DISSERTATION

LANDSCAPE ECOTOXICOLOGY: LINKING CATCHMENT-SCALE GEOLOGY TO TRACE-METAL BIOAVAILABILITY AND BENTHIC MACROINVERTEBRATE POPULATION AND COMMUNITY RESPONSES

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY TRAVIS SCOTT SCHMIDT ENTITLED "LANDSCAPE ECOTOXICOLOGY: LINKING CATCHMENT-SCALE GEOLOGY TO TRACE-METAL BIO-AVAILABILITY AND BENTHIC MACROINVERTBRATE POPULATION AND COMMUNITY RESPONSES" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

LANDSCAPE ECOTOXICOLOGY: LINKING WATERSHED-SCALE ATTRIBUTES TO TRACE-METAL BIOAVAILABILITY AND BENTHIC MACROINVERTEBRATE POPULATION AND COMMUNITY RESPONSES

The mobilization of trace-metals in surface water is a natural phenomenon resulting from the weathering of bedrock. However, mining and mineral extraction operations expose large volumes of newly disturbed, finely crushed rock which causes unnaturally high weathering rates and results in poor water quality, degraded stream habitat, and exposure of aquatic communities to toxic concentrations of trace-metals. Two missions of federal property managers are to manage lands for recreation (i.e., fishing) and the production of fresh water. As the population of the intermountain west grows, the demand for fishable and potable water will continue to increase. In anticipation of this need, and the knowledge that trace-metal contamination from historical land uses may impair water quality, the federal government is interested in determining to what extent mining has impaired water quality above and beyond natural background levels.

In an effort to develop baselines for geochemical and biological conditions using catchment-scale geology, we conducted a regional-scale monitoring study to quantify the relationship between geology and water chemistry in streams throughout the mineralized belt of the Central Colorado Rocky Mountains. Because total-recoverable metals is a poor predictor of toxicity to aquatic organisms and because there is no universal way to quantify the bioavailability and toxicity of any single trace-metal, we developed a model capable of predicting toxicity due to trace-metal mixtures.

This new model, the Chronic Criterion Accumulation Ratio (CCAR), is derived from the Biotic Ligand Model (BLM) and incorporates current theory about the interactions between aqueous constituents (e.g., hardness, DOC, pH) that affect trace-metal toxicity and accumulation. We first employed CCAR to determine which geologies produced water qualities that impair aquatic communities. We tested if the BLM-derived CCAR was predictive of trace-metal accumulation and population responses of three native taxa (i.e., ephemeropterans *Rhithrogena* spp. and *Drunella* spp. and the trichopteran *Arctopsyche grandis*) which are candidates as bio-indicators of trace-metal contamination. This evaluation also allowed us to compare information provided by presence/absence data vs. population density responses of *Rhithrogena* spp., *Drunella* spp., and *A. grandis* to trace-metals and to test the hypothesis that *accumulation of trace-metals in individuals results in deleterious effects that propagate to a population-level response*. We also tested the model by comparing the predictive capacities of CCAR to a traditional toxic unit model, Cumulative Chronic Units (CCU).

Geologic alteration was found to be a strong driver of dissolved trace-metal concentrations and alkalinity in Rocky Mountain streams. Primary rock-type (i.e., sedimentary, metamorphic, igneous) and geologic age (i.e., Cenazoic, Mesozoic/Paleozoic, Pre-Cambrian) interact with hydrothermal alteration and

pyrite mineralization to determine water quality and toxicity to aquatic organisms. Three levels of impairment of surface water due to geology were identified in this research: 1) geologies that produce such high concentrations of trace-metals that other water quality characteristics that determine trace-metal bioavailability were unimportant; 2) geologies that produce waters of moderate toxicity to aquatic organisms and where other water quality parameters may play a role in determining impairment and toxicity; and 3) those geologies which do not produce trace-metals at sufficient concentrations to impair water quality or aquatic organisms. We hypothesize that geologies that produce moderately toxic waters are the most susceptible to further impairment by mining activities.

CCAR was found to be correlated with whole body metal concentrations of benthic macroinvertebrates *Rhithrogena spp.*, *Drunella* spp. and *A. grandis*. The probability of detection for a given taxa, as determined from logistic regression of presence/absence data, was relatively insensitive to trace-metals as compared to population density. This disparity indicated that native populations are likely comprised of subpopulations which are differentially sensitivity to trace-metal exposure. These data support the conclusion that toxicity is not constant for all individuals of a population, thus violating an important assumption of the BLM. Also, water quality criteria based on the BLM were protective of some native taxa (e.g., *Drunella* spp.), but not others (e.g., *A. grandis* or *Rhithrogena* spp.).

Results of the comparison between CCAR and CCU indicated that CCAR explained more variation in benthic macroinvertebrate community metrics (e.g., richness, abundance and composition). More importantly, we found deleterious

effects in benthic macroinvertebrate communities at levels of trace-metal contamination previously thought to be safe. Utilizing simple linear regression equations developed in this study, we calculated the average change in macroinvertebrate community metrics between CCAR = 0.07 (i.e., background) and CCAR = 1 (i.e., the theoretical threshold of chronic toxicity). On average we observed a 27% reduction in richness, a 72% decrease in abundance, and a 15% change in community composition between background concentrations of trace-metals and the threshold of chronic toxicity.

This research was the first to investigate use of the Biotic Ligand Model (BLM) for evaluating native taxa responses to trace-metals. Because CCAR is a closer approximation of the causal processes which determine toxicity of trace-metals, we found that aquatic communities were more sensitive to trace-metals exposure than previously thought. This research was also the first to link accumulation of trace-metals in individuals to population-level responses and to relate trace-metal bioavailability to catchment-scale rock type and geologic processes. Results of these studies will be used to develop protocols for setting biological and geochemical baselines so that federal land managers can properly target and prioritize abandoned mine lands for reclamation to improve potable water quality and fisheries habitat in Colorado.

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Dr. William H. Clements has inspired my work since my undergraduate education. Motivated by his studies at Virginia Tech, I began work as an undergraduate to investigate benthic macroinvertebrate communities in local streams. His work is essential to my inspiration. His words of encouragement kept me going when I though I had nothing left. I thank Will for his words of encouragement, generous sharing of ideas, and guidance.

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My Ph.D. pursues began as a phone call, made to me, by my now and then, mentor, Dr. William H. Clements. I was working from home in my apartment in Wrigleville, Chicago when he called to inform me that I had been awarded a fellowship to attend Colorado State University and could study under his tutelage. This was a goal I set for myself many years prior as his work, which I read in an Ecotoxicology class as an undergraduate at Penn State, had inspired my interest in graduate school. Five minutes after speaking with Dr. Clements, a stranger from San Francisco called to offer me a job. I told him about the opportunity at CSU, after which he advised me to not think twice and to quit my job, decline the job offer, and leave for Colorado to attend graduate school. He then hung up immediately. I took his advice.

Upon arriving at CSU, Dr. Stanley E. Church had a random lunch meeting with Dr. Clements in Denver, where Stan informed him he was interested in finding a student interested in studying how geology influences water chemistry and aquatic communities. A few weeks later, I met Stan to discuss this project. I loved his ideas, appreciated his direct nature, and he, Will and I embarked on this journey.

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PREFACE

Hard rock mining began in the South Platte River in 1859 (Chronic & Chronic 1972). Native gold was mined from the river beds and flood plains via a technique called placer mining. Miners would shovel the stream bed into a sluice box which would allow them to trap heavy pieces of gold and separate it from lighter sediments. Over time these stores of gold were depleted and other processes were necessary to access ore deposits in Central Colorado. Deeper shaft mining was necessary to access the remaining gold. However, these deposits were rich in sulfides and required crushing and processing by a mill and smelter to remove impurities. As gold deposits became depleted, silver, lead, and zinc were sought after. In present time, mining operations are much larger than in the past, creating large open pits or removing entire mountain sides, depositing over burden and waste rock in valley bottoms. This diversity of mining impacts has left a legacy of anthropogenic influences on terrestrial landscapes and altered the geochemistry of surface waters.

Recent surveys of mountain streams in Colorado suggest that up to 25% are degraded by trace-metals (Clements et al. 2000). Generally, this degradation is assumed to be a result of the over 23,000 abandoned mines in Colorado (http://mining.state.co.us/Abandoned%20Mines.html). However, these mineral deposits were formed through hydrothermal geologic processes and likely influenced water quality prior to the extensive mineral extraction (Tooker 1963, Tweto 1968, Wanty et al. 2002). The extent to which mining has increased the rate of weathering of bedrock and transport of trace-metals to aquatic ecosystems throughout this region is largely unknown.

Exposure to elevated concentrations of trace-metals adversely affects aquatic populations and communities (Hare 1992, Clements et al. 2000, Soucek et al. 2002). Differential sensitivities of individuals to trace-metals result in population-level effects that culminate in an assemblage shift from a sensitive to a metal-tolerant community (Carlisle & Clements 1999, Clements et al. 2000). Trace-metal uptake by aquatic organisms can occur by association with sediment, directly from the water column, or through dietary exposure (Goodyear & McNeill 1999, Hare et al. 2003). However, aqueous exposure to trace-metals, particularly the dissolved free ion species, is thought to be the primary route of uptake and accumulation (Yu & Wang 2002, Hare et al. 2003). The bioavailability of dissolved trace-metals is influenced by several common aqueous constituents. Interactions with dissolved organic carbon (DOC) and major anions (e.g., HCO_3^-, CO_3^{2-}, Cl^-) decrease the dissolved free-ion concentrations of trace-metals, and competition with major cations (e.g., Ca^{2+}, Mg^{2+}) for the sites of toxic action on the respiratory surfaces of aquatic organisms decrease the amount of metal bound to those surfaces, thus ameliorating trace-metal toxicity (Di Toro et al. 2001, Santore et al. 2001, U.S. EPA 1999).

Landscape attributes also control trace-metal bioavailability to aquatic organisms because they influence DOC, cation and anion concentration and composition. Regional climate, hydrology, and geology control natural weathering rates and transport of trace-metals into stream channels (e.g. geoenvironmental models) (Wanty et al. 2002). In contrast, historical mining has resulted in the rapid release of trace-metals to aquatic environments. These natural and anthropogenic processes control the concentration and speciation of trace-metals in Rocky Mountain streams. The concentration and quality of ameliorative agents of trace-metal toxicity such as $CaCO_3$ and DOC are also controlled by landscape attributes. In Colorado, the concentration and composition of DOC and the concentrations of major cations and anions vary with season and landscape characteristics such as vegetation, geology, and land use (Baron et al. 1991, McKnight et al. 1997, Boyer et al. 2000, Wanty et al. 2002, Prusha & Clements 2004,). This variation in ligand quality and quantity results in changes in the bioavailability of dissolved trace-metals across the landscape.

Biological assessments such as the U.S. EPA Regional Environmental Monitoring Assessment Program (REMAP) or the USGS Central Colorado Assessment Program (CCAP) are conducted at regional spatial scales (e.g., western U.S., Colorado, the central Colorado Mountains) over which climate, vegetation, and geology change from site to site. Commensurate with these changes in landscape characteristics are changes in the concentrations of major cations, anions, and DOC that alter the bioavailability of contaminants in aquatic ecosystems (Morel 1983, Baron et al. 1991, McKnight et al. 1997, Boyer et al. 2000, Wanty et al. 2002, Prusha & Clements 2004, Playle 2004). Because these physicochemical characteristics affect each individual trace-metal differently to form a variety of metal species, there is no universal way to quantify the bioavailability and toxicity of any single trace-metal (Meyer 2002a). Large scale biological assessments of trace-metal contamination would benefit from a model that incorporates site-specific variation in aqueous chemistry and more precisely approximates the bioavailable fraction of trace-metals to aquatic organisms as it varies across the landscape.

The Biotic Ligand Model (BLM) is an algorithm that predicts acute toxicity of dissolved free ions of trace-metals to aquatic organisms (e.g., algae, cladocerans, fish, and macroinvertebrates; Di Toro et al. 2001, Santore et al. 2001). Coupled geochemical speciation models, CHESS and WHAM V, are used to quantitatively account for the influence of DOC and major cations and anions on trace-metal bioavailability (Tipping 1994, Santore & Driscoll 1995). Empirical data demonstrate that a constant amount of toxicity results from a specified accumulation of a metal on an organism's respiratory surface, which is termed the biotic ligand (e.g., the LC_{50}, the amount of metal accumulation at which 50% of the test organisms die) (Mac Rae et al. 1999, Meyer et al. 1999, 2002b). Thus, the BLM calculates the fraction of the trace-metal in water that is available to accumulate on the biotic ligand, and predicts a standard test organism's subsequent response. Because the BLM can predict the LC_{50} of trace-metals usually within a factor of 2, the U.S. EPA is adopting the model to set site-specific water quality criteria (99 FR 28314 10/28/1999, U.S. EPA, 2003). However, the BLM has a number of assumptions that are likely violated in natural ecosystems: 1) direct uptake via dissolved phases of metals is the sole exposure route; 2) toxicity to aquatic organisms is due to an acute exposure to a single trace-metal (Smith et al. 2006); 3) the model is calibrated to a limited range of chemical concentrations which may not be relevant to oligotrophic mountainous streams (Smith et al. 2006); and 4) toxicity to an individual non-indigenous aquatic organism has little ecological relevance to stream health (Clements & Kiffney 1994).

We have developed a method that utilizes the BLM (HydroQual 2003) for the purpose of ecological assessment of trace-metal contamination in natural systems. We developed a toxic unit model of additive trace-metal toxicity derived from BLM results. This new model, the Chronic Criterion Accumulation Ratio (CCAR), incorporates current theory about the interactions between aqueous constituents (i.e., hardness, DOC, pH) that affect trace-metal toxicity and accumulation of bioavailable trace-metals on the respiratory surface of aquatic organisms.

The goal of the Central Colorado Assessment Project is to develop a regional-scale geologic-based environmental assessment protocol to rank stream health for use by the U.S. Forest Service in developing their 10 year forest plan. The primary objectives of this environmental assessment are: 1) to develop environmental baselines for streams based on geologic classification and rank them; 2) validate the application of the the Biotic Ligand Model as a bioassessment tool; 3) and quantify the linkages between catchment-scale attributes (e.g. geology, land use) and local water quality, habitat, and benthic macroinvertebrate communities. Throughout this work, effort has been made to link local geochemical and biological attributes to catchment-scale land use (e.g., historical mining) so that the USGS can provide relevant data to the USFS for planning and development of effective remedial strategies to manage stressors to aquatic ecosystems.

The goal of this dissertation is to work within the framework of the Central Colorado Assessment Project to begin the development of biological and geochemical tools for the assessment of historical mine influences on catchments in Colorado. Here I present studies conducted to: 1) develop the Biotic Ligand Model for use in regional-scale bioassessments; 2) link natural geologic processes (i.e., rock type, age, hydrothermal alteration, presence/absence of pyrite) to trace-metal bioavailability as if affects aquatic organisms; and 3) test a new model of trace-metal toxicity, Chronic Criterion Accumulation Ratio (CCAR), for its ability to predict individual, population, and community responses in benthic macroinvertebrates. Results of these studies will be used to develop protocols for setting biological and geochemical baselines so that federal land managers can properly target and prioritize abandoned mine lands for reclamation to improve potable water quality and fisheries habitat in Colorado.

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CHAPTER 1 - LANDSCAPE ECOTOXICOLOGY: LINKING CATCHMENT-SCALE GEOLOGY TO WATER QUALITY AND TRACE-METAL BIOAVAILABILITY

ABSTRACT

We investigated the influence of catchment geology on water quality and trace-metal bioavailability in streams from the mineralized belt of the Central Colorado Rocky Mountains. Sixty-two catchments (n = 84 samples) underlain by a single rock type were sampled for trace-metal concentrations (i.e., Cd^{2+}, Cu^{2+}, Zn^{2+}) and water quality parameters that influence trace-metal bioavailability to aquatic organisms (i.e., alkalinity, hardness, pH, DOC). We also collected benthic communities from a sub-set of these sites (*n* = 35). Trace-metal bioavailability was modeled using the Biotic Ligand Model to derive the Chronic Criterion Accumulation Ratio (CCAR), toxic unit model. Water quality parameters and benthic macroinvertebrate metrics were contrasted by geologic classifications using one-way analysis of variance (ANOVA) and Dunnett's *post hoc* test to determine which catchments were impaired by trace-metal contamination. The relationship between trace-metal bioavailability and benthic community response was evaluated through simple linear regression. Although we did not quantify the impact of mining on water quality, we did determine which geologic classifications produced water qualities that impaired aquatic communities. These results showed that rock type (i.e., sedimentary, metamorphic, igneous) and geologic age (i.e., Cenazoic, Mesozoic/Paleozoic, Pre-Cambrian) interact with hydrothermal alteration and pyrite mineralization to determine water quality and trace-metal toxicity to aquatic organisms as

measured by CCAR. CCAR explained a significant amount of variation in EPT richness ($R^2 = 0.55$) confirming that these communities are structured by geochemistry and trace-metal bioavailability determined by geology and geologic processes. Three levels of surface water impairment were identified: 1) geologies that produced such high concentrations of trace-metals that other water quality characteristics known to determine trace-metal bioavailability were unimportant; 2) geologies that produced waters of moderate toxicity to aquatic organisms and where other water quality parameters may play a role in determining impairment and toxicity; and 3) those geologies that do not produce trace-metals at sufficient concentrations to impair water quality. Those geologies that produce moderate levels of trace-metals are most susceptible to further impairment by mining which may elevate trace-metal concentrations in surface waters and overwhelm the protective effects of constituents (i.e., alkalinity, hardness, dissolved organic carbon) in natural waters. These results support the idea that catchment geologic classifications can be used to predicted impairment of water quality and aquatic communities.

INTRODUCTION

Geology predicates ecosystems, and geologic processes, rock type, and age control the geochemistry and morphology of the earth (Figure 1; Hynes 1975, Vitousek et al. 1997, Cary et al. 2005). In combination with climate (i.e., precipitation and temperature) and vegetation (i.e., organic acids and anions) these processes determine the rate of mechanical and chemical weathering of bedrock, which influences the structure and function of terrestrial ecosystems (Drever 1994, Drever & Stillings 1997, Vitousek & Farmington 1997, Vitousek et al. 1997, Filippelli & Souch 1999, Stewart et al 2001). These same processes determine the structure and function of aquatic ecosystems (Wanty et al. 2002, Carlisle & Clements 2005).

Degradation in water quality occurs when reduced minerals are oxidized upon exposure to water and O_2 either through mining activities or natural weathering processes (Wanty et al. 2002). Oxidation of sulfide-bearing minerals produces sulfuric acid, which results in the formation of metal hydroxides and locally high concentrations of dissolved trace-metals, depending on the composition of rocks surrounding the mineral deposits (e.g., Plumlee et al. 1995). The mobilization of trace-metals in surface water is a naturally occurring chemical and physical process resulting from weathering. However, mining exposes large volumes of newly disturbed, finely crushed rock that results in high weathering rates which produce highly degraded water quality, stream habitat, and exposure of aquatic communities to toxic concentrations of trace-metals.

Trace-metal contamination has environmental and public health impacts on 21,000 km of U.S. rivers (Brezonik et al. 1993).

Exposure to elevated concentrations of trace-metals adversely affects aquatic populations and communities (Hare 1992, Clements et al. 2000, Soucek et al. 2002). Differential sensitivities of individuals to trace-metals result in population-level effects that culminate in an assemblage shift from a sensitive to a metal-tolerant community (Carlisle & Clements 1999, Clements et al., 2000). Trace-metal uptake by aquatic organisms can occur by association with sediment, directly from the water column, or through dietary exposure (Goodyear & McNeil 1999, Hare et al. 2003). Aqueous exposure to trace-metals, particularly the dissolved free ion species, is thought to be the primary route of uptake and accumulation (Yu & Wang 2002, Hare et al. 2003). The bioavailability of dissolved trace-metals is influenced by several common aqueous constituents. Interactions with dissolved organic carbon (DOC) and major anions (e.g., HCO_3^-, CO_3^{2-}, Cl^-) decrease the dissolved free-ion concentrations of trace-metals. Competition of metals with major cations (e.g., Ca^{2+} , Mg^{2+}) for the sites of toxic action on the respiratory surfaces of aquatic organisms decreases the amount of metal bound to those surfaces, thus ameliorating trace-metal toxicity (U.S. EPA 1999, Di Toro et al. 2001, Santore et al. 2001). These natural ligands are also influenced by catchment geology or other landscape factors (i.e., vegetation and/or soils) that are directly or indirectly controlled by geology.

The Biotic Ligand Model (BLM) is an algorithm that predicts acute toxicity of dissolved free ions of trace-metals to aquatic organisms (e.g., algae, cladocerans, fish, and macroinvertebrates; Di Toro et al. 2001, Santore et al. 2001). Coupled geochemical speciation models, such as CHESS and WHAM V, are used to quantitatively account for the influence of DOC and major cations and anions on trace-metal bioavailability (Tipping 1994, Santore & Driscoll 1995). Empirical data demonstrate that a constant amount of toxicity results from a specified accumulation of a metal on an organism's respiratory surface, which is termed the biotic ligand (e.g., the LC_{50}, the amount of metal accumulation at which 50% of the test organisms die; Mac Rae et al. 1999, Meyer et al. 1999, 2002b). Thus, the BLM calculates the fraction of the trace-metal in a water sample that is available to accumulate on the biotic ligand and predicts a standard test organism's subsequent response.

Recent surveys of mountain streams in Colorado suggest that up to 25% are degraded by trace-metals (Clements et al. 2000). Generally this degradation is assumed to result from the mineral extraction economy for this region that began in 1859 (Chronic & Chronic 1972). These mineral deposits were formed through natural geologic processes (i.e., hydrothermal alteration) and likely influenced water quality prior to the extensive mineral extraction (Tooker 1963, Tweto 1968, Wanty et al. 2002). However, the extent to which mining has increased the rate of weathering of bedrock and transport of trace-metals to aquatic ecosystems throughout this region is largely unknown.

Two of the missions (i.e., objectives 3 & 5) of federal land managers (i.e., U.S. Forest Service and the U.S. BLM) are to manage lands for recreation (i.e., fishing) and the production of potable water (U.S. Department of Agriculture 2004

& U.S. BLM 2004). As the population of the intermountain west grows so will the demand for fishable and potable water will continue to expand. In anticipation of this need, and the knowledge that trace-metal contamination from historical land uses may impair water quality, the federal government is interested in determining to what extent mining has impaired water quality.

We determined which geologies produce water chemistry characteristics that result in impaired water quality in streams from throughout the mineralized belt of the Central Colorado Rocky Mountains. Primary rock type, geologic age, intensity of hydrothermal alteration, and the presence or absence of pyrite were used to classify geology and determine how geology influences water quality within a stream reach. CCAR was used to determine how trace-metal bioavailability was influenced by geology and as a predictor of how aquatic communities responded to those water qualities. Because we did not explicitly quantify the impact of historic mining in these catchments, results are interpreted as the potential for different rock types to produce water qualities that impair aquatic ecosystem structure and function.

METHODS

Study Area and Sampling Strategy

The study area is the mineral belt and surrounding mountains of the central Colorado Rocky Mountains (Figure 2). Specifically, the study area includes all of Arapahoe, Roosevelt, Pike, and San Isabel National Forests, portions of the White River and Rio Grande National Forests and other public lands that are included within the area of these Forests (e.g., State, U.S. BLM, and U.S. NWR lands). Rocky Mountain National Park and Great Sand Dunes National Park also lie within the study-area boundaries.

Geographic Information Systems (ArcGIS 8.3, ArcHydro 1.2) were used to characterize the catchment habitats which were targeted for sampling (ESRI 1999, Maidment 2002). Digital elevation models (30 x 30 m) were used to define catchment boundaries. Once the catchment boundaries were defined, we used geologic data at the 1:250,000 scale to classify the presence of different primary rock types and geologic ages. New remote sensing AVIRIS (http://aviris.jpl.nasa.gov/html/aviris.task.html) and ASTER (http://aviris.jpl.nasa.gov/html/aviris.task.html) data descriptive of hydrothermally altered rock and the presence of pyrite mineralization were used to classify how intensely rock types were alteration and if pyrite was present in the bedrock. The resolutions of these data are 17m^2 for AVIRIS and 30 m^2 resolution for ASTER. All GIS data are available from USGS Geologic Division, Denver, Colorado. Small catchments (1st-3rd order) predominantly underlain by a single rock type were sampled and categorized based on mineral deposit criteria and measured cation and anion concentrations to evaluate water quality (e.g., Miller 1999).

Sample Collection

Although we collected data from > 200 catchments, for the purpose of this analysis we focused only on catchments underlain by a single rock type. This was used to directly link specific catchment lithologies to changes in surface-water geochemistry. Geochemical data collected from 62 different drainages within the study domain (Figure 2) and during base-flow conditions in the summers of 2004 and 2005 were used here. Replicate (n = 11) and duplicate (n = 11) samples were included in this analysis to ensure a robust sample size and to account for variation in water quality due to sampling crew and sampling period. These steps were taken to ensure that measured differences were due to geology and not to analytical variation, procedural error, or natural site deviances. No geologic categories were comprised of fewer than 3 independent stream sites. Benthic community samples were simultaneously collected from a sub-set (n = 35) of streams where geochemistry was collected.

Geochemical Parameters

Water samples were collected using methods described in Wilde et al. (1998) to meet the requirements of the BLM (HydroQual 2003). Routine water quality parameters (temperature, conductivity, and pH) were measured in the field using a Horiba© (USA) D-24 combination meter following methods described by Wilde et al. (1998). The probes were calibrated at the beginning of each day with certified standards, and checked periodically throughout the day. All water samples processed in the laboratory were filtered through a Acrodisc® Premium 25 mm Syringe Filter with 0.45 um Nylon Membrane at the site and stored at 4°C until analyzed. Water samples for dissolved organic carbon (DOC) were filtered through a 0.70-µm glass-fiber filter, acidified with concentrated hydrochloric acid

(12 molar) to a pH of < 2, and stored in baked amber glass bottles. A Shimadzu© TOC-5000A total organic carbon analyzer was used to measure DOC. Dissolved trace-metal samples were acidified with concentrated nitric acid (16 molar) to a pH < 2 and stored in polyethylene bottles. Samples for anions were collected and stored in polyethylene bottles. Water analyses were conducted at the analytical laboratories of the USGS Geologic Discipline Laboratory in Denver, Colorado. Concentrations of major cations (Na^+, K^-, Mg^{2+}, and Ca^{2+}) were analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Briggs 2002), whereas trace-metal concentrations (Cd^{2+}, Cu^{2+}, Zn^{2+}) were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Lamothe et al. 2002). Major anions (Cl^-, F^-, NO_3^-, SO_4^{2-}) were measured by ion chromatography (Theodorakos et al. 2002). High concentrations of SO_4^{2-} (>25 mg/L) were determined by inductively coupled plasma-atomic emission spectrometry and alkalinity was determined by titration (Theodorakos 2002).

Determination of Trace-metal Bioavailability

Biological assessments such as EPA Regional Environmental Monitoring Assessment Program (REMAP) or the USGS Central Colorado Assessment Program (CCAP) are conducted at the regional spatial scales (e.g., western U.S., Colorado, the central Colorado Mountains) over which climate, vegetation, and geology change from site to site. Commensurate with these changes in landscape characteristics are changes in the concentrations of major cations, anions, and DOC that alter the bioavailability of contaminants in aquatic ecosystems (Morel 1983, Baron et al. 1991, McKnight et al. 1997, Boyer et al. 2000, Wanty et al. 2002, Prusha & Clements 2004, Playle 2004). Because these water characteristics affect each individual trace-metal differently to form a variety of metal species, there is no universal way to quantify the bioavailability and toxicity of any single trace-metal (Meyer 2002a). Large scale biological assessments of trace-metal contamination would benefit from a model that incorporates site-specific variation in aqueous chemistry and more precisely approximates the bioavailable fraction of trace-metals to aquatic organisms as it varies across the landscape.

We have developed a method that utilizes the BLM (HydroQual 2003) for the purpose of ecological assessment of trace-metal contamination in natural systems. We developed a toxic unit model of additive trace-metal toxicity derived from BLM results. This new model, the Chronic Criterion Accumulation Ratio (CCAR), incorporates current theory about the interactions between aqueous constituents (i.e., hardness, DOC, pH) that affect trace-metal toxicity and accumulation of bioavailable trace-metals on the respiratory surface of aquatic organisms.

Because most trace-metal contaminated streams are impacted by a mixture of metals at chronic concentrations, a measure of chronic toxicity due to metal mixtures was evaluated. Water-quality criteria for individual metals represent concentrations that, when exceeded, are likely to harm aquatic organisms. Because these criterion values are only available for individual metals, alternative models are necessary to estimate toxic effects of trace-metal mixtures. Although most research investigating the toxicity of trace-metal mixtures has focused on acute effects, previous studies have shown additive effects at chronic concentrations (Newman & McCloskey 1996, Norwood et al. 2003, Playle 2004). Because the BLM predicts acute toxicity due to individual trace-metals, the endpoint must be modified to account for metal mixtures at chronic concentrations. The Chronic Criteria Accumulation Ratio (CCAR) assumes additive toxicity of trace-metal mixtures based on BLM-predicted endpoints. The CCAR is defined as the ratio of the BLM calculated accumulated trace-metal on the biotic ligand to that accumulated on the biotic ligand in water at U.S. EPA criterion value, summed for all metals at a site. More specifically, CCAR is calculated as follows:

$$\Sigma CCAR = \sum_i \frac{BLM\ calculated\ site\ specific\ [gill\ metal]}{BLM\ calculated\ [gill\ metal]\ at\ CCC}$$

where *BLM calculated site-specific [gill metal]* and *BLM calculated [gill metal] at* CCC are measurements developed from BLM endpoints. *BLM calculated [gill metal]* is the BLM predicted accumulation of the i^{th} trace-metal on the biotic ligand (gill surface), calculated by running the BLM in speciation mode using site-specific water quality parameters. *BLM calculated[gill metal] at* CCC is the BLM predicted accumulation of the i^{th} trace-metal on the gill surface, calculated using the "normalization chemistry" water quality parameters from Table 1a, U.S. EPA (2003), and the i^{th} metal Continuous Chronic Criterion (CCC) concentration (U.S. EPA 2003). The CCC is developed by averaging toxicity test data across species and genera to determine a concentration of trace-metal that will be protective of 95% of the species at a specific site. We are using CCAR to predict toxicity to benthic communities, individual species of which have differential sensitivity to trace-metals. By using the CCC and not a species specific response point such as the LA_{50} for a daphnid or fathead minnow, we can predict toxicity more generally to the community and account for the modifying effects of several water quality parameters known to determine trace-metal uptake and bioavailability (e.g., pH, DOC, alkalinity, and major cations).

