Integrative Bioassessment of Acid Mine Drainage Impacts on the Upper Powell River Watershed, Southwestern Virginia

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(ABSTRACT)

Acid mine drainage (AMD), a result of oxidation of minerals containing reduced forms of sulfur (pyrites, sulfides) upon exposure to water and oxygen, is an environmental problem associated with abandoned mined lands (AML). Numerous studies have documented the impacts of AMD upon aquatic communities within acidified stream reaches; these impacts include reduced taxonomic richness and abundance, and/or a shift from pollution sensitive to pollution tolerant species. This dissertation comprises a number of integrative assessments and experiments conducted to investigate the nature of AMD ecotoxicity in the upper Powell River watershed. Emphasis was placed upon bioassessment methodologies and AMD impacts beyond the zone of pH depression. Major findings and processes developed included: 1) an Ecotoxicological Rating (ETR) system was developed that integrates chemical, toxicological, and ecological data into a single value depicting the relative environmental integrity of a given station within a watershed; 2) water column chemistry rather than sediment toxicity was the major factor causing acute toxicity to aquatic biota in close proximity to AMD discharges; 3) solid ferric hydroxide can cause acute toxicity to standard test organisms in the absence of dissolved iron; 4) Asian clams (*Corbicula fluminea*) can be used to detect both acutely toxic AMD inputs and nutrient loading in low order streams, and clam responses of survival and growth reflect those of indigenous communities to the two contaminant types; 5) aluminum (Al) in transition from acidic to neutral pH waters can cause acute toxicity to aquatic invertebrates, and may be the cause of impaired benthic macroinvertebrate communities in neutral pH (>7.0) waters downstream of an acidic tributary; 6) in the larger river system (North Fork Powell and Powell mainstem), urban inputs appear to have a greater influence upon aquatic communities than metal loading from AMD impacted tributaries; 7) the use of individual level assessment endpoints, such as Asian clam growth in *in situ* toxicity tests, eliminates variables that may confound attribution of community level impacts to contaminants; and 8) the near elimination of predatory stoneflies (Plecoptera) downstream of the Stone/Straight Creek tributary to the North Fork Powell River was associated with water column Al concentrations.

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Preface

The upper Tennessee River system, including the Clinch, Holston and Powell Rivers in southwestern Virginia, contains aquatic habitat richer in freshwater mussel species than any other area of the United States. Of the approximately 73 species found in Virginia, 34 are either endangered or threatened, and most of those listed species reside in the tributaries of the upper Tennessee River drainage (Neves 1991). The lower Powell River system provides habitat for a number of mussel species including some endangered ones; however, all mussel fauna in the upper Powell River have been eliminated above Dryden, Virginia, ~ 265 river-km upstream of its confluence with the Clinch River (Neves et al. 1980). Some researchers have attributed the decline in Powell River mussel populations to coal waste, metal loading, and sedimentation resulting from active and abandoned coal mining operations (McCann, 1993; Temple, 1997; Wolcott, 1990). In an effort to further our understanding of factors potentially limiting the recolonization of the Powell by native mussels, this research project was developed to investigate the ecotoxicology of abandoned mined land (AML) associated inputs. In particular, acid mine drainage (AMD) impacts upon benthic macroinvertebrates were evaluated and compared to those produced by other nonpoint source-pollution inputs (i.e, nutrients and urban runoff).

Areas mined prior to the 1977 Surface Mining Control and Reclamation Act (SMCRA) and inadequately reclaimed may be considered AML. They introduce two major types of pollution to aquatic systems: sedimentation from surface runoff on exposed soils, and AMD. While sedimentation destroys aquatic habitat by filling gaps between cobbles and pebbles and homogenizing streambeds, AMD causes a variety of severe environmental problems. AMD occurs when minerals containing reduced forms of sulfur (usually pyrite, FeS_2, in the Appalachian region of the United States) are exposed to oxygenated water. This process is often associated with coal mining because pyrite is a common component of coal and associated geologic strata. The reaction between pyrite and oxygenated water results in the production of sulfuric acid, iron flocculent, and the mobilization of acid soluble metals (e.g., aluminum, copper, zinc, and lead) leached from minerals exposed to the acid or from the initial pyrite reaction. This complex mixture of pollutants creates the potential for a variety of environmental impacts.

This dissertation is divided into two major themes: 1) bioassessment techniques for small AMD impacted sub-watersheds (including chapters 1 through 3), and 2) AMD impacts upon aquatic biota beyond the zone of pH depression (including chapters 4 through 6). Integrative assessment techniques, those employing a variety of different types of assessment parameters (which are simply called endpoints throughout this work) including chemical, ecological, and toxicological parameters, were utilized throughout the research project. A special emphasis also was placed upon the use of multiple levels of biological organization, particularly individual and community level endpoints, to address questions of causality of observed impairments. The following is a brief summary of the six dissertation chapters, and a figure and table describing the locations of the sampling stations.

Chapter 1 documents the results of an integrative bioassessment conducted at 20 stations within the Puckett's Creek watershed, Lee County, Virginia (Figure 1), a tributary to the North Fork of the Powell. A number of different assessment techniques were employed including chemical/metals analysis, benthic macroinvertebrate community sampling, and water column, sediment, and *in situ* toxicity testing. The significant contribution of this study was the development of a technique to combine these different types of data into a single relative value called the Ecotoxicological Rating (ETR). The ETR was based upon a similar one developed by Cherry et al. (1999) and used two chemical, two ecological, and two toxicological parameters to rank stations within the watershed according to their relative environmental integrity. The ETR was highly sensitive to the various types of AMD inputs in the watershed (i.e., intermittent, continuously acidic, neutralized, etc.), and the approach was adopted by the U.S. Army Corps of Engineers to assess other sub-watersheds within the North Fork of the Powell.

