and PAHs in estuarine sediments, these two studies show relationships between effects at the organism level, as identified by criteria or other benchmarks for surface water or sediments, or by ambient toxicity tests of surface water or sediments and effects at the community level, as assessed with community metrics for macroinvertebrates or fish that are sensitive to the effects of these toxicants. Although effects at the organism level observed in toxicity tests can be linked conceptually to the effects measured by community metrics at the community level, these relationships are not necessarily simple. Furthermore, these relationships are obscured by technical differences among the methods beyond the differences in the levels of biological organization represented by their measurement endpoints. These technical differences affect the methods' specificity and sensitivity to the stressors being assessed. This is why the organism-level effects are only predictive to a limited extent of the community-level effects at individual sites and why these methods frequently differ in their assessment of individual sites. The value of our assessment is that we were able to use much larger data sets to show the statistical relationships among these methods, as opposed to the comparisons of relatively few individual sites in previous studies.

Criteria or guidelines are specific to the contaminants of interest in each ecosystem and environmental medium. However, criteria or guidelines cannot assess contaminants or stressors that are not measured or that lack guidelines for comparison. Ambient toxicity tests are less specific to individual contaminants because they should detect effects of any toxicants present and bioavailable in either surface water and sediments. However, ambient toxicity tests do not assess other characteristics of a site that can affect the biotic community.

Community metrics are the least specific of the three methods, because they directly measure community-level effects in the native assemblages. Although metrics may be selected that are sensitive to a specific stressor (Norton et al., 2000; Ofenbock et al., 2004), those metrics will not be necessarily sensitive only to that stressor and will respond to other stressors, such as alterations in physical habitat. Other community metrics will be insensitive to the specific stressors of interest, because they may not measure alterations in assemblage structure characteristic of the stressor of interest. Therefore, metrics alone probably cannot be used to establish stressor-specific causality but might be used to indicate likely stressors at particular sites. Moreover, data sets similar to those analyzed in this study that include both measurements of

biological assemblages and of stressors might be used to assess stressor-specific, response relationships and identify thresholds for effects associated with specific stressors. The segmented regression technique used in the analysis of the Colorado REMAP data could be used to identify such thresholds for effects.

Other factors also affect the relative sensitivity of these different methods. Toxicity tests that are designed to measure endpoints that are chronic in duration and chemical criteria or benchmarks that are based on chronic measurement endpoints should be more predictive of community-level effects than those based on acute measurement endpoints, because community metrics reflect longer-term changes in communities (Karr and Chu, 1998). Toxicity tests often use one or two standard species, which can be more tolerant of specific contaminants than other indigenous species. In such cases, toxicity tests would be less predictive of community-level effects. A chemical benchmark based on a species sensitivity distribution composed of many species is likely to be more predictive of community-level effects. Because of these limitations and because these methods are complementary, the policy of independent application remains appropriate.

These differences in specificity that make these methods complementary might be used in a strength of evidence analysis (U.S. EPA, 2000c). Low values of metrics known to be sensitive to particular stressors could be used to suggest that those stressors have influenced the community at a site. Subsequently, ambient toxicity tests of site media may be used to verify whether these stressors are toxic contaminants present water or sediments. Chemical analyses would verify whether such media contained toxic concentrations of the contaminants.

Because of the technical differences between these methods, their relative protectiveness, even when considering specific contaminants, such as metals in freshwater or sediments or metals and persistent organics in estuarine sediments, is variable and difficult to quantify with certainty. In some cases, such as the AWQCs for metals in freshwater and the thresholds identified by piecewise regression for various metrics, the protectiveness may be similar. However, in other cases, such as the TELs for metals in freshwater sediments, the guidelines may not estimate values that are related to distinct changes in the biotic assemblages as quantified by the metrics. Moreover, this protectiveness is dependent on how the point where adverse effects are considered significant is estimated. This point can be based on acute effects or chronic effects. A point can also be based on a statistically significant change relative to control tests or reference conditions or based on a specified percent change relative to a control tests or reference conditions. Field et al. (2002) state that maximum p from their

logistic regressions may be selected by a user to "match the level of protectiveness appropriate for the objectives of their assessment." Techniques, like piecewise regression, may be used to identify true thresholds, which represent levels of contaminants or other stressors above which biotic assemblages exhibit significant changes. However, a threshold model may not be appropriate in cases where both the contaminant and response change in a more linear fashion.

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