Mechanically, CCAR is calculated by using the BLM software provided by HydroQual (2003). First all water quality parameters are entered into a spreadsheet (i.e., temperature, pH, DOC, Ca^{2+}, Mg^{2+}, Na^{+}, K^{-}, SO_4^{2-}, alkalinity, S^{-}) along with the trace-metal concentration measured at a site. The BLM is then run in speciation mode where the speciation of a trace-metal is calculated by geochemical speciation models and that fraction which can accumulate on the biotic ligand surface is determined. This data is provided to the user in a spreadsheet where it can be extracted and subjected to the equation defined above.

Biological Sample

Five replicate benthic samples (*n*=5) were collected using a 0.1-m^2 Hess sampler (350-μm mesh net) from shallow riffle areas (<0.5 m) at sites selected by the GIS analysis. Representative sample locations were selected based on the following criteria: location was a riffle or run habitat unit, depth was 0.10-0.25 (m) and substrate was representative of the reach. Overlying substrate was scrubbed of all algae and diatoms, and inorganic debris was removed. All individual substrate particles larger than 22.6 mm were removed from the Hess sampler and measured along the intermediate axis. Underlying substrate was disturbed to a depth of approximately 10 cm and the remaining material was sieved using a 350-μm mesh sieve. All organisms retained were preserved in 80% ethanol in the field.

In the laboratory, samples were processed to remove debris, and sub-sampled until 300 organisms (±10%) were removed from the sample following methods described by Moulton et al. (2000). Invertebrates were identified to the lowest practical taxonomic level (genus or species for most taxa; subfamily for chironomids) (Merritt & Cummings 1996, Ward et al. 2002). Means of the five replicate benthic samples were used to calculate Ephemeroptera, Plecoptera, Trichoptera (EPT) richness and Heptigeniidae density.

Data Analysis

One-way analysis of variance (ANOVA) and Dunnett's multiple range *post hoc* tests were used to make comparisons between primary rock type classifications, geologic age, hydrothermal alteration states, and presence/absence of pyrite (Sokal & Rohlf 2003). We used a Bonforroni correction to reduce experiment-wise error rate due to multiple comparisons and did not interpret results to be statistically significant unless p-values were less than 0.006 (Sokal & Rohlf 2003). However, this does not mean results were not deemed biologically meaningful despite not being statistically significant. We used this approach to elucidate differences between mean geochemical parameters for primary and secondary geologic rock type classifications. Geochemical data (except pH) were $\log_{10}$ transformed. Q-Q plots were evaluated to determine whether the data were normally distributed and a Levene's test was used to determine homogeneity of variance. Whereas most data approached a normal distribution upon transformation, heterogeneity of variance complimented by un-equal sample sizes required the use of a Dunnett's *post hoc* test which does not assume homogeneity of variance. Simple linear regression was used to determine if benthic macroinvertebrate metrics were related to the trace-metal gradient observed in these data. All statistical analysis were conducted in SPSS© version 14 for Windows© (2004).

RESULTS

Geologic classifications

Seven different geologic parameters were evaluated: 1) primary rock types, 2) geologic age, 3) hydrothermal alteration, 4) presence/absence of pyrite, 5) igneous subdivisions, 6) metamorphic subdivisions, and, 7) sedimentary subdivisions. For the primary rock type classification (n = 84 samples), comparisons were made among three general categories: sedimentary (n = 18), metamorphic (n = 22) and igneous rocks (n = 42) (Figures 3A – 7A, Tables 1-2). Three geologic age classes (n = 84) were evaluated: Cenozoic (65 Ma to present, n = 28), Mesozoic/Paleozoic (543 to 65 Ma, n = 18), and Pre-Cambrian age rocks (Early and Middle Proterozic age rocks from 2,500 to 900 Ma, n = 38)

(Figures 3B – 7B, Table 1) (Palmer & Geissman 1996). Three classes of hydrothermal alteration (n = 27) were compared with unaltered (i.e., un-affected) rock types (n = 57 catchments). Listed in order of increasing alteration intensity, these three alteration types are: propylitic (n = 6), argilic (n = 7), and quartz-sericite-pyrite (QSP, n = 14) (Figures 3C – 7C, Table 1). Two classes that contrast the presence (n = 20) and absence (n = 64) of pyrite in catchment geology (Figure 3D - 7D).

Sedimentary rock types (n = 17) were subdivided into two classes: class 1 – fluvial (i.e., terrestrial) and some marine sedimentary rocks comprised of clastic rock containing some conglomerate, fine-grained sandstone, and shale (n = 18); and class 2 – marine rocks comprised primarily of marine shale, limestone, and evaporite (n = 8) (Figures 3G – 7G, Table 2). The primary distinction between these two groups is the depositional environment (i.e. non-marine versus marine rocks). The primary rock type classification for the metamorphic rocks (n = 21) was subdivided into 3 classes: mafic-metavolcanic rock (n = 9), metapelite (n = 6), and migmatite (n = 7) (Figures 3F – 7F, Table 2). The migmatite classification is a broad mapping term that will include some metapelite, but it is not sufficiently large that it was broken out as a distinct map unit by field geologists. The primary igneous rock type classification (n = 42) was subdivided into 4 classes: tuff (n = 3), shallow intrusive rocks (n = 6), deep-seated plutons (n = 17), and the Pikes Peak Granite (n = 16) (Figures 3E – 7G, Table 2).

Trace-metal bioavailability

Some significant differences in CCAR were found between geologic classifications (Figure 3). There were substantial differences between primary rock types with igneous and metamorphic rocks producing bioavailable trace-metals greater than sediment rocks (Figure 3A). However, sedimentary rocks produced bioavailable trace-metal concentrations below the threshold of chronic toxicity whereas igneous and metamorphic rock types produced mean CCAR values above that threshold, suggesting these geologic classifications may impair aquatic communities. Large differences between geologic age classifications were also observed with rocks of Mesozoic/Paleozoic origins averaging substantially lower CCAR values than those rocks of Cenozoic and Pre-Cambrian origins which produced mean CCAR values excessive of water quality criterion (Figure 3B). Significant differences were observed between mean CCAR values for hydrothermal alteration and the presence/absence of pyrite classifications (Figure 3C-D). Quartz-sericite-pyrite (QSP) and argilic altered rocks and rocks containing pyrite produced water quality that exceeded the threshold for chronic toxicity (Figures 3C-D).

Specific igneous rock types driving the high level of bioavailable trace-metal observed in the comparison of primary rock types (figure 3A) were tuffs and intrusive rocks (Figure 3E). By comparison, metamorphic rock types (i.e., the mafic-metavolcanic rocks and the metapelite) (Figure 3F) influenced the high primary metamorphic classification (Figure 3A) values for CCAR. It is interesting to note that rocks of volcanic origin exceeded the threshold of chronic toxicity despite being classified as igneous (i.e., tuff, CCAR = 67.7) or metamorphic (i.e., mafic-metavolcanic, CCAR = 1.08).

Dissolved trace-metal concentrations

Although no statistically significant differences were observed in dissolved trace-metal concentrations (Cd^{2+}, Cu^{2+} Zn^{2+}) among the primary rock type classifications, substantially higher concentrations of these trace-metals were observed in igneous and metamorphic rocks as compared to sedimentary rocks (Tables 1). Significant differences in dissolved trace-metal concentrations were found among the three hydrothermal alteration classes (Table 1) and the presence of pyrite (Table 1). Hydrothermal alteration intensity caused a substantial increase in Cd^{2+}, Cu^{2+}, Zn^{2+} concentrations (Table 1). Argilic and QSP-altered rocks had substantially higher Cd^{2+} concentrations when compared to un-altered or propylitic altered rocks. QSP-altered rocks produced higher concentrations of Zn^{2+} and Cu^{2+} than argilic, propylitic and unaltered rocks. The concentration of Zn^{2+} in argilic altered rocks was not significantly higher when compared to unaltered or propylitic altered rocks because of the high variance observed in Zn^{2+} derived from argilic altered rocks (Table 1) However, the mean Zn^{2+} concentration for argilic altered rocks was 10 times that of unaltered or propylitic altered rocks. Significantly more dissolved trace-metal concentrations (Cd^{2+}, Cu^{2+}, Zn^{2+}) were also found between rocks with pyrite as compared to those without pyrite (Table 1).

Although the ANOVA was significant for comparisons within the igneous rock types (p-values < 0.001; Table 2) no significant differences were observed among categories on the basis of the results from the Dunnett's *post hoc* test. However, substantially higher concentrations of trace-metals were observed in the tuff and intrusive rock types when compared to plutons and the Pikes Peak Granite (Table 2). Generally no differences were observed in dissolved trace-metal concentrations among the metamorphic rock type sub-divisions (p-values > 0.006; Table 2); however, catchments underlain by mafic-metavolcanic and metapelite rocks have much higher trace-metal concentrations than catchments in the migmatite rock class. Catchments underlain by sedimentary rocks sub-divisions produced Zn^{2+} at very low concentrations and Cd^{2+} and Cu^{2+} were below the detectable limit (Tables 2).

pH

Differences in mean pH were not substantial among most geologic classifiers except for altered rocks, igneous and sedimentary rock types (Figure 4B & 4F). Mean pH values in streams underlain by argilic altered rocks were higher than all other altered rock types. A large degree of variance observed in the QSP category indicates that this classification is capable of producing acidic pH values that are dependent upon the amount of pyrite present in the QSP-altered rocks (e.g., Plumlee et al. 1995). Other rock types that have the potential for producing acidic waters (e.g., < 5.0) were the metamorphic rock types (Figure 4A) driven primarily by mafic-metavolcanic rock (Figure 4F), rocks of Pre-

Cambrian age (Figure 4B), tuffs (Figure 4E) or/and rocks containing pyrite (Figure 4D). Class 2 – marine sedimentary rocks produced alkaline pH water which were significantly greater than class 1- fluvial (Figure 4G).

Alkalinity

Catchments underlain with sedimentary rocks were found to have significantly higher alkalinity (Figure 5A) than streams with metamorphic or igneous rocks (Figure 5G). Streams with Mesozoic/Paleozoic rocks, which comprise the bulk of the sedimentary rock types, have nearly twice the alkalinity as streams with Cenozoic or Precambrian age rocks (Figure 5B). Hydrothermal alteration and the presence of pyrite resulted in significant declines in alkalinity as compared to unaltered (i.e., un-affected) or non-pyritic rocks (Figures 5C-D). Statistically significant differences were observed within the primary rock type sub- divisions with sedimentary rocks producing on average 80 mg/L alkalinity and igneous and metamorphic rocks producing less than 30 mg/L alkalinity on average (Figure 5E). Likewise, the metamorphic mafic-metavolcanic rocks and metapelite have low alkalinity (Figures 5A, 5F). Sedimentary rocks sub-divided into fluvial and marine classes showed significant differences with the marine class having substantially higher alkalinity as compared to the fluvial class (Figure 5G), and driving the significantly higher primary sedimentary rock type alkalinity value (Figure 5A).

Hardness

Sedimentary rock types were found to have significantly higher mean hardness values than metamorphic and igneous rock types (Figure 6A). Rocks of Mesozoic/Palezoic age in the study area had significantly higher mean hardness as compared to rocks of Cenozoic and Precambrian age (Figure 6B). No significant differences were observed between different hydrothermal alteration classes or presence/absence of pyrite (Figures 6C & 6D). However, mean hardness in QSP-altered rock was generally higher than the other three hydrothermal alteration classes. Although no differences were observed between the igneous and metamorphic rock types sub-divisions (Figures 6E & 6F), mean hardness in catchments underlain by tuff was 3-times greater than values of any other igneous or metamorphic rock type. There was also significant variation in water hardness values among the igneous and sedimentary sub-divisions, with catchments underlain by tuff and sedimentary class-2 marine having greater water hardness (Figure 6E & 6G). Marine sedimentary rocks were found to derive substantially more alkalinity as compared to the fluvial sub-division (Figure 6G). This marine sub-division caused the significantly higher alkalinity in the primary rock classification sedimentary rocks observed in the primary rock classification comparison (Figure 6A).

Dissolved organic carbon

Although no statistically significant differences in dissolved organic carbon concentrations were observed among rock types (Figures 7A -7G), some interesting trends were apparent. Streams underlain by igneous tuff had much less DOC compared to all other igneous rocks (Figure 7E) and streams with predominantly mafic-metavolcanic rocks had much less DOC compared to those with other metamorphic rock types (Figure 7F). Migmatite and class 1-fluvial sedimentary rocks appear to derive higher concentrations of DOC than all other rock types (Figure 7F & G). In contrast, argilic alteration and the presence of pyrite in a catchment reduced DOC concentrations (Figures 7C & D).

Biological data

In general, benthic macroinvertebrate communities were less sensitive to our geologic classifications than measures of water chemistry or metal bioavailability (Figure 8A -G). The mean number of EPT taxa was reduced in QSP-altered catchments and in catchments with rocks containing pyrite. EPT richness was also low in catchments underlain by igneous tuff, metamorphic metapelites, and metamorphic mafic-metavolcanics, but these differences were not statistically significant. However, EPT richness was significantly related to the CCAR metric (Figure 9), indicating that benthic macroinvertebrate metrics were responsive to a trace-metal gradient in these streams. The lack of significant differences in EPT between some of our geologic categories was likely a result of the low number of individual catchments in some categories and the fact that hydrothermal alteration types are nested within primary geologic classifications.

DISCUSSION

This research represents the first attempt to link geology and geological processes to trace-metal bioavailability and benthic macroinvertebrate community responses to water quality, at a regional scale. Because all ecosystems are dependent on and intimately connected with their underlying bedrock, it is essential to understand how ecosystem health changes across geological classifications. Of particular importance is the fact that water-quality criteria are established and applied to all landscapes, despite the fact that landscapes change greatly within the geo-political units where they are applied (i.e., United State Federal Water Quality Criteria and State Water Quality Criteria). For example, it would be inappropriate to apply water quality criteria as baselines in undisturbed ecosystems that naturally produce trace-metals in excess of these standards. Water quality criteria are established to protect against the degradation of aquatic resources, not natural processes (Clean Water Act; 33 United States Code § 1251).

We quantified the geochemistry derived from catchments underlain by a single rock type from the mineralized belt of the Central Colorado Rocky Mountains. We also quantified how rock characteristics such as age, hydrothermal alteration, and the presence/absence of pyrite influenced concentrations of trace-metal and constituents that influence bioavailability to aquatic organisms. Because we did not explicitly quantify the impact of historic mining in these catchments, results are interpreted as the potential for different rock types to produce water qualities that impair aquatic ecosystem structure and function. This information will help us identify those rock types that do not limit water quality, and thus focus future research efforts on those rock types that do impair stream health. By focusing future efforts on those rock types that naturally impair water quality and aquatic communities, we can better develop appropriate experimental designs to determine how historical mining has influenced water quality.

We determined that only metamorphic and igneous rock types from the mineralized belt of central Colorado produced water quality characteristics that may impair aquatic communities (Figure 3A). Of particular interest were igneous tuffs and intrusive plutons, metapelites, and mafic-metavolcanic rocks. These rock types produce high dissolved trace-metal concentrations and control other aqueous constituents that ameliorate trace-metal toxicity (i.e., DOC, alkalinity, pH, hardness) to aquatic organisms. However, not all metamorphic or igneous rock types produced elevated dissolved trace-metal concentrations.

The geologic process of hydrothermal alteration, which can result in the formation of pyrite, is primarily responsible for the impairment of surface waters in the mineralized belt of Colorado. Hydrothermal alteration removes alkali elements and carbonate minerals, replaces iron-bearing silicate minerals with pyrite, and forms layered alumino-silicates and quartz as the alteration intensity increases. This process results in the depression of alkalinities derived from hydrothermally altered rock types (Figure 6C). Pyrite mineralization and hydrothermal alteration are important classifications but are not totally independent measures of the effects of hydrothermal alteration. Hydrothermal alteration often results in the formation of pyrite (i.e., QSP alteration), but may not result in substantial mineralization, (i.e. formation of sphalerite, chalcopyrite, or other sulfide minerals) that, upon weathering (DuBray et al. 1995, Seal & Foley 2002) releases potentially toxic trace-metals (Table 1).

Argilic and QSP alteration and pyrite were found in all primary rock types. QSP alteration in igneous tuffs and intrusive rocks, and in metamorphic mafic-metavolcanic and metapelite rocks, produced elevated trace-metal concentrations and depressed alkalinities (Table 1 & Figure 5). These rocks are of Cenozoic age. However, QSP and argilic alteration and/or the presence of pyrite in sedimentary rocks do not produce elevated concentrations of trace-metals or depressed alkalinities (Table 1, Figure 5). Alkalinities were high in sedimentary rocks because many of them are comprised of carbonates derived from marine deposition. These rocks are of Mesozoic or Paleozoic age. QSP/argilic alteration and/or the presence of pyrite in igneous plutons, Pikes Peak Granite, or in metamorphic migmatitic rocks do not produce elevated concentrations of trace-metals. These rocks are of Precambrian age.

In general, pH was not as sensitive to our geologic classifications as alkalinity. This may be due to the fact that oligotrophic waters are generally considered circumneutral to moderately acidic and hydrothermal alteration and mineralization has the potential to further depress pH.

As with pH, DOC does not appear to be strongly influenced by our primary rock type classifications or geologic age. With the exceptions of class 1- fluvial sediments and migmatite, geology does not seem to be a major source of DOC in the Central Colorado Rocky Mountains. However, geology may have an indirect effect on DOC. Other researchers have found that slope and elevation are strongly controlled by rock type and age, and these physical attributes regulate plant communities, soil development and thus the export of DOC to surface waters (Vitousek et al. 1997, Vitousek & Farrington 1997, Prusha & Clements 2004, Cary et al. 2005, Frost et al. 2006). Rocks that occur at lower elevations where the growing season is longer and form lower slopes where mature soil profiles develop should result in higher surface water DOC concentrations. However, volcanic processes result in high elevations and steep physiographic features. The young tuffs found in these areas erode at a rapid rate limiting the development of soil horizons and plant communities and result in low DOC concentrations produced by catchments underlain by tuffs (Figure 8E).

It is interesting to note that rock types producing the most bioavailable trace-metals as measured by CCAR (i.e., tuff, metapelite, and QSP-altered) also produce the highest trace-metal concentrations, lowest alkalinities, modest DOC concentrations, but the highest water hardness (Figures 3 - 8). Normally, high water hardness protects aquatic organisms by competing with binding sites and influencing gill permeability, thus ameliorating toxicity due to trace-metals (Morel 1983, U.S. EPA 1999, Di Toro et al. 2001, Santore et al. 2001). However, QSP-altered rocks, tuffs, and metapelite produced trace-metal concentrations so high that the ameliorative effects of hardness, DOC, or alkalinity were negligible. Hydrothermal alteration reduces alkalinity, resulting in increased activity of trace-metals in the water. Furthermore, because DOC was relatively low in streams with QSP-altered rocks, tuffs, and metapelite, most of the metals were not bound to organic materials. As a result, adjusting dissolved trace-metal concentrations to account for the protective effects of DOC, hardness and alkalinity in these catchments will have little practical effect due to the exceedingly high concentrations of trace-metals. However, this was not true for all rock types.

Cenozioc age rocks, igneous intrusive rocks, metamorphic mafic-metavolcanic, argilic-altered rocks and those rocks containing pyrite produce trace-metal concentrations that were only marginally or within a factor of 10 above the threshold of chronic toxicity (Figure 3). We predict that site-specific variation in pH, alkalinity, DOC or hardness in these catchments would greatly influence the level of impairment of benthic communities. Further, increases in dissolved trace-metal concentrations or alterations in water quality characteristics due to mining activities will increase the likelihood of impairment to aquatic communities in these geologies.

Migmatite, Pluton, and Pikes Peak Granite rocks produced high quality water with low dissolved trace-metal concentrations and should support robust aquatic communities (Figures 3 – 7, Tables 1 & 2). pH, alkalinity, hardness, and DOC (Figures 4 -7) were consistently higher in catchments underlain by these rock types. These results support the hypothesis that if elevated trace-metal concentrations occurred in these streams, aquatic organisms would likely be protected from toxicity.

Not surprisingly, benthic macroinvertebrate communities showed less concordance to these geologic classifications than did the geochemistry. The lack of differences in EPT richness among many geologic classifications was influenced by the low number of replicates for some categories and the general complexity of our classification scheme. For example, igneous tuff produced relatively high concentrations of bioavailable trace-metals, and we observed a low number of EPT taxa in these catchments; however, we only collected benthic macroinvertebrates from two streams within the igneous tuff classification. The geology of one of these streams was QSP-altered and contained pyrite, while the other stream was unaffected by hydrothermal alteration and lacked pyrite. These data demonstrate that relationships between geological processes, water chemistry and aquatic communities are complex and likely influenced by interactions between hydrothermal alteration and pyrite mineralization.

Despite the lack of a strong influence of geological classification, macroinvertebrate communities were significantly related to trace-metal bioavailability as measured by the CCAR (Figures 9). This suggests that benthic communities are structured by trace-metals derived from specific rock type which have been influenced by age, alteration intensity, and the presence of pyrite. In general, EPT richness was lower at sites with high trace-metal concentrations and low concentrations of aqueous constituents known to affect trace-metal toxicity (i.e., DOC, alkalinity, pH, hardness). Refinement of the geological classifications and the addition of more catchments within the underrepresented categories may allow us to quantify the direct effects of geologic processes on aquatic communities.

CONCLUSIONS

We investigated how geology controls geochemistry and benthic macroinvertebrate communities. Primary rock type (i.e., sedimentary, metamorphic, igneous) and geologic age (i.e., Cenazoic, Mesozoic/Paleozoic, Pre-Cambrian) interact with hydrothermal alteration and pyrite mineralization to determine water quality and toxicity to aquatic organisms (Figure 10). Although we did not quantify the impact of mining on water quality, we did determine which geologies and geologic processes would produce conditions likely to cause toxicity to aquatic communities. In general, we found that by classifying catchment geology based on primary rock type, age, hydrothermal alteration, and presence/absence of pyrite, three classes of water quality were generated: 1) those that have such high concentrations of trace-metals that other water characteristics known to determine trace-metal bioavailability relatively are unimportant; 2) geologies that produce waters of moderate toxicity to aquatic organisms and where other water quality characteristics (DOC, pH, alkalnity) may play a role in determining trace-metal toxicity; and 3) those geologies that do not produce trace-metals at sufficient concentrations to impair water quality of aquatic organisms. Those geologies that yield moderate levels of dissolved trace-metals and relatively dilute concentrations of amending agents may be further impaired by the influences of mining.

This work is the foundation to the development of geo-environmental models descriptive of the surface water chemistry in the Colorado mineral belt. As work progresses, we aim to develop models that predict water quality and benthic macroinvertebrate communities throughout this region based on catchment geology. We made a first step toward developing a model of trace-metal toxicity to aquatic organisms that integrates the complexities of aqueous chemistry which control trace-metal bioavailability. These models will be provided to federal land managing agencies that will apply them in the remediation of abandoned mine lands on federal properties.

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TABLES

Table 1. ANOVA and Dunnett's *post hoc* results depicting differences in trace-metal concentrations (mean ± S.D.) between primary rock type, geologic age, hydrothermal alteration, and the presence/absence of pyrite classifications. Statistical tests were conducted on log transformed data however reported values are nominal.

	Class	N	Cd^{2+} (µg/L)		Cu^{2+} (µg/L)		Zn^{2+} (µg/L)	
Primary rock type	Sedimentary	18	0.01 ± 0.00		0.3 ± 0.0		1.2 ± 0.8	
	Metamorphic	22	0.34 ± 0.98		6.1 ± 21.6		71.7 ± 173.3	
	Igneous	44	0.34 ± 1.37		130.2 ± 860.8		63.8 ± 215.7	
	ANOVA		$p = 0.057$		$p = 0.007$		$p = 0.054$	
Geologic age	Cenozoic	28	0.54 ± 1.70		204.5 ± 1079.0		99.2 ± 265.6	
	Meso/Paleozoic	18	0.01 ± 0.00		0.3 ± 0.0		1.2 ± 0.8	
	Pre-Cambrian	38	0.20 ± 0.75		3.7 ± 16.5		42.2 ± 135.2	
	ANOVA		$p = 0.172$		$p = 0.167$		$p = 0.123$	
Alteration	Un-affected	57	0.01 ± 0.00	B	0.3 ± 0.2	B	1.4 ± 1.3	B
	Propylitic	6	0.01 ± 0.00	B	0.4 ± 0.4	AB	1.7 ± 0.6	B
	Argilic	7	0.24 ± 0.62	AB	1.2 ± 2.6	AB	47.7 ± 123.6	B
	QSP	14	1.46 ± 2.42	A	417.1 ± 1523.6	A	284.5 ± 359.8	A
	ANOVA		$p < 0.001$		$p < 0.001$		$p < 0.001$	
Presence/ Absence of Pyrite	Absence	64	0.01 ± 0.00	B	0.3 ± 0.2	B	1.4 ± 1.2	B
	Presnece	20	1.11 ± 2.11	A	292.4 ± 1275.4	A	215.8 ± 323.8	A
	ANOVA		$p < 0.001$		$p < 0.001$		$p < 0.001$	

Table 2. . ANOVA and Dunnett's *post hoc* results depicting differences in trace-metal concentrations (mean ± S.D.) between primary rock type, geologic age, hydrothermal alteration, and the presence/absence of pyrite classifications. Statistical tests were conducted on log transformed data however reported values are nominal.

	Class	N	Cd^{2+} (µg/L)	Cu^{2+} (µg/L)	Zn^{2+} (µg/L)	
Sedimentatry	Class 1-fluvial	10	0.01 ± 0.00	0.3 ± 0.0	1.4 ± 0.9	
	Class 2-marine	8	0.01 ± 0.00	0.3 ± 0.0	1.0 ± 0.6	
	ANOVA		*NA*	*NA*	*p = 0.361*	
Metamorphic	Mafic-metavolcanic	9	0.12 ± 0.20	2.6 ± 3.9	43.0 ± 78.3	A
	Metapelites	6	1.05 ± 1.76	17.7 ± 41.3	197.5 ± 298.0	AB
	Migmatite	7	0.01 ± 0.00	0.7 ± 0.4	0.9 ± 0.4	B
	ANOVA		*p = 0.066*	*p = 0.397*	*p = 0.042*	
Igneous	Tuff	3	3.39 ± 4.50	1905.8 ± 3294.6	440.4 ± 504.9	
	Intrusive	6	0.77 ± 1.25	0.5 ± 1.3	236.6 ± 387.9	
	Pluton	17	0.01 ± 0.01	0.3 ± 0.01	2.2 ± 2.7	
	Pikes Peak	16	0.01 ± 0.00	0.3 ± 0.00	1.7 ± 1.3	
	ANOVA		*p < 0.001*	*p < 0.001*	*p < 0.001*	

FIGURES

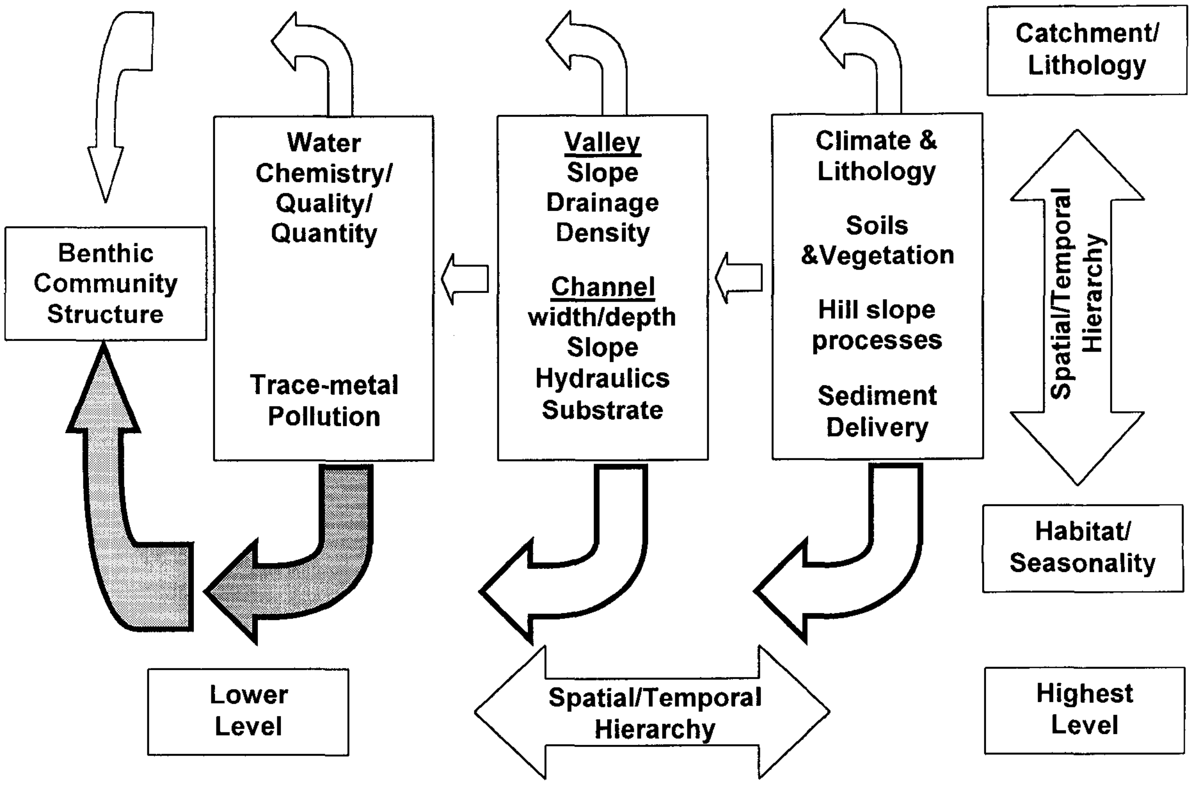

Figure 1. Conceptual diagram of the hierarchical interactions between landscape and fluvial forms and processes, and water quality. These patterns and processes create the template by which benthic communities are structured. Trace-metal contamination influences benthic communities but within the context of a landscape and fluvial disturbance regime. Color and size of arrows depict that factors lower in the hierarchy and are more directly related, occur at higher frequencies, and therefore exhibit great influence on benthic community structure.