Chapter 2 compared transplanted Asian clam (*Corbicula fluminea*) responses to AMD and nutrient inputs in the Puckett's Creek watershed to those of indigenous benthic macroinvertebrate communities. The experimental use of individuals (clams) isolated variables that can confound community level studies, while examination of invertebrate community impacts provided ecological relevance to the clam responses. Clam survival distinguished between two different levels of impact due to acidic, neutralized, and intermittent AMD inputs, and was positively correlated with water column pH and negatively correlated with conductivity and metal concentrations. Survival also was positively correlated with abundance of the most sensitive macroinvertebrate taxonomic group to AMD in this system. Clam growth was not related to AMD inputs, but was positively correlated with nitrate concentrations and the relative abundance of organisms indicative of nutrient loading. These results suggest that transplanted clam studies accurately reflect benthic macroinvertebrate community responses to multiple stressors from nonpoint sources in low order streams.

Chapter 3 was an investigation into the relative contributions of water column and sediment toxicity within a severely AMD impacted reach of Puckett's Creek. Water chemistry (pH, conductivity and metal concentrations) was found to be the strongest correlate with both water column and sediment acute toxicity test survival of *Daphnia magna*. This suggested that water column pH and metals were largely responsible for impacts within this stretch, and sediment bound metals were not as important, even at sites with neutral pH values. Acidic pore water collected along with sediment samples was suggested as being responsible for toxicity in sediment tests. An additional finding documented in this chapter was that solid ferric hydroxide can be a cause of direct toxicity (through ingestion or suffocation) to aquatic organisms in addition to its probable indirect effects due to smothering of habitat and preventing primary production.

Chapter 4 was the first study to document benthic macroinvertebrate community impacts due to aluminum in neutral pH surface waters. As in chapter 2, experimental responses of individuals, in this case *Ceriodaphnia dubia*, were used to provide confidence in determining the cause of benthic macroinvertebrate community impairment. Whole Effluent Toxicity (WET) tests were conducted with simulated AMD effluents containing combinations of acid and metals (Al, Fe, Cu, and Zn) in concentrations similar to those found in the field located AMD discharge. Aluminum was found to be the major toxic component of the effluent, and possible toxic mechanisms were suggested.

The objective of chapter 5 was to compare the effects of metal loading from AMD impacted tributaries of the Powell system to those produced by inputs from three urban centers using both individual (growth of *Corbicula fluminea*) and community level (benthic macroinvertebrate community richness and balance) endpoints. Contrary to our initial hypothesis, urban runoff had a greater adverse impact than AMD inputs, and benthic macroinvertebrate community impairment observed downstream of urban areas (i.e., reduced diversity and richness) was corroborated by reduced clam growth. The use of two levels of biological organization provided confidence in attributing community impairment downstream of urban inputs to contaminants rather than to other factors that may confound studies aimed only at higher levels of organization. These findings implicate urban inputs as potential factors in limiting recolonization of endangered freshwater mussels.

Finally, chapter 6 was a quantification of impairment to common stoneflies, downstream of the AMD impacted Stone/Straight Creek in the North Fork of the Powell River. In the course of the two-year study common stoneflies were found at one site 6 miles downstream of the tributary on one occasion. We conducted metals analysis of the water, sediments and resident invertebrate tissues (predators and primary consumers), and found that water column Al concentration was the strongest descriptor of impacts to the stonefly predators. These data indicate that further work should be conducted to determine the chronic toxicity of Al to stoneflies at neutral pH values.