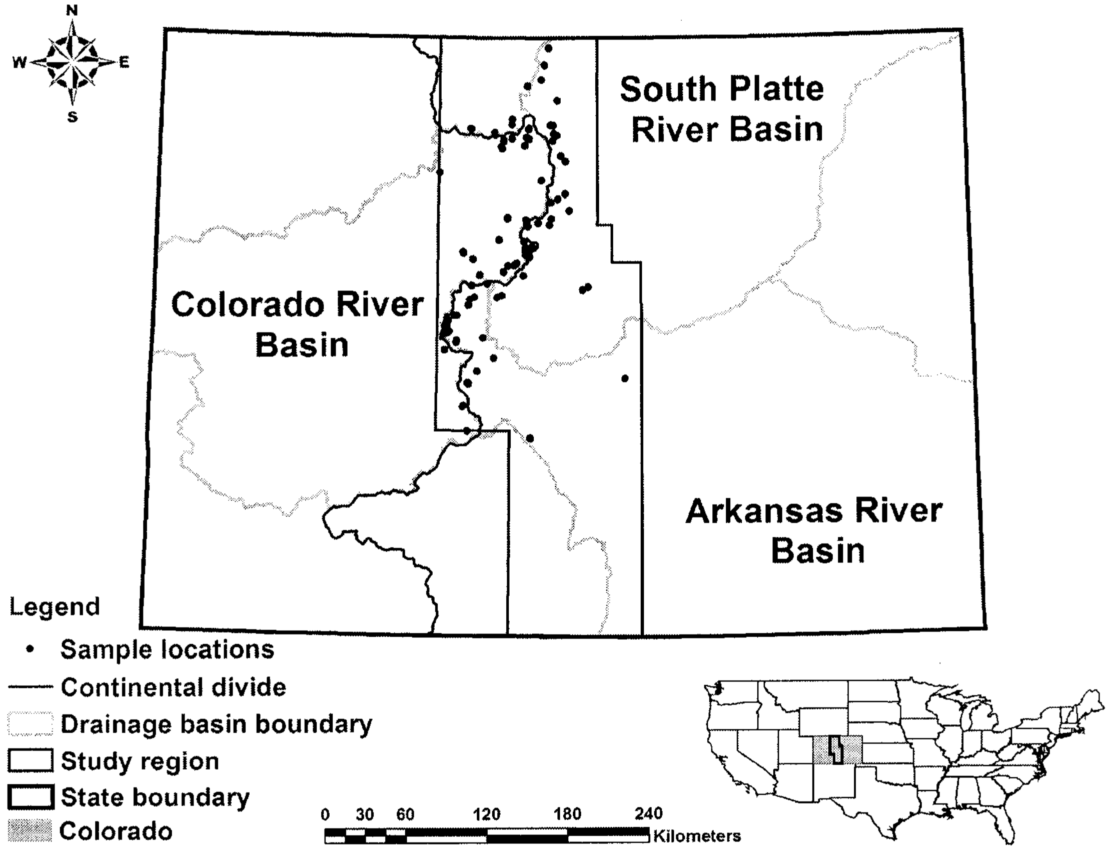

Figure 2. Map of the United States highlighting Colorado and the study domain of this project including the central Colorado Rocky Mountains from New Mexico to Wyoming.

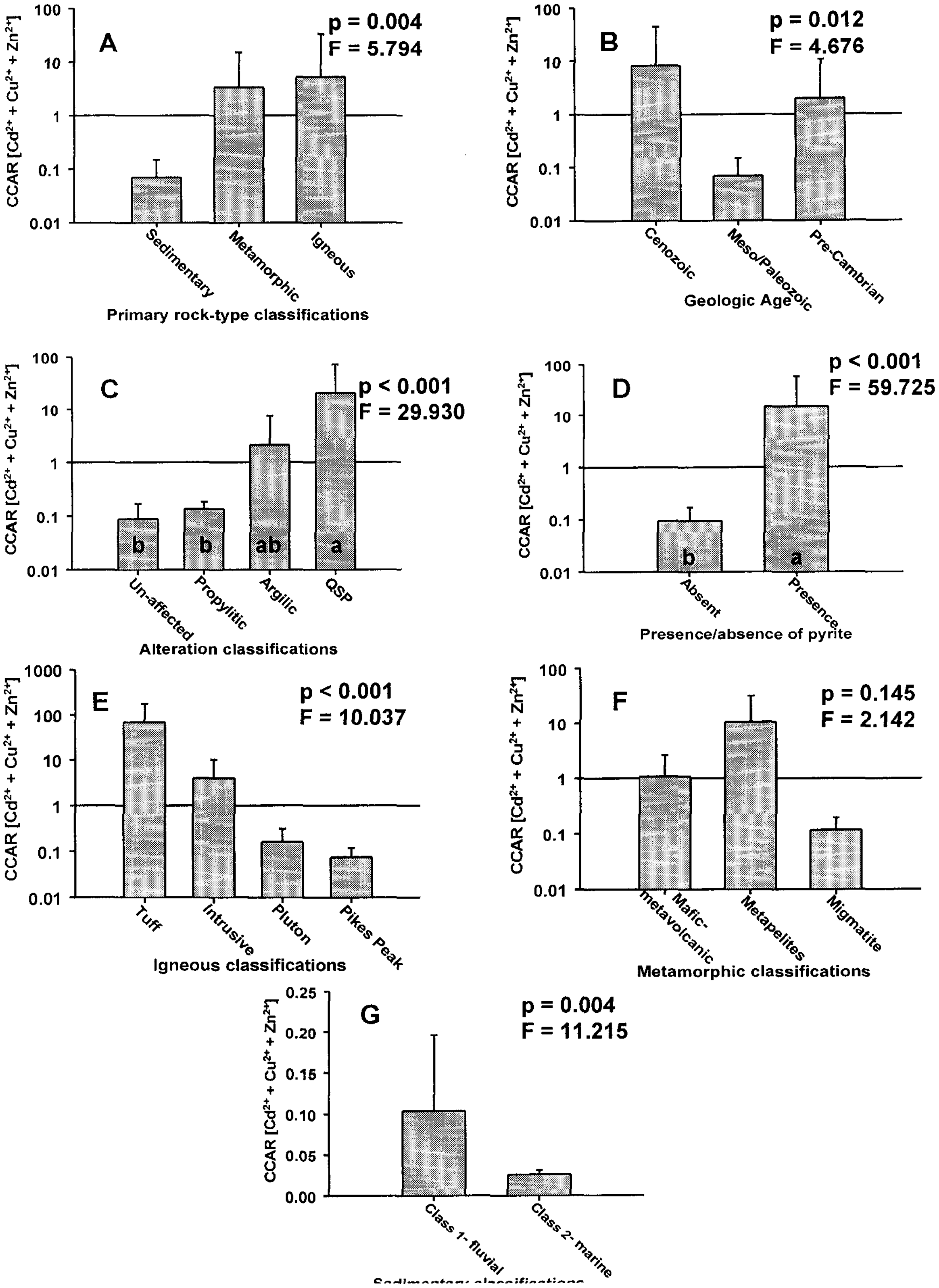

Figure 3. ANOVA and Dunnett's post hoc multiple range test results depicting statistical differences between mean (CCAR) values (plotted on a log scale) for geologic classifications. ANOVA significant to the $p \leq 0.006$ were subjected to the Dunnett's *post hoc* multiple range test. Lower case letters designate results of Dunnett's test where means with the same letter are not significantly different. Bars depict mean CCAR ($\sum Cd^{2+} + Cu^{2+} + Zn^{2+}$) values (±S.D.); Solid horizontal lines indicate the limit at which chronic effects in aquatic species are expected.

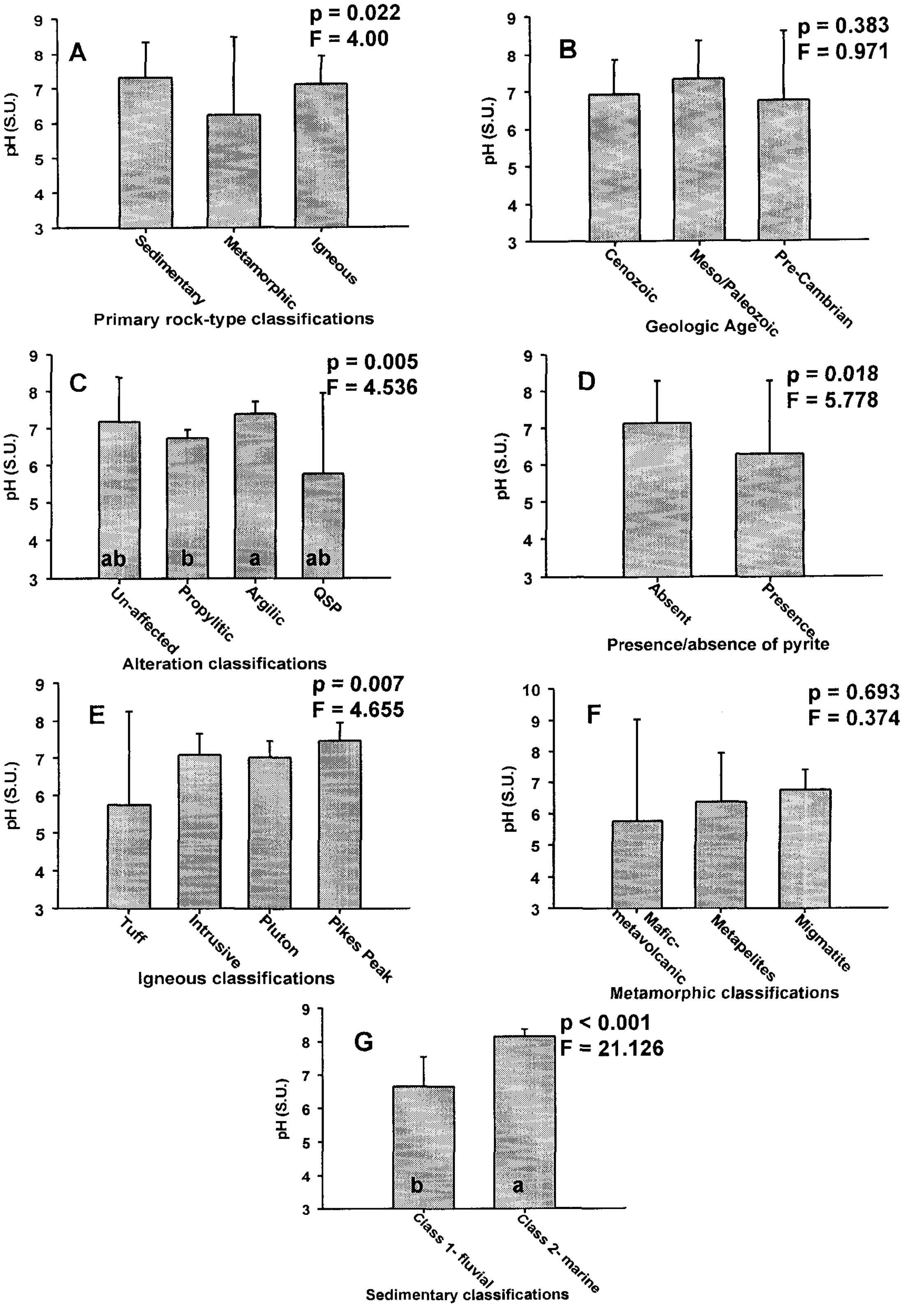

Figure 4. ANOVA and Dunnett's post hoc multiple range test results depicting statistical differences between mean pH values for geologic classifications. ANOVA significant to the $p \leq 0.006$ were subjected to the Dunnett's *post hoc* multiple range test. Lower case letters designate results of Dunnett's test where means with the same letter are not significantly different. Bars depict mean pH values (±S.D.).

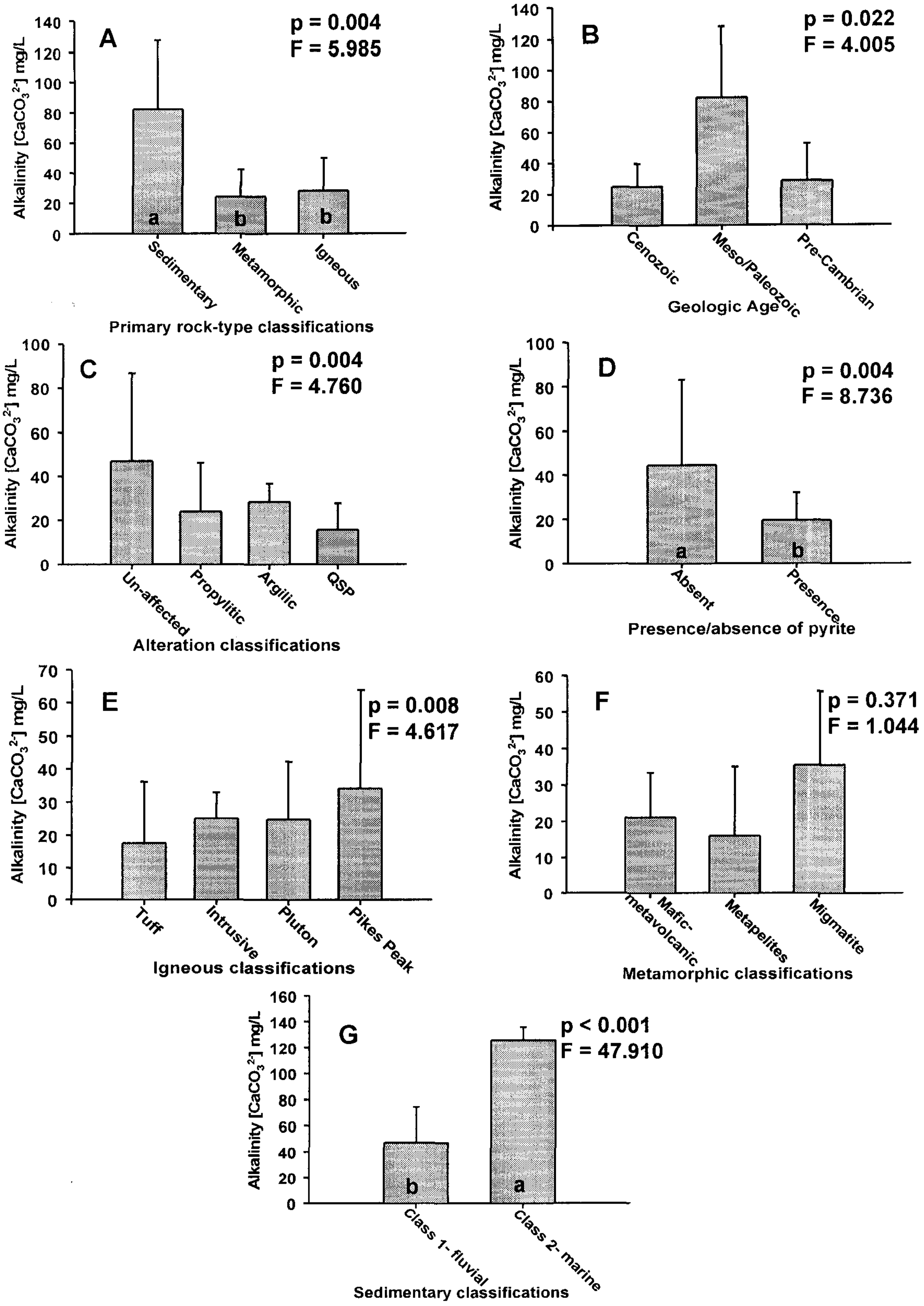

Figure 5. ANOVA and Dunnett's post hoc multiple range test results depicting statistical differences between mean alkalinity values for geologic classifications. ANOVA significant to the $p \leq 0.006$ were subjected to the Dunnett's *post hoc* multiple range test. Lower case letters designate results of Dunnett's test where means with the same letter are not significantly different. Bars depict mean alkalinity values (±S.D.).

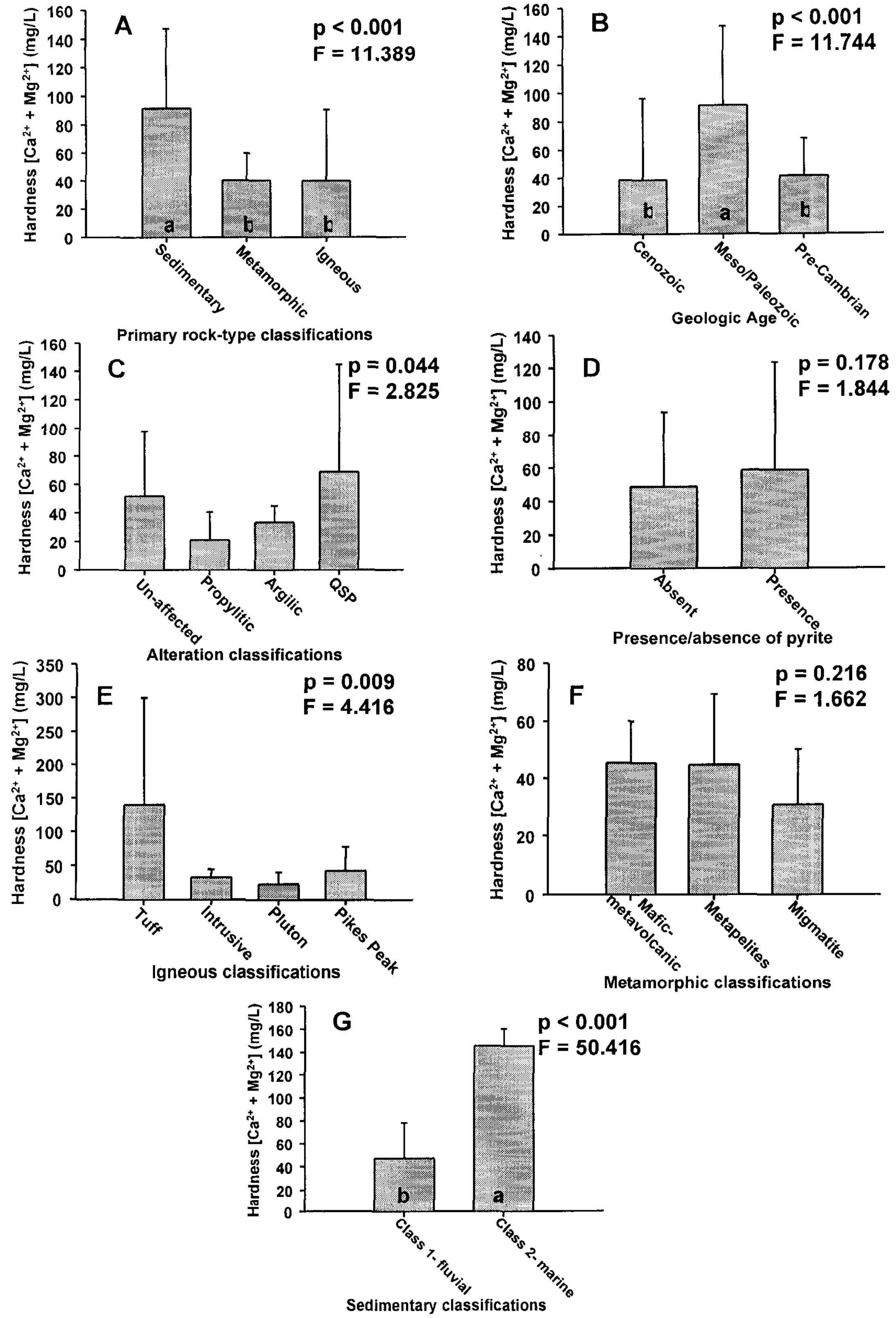

Figure 6. ANOVA and Dunnett's post hoc multiple range test results depicting statistical differences between mean hardness values for geologic classifications. ANOVA significant to the $p \leq 0.006$ were subjected to the Dunnett's *post hoc* multiple range test. Lower case letters designate results of Dunnett's test where means with the same letter are not significantly different. Bars depict hardness values (+S.D.).

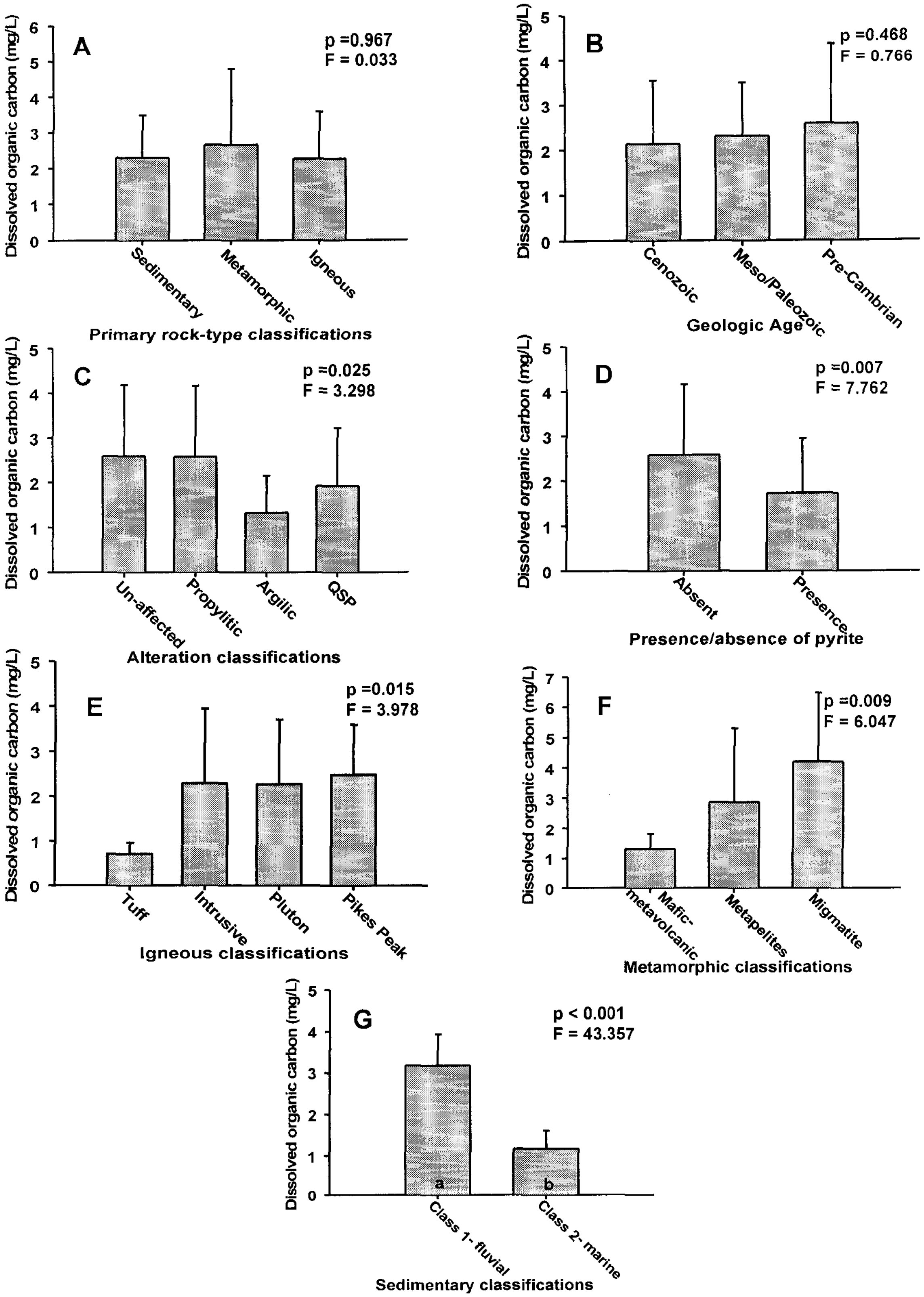

Figure 7. ANOVA and Dunnett's post hoc multiple range test results depicting statistical differences between mean DOC values for geologic classifications. ANOVA significant to the $p \leq 0.006$ were subjected to the Dunnett's *post hoc* multiple range test. Letters designate results of Dunnett's test where means with the same letter are not significantly different. Bars depict DOC values (±S.D.).

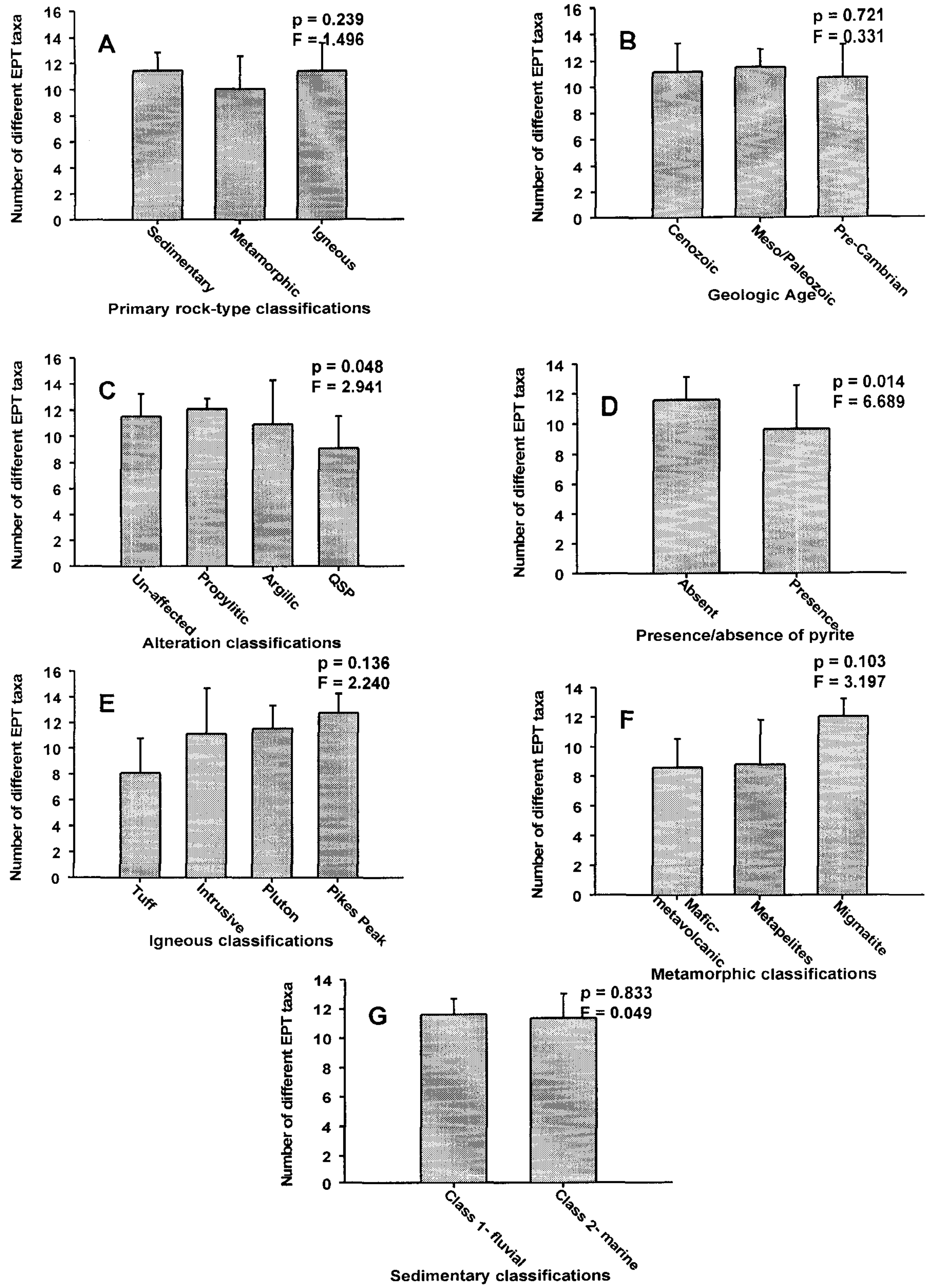

Figure 8. ANOVA and Dunnett's post hoc multiple range test results depicting statistical differences between mean EPT richness for geologic classifications. ANOVA significant to the $p \leq 0.006$ were subjected to the Dunnett's *post hoc* multiple range test. Letters designate results of Dunnett's test where means with the same letter are not significantly different. Bars depict EPT richness values (±S.D.).

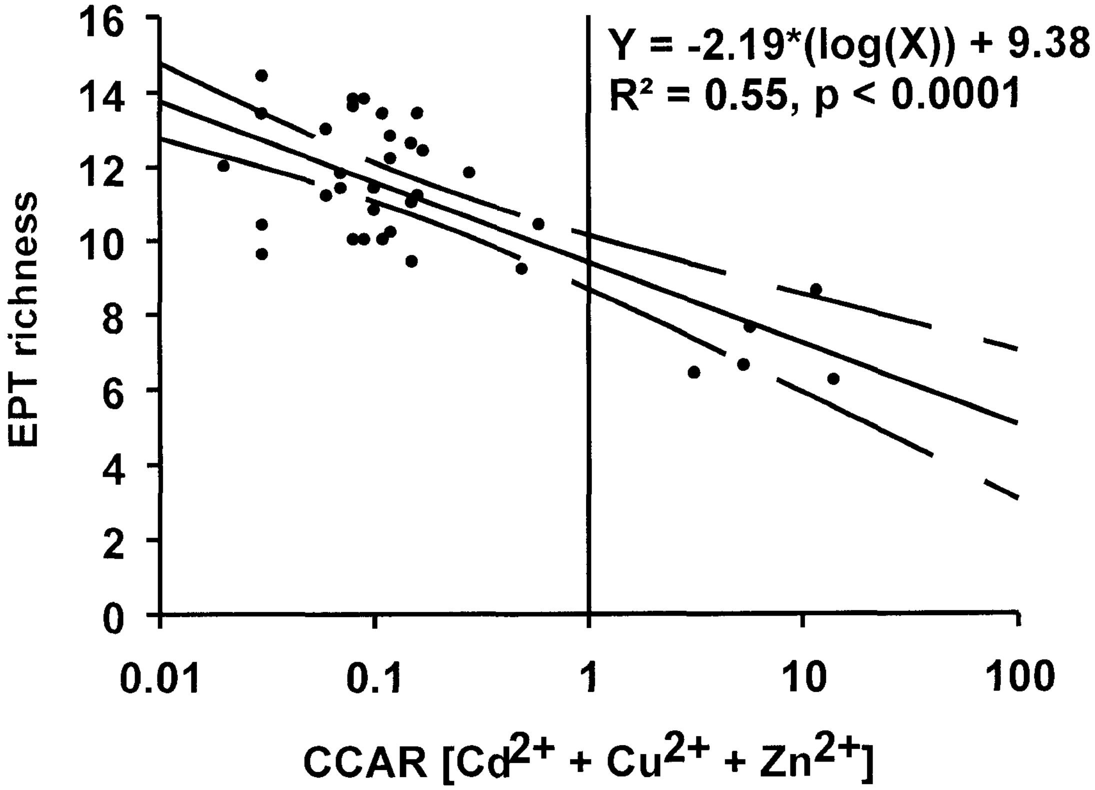

Figure 9. Simple linear regressions depicting the relationship between benthic macroinvertebrate community metric EPT richness and log(CCAR) $\sum(Cd^{2+} + Cu^{2+} + Zn^{2+})$. Solid vertical lines indicate the limit at which chronic effects in aquatic species are expected. Solid line is the linear relationship between dependant and independent variables. Dashed lines are the 95% confidence interval of regression.

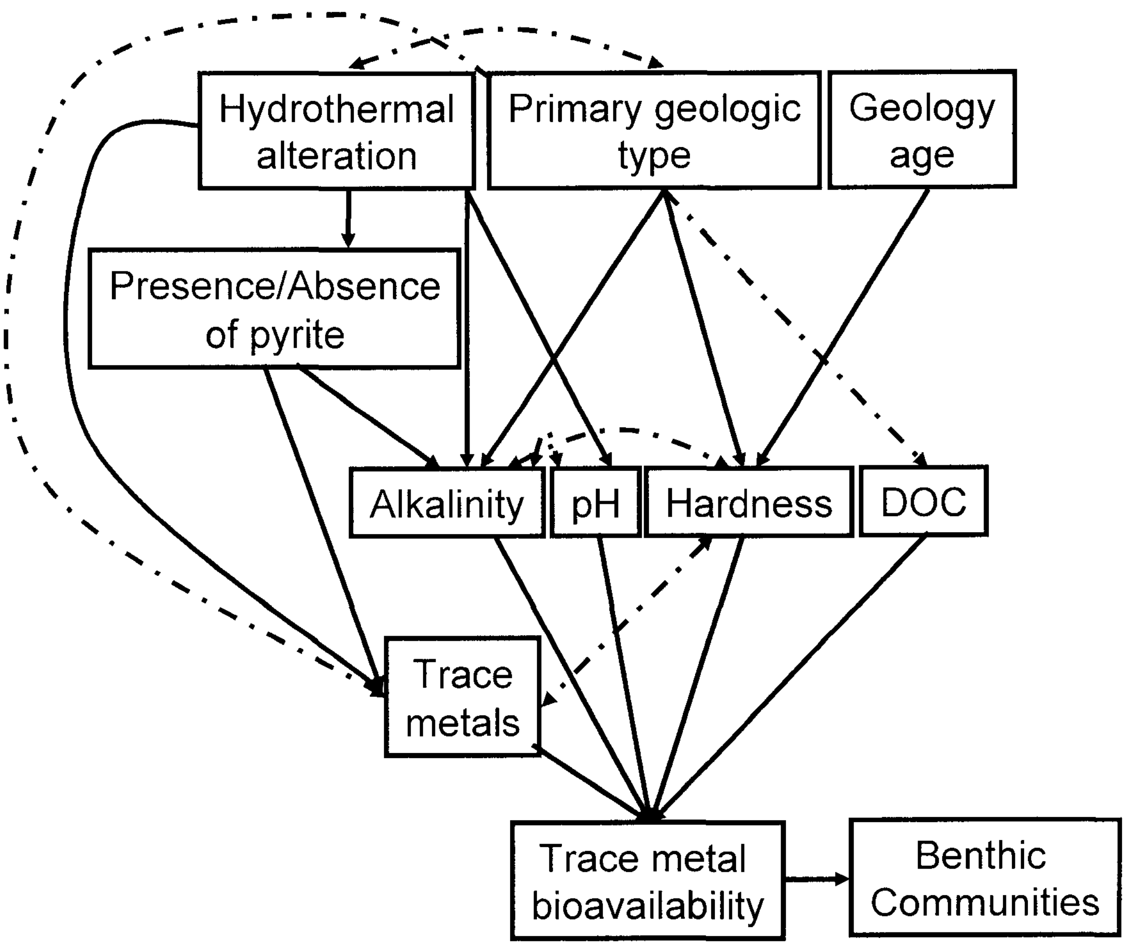

Figure 10. Conceptual diagram of the hierarchical interactions between geology and aqueous geochemical parameters that control trace-metal bioavailability derived from conclusions found in the present study. Solid one-way arrows depict direct effects between parameters. Arrow head touches subordinate parameter in hierarchy. Double-headed arrows are co-variances found between parameters. Dash-dotted arrows are interactions which are known but not evaluated in this investigation or were found to be marginal in effect.

CHAPTER 2- A FIELD TEST OF THE BIOTIC LIGAND MODEL: LINKING BIOACCUMULATION OF TRACE-METALS IN AQUATIC INSECTS TO POPULATION-LEVEL EFFECTS

ABSTRACT

We tested the hypothesis that accumulation of trace-metals in individual benthic macroinvertebrates collected from the field, results in deleterious effects that propagate to a population-level response. Accumulation, presence/absence and density responses of three taxa (i.e., ephemeropterans *Rhithrogena spp.* and *Drunella* spp. and the trichopteran *Arctopsyche grandis*) were also evaluated to determine if the unique information provided by these endpoints could elucidate how these populations respond to trace-metal contamination. We utilized a new measure of trace-metal toxicity derived from the Biotic Ligand Model (Chronic Criteria Accumulation Ratio, CCAR) to predict native taxa responses to trace-metal contamination. CCAR was significantly correlated to taxa body burden (R^2 ranging 0.47 -0.98) suggesting the model is capable of predicting that fraction of trace-metal available for accumulation. We also developed models descriptive of taxa presence/absence as a function of whole body tissue concentrations and estimated the probable decline in occurrence at increasing trace-metal concentrations.

In general, we found the caddisfly *A. grandis* is more sensitive to trace-metal contamination than previously reported while the mayfly *Rhithrogena* spp. could survive exposure to trace-metals at 270 times ambient water quality criteria. However, the population density of *Rhithrogena* spp. was reduced by 77% between background concentrations (CCAR = 0.01) of trace-metal and the

theoretical threshold at which trace-metal mixtures cause toxicity (CCAR = 1). This disparity in the data suggests that native populations are comprised of subpopulations that show differential sensitivity to trace-metals, a violation of one of the assumptions of the Biotic Ligand Model. These results suggest that water quality criteria based on the Biotic Ligand Model are protective of some native taxa (e.g., *Drunella* spp.), but not others (e.g., *A. grandis* or *Rhithrogena* spp.) and may need refinement to protect 95% of the native taxa found at any site.

INTRODUCTION

Exposure to elevated concentrations of trace-metals adversely affects aquatic communities (Hare 1992, Clements et al. 2000, Soucek et al. 2002). Differential sensitivities of individuals to trace-metals results in population-level effects that culminate in an assemblage shift from a sensitive to a metal-tolerant community (Carlisle & Clements 1999, Clements et al. 2000). Because we have little understanding about taxa specific responses to trace-metal contamination (Clements et al. 1992), we know little about the mechanisms by which population and community-level responses are manifested (Clements 1997).

The link between exposure to trace-metals and effects on individuals, populations, or communities is bioaccumulation (Hare 2003). Taxa that are sensitive to trace-metals accumulate these contaminants into tissues and specific cellular components that experience reduced function (Hare 1992, Cain et al. 2004). Accumulated metals impair an individual's fitness by inhibiting metabolic processes (i.e., detoxification, osmoregulation) or physiological performance (i.e., swimming speed) (Hare 1992 et al., Galvez et al. 1998, Alsop et al. 1999). However, tolerant organisms are thought to accumulate high levels of trace-metals by means of metabolic and cellular components that bind and render trace-metals biologically inactive (Hare 1992, Cain et al. 2004). Tolerant or not, the accumulation of trace-metals is thought to cause deleterious effects in individuals. If enough individuals are adversely affected by trace-metal accumulation, the cumulative effect will cause a population-level response.

Population densities of heptageniid and ephemerellid mayflies (*Rhithrogena* spp. and *Drunella* spp.) are sensitive indicators of trace-metal pollution in both field evaluations and in experiments (Kiffney & Clements 1993, Clements & Kiffney 1995, Besser et al. 2001, Carlisle & Clements 2003, Clark & Clements 2006). Fewer individual of these taxa are found in metal contaminated streams with moderate levels of trace-metals (i.e., 1-2 times hardness adjusted water quality criterion) relative to reference or control streams.

Some hydropsychid caddisflies (e.g., *Arctopsyche grandis*) are thought to be tolerant of trace-metal contamination (Kiffney & Clements 1993, Prusha & Clements 2004, Cain et al. 2004). *A. grandis* is often collected to determine body burdens for the purposes of validating exposure and bioavailability of trace-metals, but rarely is the number of individuals/ m^2 of this species used as an indicator of trace-metal contamination (Kiffney & Clements 1993, Cain et al. 2004, Prusha & Clements 2004). Population density (# of individuals/ m^2) however, is a generic endpoint that does not describe how subpopulations (i.e., sexes, cohorts, life stages) may differ in sensitivity to trace-metal exposure (Kiffney & Clements 1996, Clark & Clements 2006).

Researchers have postulated that the presence or absence of a species found along a chemical gradient is descriptive of the sensitivity of taxa to trace-metal contamination (Birge et al. 2000, Cain et al. 2004, Buchwalter & Luoma 2005). Presence of a species in moderately to highly contaminated waters would suggest individuals of that species are tolerant of those conditions. Absence would suggest the species is sensitive to trace-metals and has suffered deleterious effects, either lethal or sub-lethal (e.g., avoidance via drift).

In the present study, we evaluated how trace-metal accumulation is related to the presence or absence of benthic macroinvertebrate taxa along a gradient of trace-metals and compared these data to population densities (number of individuals/ m^2) for these taxa to infer how deleterious effects on an individual culminate in higher-order ecological effects (Clements 1997). Implicit in the assumptions of past investigations, these three responses were assumed to be causally linked, but were assessed separately. We propose that by using these three endpoints in a single evaluation, emergent information may be obtained to provide a better understanding of the mechanisms by which deleterious effects in an individual are manifested into a population-level response. We will explicitly test the hypothesis that accumulated trace-metals in an individual are linked to population-level responses.

The Biotic Ligand Model (BLM) is an algorithm that predicts acute toxicity of dissolved free ions of trace-metals to aquatic organisms (e.g., algae, cladocerans, fish, and macroinvertebrates) (Di Toro et al. 2001, Santore et al. 2001). Coupled geochemical speciation models (CHESS and WHAM V) are used to quantitatively account for the influence of dissolved organic carbon (DOC) and major cations and anions on trace-metal bioavailability (Tipping 1994, Santore & Driscoll 1995). Empirical data demonstrate that a constant amount of toxicity results from a specified accumulation (i.e., LA_{50}) of a metal on an organism's respiratory surface, which is termed the biotic ligand (Mac Rae et al., 1999; Meyer et al., 1999, 2002). Thus, the BLM calculates the fraction of the trace-metal in water that is available to accumulate on the biotic ligand, and predicts the response of a standard test organism (e.g., the LC_{50}, the amount of metal accumulation that results in 50% mortality). Because the BLM can predict the LC_{50} of trace-metals usually within a factor of 2, the U.S. EPA is adopting the model to set site-specific water quality criteria (99 FR 28314 10/28/1999, U.S. EPA 2003).

The BLM (HydroQual 2003) was designed to predict acute toxicity (i.e., 48-hr LC_{50}) to standard test fishes (e.g., fathead minnows and rainbow trout) and was empirically calibrated to predict toxicity to aquatic invertebrates (i.e., daphnids) (Santore et al. 2001, Niyogi et al. 2004). This assumption has merit as studies linked uptake of trace-metals to the density of regulatory cells (i.e., chloride cells) on the gill surface of macroinvertebrates and found damage to these organs that resulted from exposure (Vuori KM 1996, Buchwalter & Luoma 2005). However, the BLM ignores the fact that some taxa can regulate accumulated trace-metals (Hare 1992, Cain et al. 2006). In addition, the BLM assumes that each individual of a population is equally sensitive to trace-metals. If the BLM is to be used to set water quality criteria protective of 95% of the species at any given site, it will need to protect populations of native taxa in such a way that they can maintain sustainable population densities.

We have developed a method that utilizes the BLM (HydroQual 2003) for the purpose of ecological assessment of trace-metal pollution in mountainous streams. The method uses the BLM output to determine trace-metal toxicity under the assumption of additive toxicity similar to the toxic unit approach (Sprague 1970, Playle 2004). The new model (i.e., Chronic Criterion Accumulation Ration, CCAR) incorporates current theory about the interactions between aqueous constituents (i.e., hardness, DOC, pH) that affect trace-metal toxicity and accumulation of bioavailable trace-metals at the respiratory surface of aquatic organisms. Also, CCAR provides a better description of benthic macroinvertebrate community responses to trace-metal contamination than other toxic unit approaches (Chapter 3).

In the present study we evaluated the accumulation and population responses of ephemeropterans *Rhithrogena* spp. (Heptageniidae) and *Drunella* spp. (Ephemerellidae) and the trichopteran *A. grandis* (Hydropsychidae) to trace-metal exposure. All three taxa are thought to be cosmopolitan species in undisturbed Colorado mountainous streams and therefore, maybe good candidates as indicators of trace-metal pollution (Ward et al. 2002). Specifically, we determined if the accumulation of trace-metals in native taxa can be predicted by the BLM and linked to population responses. This allowed us to evaluate fundamental assumptions of the BLM and test the premise that the model sets site specific water quality criteria protective of native taxa. Through this approach, we also quantitatively evaluated the sensitivity of individual taxa and populations to trace-metal exposure and tested the idea that trace-metal accumulation reduces fitness of individuals and propagates into a population-level response.

METHODS

Study Area and Sampling Strategy

The study area is the mineral belt and surrounding mountains of the central Colorado Rocky Mountains (Figure 2). Specifically, the study area includes all of Arapahoe, Roosevelt, Pike, and San Isabel National Forests, portions of the White River and Rio Grande National Forests and other public lands that are included within the area of these Forests (e.g., State, U.S. BLM, and U.S. NWR lands). Rocky Mountain National Park and Great Sand Dunes National Park also lie within the study-area boundaries.

Geographic Information Systems (ArcGIS 8.3, ArcHydro 1.2) were used to delineate catchments for sampling (ESRI 1999, Maidment 2002). Digital elevation models (30 x 30 m) were used to define catchment boundaries and areas. We then used geologic data at the 1:250,000 scale to classify the presence of different primary rock types and geologic ages. New remote sensing AVIRIS (http://aviris.jpl.nasa.gov/html/aviris.task.html) and ASTER (http://aviris.jpl.nasa.gov/html/aviris.task.html) data descriptive of hydrothermally altered rock and the presence of pyrite mineralization were used to classify how intensely rock types were alteration and if pyrite was present in the primary rock. The resolution of these data are 17m^2 for resolution AVIRIS and 30 m^2 resolution for ASTER. All GIS data are available from USGS Geologic Division, Denver, Colorado. Small catchments (1st-3rd order) predominantly underlain by a single rock type were sampled and categorized based on mineral deposit criteria and measured cation and anion concentrations to evaluate water quality (e.g., Miller

1999). The purpose of this sampling strategy was to target a large variety of water qualities resultant from parent geology to test the BLM

Sample Collection

Geochemical and benthic macroinvertebrate community samples were collected from 114 catchments during base flow conditions in the summers of 2003 (n = 21), 2004 (n = 49), and 2005 (n = 44) (Figure 1). At each of these stream locations, we collected up to 9 individuals each of *Rhithrogena* spp. (*R. hageni* and *R. robusta*), *Drunella* spp. (*D. doddsii* and *D. coloradensis*) and/or *Arctopsyche grandis.*

Physicochemical Parameters

Water samples were collected using methods described in Wilde et al. (1998) to meet the requirements of the BLM (HydroQual 2003). Routine water quality parameters (temperature, conductivity, and pH) were measured in the field using a Horiba© (USA) D-24 combination meter following methods described by Wilde et al. (1998). The probes were calibrated at the beginning of each day with certified standards, and checked periodically throughout the day. All water samples processed in the laboratory were filtered through a Acrodisc® Premium 25 mm Syringe Filter with 0.45 um Nylon Membrane at the site and stored at 4°C until analyzed. Water samples for dissolved organic carbon (DOC) were filtered through a 0.70-µm glass-fiber filter, acidified with concentrated hydrochloric acid

(12 molar) to a pH of < 2, and stored in baked amber glass bottles. A Shimadzu© TOC-5000A total organic carbon analyzer was used to measure DOC. Dissolved trace-metal samples were acidified with concentrated nitric acid (16 molar) to a pH < 2 and stored in polyethylene bottles. Samples for anions were collected and stored in polyethylene bottles. Water analyses were conducted at the analytical laboratories of the USGS Geologic Discipline Laboratory in Denver, Colorado. Concentrations of major cations (Na^+, K^-, Mg^{2+}, and Ca^{2+}) were analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Briggs 2002), whereas trace-metal concentrations (Cd^{2+}, Cu^{2+}, Zn^{2+}) were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Lamothe et al. 2002). Major anions (Cl^-, F^-, NO_3^-, SO_4^{2-}) were measured by ion chromatography (Theodorakos et al. 2002). High concentrations of SO_4^{2-} (>25 mg/L) were determined by inductively coupled plasma-atomic emission spectrometry and alkalinity was determined by titration (Theodorakos 2002).

Benthic Invertebrate Population and Community Samples

Five replicate benthic samples (*n* = 5) were collected using a 0.1-m^2 Hess sampler (350-µm mesh net) from shallow riffle areas (<0.5 m). Representative sample locations were selected based on the following criteria: the location was a riffle or run habitat unit, depth was 0.10-0.25 (m) and substrate was representative of the reach. Overlying substrate was scrubbed of all algae and diatoms, and inorganic debris was removed. Underlying substrate was disturbed to a depth of approximately 10 cm and the remaining material was sieved using a

350-μm mesh sieve. All organisms retained were preserved in 80% ethanol in the field.

In the laboratory, samples were processed to remove debris, and sub-sampled until 300 organisms (±10%) were removed from the sample following methods described by Moulton et al. (2000). Invertebrates were identified to the lowest practical taxonomic level (genus or species for most taxa, subfamily for chironomids) (Merritt & Cummings 1996, Ward et al. 2002). Means of the five replicate benthic samples were used to calculate abundance of the three species used in this study.

Body Burden Samples

Drunella spp., *Rhithrogena* spp., and *A. grandis* (n = 1-9) were collected from each stream from which they could be found for whole body trace-metal tissue analysis (hereafter referred to as body burden). Large individuals were collected to ensure enough tissue was collected to limit analytical error. We assumed that trace-metal concentrations in these individuals were at steady-state. We also assumed that whole body concentrations are correlated with the concentration of trace-metal at the biotic ligand. This assumption was necessary because it was not plausible to dissect individual taxa to isolate the surfaces where the sites of toxic action (i.e., biotic ligand) are hypothesized to be located (e.g. gut and gill surfaces).

Each individual organism was placed into a 35 mL test tube filled with site water and kept at 4 °C for 24 – 48 hours to allow for depuration. Depuration was necessary to remove the gut contents which may contain metals that are not accumulated into the tissues of the organism (Hare 1992). Once depurated, individuals were removed and placed into a 15 mL test tube filled with 5 mL of 0.01 M EDTA (ethylenediaminetetraacetic acid) titrant for 30 seconds to remove any externally bound trace-metal (Hare 1992). Individuals were then placed into 15 mL polypropylene tubes and frozen until digestion. All test tubes were twice washed in a 10% nitric acid bath and rinsed clean with deionized water prior to these procedures.

Individuals were digested using methods detailed in Prusha (2002). Organisms were dried at 60 °C for 72 hours, and then placed in a desiccator until samples returned to room temperature. Individuals were weighed to the nearest tenth of a milligram using a Satorius® BP 110S balance. Concentrated nitric acid (16 M) was added to each tube for 24 hours and then heated to 60 °C for 4 hours in a water bath. Test tubes were cooled to room temperature and distilled hydrogen peroxide was added to each sample. Samples were again heated to 60 °C for 4 hours in a water bath, allowed to cool to room temperature, and diluted with double distilled nitric acid to a final volume of 2 mL. Chemical analysis followed the same methods and used the same analytical equipment as described in the physicochemical analysis above. Mean ± standard error (S.E.) whole body tissue concentrations ($\sum Cd^{2+} + Cu^{2+} + Zn^{2+}$) were developed when multiple individuals of the same taxa were collected from the same stream.

Development of the Chronic Criterion Accumulation Ratio

Because the BLM predicts toxicity due to individual trace-metals and most streams contain a mixture of metals at low concentrations which may cause sub-lethal or chronic effects, the BLM was modified to account for metal mixtures. The Chronic Criteria Accumulation Ratio (CCAR) was developed as a toxic unit model and assumes additive toxicity of trace-metal mixtures based on BLM-predicted endpoints (Sprague 1970). Research investigating the toxicity of trace-metal mixtures has shown additive effects of metal mixtures at chronic concentrations (Newman & McCloskey 1996, Norwood et al. 2003, Playle 2004).

The CCAR metric is defined as the ratio of the BLM-calculated trace-metal accumulated on the biotic ligand to that accumulated on the biotic ligand in water at U.S. EPA criterion value, summed for all metals at a site. More specifically, CCAR is calculated as follows:

$$\Sigma CCAR = \sum_i \frac{BLM\ calculated\ site\ specific\ [gill\ metal]}{BLM\ calculated\ [gill\ metal]\ at\ CCC}$$

where *BLM calculated site specific [gill metal]* and *BLM calculated [gill metal] at* CCC are metrics developed from BLM endpoints. *BLM calculated [gill metal]* is the BLM predicted accumulation of the i^{th} trace-metal on the biotic ligand (gill surface), calculated by running the BLM in speciation mode using site-specific water quality parameters. *BLM calculated [gill metal] at* CCC is the BLM predicted accumulation of the i^{th} trace-metal on the gill surface, calculated using the "normalization chemistry" water quality parameters from Table 1a, U.S. EPA (2003), and the i^{th} metal Continuous Chronic Criterion (CCC) concentration (U.S.

EPA 2003). The CCC is developed by averaging toxicity test data across species and genera to determine a concentration of trace-metal that will be protective of 95% of the species at a specific site. We are using the CCAR to predict toxicity to benthic communities, individual species of which have differential sensitivity to exposure to a trace-metal. By using the CCC instead of the response of a surrogate species (e.g., the LA_{50} for a daphnid or fathead minnow), we can predict toxicity more generally to the community and account for the modifying effects of several water quality parameters known to determine trace-metal uptake and bioavailability (e.g., pH, DOC, alkalinity, and major cations).

Statistical Analysis

Simple linear regression models were developed (SPSS® version 14 for Windows®), to determine if the Biotic Ligand Model derived CCAR could be used to predict whole body tissue concentrations of trace-metals in *Drunella* spp., *Rhithrogena* spp., and *A. grandis* (SPSS 2004). No differences were observed in body burdens between *D. doddsii or D. coloradoensis* or between *R. hageni or R. robusta* so these taxa were lumped at the genus level. CCAR was regressed against the average total metal concentration (i.e., $\sum Cd^{2+} + Cu^{2+} + Zn^{2+}$) for each species. These regression models were then used to extrapolate body burdens to sites where *Drunella* spp., *Rhithrogena* spp., and/or *A. grandis* were not collected.

The presence/absence of a taxa found along a chemical gradient may be descriptive of that organism's sensitivity to the chemical stressor (Birge et al. 2000, Cain et al. 2004, Buckwalter et al. 2005). Here we test the hypothesis that accumulated metal results in deleterious effects (e.g., mortality, loss in fitness or ability to propagate as normal) to the individual which culminates in a population response (i.e., decreased probability of detection). To link the accumulated metal in an individual to a decline in the probability of detection at a field site, we related taxa presence/absence as a function of body burden through logistic regression using SPSS® software (Hosmer & Lemeshow 1989, SPSS 2004). First we transformed density estimates of *Drunella* spp., *Rhithrogena* spp., and *A. grandis* into presence/absence data (e.g., 0 = absence, 1 = presence) and subjected these data to logistic regression analysis to determine the probability of occurrence for each taxa, given the predicted body burden.

To determine if the BLM developed criteria are protective of native taxa, we plotted the probability of taxa presence verses CCAR and determined the percent change in occurrence at CCAR = 1, the threshold where chronic toxicity is hypothesized to occur. We calculated the percent change in probable occurrence as: ((maximum probable occurrence – probable occurrence at CCAR)/ maximum probable occurrence)*100. This approach makes sound toxicological sense. Trace-metals in the water column are known to accumulate in/on tissues of aquatic organisms and cause deleterious effects which are detectable both in individuals and populations. The result is a relationship depicting the change in the probability of detection for a given taxa as a function of accumulated metal from the water column and expressed in toxic units.

To estimate the range of trace-metals where negative effects on macroinvertebrate populations were detectable, sites were categorized based on measured CCAR toxic unit values and compared using one-way analysis of variance (ANOVA) (Sokal & Rohlf 2003). A post-hoc Dunnett's test was used to compare mean densities between categories (Sokal & Rohlf 2003). Category intervals were selected based on previous work (Clements et al. 2000, Griffith et al. 2004, Clark & Clements 2006,) that predicted adverse effects in benthic macroinvertebrate communities at 1, 10, and 100 Cumulative Chronic Units (CCU) (equivalent to CCAR). Because the present study sampled streams with low levels of trace-metal contamination we were interested to determine if effects could be detected at these lower metal concentrations. Sites were grouped by order of magnitude differences in measured CCAR values (i.e., 0.01 - 0.1, 0.1 - 1, 1 - 10, 10 - 100, > 100) with the lowest toxic units category (0.01 – 0.1) representing background conditions. Densities were log transformed prior to statistical tests and were found to approximate normality in Q-Q plots, but variances were heterogeneous. Use of the Dunnett's multiple range test does not assume homogenous variances.

RESULTS

Physicochemical characteristics

Chronic Criteria Accumulation Ratio (CCAR) values for the $\sum(Cd^{2+} + Cu^{2+} + Zn^{2+})$ averaged 18.19 ± 59.37 (mean ± S.D.) and ranged from 0.02 to 270 CCAR units (Table 1). Trace-metal concentrations (µg/L) were dominated by Zn^{2+} (75.91 ± 250.16) followed by Cu^{2+} (24.74 ± 116.65) and Cd^{2+} (0.30 ± 1.0). The average hardness (47.97 ± 47.11 mg/L), pH (6.88 ± 0.97 S.U.), alkalinity (29.07 ± 30.56, mg/L $CaCO_3$) and DOC (1.73 ± 1.21, mg/L) were typical of oligotrophic mountain streams.

Table 2 depicts the mean ± S.D. for physicochemical parameters for each group evaluated in the ANOVA analysis. These data demonstrate that while trace-metal concentrations increased across categories, the other water quality parameters showed relatively little variation. Notable exceptions were mean alkalinity and pH, which were significantly reduced in the highest metal category compared to the reference streams. In contrast to these results, mean DOC was lower and SO_4^{2-} was higher in contaminated streams compared to the reference streams. NO_3^- concentrations were low and had little variance across categories, suggesting these water qualities were not affected by development other than mining.

Estimation of taxa specific body burden

On average *Rhithrogena* spp. (1055 ± 1267 mean ± standard error; 29 individuals from n = 9 streams) accumulated similar amounts of trace-metals as *Drunella* spp. (824 ± 1638, 276 individuals from n = 37 streams), while both species accumulated more metals than *A. grandis* (186 ± 18.56, 230 individuals from n = 31 streams) (Figure 3). Simple linear regression analyses of CCAR verses body burden of $\sum(Cd^{2+} + Cu^{2+} + Zn^{2+})$ for *Rhithogena* spp. ($R^2 = 0.96$), *Drunella* spp. ($R^2 = 0.53$) and *A. grandis* ($R^2 = 0.47$) were highly significant ($p < 0.0001$) (Figure 3). The slopes of these relationships also suggested that *Drunella* spp. (slope = 1641) and *Rhithrogena* spp. (slope = 1572) accumulated more trace-metals than *A. grandis* (slope = 73.80). These regressions were used to extrapolate body burden values to streams where organisms were not collected.

Probability of detection

Logistic regressions were used to convert presence/absence data into the probability of occurrence as a function of body burden based on the relationships established above (Figure 4). Model results for *Rhithrogena* spp. estimated probabilities ranging from 0.45-0.96 while those for *Drunella* spp. estimated probabilities ranging 0.01-0.99. The logistic regression model results for *Arctopsyche grandis* estimated probabilities ranging 0.11-0.81. From these relationships, point estimates were developed to determine the change in probable detection of each taxa between the highest probability of detection and that at the threshold of chronic toxicity, CCAR = 1, for each taxa. Probabilities of detection at CCAR = 1.0 for *Rhithrogena* spp., *Drunella* spp. and *Arctopsyche grandis* were 0.85, 0.95, and 0.50, respectively. Thus, the percent change in the probability of detections were *Arctopsyche grandis* (40% change), followed by *Rhithrogena* spp. (13%) and *Drunella* spp. (4%).

Estimation of population effects

Results of the one-way ANOVA and Dunnett's multiple range test found significant differences in abundance of *Rhithrogena* spp. and *Drunella* spp. among CCAR categories (Figure 5). *Arctopsyche grandis* densities were reduced with increasing trace-metal toxic units, but these differences among CCAR categories were not statistically significant (p = 0.051) due to low average abundances relative to variances. For *Rhithrogena* spp., a large reduction in the population density was observed between background (CCAR = 0.01) and CCAR = 0.1; however, statistically significant differences based on Dunnett's test were not detected until CCAR >100. Although the density of *Drunella* spp. did not show a distinct concentration-response relationship due to high variation among categories, the general trend was a decline in density with a significant reduction at the most contaminated sites.