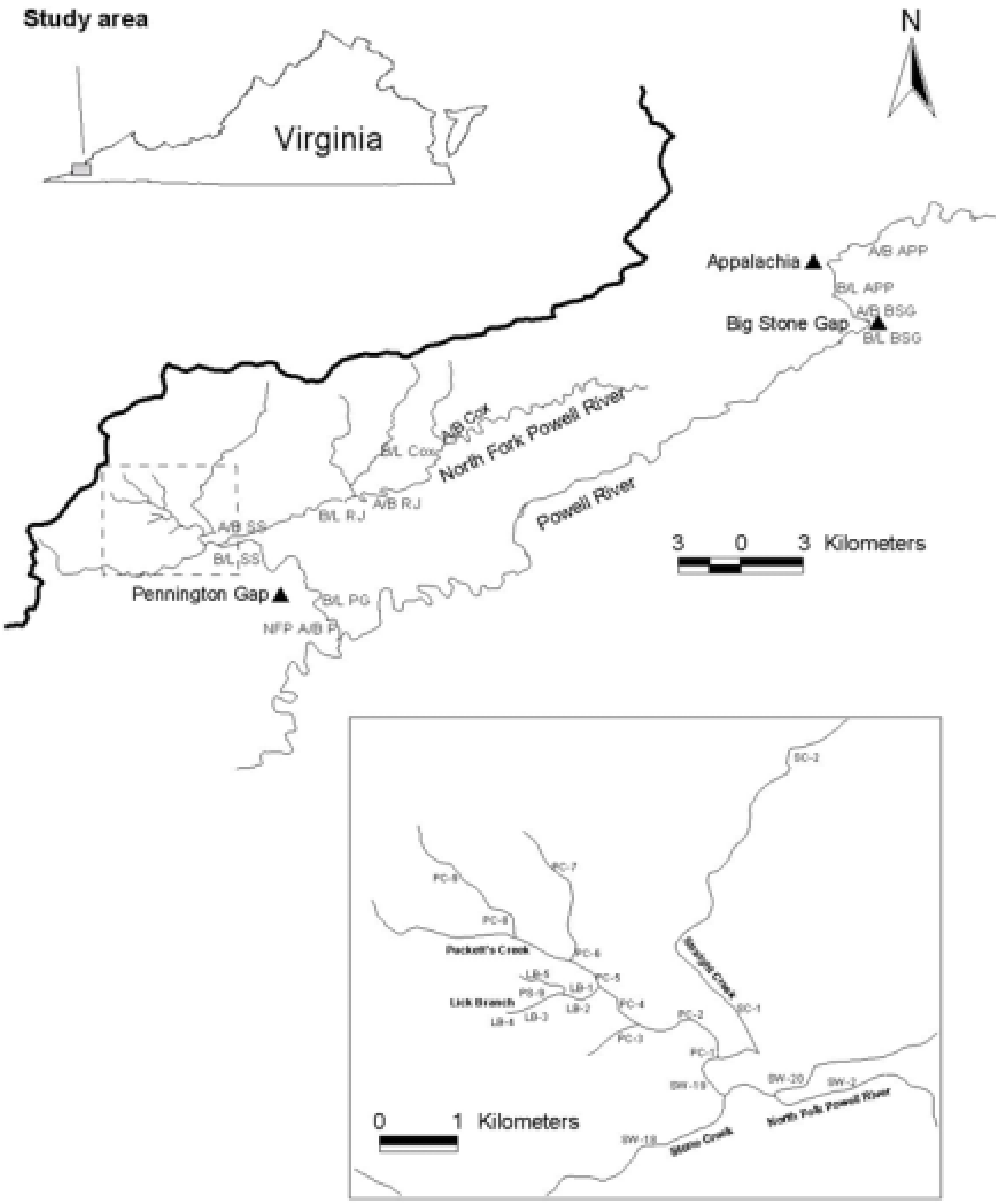

Figure 1. Map of upper Powell River watershed indicating all sampling stations used in this research.

Table 1. Master list of sampling station names, descriptions, and indication of chapter(s) in which stations were used.

Site Name	Location	Type*	Chapter
PC-9	Puckett's Creek	A/B AMD (1)	1,2
PC-8	Puckett's Creek	A/B AMD (1)	1,2
PC-7	Puckett's Creek	A/B AMD (1)	1,2
PC-6	Puckett's Creek	B/L AMD (2)	1,2
PC-5	Puckett's Creek	B/L AMD (2)	1,2
PC-4	Puckett's Creek	B/L AMD (4)	1,2,3,4
PC-3	Puckett's Creek	A/B AMD (1)	1,2
PC-2	Puckett's Creek	B/L AMD (4)	1,2,3,4
PC-1	Puckett's Creek	B/L AMD (4)	1,2,3,4
LB-5	Lick Branch	B/L AMD (2)	1,2,3
LB-4	Lick Branch	A/B AMD (1)	1,2,3
LB-3	Lick Branch	B/L AMD (3)	1,2,3,4
LB-2	Lick Branch	B/L AMD (3)	1,2,3
LB-1	Lick Branch	B/L AMD (3)	1,2,3
PS-9	Lick Branch	B/L AMD (3)	1,2,3
SC-2	Straight Creek	A/B AMD (1)	1,2
SC-1	Straight Creek	B/L AMD (5)	1,2
SW-18	Stone Creek	B/L AMD (5)	1,2
SW-19	Straight Creek	B/L AMD (5)	1,2
SW-20 = A/B SS	North Fork Powell	A/B AMD	1,5,6
SW-2 = B/L SS	North Fork Powell	B/L AMD (5)	1,2,5,6
A/B Cox	North Fork Powell	A/B AMD	5
B/L Cox	North Fork Powell	B/L AMD	5
A/B RJ	North Fork Powell	A/B AMD	5,6
B/L RJ	North Fork Powell	B/L AMD	5,6
B/L PG	North Fork Powell	B/L AMD/Urban	5,6
NFP A/B P = NFP WW	North Fork Powell	B/L AMD/Urban	6
A/B App	Powell River	A/B Urban	5
B/L App	Powell River	B/L Urban	5
A/B BSG	Powell River	A/B Urban	5
B/L BSG	Powell River	B/L Urban	5

*Numbers in parentheses under "type" column indicate station group types as described in chapters 1 and 2. A/B AMD = station located upstream of acid mine drainage inputs, B/L AMD = station located downstream of acid mine drainage inputs, A/B Urban = station located upstream of urban runoff inputs, B/L Urban = station located downstream of urban runoff inputs.

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THEME I.

Bioassessment Techniques for Small Acid Mine Drainage Impacted Watersheds

Chapter 1. Laboratory to field validation in an integrative assessment of an acid mine drainage impacted watershed.

Chapter 2. In situ studies with Asian clams (*Corbicula fluminea*) detect acid mine drainage and nutrient inputs in low order streams.

Chapter 3. Relative Acute Toxicity of Acid-Mine Drainage Water Column and Sediments to *Daphnia magna* in the Puckett's Creek Watershed, VA, USA.

Chapter 1.

Laboratory to field validation in an integrative assessment of an acid mine drainage impacted watershed.

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Abstract—An integrative assessment was conducted in the Puckett's Creek watershed, southwestern Virginia, to investigate environmental impacts of acid mine drainage (AMD) inputs. Twenty-one sampling stations were categorized based on five degrees of AMD input: (1) none, (2) intermittent acidic/circum-neutral AMD, (3) continuous acidic AMD, (4) continuous circum-neutral AMD, and (5) receiving system stations with at least two levels of dilution. Bioassessment techniques included water/sediment chemistry, benthic macroinvertebrate sampling, laboratory acute water column toxicity testing, laboratory chronic sediment toxicity testing, and in situ toxicity testing with Asian clams (*Corbicula fluminea* (Müller)). Group 3 stations had significantly altered water chemistry (low pH, high conductivity, and high water column metals) relative to the other groups, and significantly higher sediment iron concentrations. Both group 3 and group 4 had significantly decreased Ephemeroptera-Plecoptera-Trichoptera (EPT) richness and percent Ephemeroptera abundance relative to unimpacted stations. Group 3 also had decreased total taxon richness. Water column toxicity testing was sensitive to AMD impacts with samples from group 3 being significantly more toxic than those from groups 2 and 4, which in turn were more toxic than those from groups 1 and 5. Similar results were observed for in situ toxicity testing. No differences in sediment toxicity test survival and impairment results were observed among the station groups. Stepwise multiple linear regression and simple bivariate correlation analyses were used to select parameters for use in an Ecotoxicological Rating (ETR) system. The ETR was successful in differentiating between two levels of environment impact relative to stations receiving no AMD input.