DISCUSSION

Very little is known about how native taxa in stream ecosystems respond to trace-metal contamination (Clements et al. 1992). However, this knowledge is imperative to understanding the mechanisms that propagate individual responses through populations and communities (Clements 1997). Here we investigated how metal accumulation in individuals, presence/absence data, and population densities of three taxa provided unique information regarding how these populations responded to trace-metal contamination. We tested the hypothesis that accumulation is the link between deleterious effects in individuals and population-level effects. Further, we utilized a toxic unit model (CCAR) to: 1) characterize the trace-metal stressor gradient; 2) evaluate if the BLM was capable of predicting individual and population-level responses to trace-metal exposure and; 3) determine if water quality criteria are protective of these taxa.

Substantially more trace-metals were accumulated in two mayflies (*Rhithrogena* spp. and *Drunella* spp.) known to be sensitive to trace-metal contamination compared to a metal-tolerant caddisfly (*A. grandis*) (Figures 2-3). Trace-metal accumulation was significantly correlated with CCAR (R^2 range= 0.47- 0.96) which estimates the amount of trace-metal bound to the biotic ligand (Figure 3). The strong agreement between CCAR and *Rhithrogena* spp. body burden of trace-metal suggests the BLM is capable of estimating that fraction of trace-metal which *Rhithrogena* spp. accumulates and that *Rhithrogena* spp. lacks mechanisms to actively excrete accumulated trace-metal. Although CCAR did successfully predicted accumulation in *Drunnella* spp. and *A. grandis*, model agreement was modest, suggesting other mechanisms may be important in determining accumulation (i.e., accumulation modeled differentially from the BLM or biological regulation including excretion) (Hare 1992, Cain et al. 2006). These results indicate the BLM can very accurately estimate trace-metal accumulation in taxa which do not regulate accumulated trace-metals, but will need refinement to better predict accumulation in those taxa which do regulate internalized metals.

Having demonstrated that CCAR is capable of estimating the trace-metals accumulated in native taxa, we asked the question, do higher levels of accumulated trace-metal cause deleterious effects in individuals (i.e., mortality of individuals) such that the probability of occurrence in streams would decrease with increasing bioavailable water column metal concentrations (Figure 4). Surprisingly, *Rhithogena* spp., a mayfly previously described as sensitive to trace-metal pollution based on population-level responses in both field observations and laboratory experiments (Kiffney et al. 1993, Clements & Kiffney 1995, Besser et al. 2001, Carlisle & Clements 2003, Clark & Clements 2006), tolerated trace-metal concentrations 270 times water quality criteria (as measured by CCAR) and still maintained a probability of occurrence as high as 0.45 (Figure 4). *A. grandis*, a species considered relatively tolerant to trace-metal pollution (Cain et al. 2004) was more sensitive as compared to *Rhithrogena* spp., with an 81% decline in probable occurrence. *Drunella* spp.

was the most sensitive taxa, experiencing the greatest decline in overall probable occurrence (99%).

We evaluated if the BLM provided water quality criteria that were protective of these three taxa. At a CCAR = 1 (i.e., the threshold of chronic toxicity) the reductions of probable occurrences were 40% for *A. grandis*, 13% for *Rhithrogena* spp., and 4% for *Drunella* spp. More importantly, there appears to be a threshold response for *Drunella* spp. between CCAR = 1 and 10 where the probability of occurrence precipitously falls. When considering the density responses to these three taxa we found a substantial reduction in *Rhithrogena* spp. and *A. grandis* densities (individuals/m^2) below the threshold of chronic toxicity (CCAR = 1) (Figure 4). *Drunella* spp., the species found to be most sensitive based on presence/absence data, did not show deleterious effects based on population density until CCAR > 10 (albeit this response was not statistically significant). These results make clear a general disparity exists between taxa presence/absence data and density data.

Metal contamination in streams is generally not sufficiently high to eliminate all individuals within a species at a particular site. Some individuals from metal-sensitive populations may occur at contaminated sites, but at very low population densities. For example, we collected *Rhithrogena* spp. from sites where trace-metal concentrations were 270 times greater than water quality criteria while simultaneously finding a 77% decline in population density at concentrations below criteria (Figure 4). As a consequence, presence/absence data alone are relatively insensitive to metal pollution. In contrast, abundance data integrates a number of subpopulation (e.g., size, sex, cohorts, and instars) responses to trace-metal exposure (Kiffney & Clements 1993, Clark & Clements 2006). We hypothesize that this disparity between the presence/absence data and the density data is likely a result of differential sensitivity among subpopulations.

Variation in sensitivity among individuals within a population may also explain the disparity between standard laboratory toxicity tests using native taxa and field results (Buchwalter et al. in review). As highlighted by our comparison of presence/absence data and density data, differential sensitivities within aquatic invertebrate populations exist. For example, smaller, early instars of *Rhithrogena hageni* are more sensitive to metals than larger individuals (Clark & Clements 2006). It is likely that researchers conducting single species toxicity tests with aquatic insects are collecting larger, more apparent and robust individuals. These larger individuals are more tolerant of trace-metals than the remainder of the population. As a result, these laboratory toxicity tests are biased and probably estimate maximum tolerable metal concentrations, not LC_{50}. However, differential sensitivity to trace-metals among subpopulations of aquatic invertebrate may be the exception, not the rule.

The presence/absence and density data for *Arctopsyche grandis* and *Drunella* spp. *(D. doddsii and D. coloradensis)* appear more aligned. *Arctopsyche grandis* experienced a 40% reduction in probability of occurrence and a 60% reduction in density at CCAR = 1 while *Drunella* spp. experienced a 4% reduction in probability of occurrence and a 14% decrease in density. These data suggest that effects of trace-metals are more consistent among subpopulations of *Arctopsyche grandis* and *Drunella* spp.

The incredible tolerance of individual (probably large) *Rhithrogena* spp. could also be a result of acclimation or adaptation resulting from prior metal exposures (Klerks & Levinton 1989, Clements 1999, Cain et al. 2006). Larger individuals of *Rhithrogena* spp. are older and may have experienced exposure to trace-metals before. Such exposures, if sub-lethal, may allow the taxa to up-regulate physiological mechanisms which allow for acclimation to trace-metals and thus, enhanced tolerance (Weis & Weis 1989, Cain et al. 2006). As a result, the simple presence of taxa at a site with very high concentrations of trace-metals may not indicate its sensitivity. The presences of these tolerant individuals suggest that populations can be plastic in their tolerance to trace-metals and capable of persisting in highly toxic environments at much reduced densities. This fact has implications on the future use of species richness metrics when assessing the ecological impacts of trace-metals in streams. The presence of these tolerant members of a population of aquatic insects at all sites, toxic or not, could weaken the ability of richness metrics to differentiate between sites adversely affected by trace-metals and those that are not.

CONCLUSION

This research demonstrated that accumulation, presence/absence, and population density are all metrics that contribute information about how individual taxa respond to trace-metal contamination. We found that trace-metal accumulation in individuals caused deleterious effects in populations by reducing individual fitness such that the probability of occurrence declined. Our results suggest that taxa previously thought to be tolerant of trace-metals (i.e., *A. grandis*) were in fact relatively sensitive. Some individuals of *Rhithrogena* spp., a taxa previously reported to be a sensitive indicator of trace-metal pollution actually survived exposure to mixtures of trace-metals 270 times greater than water quality standards, despite the fact the population decreased significantly at trace-metal concentrations below water quality criteria. This disparity between the information provided by presence/absence data and density data was likely a result of differential sensitivities of subpopulations to trace-metals exposure.

We also applied the accumulation, presence/absence and population density data to assess the ability of the BLM, through the use of CCAR, to predict individual taxa responses to trace-metals. We found the BLM was capable of predicting accumulation of trace-metals in native taxa. By characterizing the trace-metal gradient using CCAR, we calculated the probability of occurrence vs. absence, and density responses to increasing levels of trace-metal contamination. The toxicity of trace-metals was not consistent for all taxa, as subpopulations of *Rhithrogena* spp. appeared to express differential sensitivities to trace-metal exposure. This finding is in contrast to assumptions of the Biotic Ligand Model, which requires that toxicity is constant within a species. Further, we found that some taxa (e.g., *Rhithrogena sp* and *A. grandis*) experienced significant deleterious effects at concentrations below ambient water quality criteria as predicted by the Biotic Ligand Model.

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TABLES

Table 1. Summary of water quality data collected from 114 sites in Colorado, 2003-2005.

Parameters	Average ± S. D.
Alkalinity as $CaCO_3^{2-}$ (mg/L)	29 ± 31
Ca^{2+} (mg/L)	14 ± 13
CCAR	18 ± 59
Cd^{2+} (μg/L)	0.30 ± 1.00
Cl^- (mg/L)	1.14 ± 1.39
Cu^{2+} (μg/L)	24.7 ± 116.7
DOC (mg/L)	1.7 ± 1.2
Hardness	48 ± 47
K^+ (mg/L)	0.74 ± 1.19
Mg^{2+} (mg/L)	3.4 ± 4.4
Na^+ (mg/L)	1.7 ± 1.4
NO_3^- (mg/L)	0.52 ± 1.12
pH (S.U.)	6.9 ± 1.0
SO_4^{2-} (mg/L)	22 ± 38
Temperature (°C)	9 ± 3
Zn^{2+} (μg/L)	76 ± 250

Table 2. Mean ± S.D. of water quality parameters for categories of CCAR associated with the ANOVA analysis.

Categories	0.01-0.10	0.10-1	1-10	10.0-100	>100
Number of sites	29	60	11	5	9
Temperature (°C)	10 ± 3	8 ± 3	9 ± 2	7 ± 3	11 ± 2
pH (S.U.)	7.5 ± 0.6	6.9 ± 0.5	7.1 ± 0.8	6 ± 1.6	4.7 ± 0.6
Alkalinity as $CaCO_3^{2-}$ (mg/L)	512 ± 45	22 ± 17	33 ± 21	12 ± 10	1 ± 2
SO_4^{2-} (mg/L)	12 ± 18	12 ± 36	35 ± 23	40 ± 22	93 ± 39
NO_3^- (mg/L)	0.26 ± 0.37	0.46 ± 0.36	0.61 ± 0.36	0.35 ± 0.29	0.52 ± 0.24
Cl^- (mg/L)	1.20 ± 0.67	1.02 ± 1.00	2.22 ± 3.32	0.20 ± 0.25	1.01 ± 1.45
Ca^{2+} (mg/L)	17 ± 14	10 ± 14	20 ± 5	13 ± 3	20 ± 3
Cd^{2+} (ug/L)	0.01 ± 0.00	0.04 ± 0.09	0.60 ± 0.38	1.29 ± 0.38	2.04 ± 3.00
Cu^{2+} (ug/L)	0.3 ± 0.1	0.7 ± 1.1	1.4 ± 1.8	5.7 ± 5.6	302.7 ± 312.7
K^+ (mg/L)	0.74 ± 0.34	0.79 ± 1.64	0.73 ± 0.20	0.48 ± 0.21	0.61 ± 0.14
Mg^{2+} (mg/L)	5.3 ± 7.4	1.9 ± 2.0	4.9 ± 2.3	2.9 ± 1.7	5.1 ± 2.3
Na^+ (mg/L)	1.9 ± 1.0	1.7 ± 1.6	1.9 ± 1.3	1.5 ± 0.6	1.3 ± 0.3
Zn^{2+} (ug/L)	1.1 ± 0.7	7.4 ± 12.1	177.7 ± 111.3	408.0 ± 128.4	465.8 ± 741.2
Hardness (mg/L)	64 ± 63	33 ± 41	70 ± 19	44 ± 6	71 ± 17
DOC[a] (mg/L)	2.1 ± 1.9	1.8 ± 0.7	1.4 ± 0.8	1.2 ± 0.8	0.6 ± 0.3

[a] Dissolved Organic Carbon

FIGURES

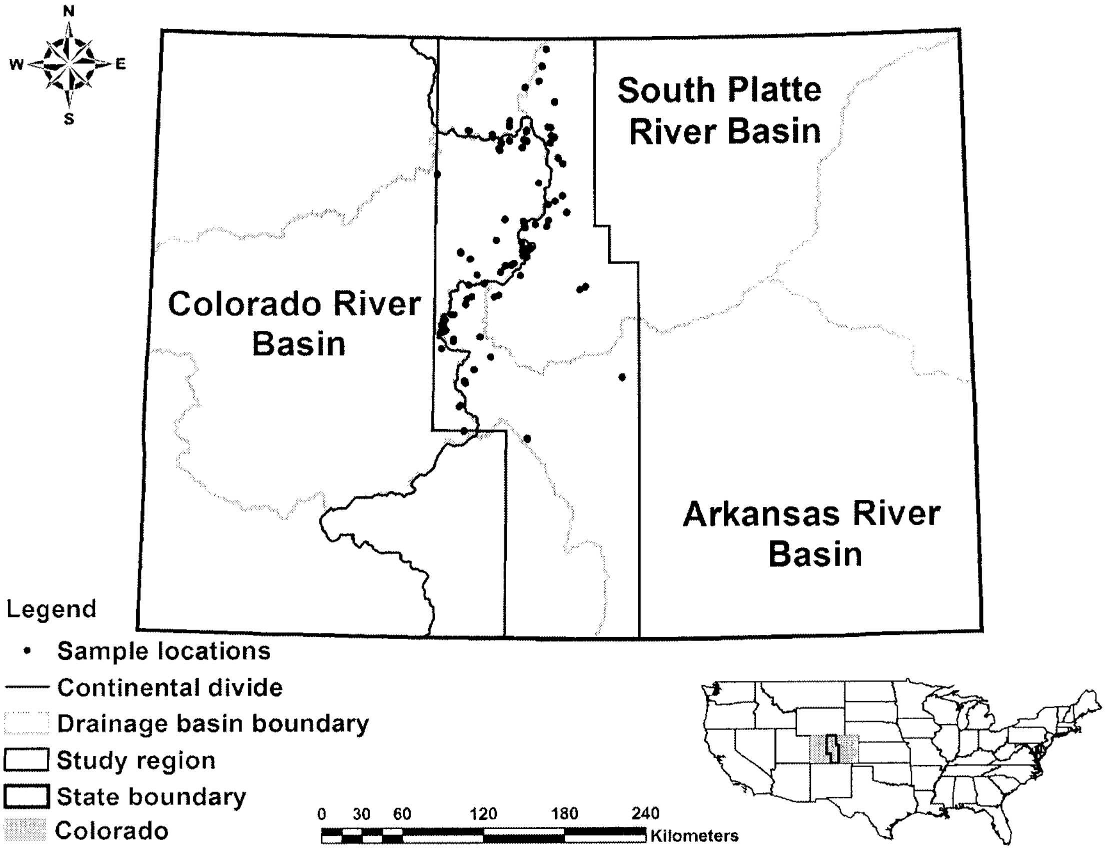

Figure 1. Map of the United States highlighting Colorado and the study domain of this project including the central Colorado Rocky Mountains from New Mexico to Wyoming.

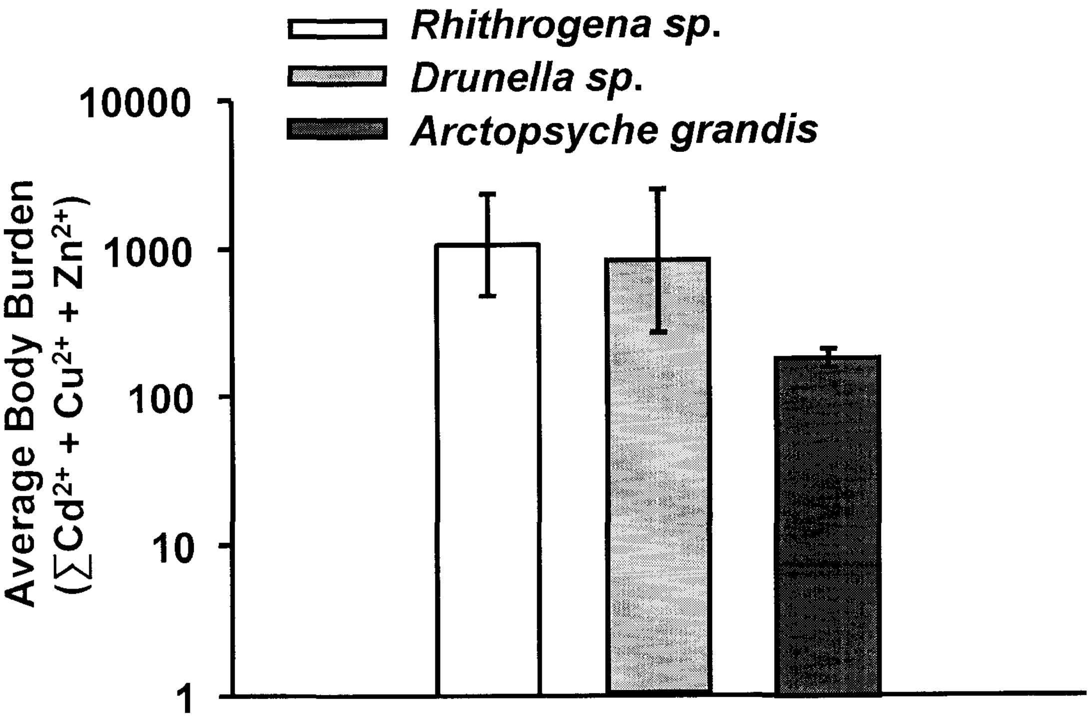

Figure 2. Mean (± S.D.) metal body burden for *Rhithrogena sp.*, *Drunella sp.*, and *Arctopsyche grandis* collected from field sites in Colorado, 2003-2005.

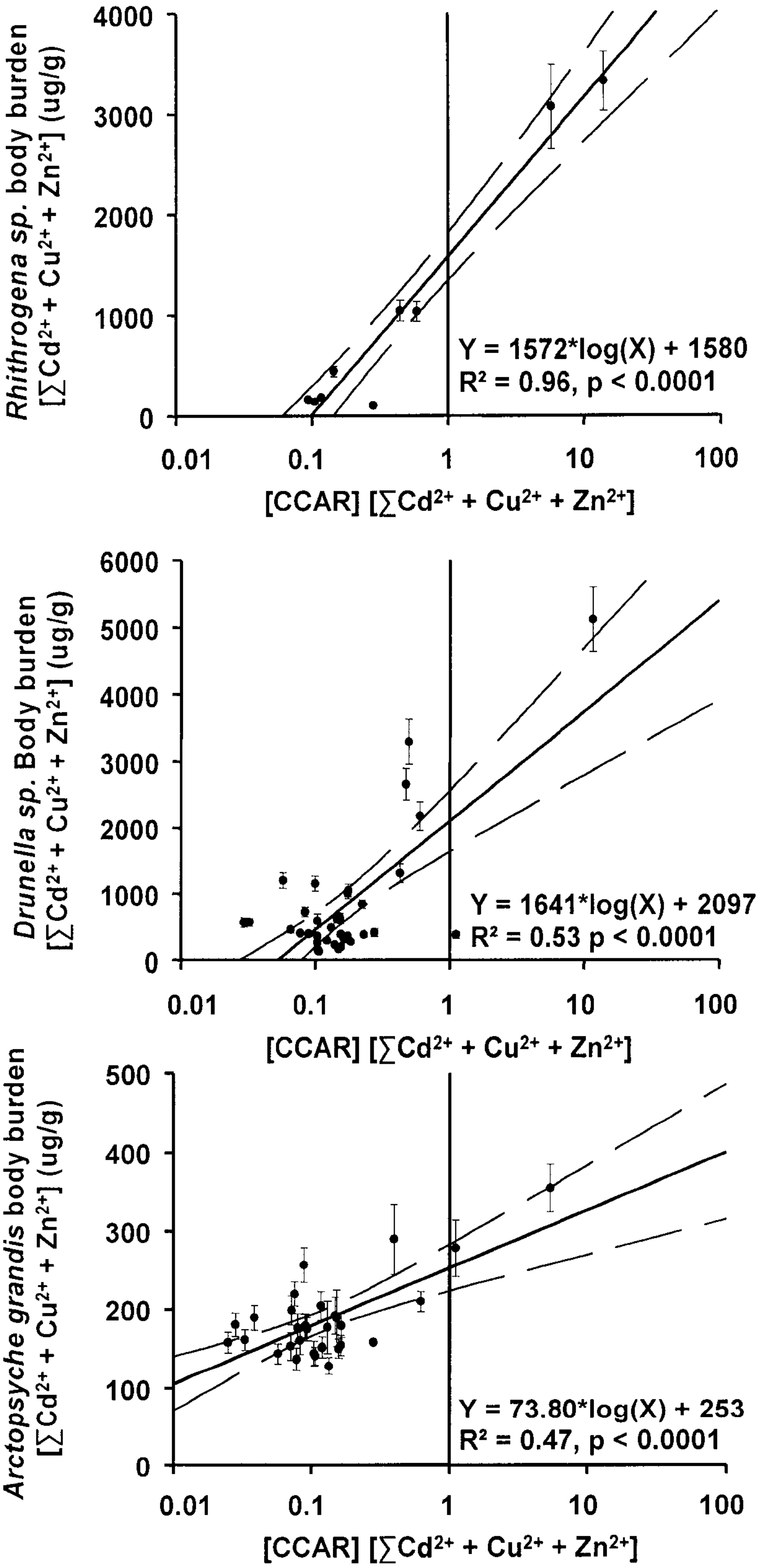

Figure 3. Simple linear regressions depicting the relationship between body burden and $\log_{10}$(CCAR). Each point represents the mean (± S.E.) metal concentration for each stream. The solid vertical line is the hypothesized threshold of chronic toxicity. The figure also shows the 95% confidence interval for the regression relationships

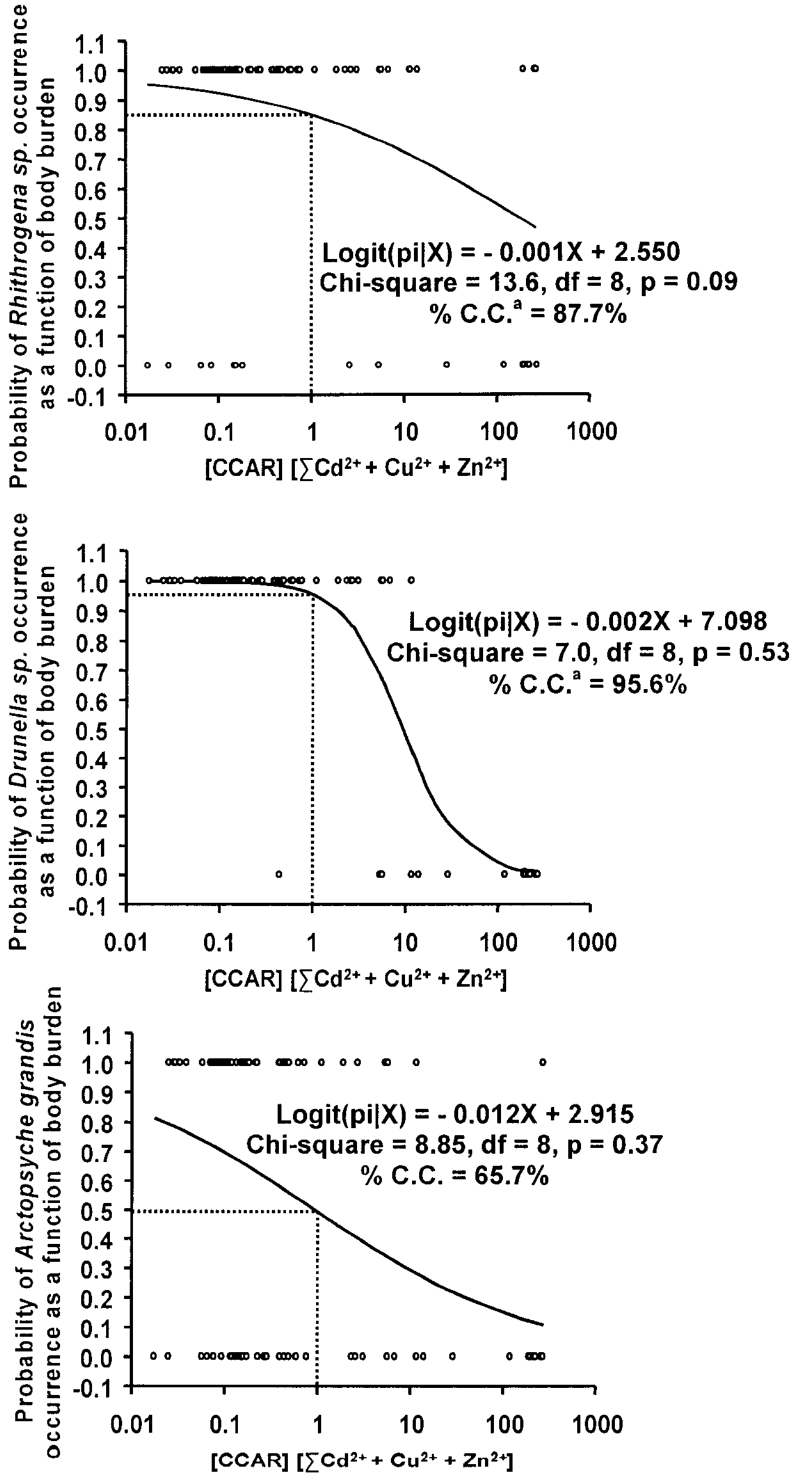

Figure 4. The relationships between the probability of taxa presence and the BLM derived Log_{10}(CCAR) endpoint of trace-metal toxicity. The probability of occurrence was calculated with binary logistic regression using body burden as the covariate. The solid curve shows the estimated probability of detection and the dotted lines represent a point estimate of probability of occurrence at the threshold of chronic toxicity. The open circles show the observed presence (1.0) or absence (0.0) of taxa. The %C.C. is the percent of correct classification based on results of logistic regression. pi – the probability of taxa occurrence. X – the covariate body burden.

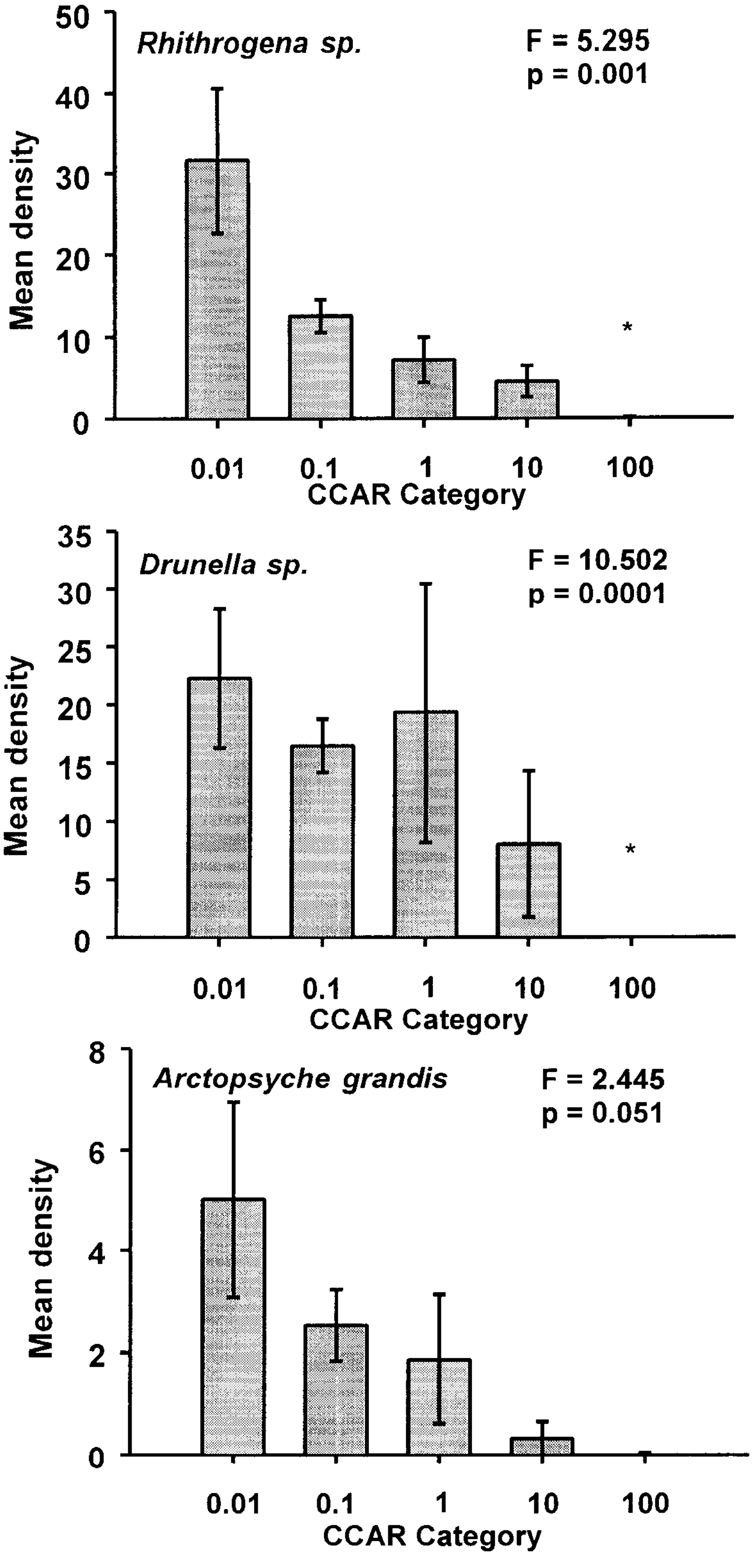

Figure 5. Mean densities (# of individuals/ 0.1 meter^2) of *Rhithrogena sp.*, *Drunella sp.*, and *Acrtopsyche grandis* at BLM derived (CCAR) toxic unit categories where differentiation of adverse effects is thought to occur. Data are means ± S.E. Results of one-way ANOVA for each variable are also shown (df = 4, 108). Asterisk indicates category found to be significantly different from CCAR = 0.01 (background toxic unit values) ($p \leq 0.001$), based on Dunnett's multiple-comparisons test.

CHAPTER 3 - FIELD TEST OF THE BIOTIC LIGAND MODEL IN MONTAIN STREAMS IN COLORADO, USA.