Introduction

The United States Environmental Protection Agency (U.S. EPA) has singled out acid mine drainage (AMD) from abandoned coal mines as being the greatest water quality problem in Appalachia (Office of Surface Mining, 1995). Acid mine drainage water is produced when pyrite (FeS_2) rich coal and/or bedrock are exposed to oxygen and water. Through a number of reactions (some catalyzed by iron oxidizing bacteria), pyrite is oxidized, eventually to form ferric hydroxides and sulfuric acid. If produced in sufficient quantity, iron precipitate, heavy metals (depending on soil mineralogy) and sulfuric acid will contaminate surface and groundwater. There are 557,650 abandoned mines throughout the US, and the potential environmental impact of AMD is immense (U.S. Water News, 1993).

Several investigators have examined the effects of AMD on benthic macroinvertebrates (Armitage, 1980; Cherry et al., 1995; Herricks and Cairns, 1974; Roback and Richardson, 1969; Rutherford and Mellow, 1994; Smith and Frey, 1971; Winterbourn and McDiffet, 1996) and fish (Rutherford and Mellow, 1994), generally revealing reduced diversity in impacted areas relative to unimpacted ones and/or species shifts from intolerant to tolerant taxa. Compared to field investigations, fewer researchers have examined acid mine drainage water column and/or sediment toxicity in the laboratory (Fucik et al., 1991; Kemble et al., 1994; Tan and Coler, 1986;Vinyard, 1996).

The present study employed three different bioassessment techniques in addition to water/sediment chemistry and benthic macroinvertebrate sampling: (1) laboratory water column toxicity testing with *Ceriodaphnia dubia*, (2) sediment toxicity testing with *Daphnia magna* and *Chironomus tentans* and (3) in situ toxicity testing with the Asian clam (*Corbicula fluminea*). The purpose of the research was to determine the sensitivity of ecological, toxicological, and chemical assessment techniques to several different levels of AMD impact. In addition, a multiple linear regression analysis (MLRA) was employed to determine which combination of chemical and toxicological variables constructed the best models to predict benthic macroinvertebrate assemblages. The MLRA, along with simple bivariate correlation analysis was further used to develop an Ecotoxicological Rating (ETR) system which assigned numerical values that characterized the relative degree of environmental impact at each station.

Materials and methods

Sampling stations/Station groups

Twenty-one sampling stations were selected in the Puckett's Creek watershed and its receiving system in Lee County, southwestern Virginia. Puckett's Creek flows via Straight Creek and Stone Creek into the North Fork of the Powell River, Tennessee River drainage. Stations were selected to provide upstream and downstream representation of the major AMD inputs in the watershed. Fifteen stations were in either Puckett's Creek or its tributary, Lick Branch, which contained two AMD point sources. The remaining six stations were at sites upstream and downstream of Puckett's Creek in Stone Creek, Straight Creek and the North Fork of the Powell, which made up the receiving system.

For ecotoxicological analysis, the 21 stations were categorized based on level of AMD input as determined by location and mean pH. The first group (hereafter called group 1, n=7) consisted of stations that were upstream of all known AMD inputs either in Puckett's Creek, Lick Branch, Straight Creek, or the North Fork of the Powell. The second group (2, n=3) consisted of three stations subjected to intermittent AMD input, designated based on having wide pH ranges over time (e.g., 3.17 – 6.30 for station LB-5), or being downstream of such a station but upstream of any continuous AMD input. The third group (3, n=4) consisted of those stations in Lick Branch continuously subjected to AMD input and having acidic mean pH values (≤ 4.50), while the fourth group (4, n=3) consisted of stations in Puckett's Creek below the confluence of Lick Branch. These stations were continuously subjected to AMD input but had circum-neutral mean pH values (6.11 – 7.42) because of dilution. Therefore, group 4 stations will be referred to as neutral AMD stations rather than neutral mine drainage stations. The final group (5, n=4) consisted of stations downstream of Puckett's Creek in Straight Creek, Stone Creek and the North Fork of the Powell. While group 5 stations were downstream of AMD inputs, they were differentiated from group 4 as having a higher level of dilution. Group 4 stations were in a third order stream (Puckett's Creek), while group 5 stations all were in greater than third order streams.

Water column and sediment chemistry

Water samples from the 21 stations were collected for analysis of selected water quality parameters in October 1997, January, February, May, and July 1998. Samples either were brought to the laboratory, stored for 24 h at 4° C and measurements taken under laboratory conditions, or measurements were taken under field conditions. The pH was measured using an Accumet® (Fisher Scientific, Pittsburgh, PA, USA) pH meter equipped with an Accumet® gel-filled combination electrode (accuracy < ± 0.05 pH at 25 °C). A Yellow Springs (RDP, Dayton, OH, USA) model 54A meter was used to measure dissolved oxygen. Conductivity measurements were made using a Hach® (Hach, Loveland, CO, USA) conductivity/TDS meter. Alkalinity and hardness were measured by titration as described in APHA et al. (1992). In addition, water column and sediment samples were collected for metals analysis by Spectrum Laboratories, Coeburn, Virginia. Filtered (0.45 μm pore size) water samples were analyzed for aluminum (Al), iron (Fe), and manganese (Mn) by atomic absorption spectrophotometry or inductively coupled plasma spectrometry as appropriate. Sediment samples (collected when samples were collected for sediment toxicity testing) were digested using 50% (v/v) nitric and 20% (v/v) hydrochloric acid (Fisher Scientific, Pittsburgh, PA, USA) with reflux heating according to U.S. EPA (1991) methods. Samples then were analyzed for total Al, copper (Cu), Fe, Mn, and zinc (Zn) using atomic absorption spectrophotometry or inductively coupled plasma spectrometry as appropriate. Lower detection limits (mg/L) for metals were 0.001, 0.002, 0.002, 0.001, and 0.002 for Al, Cu, Fe, Mn, and Zn, respectively.

For statistical analysis, mean values for individual sampling stations were calculated for parameters of which more than one measurement was taken (i.e., pH and conductivity). Then, mean values for station groups 1 through 5 were compared by analysis of variance (ANOVA) using JMP IN ® software (Sall and Lehman, 1996). The Tukey-Kramer Honestly Significant Difference (HSD) post-hoc test was used for pair-wise analysis at the $\alpha = 0.05$ level.