ABSTRACT

Two toxic unit models that estimate toxicity of mixtures of trace-metals to aquatic organisms were compared in a regional-scale assessment of the geochemical and biological impacts of trace-metals on mountain streams from Colorado. We developed the Chronic Criterion Accumulation Ratio (CCAR) derived from the biotic ligand model of trace-metal toxicity. This model accounts for the modifying and competitive influences of major cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, H^{+}), anions (HCO_3^-, CO_3^{2-},SO_4^{2-}, Cl^-, S^-) and dissolved organic carbon (DOC) in determining that fraction of trace-metal that is available for accumulation on the site of toxic action, the biotic ligand. The Cumulative Criterion Unit (CCU) model of trace-metal toxicity only considers the ameliorative properties of Ca^{2+} and Mg^{2+} (hardness) in determining the toxicity of dissolved trace-metals and is based on empirical observations which are not mechanistic in nature. In most cases, CCAR under-predicted toxicity relative to CCU by accounting for that fraction of trace-metal that is bound to DOC (37% average reduction in trace-metal concentration), whereas CCAR over predicted toxicity at 12 sites where pH was $<$ 5.8. CCAR explained more variance in benthic macroinvertebrate community metrics of richness metrics (R^2 ranging 0.32-0.69), abundance (R^2 ranging 0.32-0.72) and composition (R^2 ranging 0.00-0.46) as compared to CCU (R^2 ranging 0.28-0.56 for richness metrics, 0.24-0.57 for abundance metrics, and 0.00-0.40 for compositional metrics). Alterations of benthic communities in this study were found to occur at levels of trace-metal contamination previously thought to be

safe. Specifically, when comparing average benthic macroinvertebrate community metric responses to trace-metal concentrations at median background concentrations (CCAR = 0.07) to the threshold of chronic toxicity based on continuous chronic criteria (CCAR =1) a 27% reduction in richness metrics, 72% decrease in abundance metrics, and a 15% change in composition metrics were observed. Negative effects in a multi metric (Index of Biotic Condition, U.S. EPA 2006), developed to detect the effects of generic ecological disturbances in stream communities, was observed between CCAR category ([CCAR] = 0.01 - 0.1, background concentrations) and [CCAR] category = 0.1 – 1.0. These data suggest the biotic ligand model can be used to predict responses of native communities to trace-metal mixtures in natural waters. Further, alterations in benthic communities were observed below current water quality criteria.

INTRODUCTION

Elevated concentrations of trace-metals in surface waters draining mineralized and mined geologies are common phenomena in Colorado (Clements et al. 2000, Wanty et al. 2002). Exposure to elevated concentrations of trace-metals adversely affects aquatic populations and communities (Hare 1992, Clements et al. 2000, Soucek et al. 2002). Differential sensitivities of individuals to trace-metals result in population-level effects that culminate in an assemblage shift from a sensitive to a metal-tolerant community (Carlisle & Clements 1999, Clements et al. 2000). These properties of benthic macroinvertebrate communities make them a useful tool for evaluating the ecological effects of trace-metal pollution in streams.

Trace-metal uptake by aquatic organisms can occur by association with sediment, directly from the water column, or through dietary exposure (Goodyear & McNeill 1999, Hare et al. 2003). However, total-recoverable metals (i.e., the sum of dissolved, colloidal, and solid metal that can be liberated via extraction with mineral acid) from a water sample are not good predictors of toxicity to aquatic organisms (Morel 1983, Campbell & Stokes 1985, Meyer 2002a, U.S. EPA 2003). Aqueous exposure to the dissolved free ion species is thought to be the primary route of uptake, accumulation and toxicity to aquatic organisms (Campbell & Stokes 1985, Yu & Wang 2002, Hare et al. 2003).

The bioavailability of a dissolved free-ion trace-metal is affected by a suite of constituents found in natural surface waters (Campbell & Stokes 1985, Meyer

2002a). The activities of dissolved free-ionic species of trace-metals are controlled by pH and alkalinity (Meador 1991). Interactions with dissolved organic carbon (DOC) and major anions (e.g., HCO_3^-, CO_3^{2-}, Cl^-) decrease the dissolved free-ion concentrations of trace-metals (Tipping 1994, Santore & Driscoll 1995). Competition with major cations (e.g., Ca^{2+} , Mg^{2+}) for the sites of toxic action decrease the amount of metal bound to those sites, thus ameliorating trace-metal toxicity (Morel 1983, U.S. EPA 1999, Di Toro et al. 2001, Santore et al. 2001). Because each individual trace-metal interacts with these modifying water quality parameters and sites of toxic action differently to form a variety of metal species, there is no universal way to quantify the bioavailability and toxicity of any single trace-metal (Meyer 2002a).

More frequently than not, streams are impaired by a mixture of trace-metals at chronic concentrations that act additively to cause toxicity to aquatic organisms (Sprague 1970, Newman & McCloskey 1996, Norwood et al. 2003, Playle 2004). Cumulative Criterion Units (CCU) is a toxic unit approach to model the additive toxicity of trace-metal mixtures to aquatic organisms (Birge et al. 2000, Clements et al. 2000, Playle 2004). CCUs relate the dissolved concentration of a trace-metal to the Ambient Water Quality Criterion Continuous Chronic Concentration (CCC) for that metal. The criterion for each metal is hardness adjusted to account for the protective effect of Ca^{2+} and Mg^{2+} using an equation derived from empirical laboratory observations (U.S. EPA 1980, 1985, 1996). Incidentally, in these observations it was found that pH and alkalinity co-varied with hardness and, as a result, these hardness adjustment equations indirectly account for the role of pH and alkalinity (pH, alkalinity, and hardness are covariates) on trace-metal toxicity. However, this is not a mechanistic approach to approximating the toxicity of trace-metals to aquatic organisms and may not be appropriate for waters where pH, alkalinity and hardness do not co-vary (U.S. EPA 1980, 1985, 1996). More importantly, these correction factors also do not adjust for the role of other aqueous constituents found in natural waters (e.g., DOC, Cl^-, SO_4^{2-}).

The Biotic Ligand Model (BLM; HydroQual 2003) is an algorithm that predicts acute toxicity of dissolved free ions of trace-metals to aquatic organisms (e.g., algae, cladocerans, fish, and macroinvertebrates; Di Toro et al. 2001, Santore et al. 2001). Coupled geochemical speciation models (CHESS and WHAM V) are used to quantitatively account for the influence of DOC and major cations and anions on trace-metal bioavailability (Tipping, 1994, Santore & Driscoll 1995). Empirical data demonstrate that a constant amount of toxicity results from a specified accumulation of a metal on an organism's respiratory surface, which is called the biotic ligand (Mac Rae et al. 1999, Meyer et al. 1999, 2002b). Thus, the BLM calculates the fraction of the trace-metal in a water sample that is available to accumulate on the biotic ligand, and predicts the response of a standard test organism (e.g., the LA_{50}, the amount of metal accumulation at the ligand site at which 50% of the test organisms die). Because the BLM can predict the LC_{50} (i.e., the concentration at which 50% of the test organisms die) of trace-metals usually within a factor of 2, the U.S. EPA has adopted the model to establish site-specific water quality criteria (99 FR 28314 10/29/1999; U.S. EPA, 2003).

Biological assessments such as Environmental Monitoring Assessment Program (EMAP), Regional Environmental Monitoring Assessment Program (REMAP), Wadable Streams Assessment (WSA) and the present study, Central Colorado Assessment Program (CCAP), are conducted at large spatial scales (e.g., continental U.S., Colorado, the central Colorado Mountains) over which climate, vegetation, and geology change from site to site. Commensurate with these changes in landscape characteristics are changes in the concentrations of major cations, anions, and DOC that alter the bioavailability of contaminants in aquatic ecosystems (Baron et al. 1991, McKnight et al. 1997, Boyer et al. 2000, Wanty et al. 2002 Prusha & Clements 2004). Regional-scale biological assessments of trace-metal contamination would benefit from a model that incorporates site-specific variation in aqueous chemistry and more precisely approximates the bioavailable fraction of trace-metals to aquatic organisms.

The BLM is capable of modeling the bioavailable fraction of dissolved trace-metals in natural waters. However, the BLM was designed to predict acute toxicity (i.e., 48-hr LC_{50}) to standard test fishes (e.g., fathead minnows and rainbow trout) and was empirically calibrated to predict toxicity to aquatic invertebrates (i.e., daphnids) (Santore et al. 2001, Niyogi & Wood 2004). As a result, some of the mechanistic approaches, although robust, are not specific to the physiology of aquatic insects (Heijerick et al. 2002, Niyogi & Wood 2004). Specifically, the current BLM may not appropriately model Mg^{2+} and Ca^{2+} in competitive interactions with trace-metals for the biotic ligand in aquatic invertebrates (Heijerick et al. 2002, Niyogi & Wood 2004). The primary assumption is that all aquatic organisms have a biotic ligand that responds to trace-metals in the same general way. However, the BLM has not been tested to determine whether the criteria set by the model are protective of native taxa in field conditions (Adams et al. 2002, Paquin et al. 2002).

The primary endpoint of the BLM (i.e., LC_{50}) is not especially useful in systems contaminated by mixtures of trace-metals (Smith et al. 2006). Nor is the LC_{50} an ecologically meaningful endpoint as it does not describe the possible consequences to higher levels of biological organization (e.g., populations, communities, ecosystems) resulting from exposure to toxic levels of trace-metals (Clements 1997). If the BLM is to be employed to set site-specific water quality criteria protective of aquatic communities (e.g., 95% of the organisms at a specified site) it should be capable of predicting the responses of native populations and communities.

We have developed a method that utilizes the BLM for the purpose of ecological assessment of trace-metal pollution in natural systems. We developed a toxic unit model of additive trace-metal toxicity derived from BLM outputs and compared it with a traditional toxic unit model, Cumulative Chronic Units (CCU). This new model, the Chronic Criterion Accumulation Ration (CCAR), is derived from the BLM and incorporates current theory about the interactions between aqueous constituents (i.e., hardness, DOC, pH) that affect trace-metal toxicity and accumulation of bioavailable trace-metals on the respiratory surface of aquatic organisms. In contrast, the CCU accounts only for the influence of hardness on trace-metal toxicity through the use of equations derived from single species toxicity tests. These two models of trace-metal toxicity allow for a comparison of model predictive capabilities.

The primary objective of this research is to explore the use of the BLM as a bioassessment tool to predict responses of benthic macroinvertebrate communities to trace-metals in natural systems. Further, we make comparisons between this new BLM-derived estimate of trace-metal toxicity and the traditional model of additive toxicity based on hardness-adjusted chronic criterion values.

METHODS

Field Validation of the Biotic Ligand Model

Because most trace-metal contaminated streams are impacted by a mixture of metals at chronic concentrations, a measure of chronic toxicity due to metal mixtures was evaluated. Water-quality criteria for individual metals represent concentrations that, when exceeded, are likely to harm aquatic organisms. Because criterion values are only available for individual metals, alternative models are necessary to estimate toxic effects of trace-metal mixtures. Although most research investigating the toxicity of trace-metal mixtures has focused on acute effects, previous studies have shown additive effects at chronic concentrations (Newman & McCloskey 1996, Norwood et al. 2003, Playle 2004). Cumulative Criterion Units (CCU) will be used to evaluate toxicity due to trace-metal mixtures and will be calculated as described in

Clements et al. (2000), with modification from the method described originally in U.S. EPA water-quality criteria documents (Sprague 1970, NAS/NAE, 1973). Therefore, it is assumed that interactions among metals are additive and the CCU is defined as the ratio of the measured metal concentration to the U.S. EPA criterion value, summed for all metals at a station. The Cumulative Criterion Unit is calculated as:

$$CCU = \sum m_i/c_i$$

where m_i is the total dissolved trace-metal concentration and c_i is the criterion value for the i^{th} metal. Because water hardness affects toxicity and bioavailability of some trace-metals, criterion values for Cd^{2+}, Cu^{2+}, and Zn^{2+} were modified to account for variation in water hardness among streams (U.S. EPA, 2003). For example, at a water hardness of 100 mg/L ($CaCO_3$), criterion values for these three trace-metals would be 0.25, 9.0, and 120 µg/L, respectively. Under the current criteria a CCU value of $\leq$ 1.0 represents a mixture of metal concentrations that would not be predicted to cause chronic effects to at least 95% of the organisms at a specified site. This model is the current approach for assessing trace-metal toxicity due to mixtures and will be used to evaluate the BLM predictions.

Because the BLM predicts acute toxicity due to individual trace-metals, it must be modified to account for metal mixtures and for comparison with the CCU model. The Chronic Criteria Accumulation Ratio (CCAR) was developed similar to the CCU and assumes additive toxicity of trace-metal mixtures based on BLM-predicted endpoints. The CCAR is defined as the ratio of the BLM calculated accumulated trace-metal on the biotic ligand to that accumulated on the biotic ligand in water at U.S. EPA criterion value, summed for all metals at a site. More specifically, CCAR is calculated as follows:

$$\Sigma CCAR = \sum_{i} \frac{BLM\ calculated\ site\ specific\ [gill\ metal]}{BLM\ calculated\ [gill\ metal]\ at\ CCC}$$

where *BLM calculated site-specific [gill metal]* and *BLM calculated [gill metal] at* CCC are measurements developed from BLM endpoints. *BLM calculated [gill metal]* is the BLM predicted accumulation of the i^{th} trace-metal on the biotic ligand (gill surface), calculated by running the BLM in speciation mode using site-specific water quality parameters. *BLM calculated[gill metal] at* CCC is the BLM predicted accumulation of the i^{th} trace-metal on the gill surface, calculated using the "normalization chemistry" water quality parameters from Table 1a, U.S. EPA (2003), and the i^{th} metal Continuous Chronic Criterion (CCC) concentration (U.S. EPA 2003). The CCC is developed by averaging toxicity test data across species and genera to determine a concentration of trace-metal that will be protective of 95% of the species at a specific site. We are using CCAR to predict toxicity to benthic communities, individual species of which have differential sensitivity to trace-metals. By using the CCC and not a species specific response point such as the LA_{50} for a daphnid or fathead minnow, we can predict toxicity more generally to the community and account for the modifying effects of several water quality parameters known to determine trace-metal uptake and bioavailability (e.g., pH, DOC, alkalinity, and major cations).

Study Area and Sampling Strategy

The study area is the mineral belt and surrounding mountains of the central Colorado Rocky Mountains (Figure 2). Specifically, the study area includes all of Arapahoe, Roosevelt, Pike, and San Isabel National Forests, portions of the White River and Rio Grande National Forests and other public lands that are included within the area of these Forests (e.g., State, U.S. BLM, and U.S. NWR lands). Rocky Mountain National Park and Great Sand Dunes National Park also lie within the study-area boundaries.

Geographic Information Systems (ArcGIS 8.3, ArcHydro 1.2) were used to delineate catchments for sampling (ESRI 1999, Maidment 2002). Digital elevation models (DEM, 30 x 30 m) were used to define catchement boundaries, area, slope, and relief ratio. Small catchments (1^{st}-3^{rd} order) predominantly underlain by a single rock type were sampled and categorized based on mineral deposit criteria and measured cation and anion concentrations to evaluate water quality (e.g., Miller 1999). The purpose of this sampling strategy was to target a large variety of water quality conditions resultant from parent geology to test the BLM. The Colorado Vegetation Model (30 m x 30 m resolution) was used to determine vegetation and land use types to make sure we were sampling catchments which were not influenced by anthropogenic factors other than the effects of mining (Theobald et al. 2004).

Sample Collection

Geochemical and benthic macroinvertebrate samples were collected from 114 catchments during base flow conditions in the summers of 2003 (n = 21), 2004 (n = 49), and 2005 (n = 44) (Figure 1).

Physicochemical Parameters

Stream discharge was measured with USGS Price pygmy current meter and depth was measured across the stream channel at 15 – 25 intervals, depending upon stream width. Stream discharge (m^3/s) was calculated using the continuity equation. Wetted perimeter and densiometer (Forest Densiometers©, Model A, Bartlesville, OK, USA) readings of canopy cover were collected from each point where benthic samples were collected.

Water samples in 2004-2005 were collected using methods described in Wilde et al. (1998) to meet the requirements of the BLM (HydroQual 2003). Routine water quality parameters (temperature, conductivity, and pH) were measured in the field using a Horiba D-24 combination meter following methods described by Wilde et al. (1998). The probes were calibrated at the beginning of each day with certified standards, and checked periodically throughout the day. All water samples processed in the laboratory were filtered through a Acrodisc® Premium 25 mm Syringe Filter with 0.45 um Nylon Membrane at the site and stored at 4°C until analyzed. Water samples for dissolved organic carbon (DOC) were filtered through a 0.70-µm glass-fiber filter, acidified with concentrated hydrochloric acid (12 molar) to a pH of <2, and stored in baked amber glass bottles. A Shimadzu TOC-5000A total organic carbon analyzer was used to measure DOC. Dissolved trace-metal samples were acidified with concentration nitric acid (16 molar) to a pH < 2 and stored in polyethylene bottles. Samples for anions were collected and stored in polyethylene bottles. Water analyses were conducted at the analytical laboratories of the USGS Geologic Discipline Laboratory in Denver, Colorado. Concentrations of major cations (Na^+, K^-, Mg^{2+}, and Ca^{2+}) were analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Briggs 2002), whereas trace-metal concentrations (Cd^{2+}, Cu^{2+}, Zn^{2+}) were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Lamothe et al. 2002). Major anions (Cl^-, F^-, NO_3^-, SO_4^{2-}) were measured by ion chromatography (Theodorakos et al. 2002). High concentrations of SO_4^{2-} (>25 mg/L) were determined by inductively coupled plasma-atomic emission spectrometry and alkalinity was determined by titration (Theodorakos 2002).

Analysis methods for 2003 differed from above in that major cations and trace-metals were analyzed by flame (Zn^{2+}) and furnace atomic absorption (Cd^{2+} and Cu^{2+}) spectrophotometry (Perkin-Elmer, Perkin-Elmer model 372). However, the only significant difference in minimum reported detection limits between sampling periods was for Zn^{2+} (5 µg/L in 2003 vs. 0.25 µg/L in 2004-2005).

Biological Sample

Five replicate benthic samples (*n*=5) were collected using a 0.1-m^2 Hess sampler (350-µm mesh net) from shallow riffle areas (<0.5 m) at targeted catchments determined from the GIS analysis. Representative sample locations were selected based on the following criteria: location was a riffle or run habitat unit, depth was 0.10-0.25 (m) and substrate was representative of the reach. Overlying substrate was scrubbed of all algae and diatoms, and inorganic debris was removed. All individual substrate particles larger than 22.6 mm were removed from the Hess sampler and measured along the intermediate axis. Underlying substrate was disturbed to a depth of approximately 10 cm and the remaining material was sieved using a 350-µm mesh sieve. All organisms retained were preserved in 80% ethanol in the field.

In the laboratory, samples were processed to remove debris, and sub-sampled until 300 organisms (±10%) were removed from the sample following methods described by Moulton et al. (2000). Invertebrates were identified to the lowest practical taxonomic level (genus or species for most taxa; subfamily for chironomids) (Merritt & Cummings 1996, Ward et al 2002). Means of the five replicate benthic samples were used to calculate benthic macroinvertebrate community metrics in the Invertebrate Data Analysis System (IDAS) version 3.9.5 (Cuffney 2003) including the following richness metrics: total taxa richness, intolerant taxa richness, EPT taxa richness, Ephemeroptera richness, collector-gatherer richness, shredder richness, scraper richness, predator richness, and the following abundance metrics: EPT abundance, Ephemeroptera abundance, Heptageniidae abundance, collector-gatherer abundance, shredder abundance, scraper abundance, predator abundance. IDAS was also used to calculate the following composition metrics: percent EPT taxa abundance, percent individuals in top 5 dominant taxa, percent clinger abundance, and percent tolerant taxa abundance. Trait metrics were developed based on data from the U.S. EPA Rapid Bioassessment Protocol (Barbour et al. 1999) while tolerance/intolerance values were taken assigned the national average tolerance values as determined by Cuffney (2003) descriptive of taxa tolerance to organic pollution.

The Western Mountainous Ecoregion Index of Biotic Condition (IBC) was also developed (U.S. EPA 2006). This multi-metric is a composite of 6 individual metrics (EPT richness, scrapper richness, percent EPT abundance, percent abundance of the 5 most dominant taxa, percent tolerant taxa abundance, and percent clinger abundance). Because each metric is in a different scale, normalization is required to create a multi-metric comprised of 6 equally weighted metrics into a final multi-metric score. Individual metrics were put into two groups for normalization, those that increase in response to anthropogenic stress (i.e., percent tolerant taxa and percent abundance of the 5 most dominant taxa) and those that decrease in response to anthropogenic responses (i.e., EPT richness, scraper richness, percent EPT abundance, percent clinger abundance). For metrics which increase in response to anthropogenic stress, sites with metric scores equal to and below the 5th percentile were assigned a value of 10 while those equal to or above the 95th percentile were assigned a 0. All other scores between the 5th and 95th percentiles were assigned a value which was linearly interpolated between the 5th and 95th percentiles. For those metrics which decrease in response to anthropogenic stress, the 5th percentile score was assigned a 0 while the 95th percentile score was assigned a value of 10. All other scores were assigned by linear interpolation between these two values.

To develop the final multi-metric value the normalized scores for the individual metrics were summed and multiplied by 1.66 to result in values that range from 0-100. The IBC was designed as a generic descriptor of benthic macroinvertebrate community responses to any anthropogenic disturbance and used here as a check to make sure no other impacts other than those of trace-metals influences the study field sites. We are also using the IBC as a comprehensive metric capable of describing alterations in benthic macroinvertebrate community structure in responses to exposure to trace-metals.

Data Analysis

All statistical analysis were conducted in SPSS© version 14 (2005). To evaluate our ability to predict trace-metal toxicity to natural populations and communities we performed simple linear regression between benthic macroinvertebrate metrics (e.g., total taxa richness, intolerant taxa richness), and the measures of trace-metals toxicity, CCAR and CCU. These benthic macroinvertebrate community metrics were selected because previous research suggested they were sensitive to trace-metal exposure (Carlisle & Clements 1999, Clements et al. 2000, Griffith et al. 2004, Soucek et al. 2002). Incidentally, piecewise regressions were initially fit to detect thresholds in benthic community responses to trace-metals in an attempt to make comparisons with data previously published by Griffith et al. (2004). However, piecewise models did not improve overall fit compared to general linear models and therefore are not presented.

Under the assumption of additive toxicity of metal mixtures, we evaluated all possible models (i.e., CCU or CCAR) to determine which were best at predicting benthic macroinvertebrate taxa richness. Taxa richness was selected for this analysis because it is ecologically relevant, as water quality criteria are intended to protect 95% of all species found at a site. In addition, taxa richness is consistently used as a sensitive indicator of trace-metal contamination (Carlisle & Clements 1999, Clements et al. 2000, Griffith et al. 2004). Akaike Information Criteria (AIC) values were calculated to determine which of the 14 competing models had the highest probability of being the best model (Burnham & Anderson 1998). We used a version of AIC (AIC_c) which corrects for a small ratio of model parameters (K) to observations (n). AIC_c was standardized by subtracting the minimum AIC_c score from each of the candidate model AIC_c values to derive Δ_I and allow us to rank all candidate models (Burnham & Anderson 2001). Akaike weights (ω_i), the probability of the candidate model being the best model among all included in the candidate set, were also calculated.

To estimate the range of trace-metal mixtures where negative effects on benthic communities were detectable, sites were categorized based on measured CCAR and CCU toxic unit values and compared using one-way analysis of variance (ANOVA). A post-hoc Least Significant Difference (LSD) test was used to compare mean IBC scores between categories (Sokal & Rohlf 2003). Category intervals were selected based on previous work (Clements et al. 2000, Clark & Clements 2006, Griffith et al 2004) suggesting differentiation in expected adverse effects due to exposure to trace-metals at 1, 10, and 100 CCU units. Because the present study sampled streams with low levels of trace-metal contamination, we were interested to determine whether effects could be detected at these lower metal concentrations. Sites were grouped by order of magnitude of measured CCAR or CCU values (i.e., 0.01 - 0.1, 0.1 - 1, 1 - 10, 10 - 100, > 100) with the lowest toxic units category (0.01 – 0.1) representing a control descriptive of streams of low trace-metal concentrations for comparison to higher values of CCAR and CCU. This methodology resulted in 5 categories of sites using CCAR and 4 categories of sites for the CCU.

RESULTS

Physicochemical characteristics

To minimize the influence of confounding variables, our study sampled catchments with little to no residential, commercial, or agricultural development and in relatively remote areas. Habitat characteristics of these catchments were typical of small to mid-sized headwater streams (i.e., 1st – 3rd order) of the Rocky Mountains and indicated that anthropogenic influences other than mining (e.g., pasture/hay mean percent-area = 0% ± 0.2%, development mean percent-area = 0% ± 0.2%) had little direct effect on water quality and benthic macroinvertebrate communities (Table 1). The large variation observed in some local (e.g. D_{50} and percent cover ranged 22.6-90, and 1-96% respectively) and landscape-level habitat parameters (e.g., percent forest area and basin relief ratio ranged 2-98% and 0.06-0.35 respectively) was expected because we sampled catchments underlain by different geologies to capture a wide range of both chemical and physical differences of streams within the study area (Table 1).

Physicochemical characteristics of our field sites showed a broad range of variation in water quality; however, these values were within the range of water quality parameters used to develop the BLM (Table 2) (Fey et al. 2007). Exceptions included temperature (ranging 3-18 °C), pH (ranging 3.5 - 8.6 pH), alkalinity (0 – 190 mg/L), and Cl^- (0.04 – 10) which were generally below the specified limits of the BLM.

Comparison of models of trace-metal toxicity

Chronic Criteria Accumulation Ratio (CCAR) values for the sum of Cd^{2+}, Cu^{2+} and Zn^{2+} ranged from 0.02 to 270 while Cumulative Chronic Unit (CCU) values at these stations ranged from 0.04 to 118. A direct comparison of the two models of trace-metal toxicity shows that CCAR under predicts toxicity compared to CCU in most cases (Figure 2). By including DOC in determining the bioavailable fraction of trace-metal as in CCAR, the average concentration of dissolved trace-metals was decreased by 37%. In 12 cases CCAR substantially over predicted toxicity relative to CCU. In each of these cases it was found that pH was below 6 (out of a total of 14 sites where pH was below 6) or alkalinity was below 6 mg/L. Excluding these 12 observations and assuming that 1.0 CCU

= 1.0 CCAR, the average decline in toxic units that resulted from using the BLM was 0.68.

Simple linear regression analyses of CCAR verses benthic macroinvertebrate community metrics resulted in R^2 ranging between 0.32-0.69 for richness metrics (Figures 3-4), 0.32-0.72 for abundance metrics (Figures 5-6), and 0.00-0.46 for compositional metrics (Figures 7) ($p < 0.0001$ for all metrics except percent EPT abundance ($p = 0.001$) and percent tolerant taxa abundance $p = 0.626$). Simple linear regression analyses of CCU verses benthic macroinvertebrate community metrics resulted in R^2 ranging between 0.28-0.56 for richness metrics (Figures 3-4), 0.24-0.57 for abundance metrics (Figures 5-6), and 0.00-0.40 for compositional metrics (Figures 7) ($p < 0.0001$ for all metrics except percent tolerant taxa abundance $p = 0.848$

Akiake Information Criteria (AIC_c) was used to determine which models were best at predicting effects of trace-metals on total taxa richness (Table 3). A total of 14 possible additive models were evaluated that utilized between 2 and 3 estimated parameters (exclusive of regression constant). The parameters were comprised of individual metal metrics (e.g., CCU Zn^{2+}) and combinations of these metal metrics (e.g., CCAR $\sum(Cd^{2+} + Cu^{2+} + Zn^{2+})$ or CCAR (Cd^{2+}) + CCAR (Cu^{2+})). The most probable model given the model set was the additive combination of the CCAR metric for Cu^{2+} and Zn^{2+} ($\omega_i = 0.97$) followed by CCAR $\sum(Cd^{2+}+ Cu^{2+} + Zn^{2+})$ ($\omega_i = 0.03$). No other models, including any CCU model, were probable descriptors of total taxa richness given this set of models.

Community-level responses to metals

Visual inspection of Figures 3 - 7 indicates that trace-metal concentrations (characterized as CCAR and CCU) caused deleterious effects in benthic macroinvertebrate communities from central Colorado mountainous streams. Specifically, CCAR and CCU were found to be linearly related to measures of benthic macroinvertebrate community richness, abundance, and composition, with negative effects observable below the chronic threshold of toxicity (i.e., CCU or CCAR = 1) (Figures 3 - 7). Utilizing the regression equations describing the relationship between CCAR and the benthic macroinvertebrate community metrics, we determined the percent differences in mean metric response between CCAR = 0.07 (i.e., median background value) and 1.0 (the hypothetical safe concentration) to determine the extent to which deleterious effects were observed, below criteria. On average, for all 8 richness metrics a 27% reduction was observed (Figures 3 - 4). While for abundance metrics (7 metrics) a 72% decline in abundance was found (Figures 5 - 6), and an average 15% change in the 4 composition metrics (Figure 7) was observed.

Figure 8 depicts the relative contribution of trace-metals to each category for both the CCAR (Figure 8 A) and the CCU (Figure 8 B). For CCAR, Cd^{2+} dominated the lower concentration groups (78%, 64%, 45%, 31%, 0.5%), but as the level of contamination increased, the contribution of Cu^{2+} (13, 20, 16, 41, 99.3%) and Zn^{2+} (9, 16, 39, 28, 0.2 %) increased. For CCU, the contribution of Cd^{2+} decreased with increasing trace-metal concentrations (51, 44, 42, 31%), while Cu^{2+} dominated the highest metal category (35, 39, 33, 48%) and Zn^{2+}

increased in contribution to total CCU as trace-metal concentrations also increased (14, 17, 25, 21%).

Tables 4 and 5 summarize the differences in BLM water quality parameters for the CCAR and CCU toxic unit categories used with the ANOVA analysis of the IBC metric. These data demonstrate that whereas trace-metal concentrations increased across toxic unit categories, the other water quality parameters showed relatively little variation. Notable exceptions were mean alkalinity and pH, which were significantly lower in the highest toxic unit category (alkalinity = 51 vs 1; pH = 7.5 vs 4.7, for CCAR (0.01-0.1) and CCAR (> 100) respectively). In contrast to these results, mean DOC and SO_4^{2-} increased in severely contaminated streams (DOC = 2.1 vs 0.6 mg/L; SO_4^{2-} = 12 and 93 mg/L, respectively). NO_3^- concentrations were low and similar in concentration across categories, supporting the hypothesis that agriculture or commercial/residential development had little impact on these field sites.

The IBC is comprised of 6 metrics: EPT richness (Figure 3), scrapper richness (Figure 4), percent EPT abundance, percent abundance of the 5 most dominant taxa, percent tolerant taxa abundance, and percent clinger abundance (Figure 7). Five of the metrics were found to be significantly related to the metals gradient as described by the CCAR and CCU. Percent tolerant taxa abundance, a metric used to detect the influence of organic pollution, was not significantly related to either CCAR or CCU (Figure 6). The mean value and standard deviation of the percent tolerant taxa abundance metric was 0.48 ± 1.22, suggesting that the community at any given site sampled as part of this study was comprised of < 1% of taxa known to be tolerant to organic pollution. Organic pollution from agriculture or urban development was not a factor in these streams.

Results of one-way ANOVA and LSD tests showed highly significant differences in IBC scores among CCAR and CCU categories ($p < 0.000$). Results of the multiple range tests showed that the IBC was significantly reduced at 0.1 CCAR and 1.0 CCU, metal concentrations that are presumed to be benign to aquatic organisms.

DISCUSSION

The intent of this work was to develop and evaluate the BLM as a bioassessment tool capable of predicting benthic macroinvertebrate community responses to mixtures of trace-metals. This is an important step in the evolution of the BLM as its purpose is to develop site-specific water quality criteria with the intent of protecting 95% of aquatic species (U.S. EPA 2003). However, the model was developed to predict toxicity to standard test organisms and has not been tested to determine whether it can predict the response of native taxa to trace-metals in natural waters (Adams et al. 2002, Paquin et al. 2002). We developed an estimate of metal pollution (CCAR) that uses BLM-derived endpoints to predict responses of benthic macroinvertebrate communities to mixtures of trace-metals and evaluated its performance relative to a traditional toxic unit model, CCU. This evaluation was conducted as part of a regional-scale environmental assessment of the impacts of trace-metals throughout Colorado, which provided a diversity of habitats, geologies, and physicochemical conditions to test this new model of trace-metal toxicity.

Natural surface waters in the Colorado Rockies are generally oligotrophic with low concentrations of dissolved solutes. This condition increases the bioavailability of trace-metals as compared to waters of high ionic strength and DOC (Morel 1983, Pagenkopf 1983, Baron et al. 1991). Although most physicochemical characteristics of streams in this study were within the range of those used to develop the BLM, a number of cations and anions (e.g., Mg^{2+} Na^{+}, K^{+}, Cl^{-}) were found to be on the lower end of the design range (Table 2). In contrast, the range of DOC was larger than anticipated and accounted for a 37% decrease in nominal dissolved trace-metal concentrations as calculated by the BLM. The greater range of DOC resulted in the general under-estimation of trace-metal bioavailability relative to that estimated by CCU (figure 2). It is also important to note that the CCU predicted metal levels at 39 sites exceeding the theoretical threshold of chronic toxicity of 1.0, as compared to 25 sites using CCAR (Figures 3 - 7). At 12 sites low pH (< 5.8) resulted in a relatively large increase in trace-metal bioavailability as compared to that found by CCU. This increase in trace-metal bioavailability was substantial and demonstrates that low pH can overwhelmingly increase trace-metal toxicity despite the influence of DOC (Sciera et al. 2004). These results highlight the need to incorporate a suite of water quality parameters that can affect trace-metal bioavailability and illustrate there is no universal way to estimate trace-metal toxicity to aquatic organisms (Meyer 2002a).

CCAR described more variation in benthic macroinvertebrate community metrics than CCU in 17 of 19 cases (Figures 2-7). It was anticipated that CCAR would out perform the CCU model due to the fact that the CCAR utilizes the BLM to predict toxicity by including more chemical parameters that modify trace-metal bioavailability as compared to CCU. However, the extent of alteration observed in benthic macroinvertebrate communities occurred at very low trace-metal levels not previously reported. At least a 27% reduction in community richness, 72% reduction in abundances, and a 15% change in composition metrics were observed between background concentration (i.e., 0.07 = CCAR) and the threshold of chronic toxicity (i.e., CCAR = 1.0). These data suggest that toxicity to native populations and communities in Rocky Mountain streams may occur at trace-metal concentrations below those previously thought safe.

Further evidence that alteration in benthic communities occurs at trace-metal concentrations historically thought to be safe were observed in the IBC multi-metric index of benthic community and stream health. The U.S. EPA watershed assessment IBC is a multi-metric index developed to assess the generic ecological health of streams and is not generally thought to be sensitive to trace-metal pollution. However, 5 of the 6 metrics that comprise the IBC were significantly related to CCAR or CCU. Negative effects in the IBC multi-metric were observed between (0.01 - 0.1) CCAR or (0.01 – 1) CCU, suggesting a general decline in benthic communities is observed at trace-metal concentrations below water quality criteria (Figure 9).

Previously published results have reported significant reduction in community richness and abundances of sensitive taxa between 1-2 CCU (Clements & Kiffney 1995, Clements et al. 2000). Other researchers suggested that benthic macroinvertebrate communities only responded negatively to increasing trace-metal toxicity after concentrations exceeded the chronic threshold of 1.0 CCU (Griffith et al. 2004). The results of this study corroborate earlier findings that trace-metals negatively affect benthic macroinvertebrate communities at or near water quality criterion; however, the data presented here strongly suggest that these criteria are not protective against losses in biodiversity and biomass in Colorado mountainous streams.

Model selection based on AIC demonstrated that an additive model of trace-metal toxicity using BLM-derived endpoints (CCAR Cu^{2+} + CCAR Zn^{2+}) was the most probable predictor of the total species richness. Although not presented here, this trend was observed in most other richness and abundance metrics. This analysis highlights the role that Cu^{2+} and Zn^{2+} play in structuring aquatic communities in Colorado mountain streams. However, this result does not suggest that Cd^{2+} does not play a major role in these ecosystems. Cd^{2+} is iso-chemical to Zn^{2+} and found in the same mineralogy but in much smaller quantities. Also, simple linear regression tends to bias towards selecting variables which vary greatly as compared to those which do not (i.e., Zn^{2+} as compared to Cd^{2+}). Because of these geochemical and statistical covariance issues, Zn^{2+} may mask the influence of Cd^{2+} in the mineral belt of Colorado. Despite this issue, AIC indicated that CCAR is more consistent with the observed benthic data as compared to the CCU. This was an expected outcome because the BLM is a more mechanistic approach to quantifying toxicity and accounts for the effects of numerous physicochemical variables that control trace-metal bioavailability. In contrast, the CCU, an empirically derived model, only corrects for water hardness, which is relatively low in most Colorado streams. We conclude that CCAR is capable of predicting responses of native communities to trace-metal mixtures and is a significant improvement over the traditional toxic unit model.

CONCLUSIONS

In summary, we developed a toxic unit model utilizing the BLM to predict benthic macroinvertebrate community responses to mixtures of trace-metals and compared it to a traditional toxic unit model, the CCU. CCAR was found to be an improvement over the traditional hardness adjusted toxic unit model and capable of predicting native community responses to trace-metal contamination. CCAR uses a mechanistic approach to model trace-metal bioavailability by using the latest knowledge in aqueous geochemistry and physiology of aquatic organisms to predict metal toxicity. Low levels of trace-metals, between 0.01 and 0.1 CCAR units, resulted in negative effects on benthic macroinvertebrate community metrics, resulting in an average loss of 27% of richness and 72% loss of abundance. These results suggest that water quality criteria may not be sufficiently low to protect against losses in aquatic insect diversity and biomass in Rocky Mountain streams.

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TABLES

Table 1. Habitat parameter range, mean ± SD from field sites showing little residential or commercial or agricultural development in targeted catchments.

Parameter	Range	Mean ± SD
D_{50} (mm)[a]	22.6-90	38 ± 14
CFS[b]	0.5 - 137	11 ± 16
Cover (%)[c]	1 - 96	58 ± 29
Alpine Area (%)	0 - 99	41 ± 27
Forest Area (%)	2 - 98	50 ± 27
Pasture/Hay (%)	0-1	0 ± 0.2
Developed Area (%)	0 - 2	0 ± 0.2
Maximum Elevation (m.)[d]	2966 - 4412	3954 ± 293
Minimum Elevation (m.)	2230 – 3408	2903 ± 294
Basin Relief Ratio[e]	0.06 - 0.35	0.17 ± 0.07
Watershed Area (km^2)	2 - 481	37 ± 63

[a] Median particle size in millimeters

[b] Flow in cubic feet/second

[c] Percent area

[d] Meters

[e]((Maximum elevation – minimum elevation)/basin length)- surrogate for basin slope

Table 2. Range of chemical parameters measured in this study and the design range of the biotic ligand model.

Parameter	Measured Range	BLM Design Range[a]
Temperature (°C)	3 - 18	10 - 25
pH (S.U.)	3.5 - 8.6	4.9 - 9.2
DOC (mg/L)	0.3 – 8	0.05 - 29.65
Humic Acid Content (%)	10	10 - 60
Ca^{2+} (mg/L)	1.2 - 105	0.204 - 120.24
Mg^{2+} (mg/L)	0.2 - 40	0.024 - 51.9
Na^{+} (mg/L)	0.2 - 10	0.16 - 236.9
K^{+} (mg/L)	0.15 - 13	0.039 - 156
SO_4^{2-}(mg/L)	0.7 - 270	.096 - 278.4
Cl^{-} (mg/L)	0.04 - 10	0.32 - 279.72
Alkalinity (mg/L)	0 - 190	1.99 - 360
DIC (*mmol*/L)[b]	Estimated	Estimated
S^{2-} (mg/L)	NM[c]	0 - 0
Cd^{2+} (ug/L)	0.01 - 8	NL[d]
Cu^{2+} (ug/L)	0.15 – 940	NL
Zn^{2+} (ug/L)	0.25 - 1900	NL

[a] (HydroQual 2003)

[b] Dissolved inorganic carbon - BLM estimates DIC from measured values of alkalinity and pH.

[c] Not measured but input as recommended, 0.

[d] No limits are set for trace-metal concentrations.

Table 3. Akaike Information Criteria for all possible additive models using CCAR to predict total taxa richness.

Candidate models	K	SSE	AIC$_C$	Δ_i	ω_i
CCAR Cu^{2+} + Zn^{2+}	3	1152.90	270.00	0.00	0.97
CCAR ($\sum Cd^{2+}$ + Cu^{2+} + Zn^{2+})	2	1246.28	276.76	6.77	0.03
CCAR Cd^{2+} + Cu^{2+}	3	1282.01	282.10	12.10	0.00
CCAR Cu2+	2	1444.59	293.60	23.60	0.00
CCU ($\sum Cd^{2+}$ + Cu^{2+} + Zn^{2+})	2	1905.35	325.16	55.16	0.00
CCU Zn^{2+} + Cu^{2+}	3	1898.01	326.83	56.83	0.00
CCU Cd^{2+} + Cu^{2+}	3	1982.73	331.81	61.81	0.00
CCU Cd^{2+}	2	2321.45	347.68	77.68	0.00
CCU Cd^{2+} + Zn^{2+}	3	2288.70	348.17	78.17	0.00
CCU Zn^{2+}	2	2395.91	351.27	81.28	0.00
CCU Cu^{2+}	2	2489.13	355.63	85.63	0.00
CCAR Zn^{2+}	2	2902.91	373.16	103.16	0.00
CCAR Zn^{2+} + Cd^{2+}	3	2881.99	374.44	104.45	0.00
CCAR Cd^{2+}	2	3240.97	385.71	115.72	0.00

K - number of parameters estimated in the model
RSS - Residual sum of squares
AIC$_C$-Akaiki information criteria (second-order)
Δ_I – standardized AIC$_C$ used to rank models
ω_i – probability candidate model is the best model in set.

Table 4. Mean ± SD of water quality parameters for categories of CCAR associated with the ANOVA of the U.S. EPA Western Regional Index of Biotic Condition (2006).

Categories for CCAR[a]	0.01-0.10	0.10-1	1-10	10.0-100	>100
Number of sites	29	60	11	5	9
Temperature (°C)	10 ± 3	8 ± 3	9 ± 2	7 ± 3	11 ± 2
pH (S.U.)	7.5 ± 0.6	6.9 ± 0.5	7.1 ± 0.8	6 ± 1.6	4.7 ± 0.6
Alkalinity (mg/L)	51 ± 45	22 ± 17	33 ± 21	12 ± 10	1 ± 2
SO_4^{2-} (mg/L)	12 ± 18	12 ± 36	35 ± 23	40 ± 22	93 ± 39
NO_3^- (mg/L)	0.26 ± 0.37	0.46 ± 0.36	0.61 ± 0.36	0.35 ± .29	0.52 ± 0.24
Cl^- (mg/L)	1.20 ± 0.67	1.02 ± 1.00	2.22 ± 3.32	0.20 ± 0.25	1.01 ± 1.45
Ca^{2+} (mg/L)	17 ± 14	10 ± 14	20 ± 5	13 ± 3	20 ± 3
Cd^{2+} (ug/L)	0.01 ± 0.00	0.04 ± 0.09	0.60 ± .38	1.29 ± 0.38	2.04 ± 3.00
Cu^{2+} (ug/L))	0.3 ± 0.1	0.7 ± 1.1	1.4 ± 1.8	5.7 ± 5.6	302.7 ± 312.7
K^+ (mg/L)	0.74 ± 0.34	0.79 ± 1.64	0.73 ± 0.20	0.48 ± 0.21	0.61 ± 0.14
Mg^{2+} (mg/L)	5.3 ± 7.4	1.9 ± 2.0	4.9 ± 2.3	2.9 ± 1.7	5.1 ± 2.3
Na^+ (mg/L)	1.9 ± 1.0	1.7 ± 1.6	1.9 ± 1.3	1.5 ± 0.6	1.3 ± 0.3
Zn^{2+} (ug/L)	1.1 ± 0.7	7.4 ± 12.1	177.7 ± 111.3	408.0 ± 128.4	465.8 ± 741.2
Hardness (mg/L)	64 ± 63	33 ± 41	70 ± 19	44 ± 6	71 ± 17
DOC (mg/L)	2.1 ± 1.9	1.8 ± 0.7	1.4 ± 0.8	1.2 ± 0.8	0.6 ± 0.3

[a] Chronic Concentration Metal Accumulation Ratio.

[b] Dissolved Organic Carbon

Table 5. Mean ± SD of water quality parameters for categories of CCU associated with the ANOVA analysis of the IBC.

Categories for CCU	0.01-0.10	0.10-1.0	1.0-10	>10.00
Number of sites	7	68	26	13
Temperature (°C)	9 ± 2	9 ± 3	9 ± 2	9 ± 3
pH (S.U.)	8.3 ± 0.3	7.0 ± 0.6	7.1 ± 0.6	5.1 ± 1.2
Alkalinity (mg/L)	125 ± 37	25 ± 12	26 ± 24	5 ± 8
SO_4^{2-} (mg/L)	28 ± 34	7 ± 6	32 ± 54	77 ± 43
NO_3^- (mg/L)	0.21 ± 0.18	0.41 ± 0.39	0.49 ± 0.37	0.48 ± 0.26
Cl^- (mg/L)	1.0 ± 0.5	1.1 ± 1.0	1.5 ± 2.3	0.8 ± 1.2
Ca^{2+} (mg/L)	38 ± 13	9 ± 6	18 ± 20	18 ± 5
Cd^{2+} (ug/L)	0.01 ± 0.00	0.02 ± 0.02	0.30 ± 0.70	1.90 ± 2.45
Cu^{2+} (ug/L)	0.3 ± 0.0	0.4 ± 0.2	2.8 ± 6.2	209.3 ± 293.9
K^+ (mg/L)	0.89 ± 0.14	0.65 ± 0.53	1.03 ± 2.34	0.57 ± 0.18
Mg^{2+} (mg/L)	15.2 ± 10.6	1.9 ± 1.3	3.4 ± 3.1	4.5 ± 2.3
Na^+ (mg/L)	1.4 ± 0.6	1.8 ± 1.3	1.9 ± 1.9	1.4 ± 0.4
Zn^{2+} (ug/L)	1.3 ± 0.6	3.7 ± 7.5	83.9 ± 108.8	477.9 ± 596.9
Hardness (mg/L)	157 ± 72	30 ± 18	58 ± 58	62 ± 19
DOC (mg/L)	1.0 ± 0.2	2.0 ± 1.4	1.7 ± 0.9	0.9 ± 0.6

FIGURES

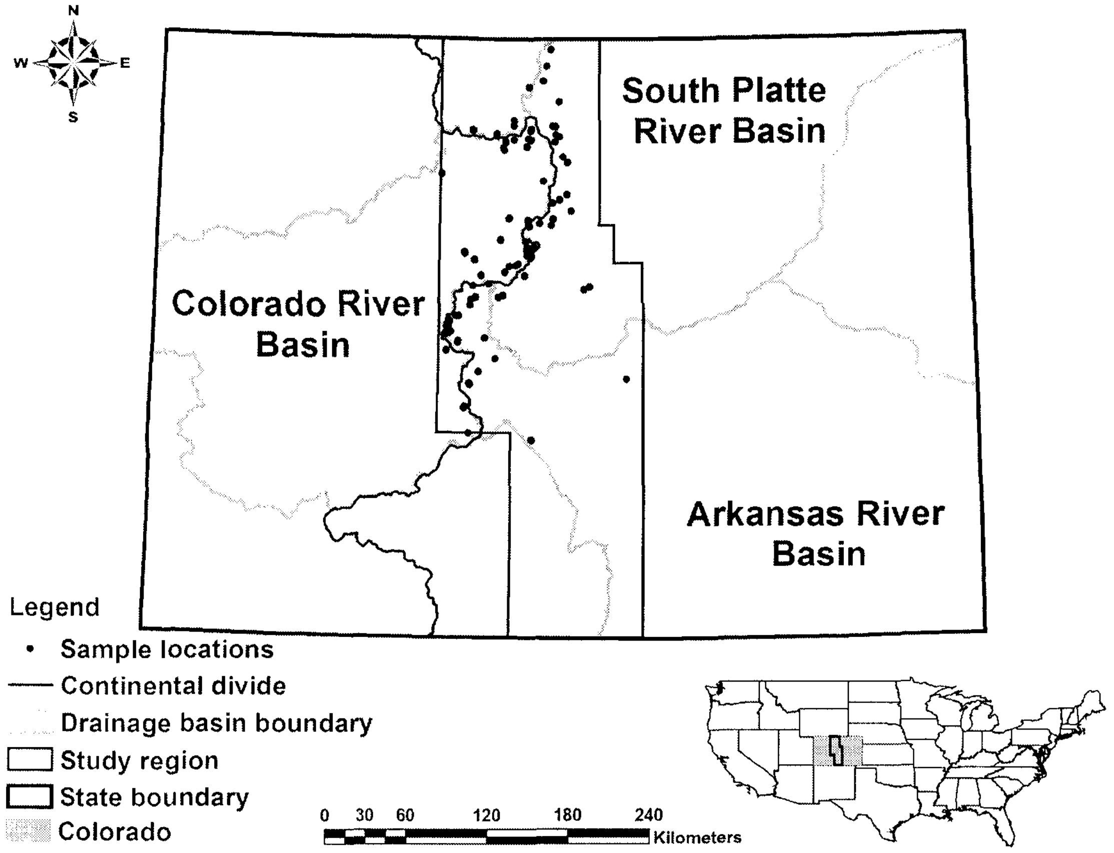

Figure 1. Map of the United States highlighting Colorado and the study domain of this project including the central Colorado Rocky Mountains from New Mexico to Wyoming.

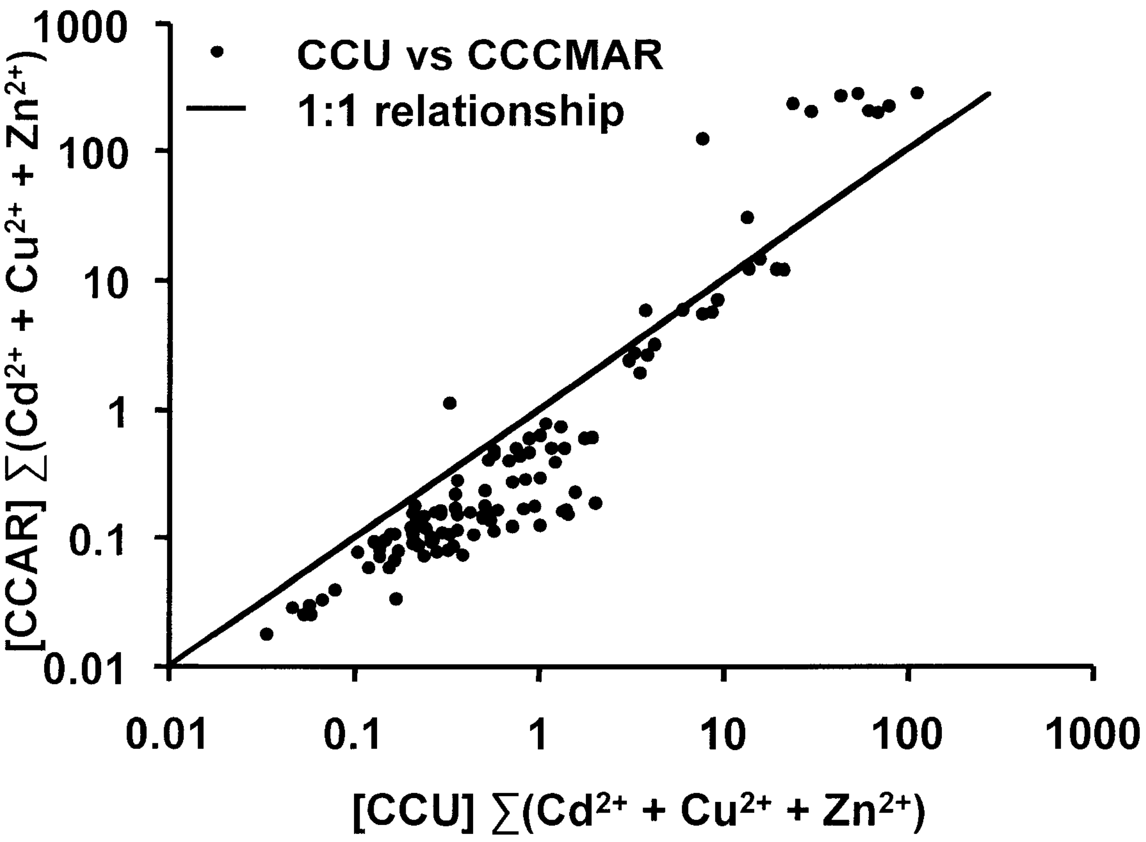

Figure 2. Graph depicts the relationship between $\log_{10}$(CCU) and $\log_{10}$(CCAR) at field sites showing that CCAR generally under-predicted trace-metal toxicity relative to CCU, except at high trace-metal concentrations where pH was < 6.0.

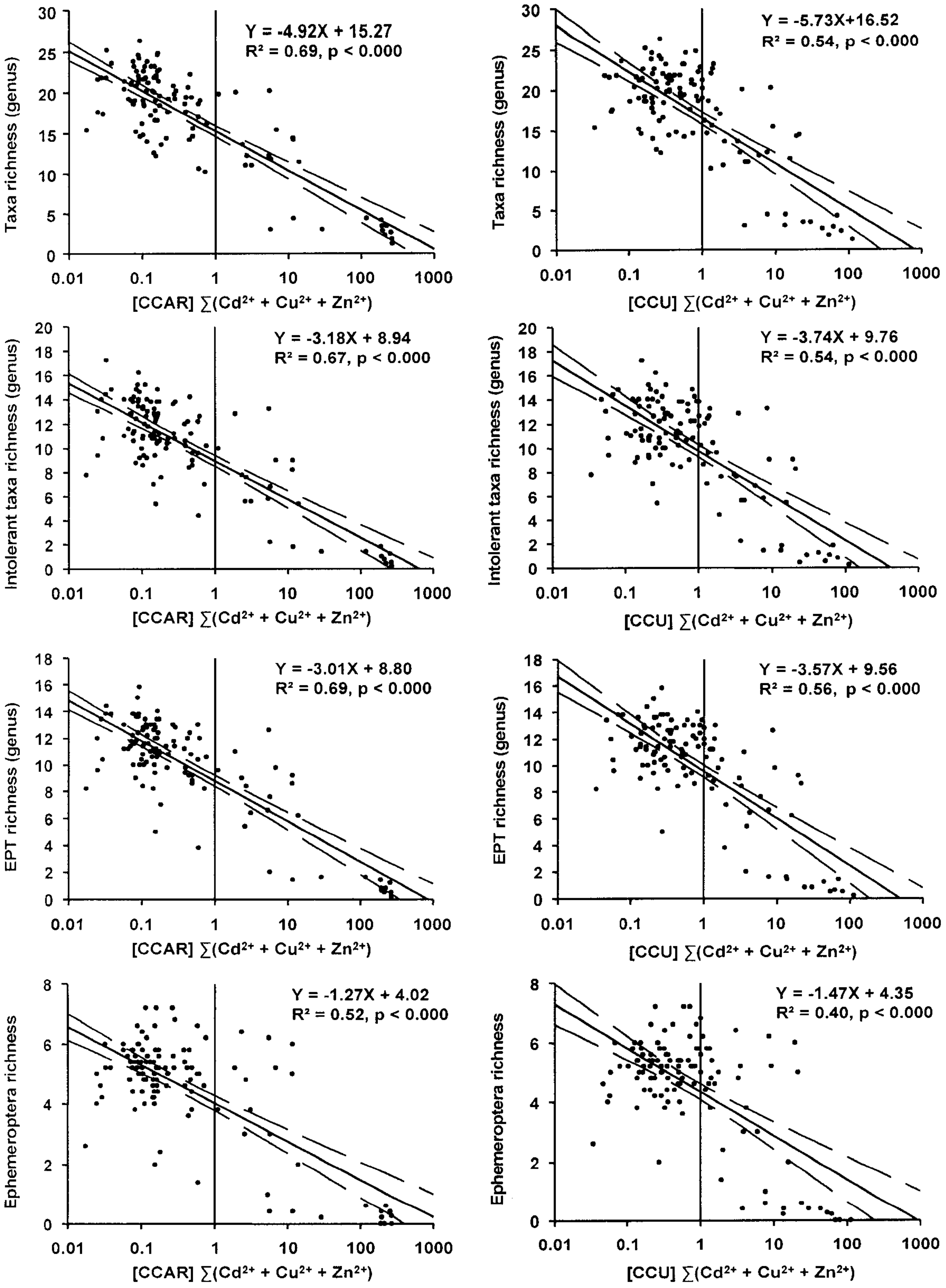

Figure 3. Simple linear regressions depicting the relationship between benthic macroinvertebrate community metrics and trace-metals measured by $\log_{10}$(CCAR) and hardness adjusted water quality criterion $\log_{10}$(CCU) for the sum of $\sum(Cd^{2+} + Cu^{2+} + Zn^{2+})$. Solid vertical lines indicate the limit at which chronic effects in aquatic species are expected. Solid line is the linear relationship between dependant and independent variable. Dashed lines are the 95% confidence interval of regression.

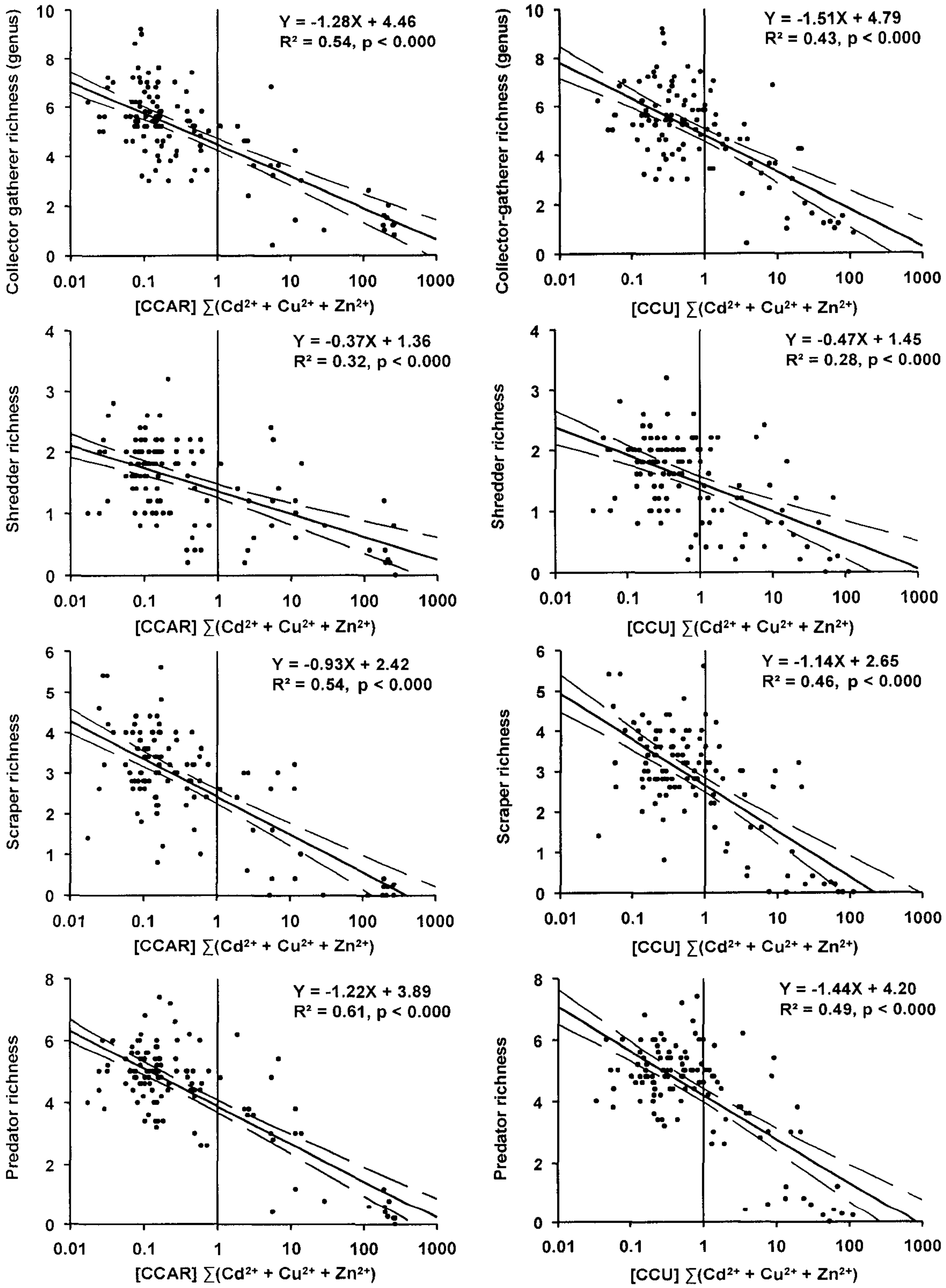

Figure 4. Simple linear regressions depicting the relationship between benthic macroinvertebrate community metrics and trace-metals measured by $\log_{10}$(CCAR) and hardness adjusted water quality criterion $\log_{10}$(CCU) for the sum of $\sum(Cd^{2+} + Cu^{2+} + Zn^{2+})$. Solid vertical lines indicate the limit at which chronic effects in aquatic species are expected. Solid horizontal line is the linear relationship between dependant and independent variable. Dashed lines are the 95% confidence interval of regression.