Benthic macroinvertebrate sampling

Benthic macroinvertebrate surveys were conducted according to the US Environmental Protection Agency (U.S. EPA/444 (4-89-001)) Rapid Bioassessment Protocols (RBP) (Plafkin et al., 1989). Riffle, run, pool and shoreline rooted areas were thoroughly sampled for 20 min per site using dip nets with an 800-µm mesh. Two replicate samples were collected per site. Organisms were identified to the lowest practical taxonomic level (usually genus) using standard keys (Merritt and Cummins, 1996; Pennak, 1989). Community indices calculated included total abundance, total taxon richness, Ephemeroptera-Plecoptera-Trichoptera (EPT) richness, percent EPT abundance, and percent Ephemeroptera abundance. Index values for replicate samples were combined to obtain mean index values per station (i.e. mean taxon richness etc.). As with statistical analyses for water and sediment chemistry, mean community index values for station groups 1 through 5 were compared using ANOVA and pair-wise analyses as described previously at the $\alpha = 0.05$ level.

In situ clam toxicity testing

Asian clams (*Corbicula fluminea* (Müller)) used for testing were collected from the New River near Ripplemead, Virginia, using clam rakes. Clams were held in Living Streams® (Toledo, OH) at the Ecosystem Simulation Laboratory, Virginia Tech, Blacksburg, VA, until use in toxicity testing. Testing procedures consisted of tying five mesh bags, each bag containing five clams, to stakes at each sampling station. Bags were 18 cm wide by 36 cm long with a mesh size of ~0.5 cm^2. At the end of 31 d, clam bags were collected from each testing station and transported on ice to the laboratory at

Virginia Tech. Clams were counted as dead or alive; clams found with valves separated or that were easily opened were considered dead. Mean clam survival values for station groups 1 through 5 were compared using ANOVA and pair-wise analyses as described previously at the $\alpha = 0.05$ level.

Water column toxicity testing

Water column samples from the stations were tested on three occasions for 48-h acute toxicity to the cladoceran, *Ceriodaphnia dubia*. Samples were collected in February, May and July of 1998. Test organisms, raised by Virginia Tech personnel, were cultured in filtered water from a non-toxic reference stream near Newport, Virginia (Sinking Creek). Analysis of the culture water indicated that metal concentrations were low to undetectable (1.6 μg Al/L, 14.3 μg Fe/L, and Cu and Zn were below detection limits). Average pH, conductivity, alkalinity, and hardness for culture/diluent water was 8.01 ± 0.10, 225 ± 5.48 μmhos/cm, 131 ± 9.45 mg/L as $CaCO_3$, and 122.6 ± 4.16 mg/L as $CaCO_3$, respectively.

Prior to testing, organisms were fed a diet of *Selenastrum capricornutum* and a Yeast-Cereal Leaves-Trout Chow (YCT) mixture at a rate of 0.18 ml each per 30-ml water, daily. Five organisms were placed into each of four replicate 50-ml beakers containing water collected from each station for 48 h. Sinking Creek water was used as a control group. At the end of the test period, percent survival for each station was recorded. Mean percent survival was calculated for each individual sampling station over all three sampling periods, then mean values for station groups 1 through 5 were compared using ANOVA and pair-wise analyses at the $\alpha = 0.05$ level.

Sediment toxicity testing

Sediment samples collected from the 21 sampling stations were returned to the laboratory for chronic impairment testing of the cladoceran, *Daphnia magna,* and the midge, *Chironomus tentans*. Samples were collected with a polyurethane dipper, placed in plastic freezer bags and stored on ice at 4° C for no more than 14 d. Tests were conducted according to Nebeker et al. (1984), U.S. EPA (1994) and ASTM (1995) methods with modifications, using 1-L beakers as test containers with 200 ml sediment and 800 ml water, and placing both organisms into the same set of test containers. Test organisms came from Virginia Tech cultures. Five daphnids and ten chironomids were placed into each beaker with five replicate beakers per station. Overlying water was non-toxic reference water (Sinking Creek), and the test containers were lightly aerated throughout the test period. Organisms were fed daily a diet of *Selenastrum*/YCT for daphnids and a Tetra Min® (TetraWerke, Melle, Germany) flake food/cereal leaves mixture for chironomids. At the end of the 10-d test period, percent survival of adult organisms of both species was recorded. In addition, the number of *D. magna* neonates produced per surviving adult and the mean weight (to the nearest 0.001 mg using a Sartorius ® microbalance) of *C. tentans* survivors was recorded. For use as a control, a non-toxic sediment was formulated using a mixture of sand and potting soil (4:1, w/w). Preliminary tests with the sediment showed that it consistently met U.S. EPA/ASTM requirements for control sediments (at least 80% survival of daphnids and 70% survival of midges). Mean survival and impairment values for station groups 1 through 5 were compared using ANOVA and pair-wise analyses at the $\alpha = 0.05$ level.

Ecotoxicological rating system

With the goal of producing a single numerical value that summarized the relative environmental status of a given station, an Ecotoxicological Rating (ETR) system was developed. The ETR was based on ecological, toxicological and chemical parameters. Ecological (benthic macroinvertebrate community index) parameters were selected based on sensitivity to AMD inputs as determined by ANOVA and post-hoc test results. Other parameters were selected by subjecting toxicological and chemical data for all 21 stations to stepwise multiple linear regression analysis (MLRA), followed by bivariate correlation analyses with ecological data. Five different analyses were conducted with all five different benthic macroinvertebrate community indices serving individually as dependent variables. A stepwise selection process was conducted using SAS ® software (1996) to determine the model that best explained the variation in each dependent variable. Only models in which all independent variables had a significant effect ($\alpha = 0.05$) on the overall model were considered, and the one with the highest R^2 value was selected as the best model for a given dependent variable. To avoid using models in which multicolinearity between variables existed, bounds on condition numbers were calculated using the SAS ® (SAS, 1996) program. If these values were less than 1000, multicolinearity was not considered a problem.

Toxicological and chemical parameters that appeared in multiple regression models then were compared with benthic community indices by simple bivariate correlation analysis using JMP IN ® software (Sall and Lehman, 1996). The purpose of the bivariate analyses was to eliminate variables that appeared in MLRA models but did not truly correlate with ecological parameters; therefore, variables that both appeared in the MLRA models and had significant bivariate correlations with ecological parameters were used as parameters in the ETR.

To uphold data normality requirements for both the bivariate and the multiple regression analyses, toxicity testing data were log or log $(x + 1)$ transformed as appropriate. Log transformed benthic macroinvertebrate community data still were not normal so a $(-1/x)$ transformation was conducted to achieve normality. All water column and sediment chemistry data were normal prior to transformation and, therefore, were not manipulated.

Once ETR parameters were selected, each individual station was given a relative rating for each parameter. For example if total taxon richness was selected, the station with the highest value for taxon richness would receive a 1.0 for that parameter while all others would receive values equal to their proportion (0 to 1.0) of the highest value. This process was used for all ecological parameters. For toxicological endpoints, either proportion surviving would be used, or proportion of the highest value in the case that sediment test daphnid reproduction or chironomid weight was selected. Stations would be rated for chemical values such as pH, conductivity and sediment metals based on percent of the highest value at any station (or 1 minus percent of highest in the case of conductivity). Finally, if a water column metal was selected as a parameter, stations would receive either a 1.0 or an 0.1 based on whether or not the station had concentrations above the Ambient Water Quality Criterion (WQC) for that metal.

The ratings for all selected parameters for a given station then were added up, divided by the number of parameters and multiplied by one hundred. This process gave each station a relative percentile score, which was designated as its ETR. Mean ETR values for station groups 1 through 5 then were compared using ANOVA and pair-wise analyses at the $\alpha = 0.05$ level.

Results

Water column and sediment chemistry

Significant differences ($p < 0.05$) among station groups were observed for mean pH, conductivity, and water column concentrations of Al, Fe, and Mn (Table 1). For all of the above parameters except Fe, group 3 (stations with acidic AMD input) values were significantly different from all other groups with lower mean pH (3.71), higher mean conductivity (1216 μmhos/cm), and higher water column Al (30.7 mg/L) and Mn (3.29 mg/L). Mean water column Fe concentration for group 3 was significantly higher than that for group 1 (no AMD), but was only substantially higher than those for the other groups.

Table 1. Mean (± SD) water and sediment chemistry data for station groups in the Puckett's Creek watershed. Cond. = conductivity. Group 1 = no AMD impact (n=7), 2 = intermittent AMD (n=3), 3 = acidic AMD (n=4), 4 = neutral AMD (n=3), 5 = receiving system (n=4 for pH and cond., 3 for metals). Means followed by the same letter are not significantly different ($\alpha = 0.05$).

Water Chemistry					
Station Group	pH mean	Cond. (µmhos/cm)	Al (mg/L)	Fe (mg/L)	Mn (mg/L)
1	7.41 ± 0.48 a	243 ± 142 a	0.2 ± 0.1 a	0.04 ± 0.03 a	0.05 ± 0.06 a
2	6.63 ± 1.35 a	525 ± 180 a	9.2 ± 10.8 a	4.32 ± 4.60 ab	1.27 ± 1.76 a
3	3.71 ± 0.69 b	1216 ± 365 b	30.7 ± 13.4 b	17.09 ± 19.12 b	3.29 ± 0.85 b
4	6.84 ± 0.66 a	559 ± 6 a	2.2 ± 1.9 a	0.09 ± 0.07 ab	0.39 ± 0.29 a
5	7.76 ± 0.30 a	469 ± 187 a	0.3 ± 0.2 a	0.8 ± 0.11 ab	0.07 ± 0.04 a

Sediment Chemistry					
Station Group	Al (mg/kg)	Cu (mg/kg)	Fe (g/kg)	Mn (mg/kg)	Zn (mg/kg)
1	5080 ± 1327 a	11.03 ± 5.47 a	25.9 ± 13.4 a	956 ± 580 a	48.9 ± 13.9 a
2	4050 ± 150 a	6.09 ± 1.23 a	29.1 ± 18.5 ab	324 ± 235 ab	50.6 ± 16.5 a
3	4686 ± 2771 a	8.84 ± 5.22 a	86.1 ± 58.8 b	111 ± 97 b	45.0 ± 31.8 a
4	5300 ± 826 a	8.02 ± 1.79 a	24.4 ± 1.6 ab	779 ± 120 ab	80.6 ± 7.9 a
5	4210 ± 1142 a	10.82 ± 2.75 a	23.1 ± 9.5 ab	672 ± 354 ab	70.4 ± 18.3 a

Dissolved oxygen was at or near saturation for each sampling station on every sampling occasion, and will not be discussed further. Alkalinity values in the watershed were generally low to moderate (<160 mg/L) where it could be measured (stations having pH > 4.50). Hardness ranged from 20 to 219 mg/L for stations either upstream of or far downstream of AMD inputs. Hardness values were not obtained for the severely AMD impacted stations due to interference in the titration method.

No differences were observed among stations groups ($p > 0.05$) for sediment Al, Cu and Zn concentrations (Table 1). Mean sediment Fe concentrations were significantly higher ($p < 0.05$) for group 3 stations (86.1 g/kg) as compared to group 1 (25.9 g/kg). Sediment Fe concentrations for groups 2, 4, and 5 averaged from 23.1 to 29.1 g/kg, but were not significantly different from group 3 due to smaller numbers of stations per group. Mean sediment Mn was lower ($p < 0.05$) for group 3 (111 mg/kg) than for group

1 (956 mg/kg). The remaining three groups had intermediate mean Mn concentrations which were not different from either group 1 or group 3.

Benthic macroinvertebrate sampling

AMD had an impact on the benthic macroinvertebrate communities of Puckett's Creek as significant differences ($p < 0.05$) were observed among station groups for all five community indices (Table 2). Total abundance, EPT richness and percent Ephemeroptera abundance were the most sensitive parameters with at least two station groups (three in the case of total abundance) having significantly lower values than group 1. For total abundance, groups 3, 4 (circum-neutral AMD), and 5 (receiving system) had significantly fewer (15.3 to 30.3) organisms per sample as compared to group 1 (163.1). For both EPT richness and percent Ephemeroptera abundance, groups 3 (1.0 and 3.5, respectively) and 4 (4.3 and 1.3, respectively) were significantly different from group 1, while groups 2 (intermittent AMD) and 5 were not significantly different from any groups. Total taxon richness and percent EPT abundance were less sensitive with only group 3 having significantly lower values than group 1.