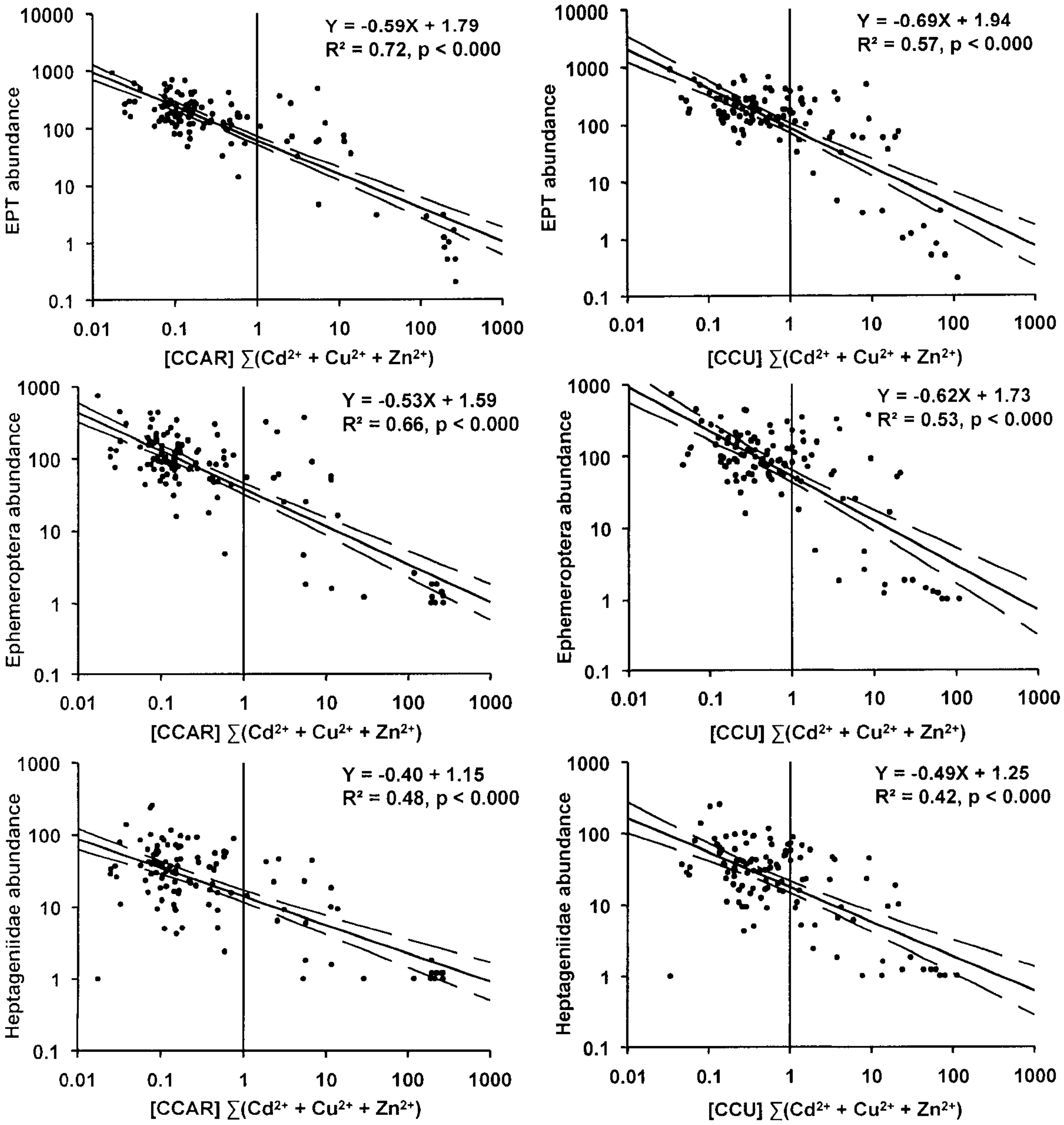

Figure 5. Simple linear regressions depicting the relationship between benthic macroinvertebrate community metrics and trace-metals measured by $\log_{10}$(CCAR) and hardness adjusted water quality criterion $\log_{10}$(CCU) for the sum of $\sum(Cd^{2+} + Cu^{2+} + Zn^{2+})$. Solid vertical lines indicate the limit at which chronic effects in aquatic species are expected. Solid line is the linear relationship between dependant and independent variable. Dashed lines are the 95% confidence interval of regression.

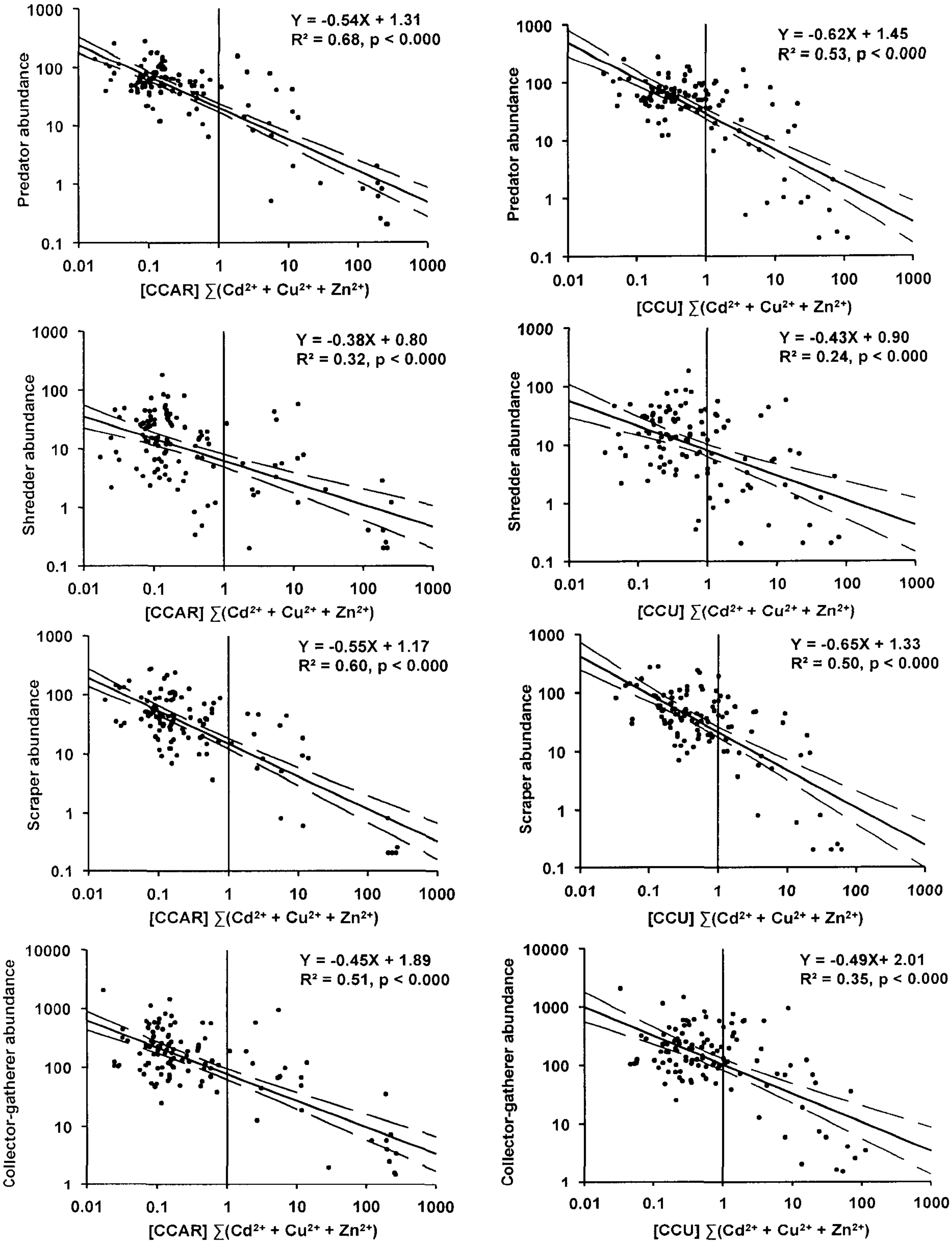

Figure 6. Simple linear regressions depicting the relationship between benthic macroinvertebrate community metrics and trace-metals measured by $\log_{10}$(CCAR) and hardness adjusted water quality criterion $\log_{10}$(CCU) for the sum of $\sum(Cd^{2+} + Cu^{2+} + Zn^{2+})$. Solid vertical lines indicate the limit at which chronic effects in aquatic species are expected. Solid line is the linear relationship between dependant and independent variable. Dashed lines are the 95% confidence interval of regression.

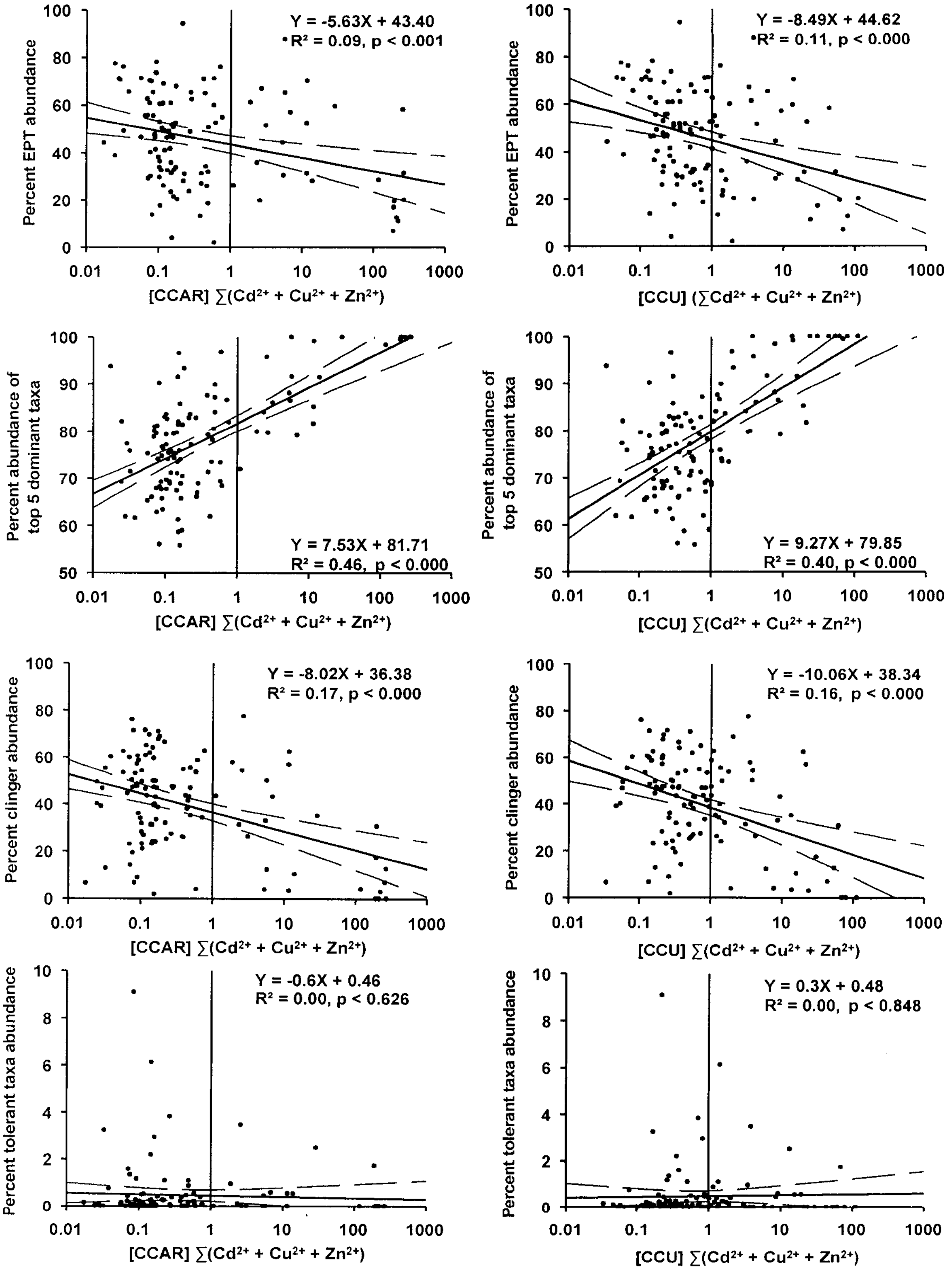

Figure 7. Simple linear regressions depicting the relationship between benthic macroinvertebrate community metrics and trace-metals measured by $\log_{10}$(CCAR) and hardness adjusted water quality criterion $\log_{10}$(CCU) for the sum of $\sum(Cd^{2+} + Cu^{2+} + Zn^{2+})$. Solid vertical lines indicate the limit at which chronic effects in aquatic species are expected. Solid line is the linear relationship between dependant and independent variable. Dashed lines are the 95% confidence interval of regression.

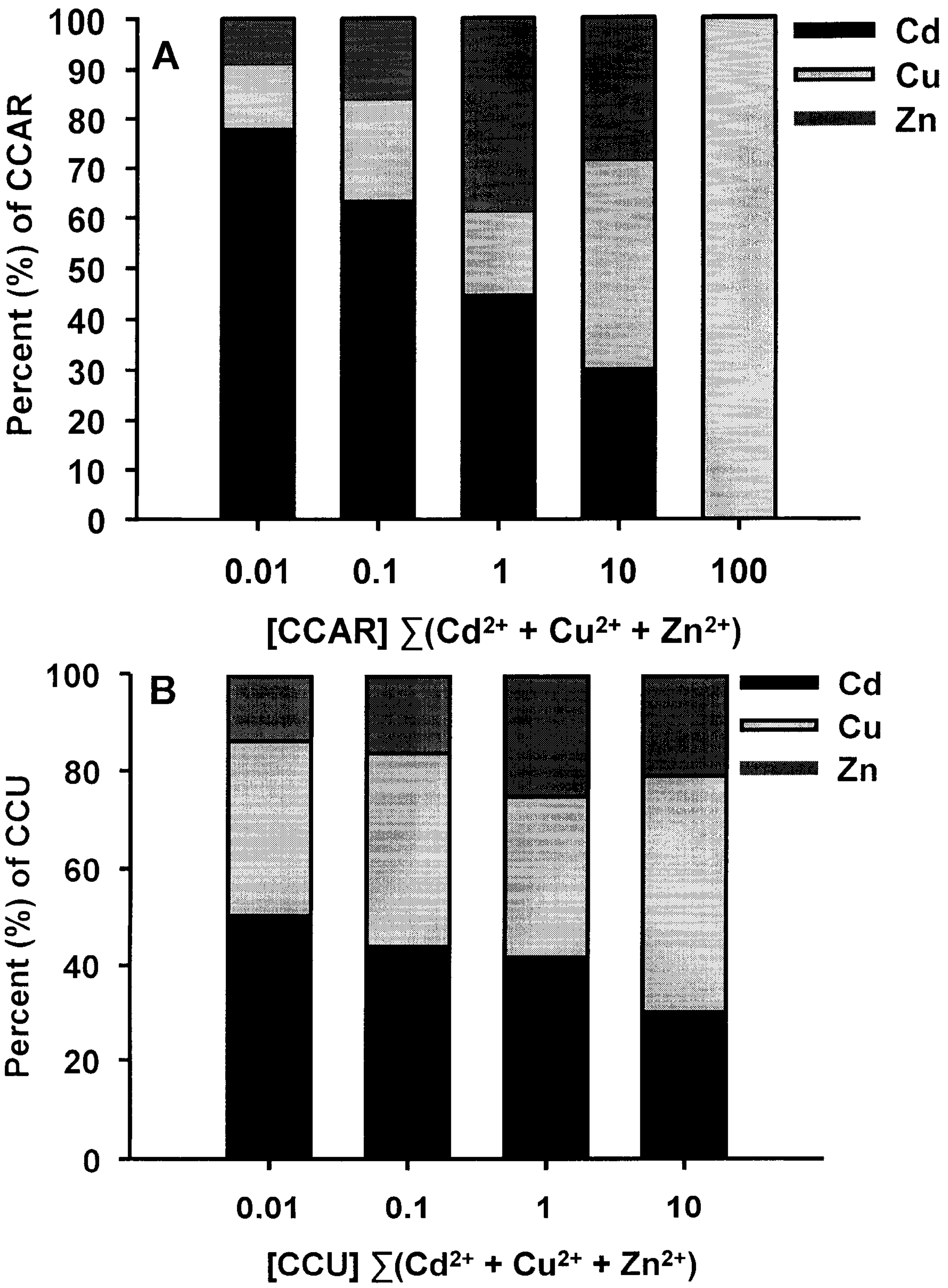

Figure 8. Relative contribution of each metal (Cd^{2+}, Cu^{2+}, Zn^{2+}) to (CCAR) (A) and hardness adjusted (CCU) (B).

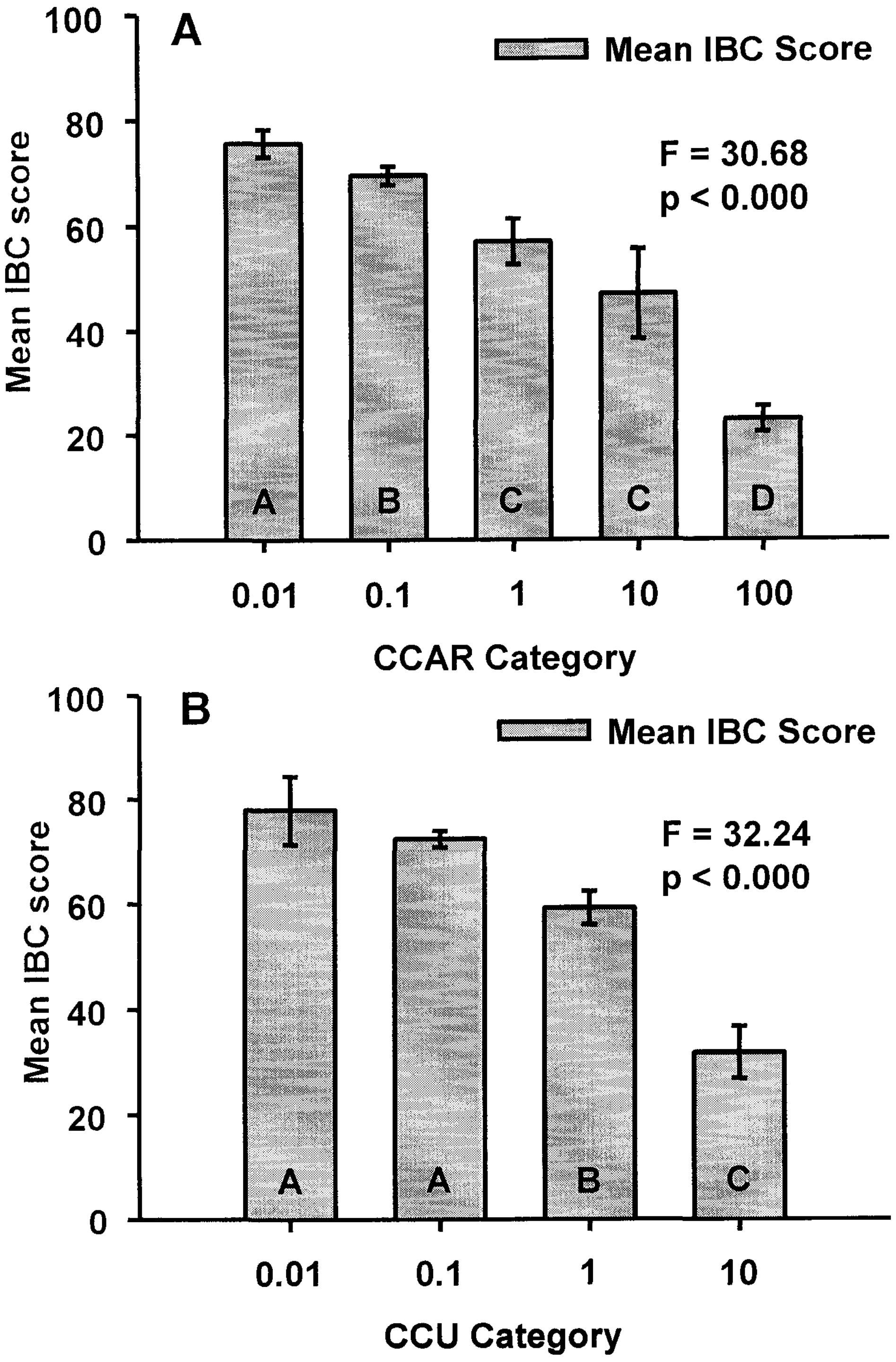

Figure 9. ANOVA and LSD results depicting statistical differences between mean IBC for categories of Continuous Criterion Accumulation Ratio (CCAR) (A) or Cumulative Chronic Units levels (B). Bars depict mean IBC scores (±S.D.); letters designate results of LSD tests where means with the same letter are not significantly different.

EPILOGUE

The goal of this dissertation was to work within the framework of the Central Colorado Assessment Project to develop and validate biological and geochemical tools for the assessment of historical mine influences on catchments in Colorado. Here I presented studies conducted to: 1) develop the Biotic Ligand Model (HydroQual 2003) for use in regional-scale bioassessments; 2) link natural geologic processes (i.e., rock type, age, hydrothermal alteration, presence/absence of pyrite) to trace-metal bioavailability as it affects aquatic organisms; and 3) test the new model of trace-metal toxicity, Chronic Criterion Accumulation Ratio (CCAR), for its ability to predict individual, population, and community responses in benthic macroinvertebrates. Results of these studies will be used to develop protocols for setting biological and geochemical baselines so that federal land managers can properly target and prioritize abandoned mine lands for reclamation to improve potable water quality and fisheries habitat in Colorado.

We developed a toxic unit model of additive trace-metal toxicity derived from BLM (HydroQual 2003). This model, CCAR, incorporates current theory about the interactions between aqueous constituents (i.e., hardness, DOC, pH) that affect trace-metal toxicity and the physiology of aquatic organisms which results in the accumulation of bioavailable trace-metal on an aquatic organism's respiratory surface, thereby causing toxicity. CCAR is capable of predicting toxicity due to trace-metals in natural waters derived from different geologies. In this way, we are capable of determining how the toxicity of a trace-metal changes from landscape to landscape.

CCAR is capable of predicting responses in benthic macroinvertebrates (e.g., individual, population, and community) to exposure to trace-metals. Because CCAR can predict ecologically-meaningful endpoints, it may be used for the assessment of the ecological integrity of mountain streams in Colorado (Clements 1997). We believe that CCAR can be used by federal land managers to describe the ecological condition of a stream influenced by trace-metals. CCAR can be used to determine to what extent stream geochemistry has been altered by mining activities, and how these changes in geochemistry affect aquatic organisms' exposure to trace-metals.

The goal of the Central Colorado Assessment Project is to develop a regional-scale geologic-based environmental assessment protocol to rank stream health for use by the U.S. Forest Service in developing their 10 year forest plan. The primary objectives of this environmental assessment are: 1) to develop environmental baselines for streams based on geologic classification and rank them; 2) to develop the Biotic Ligand Model as a bioassessment tool; 3) and to quantify the linkages between catchment-scale attributes (e.g. geology, land use) to local water quality, habitat, and benthic macroinvertebrate communities.

The next step in this project will be to add to these basic studies so that the appropriate protocols may be developed for the regional-scale environmental assessment. We need to augment the biological data to fully develop these environmental assessment protocols. We observed strong trends in the bioavailability of trace-metals among geologic classifications and determined that benthic macroinvertebrate communities were strongly correlated with geochemical gradients. However, we did not observe these same differences in benthic macroinvertebrate communities across geologic classifications. Because the classification scheme necessary to differentiate among geologic units was complex our limited biological data did not provide enough statistical power to differentiate among these geologic classifications. Additional biological data are necessary to develop clearer differences in benthic macroinvertebrate community responses to trace-metal bioavailability resultant from each geologic classification.

Although we identified those geologies with the potential to cause impairment to aquatic ecosystems, we did not measure the influence of mining in these catchments. Mining accelerates the natural weathering processes of rocks and can result in the altering of stream geochemistry and habitats (Wanty et al. 2002). However, mining in Colorado has been conducted using various methods (i.e., placer mining, shaft mining, pit mining) and intensities which may differentially impact stream geochemistry and habitat. The degree to which mining influences the geochemistry and habitat of streams above and beyond these natural processes is unknown and likely influenced by the type and intensity of mining in the catchment. Future work will be required to determine how to characterize mining effects on stream chemistry and habitat in order to properly develop geochemical and biological baselines. This information could then be used to determine the extent of mining affects on aquatic resources in Colorado.

In this research, we gained considerable insight into how geology directly affects water quality, but also gained a better understanding into the indirect effects of geology on water quality (Figure 10 of chapter 1). Rock-type, geologic age, and hydrothermal alteration are geologic factors operating at temporally and spatially different scales to control trace-metal bioavailability. Some of these factors are nested within geologic classifications, thus enhancing trace-metals bioavailability under certain combinations while other times not causing obvious changes in water quality. We also found sufficient evidence to suggest some geologic units may be sources of DOC which can ameliorate trace-metal toxicity (Figure 7, chapter 1). However, past researchers have found stronger relationships between vegetation, soil, and basin morphology and the concentration of DOC in surface waters (Prusha & Clements 2004, Frost et al. 2006, Baron et al. 1991). Vegetation and soil development and basin morphology are all indirect characteristics of catchments controlled by parent geology and geologic processes. Future investigations should focus some effort on determining the indirect effects of catchment geology on stream geochemistry.

Geology also indirectly influences stream habitat. We have observed that hydrothermal alteration and rock-type can affect the weathering of catchment bedrock. It is likely that the weathering rate of catchment bedrock may be linked to the size distribution of the cobble substrate across these geologic classifications. Changes in substrate composition are known to influence the structure and function of benthic macroinvertebrate communities (Richards et al. 1996, McRae et al. 2004, Cannan & Armitage 1999, Pouilly et al. 2006). Future investigations should determine to what extent geologic weathering alters stream habitat thereby causing changes in benthic macroinvertebrate communities.

Elevation and stream size cause important changes in benthic macroinvertebrate community structure and function and are catchment-scale attributes indirectly influenced by catchment geology (Kiffney & Clements 1994, 1996, Clements & Kiffney 1995). Changes in life-history between habitat types were hypothesized to cause changes in species sensitivities across sites which can cause confounding results in regional-scale bioassessments in mountainous regions. However, the spatial relationships between elevation, stream order, and trace-metal bioavailability were not evaluated in this research. Future investigations will need to determine: 1) if differences in life histories caused by these longitudinal gradients cause changes in species sensitivity to trace-metals; 2) if the spatial orientation of geologies which produce elevated trace-metals and other aqueous constituents that control trace-metal bioavailability vary with elevation; and/or 3) if these processes work together to confound bioassessments at regional-scales.

Now that we have identified potential direct and indirect effects of geology on the structure and function of aquatic communities in the mineralized belt of Colorado, future investigations should use modeling approaches which can accurately quantify these relationships. Here I have presented a diagram (Figure

10, chapter 1) depicting the direct effects of catchment geology on geochemistry and benthic macroinvertebrates including some hypothesized indirect effects. This conceptual diagram could be combined with the results of future investigations into the indirect effects of geology on stream geochemistry, habitat, and benthic macroinvertebrates. These conceptual models could quantify the total, indirect, and direct effects of geology on both water quality parameters and benthic macroinvertebrate communities through the use of path analysis (Wright 1921).

Similar to regression, path analysis partitions simple correlations among variables based on *a priori* hypothesized relationships (Wright, 1921, 1934). Development of a path model translates an *a priori* hypothesis into a mechanistic conceptual model known as a path diagram (Figure 10, Chapter 1). Path analysis is capable of detecting hierarchical interactions among variables and measuring the direct and indirect interactions among model descriptors and the response variables. This modeling approach more accurately depicts the relationships between multiple causal and response variables which comprise ecosystems, than traditional regression techniques

The importance of the use of path analysis in this research is threefold: 1) path analysis produces more reliable results than regression techniques; 2) results are depicted as mechanistic conceptual diagrams easily understood by federal land managers; and 3) path analysis allows for the development of direct and indirect interactions among observed model variables which are more realistic representations of the nested-hierarchical nature of ecosystems. Path analysis will allow us to determine the importance of reach- and catchment-scale attributes and how they respond both directly and indirectly to land use. Land-use managers will in turn be able to develop land use strategies to improve stream health and incorporate monitoring data into the model to measure the appropriate response of the system.

The path models developed with this protocol can also be used to extrapolate the results to the entire domain of the project area (chapter 1, figure 1). These path models could predict local stream health based on catchment-scale variables such as vegetation, land use and geology -- all parts of the GIS component of CCAP. By re-introducing the path models into the GIS modeling environment, we can use catchment-scale predictors of local stream health to model stream health in areas within the study domain but not previously sampled. The results of this extrapolation would be a map depicting stream health throughout the Central Colorado Mineral Belt, specifically identifying areas with impaired stream health caused by natural geology, historic mining activities or land use.

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