In situ clam toxicity testing

Asian clams were highly sensitive to AMD with no clams surviving the 31-d test period at any of the group 3 stations (Table 2). Mean percent survival values for both groups 3 and 4 (36) were significantly lower ($p < 0.05$) than those for groups 1 and 5 (94 and 92, respectively) (Table 2). Group 3 mean survival also was significantly lower than that for the intermittent AMD group (2); however, group 4 mean survival was not.

Table 2. Mean (± SD) ecological and toxicological endpoint values for station groups in the Puckett's Creek watershed. Eph. = Ephemeroptera. *D. magna* reproduction is shown in # of neonates (% of control). Group 1 = no AMD impact (n=7), 2 = intermittent AMD (n=3), 3 = acidic AMD (n=4), 4 = neutral AMD (n=3), 5 = receiving system (n=4). Means followed by the same letter are not significantly different (α = 0.05).

Benthic Macroinvertebrate Community Indices					
Station Group	Total Abundance	Total Richness	EPT Richness	% EPT Abundance	% Eph. Abundance
1	163.1 ± 97.9 a	18.5 ± 6.7 a	11.8 ± 5.1 a	80.9 ± 13.4 a	35.0 ± 20.5 a
2	79.0 ± 82.4 ab	13.6 ± 4.3 ab	8.6 ± 2.9 ab	66.2 ± 31.9 a	24.3 ± 15.5 ab
3	24.9 ± 10.1 b	5.4 ± 1.9 b	1.0 ± 0.7 b	21.5 ± 25.2 b	3.5 ± 5.7 b
4	15.3 ± 7.9 b	9.7 ± 2.6 ab	4.3 ± 1.0 b	43.8 ± 14.0 ab	1.3 ± 2.3 b
5	30.3 ± 10.3 b	12.0 ± 1.3 ab	6.3 ± 1.7 ab	52.9 ± 10.0 ab	20.8 ± 13.4 ab

Toxicological Parameters						
Station Group	*C. fluminea* % survival	*C. dubia* % survival	*D. magna* % survival	*D. magna* reproduction	*C. tentans* % survival	*C. tentans* weight (mg)
1	94 ± 5 a	90 ± 11 a	71 ± 14 a	87 ± 36 a	63 ± 15 a	4.05 ± 0.42 a
2	55 ± 48 ab	47 ± 25 b	77 ± 23 a	57 ± 18 a	54 ± 23 a	3.68 ± 0.53 a
3	0 ± 0 c	0 ± 0 c	49 ± 58 a	49 ± 58 a	47 ± 37 a	2.76 ± 1.68 a
4	36 ± 34 bc	45 ± 33 b	83 ± 2 a	93 ± 22 a	62 ± 22 a	3.73 ± 1.42 a
5	92 ± 6 a	96 ± 1 a	76 ± 13 a	115 ± 23 a	53 ± 11 a	4.65 ± 0.60 a

Water column toxicity testing

Water column samples collected from the group 3 stations were extremely toxic to *C. dubia* (Table 2). No test organisms survived in any of the three tests for any of the four stations. Mean percent survival for group 3 was significantly lower ($p < 0.05$) than that for both groups 2 and 4, which had intermittent and circum-neutral AMD, respectively. In turn, mean survival values for groups 2 and 4 (47 and 45%, respectively) were significantly lower than those for groups 1 and 5 (90 and 96%, respectively)

Sediment toxicity testing

While samples from some individual stations caused low survival of test organisms, no significant differences ($p > 0.05$) were found among station groups for any of the four sediment toxicity test parameters (Table 2). Group 3 had substantially lower mean daphnid and chironomid survival, daphnid reproduction, and chironomid weight values than the other station groups, but differences were not significant due to high variability. Organisms in the formulated control sediments passed U.S. EPA (1994) and ASTM (1995) criteria for survival, reproduction (daphnids) and weight (weight). While none of the station groups had mean values for survival of daphnids (80%) or chironomids (70%) that surpassed criteria for consideration as good references or controls, several individual stations did have higher survival values as illustrated by standard deviations (Table 2).

Selection of parameters for Ecotoxicological Rating system

Stepwise MLRA produced models containing from two (for percent Ephemeroptera abundance and total abundance) to five (for EPT richness) independent variables to predict benthic macroinvertebrate community index values (Table 3). Models were able to predict from 48 (percent Ephemeroptera abundance) to 96 (percent EPT abundance) percent of the variation in the dependent variables from station to station. No evidence of multi-colinearity was observed, with bounds on condition numbers all being much less than 1000.

Table 3. Prediction equations for benthic macroinvertebrate community indices based on multiple linear regression analysis. The best model for each index as determined by a stepwise selection procedure is shown. For each model, all variables contribute significantly to the overall model at the $\alpha = 0.05$ level.

Richness =	-0.102 + 0.050 (*C. dubia* survival) + 0.141 (*D. magna* survival) – 0.156 (*C. tentans* survival) – 0.001 (sediment Zn). $R^2 = 0.9186$, total *d.f.* = 20, p = 0.0001.
Abundance =	0.043 – 3.48 x 10^{-3} (Conductivity) – 9.94 x 10^{-3} (sediment Zn). $R^2 = 0.6439$, total *d.f.* =20, p = 0.0001.
EPT Rich. =	0.343 – 2.55 x 10^{-3} (Conductivity) + 0.104 (*C. dubia* survival) + 0.315 (*D. magna* survival) – 0.272 (*C. tentans* survival) – 0.017 (water Fe). $R^2 = 0.9485$, total *d.f.* = 20, p = 0.0001.
% EPT Abund. =	-0.782 + 0.079 (*C. dubia* survival) + 0.494 (*D. magna* survival) – 0.224 (*C. tentans* survival) + 3.31 x 10^{-6} (sediment Fe). $R^2 = 0.9604$, total *d.f.* = 20, p = 0.0001.
% Eph. Abund. =	0.567 – 5.93 x 10^{-4} (Conductivity) – 8.88 x 10^{-3} (sediment Zn). $R^2 = 0.484$, total *d.f.* = 20, p = 0.0026.

Seven of the sixteen chemical and toxicological variables appeared in the five regression models at least once: (1) *C. dubia* water column test survival, (2) *D. magna* sediment test survival, (3) *C. tentans* sediment test survival, (4) sediment Zn concentration, (5) water column conductivity, (6) water column Fe concentration, and (7) sediment Fe concentration (Table 3). Sediment and water column Fe concentration each appeared in only one model, while the remaining five variables each appeared in three different models. These seven variables next were subjected to bivariate correlation analysis with the five ecological parameters.

Bivariate correlation analysis indicated that *C. dubia* water column percent survival was significantly ($p < 0.05$) positively correlated with four of the five ecological endpoints with correlation coefficients (r) ranging from 0.4939 for percent Ephemeroptera abundance to 0.8070 for EPT richness (Table 4). The only community index not correlated with *C. dubia* survival was total abundance. These high r-values suggest that water column toxicity testing results were well correlated with impacts observed in the field. Water column conductivity was negatively correlated ($p < 0.05$) with the same four ecological variables suggesting the higher the conductivity at a station, the fewer kinds of benthic macroinvertebrates present in general.

Both *D. magna* sediment test survival and water column Fe concentration were correlated ($p < 0.05$) with total richness, EPT richness and percent EPT abundance (Table 4). Daphnid survival was positively correlated with the ecological endpoints, while water column Fe was negatively correlated, suggesting the higher the Fe concentration in the water at a given station, the fewer kinds of aquatic biota present in general. The remaining three independent variables (*C. tentans* survival, sediment Fe, and sediment Zn) either were not significantly correlated with any of the ecological parameters or were only correlated with one.

Table 4. Correlation coefficients (r) for comparisons of benthic macroinvertebrate community indices with the seven most important ecotoxicological parameters as selected by multiple linear regression analysis. * indicates a statistically significant relationship ($\alpha = 0.05$). Abund. = Abundance, Eph. = Ephemeroptera.

Parameter	Total Richness	Total Abund.	EPT Richness	% EPT Abund.	% Eph. Abund.
C. dubia survival	0.7464*	0.3150	0.8070*	0.5463*	0.4939*
D. magna survival	0.6591*	-0.0783	0.7737*	0.8186*	0.3096
Conductivity	-0.7624*	-0.3146	-0.8429*	-0.6496*	-0.5019*
C. tentans survival	0.1545	-0.0970	0.3362	0.2830	-0.0368
Fe in sediments	-0.3493	-0.0767	-0.5146*	-0.3015	-0.1772
Fe in H2O	-0.5487*	-0.0393	-0.6409*	-0.5495*	-0.2263
Zn in sediments	0.0403	-0.6380*	0.1777	0.3296	-0.3436

Parameters for use in the ETR then were selected based on the number of dependent variables they were significantly correlated with. Four parameters were significantly correlated with at least three of the five ecological endpoints: (1) *C. dubia* water column test survival, (2) *D. magna* sediment test survival, (3) water column conductivity, and (4) water column Fe concentration. With two chemical and two toxicological endpoints selected, two ecological endpoints then were selected. These were EPT richness and percent Ephemeroptera abundance based on the fact that they were the most sensitive (Table 2) with the exception of total abundance. Total abundance was not selected because sampling conducted was not quantitative, and because abundance of tolerant species may increase in polluted areas as a result of decreased competition for resources (Wiederholm, 1984). While percent Ephemeroptera abundance depends on the number of organisms collected, it is a composition measure. These two ecological endpoints brought the total number of ETR parameters to six (Table 5).

With parameters selected, individual stations were ranked for each parameter as described previously (Table 5). Mean values for station group ETRs then were calculated and compared by ANOVA. Significant differences ($p < 0.05$) were observed among groups, with stations receiving no AMD (group 1) having the highest average ETR (80.08) (Table 6). Groups 2 and 4 (54.9 and 53.78, respectively) had significantly lower mean ETRs than group 1, while group 3 stations (15.90) had significantly lower ETRs than groups 2, 4, and 5. Group 5 (69.84) was not significantly different from either group 1, or groups 2 and 4.

Table 5. Parameters selected for inclusion in the Ecotoxicological Rating (ETR)* system. Parameters were selected based on sensitivity, multiple linear regression analysis and correlation analysis.

Parameter	Station Ranking Procedure
EPT Richness	Percent of highest value
% Eph. Abundance	Percent of highest value
C. dubia survival (H2O)	Mean percent survival
D. magna survival (sediment)	Percent survival
Conductivity	1 minus percent of highest value
Fe (H2O)	Above (0.1) or below (1.0) Water Quality Criterion

*A station's rankings for each parameter then were added together, divided by six and multiplied by 100 to give the final ETR.

Table 6. Mean (± SD) Ecotoxicological Ratings (ETRs) for station groups in the Puckett's Creek watershed and surrounding areas. Means followed by the same letter are not significantly different ($\alpha = 0.05$).

Station Group	Number of Stations In Group	Mean ETR
(1) No AMD impact	7	80.05 ± 11.10 a
(2) Intermittent AMD impact	3	54.90 ± 4.69 b
(3) Acidic AMD stations	4	15.90± 14.73 c
(4) Neutral AMD impacted stations	3	53.78 ± 4.34 b
(5) Receiving system stations	4	69.84 ± 6.04 ab

Discussion

AMD had a negative impact on the benthic communities of the Puckett's Creek watershed with significant differences between acidic and neutral AMD sites and unimpacted sites for several community indices. Numerous investigators have observed similar declines in macroinvertebrate assemblages due to acid mine drainage, acid precipitation and neutral mine drainage (Armitage, 1980; Cherry et al., 1995; Griffith et al., 1995; Griffiths and Keller, 1992; Herricks and Cairns, 1974; Kobuszewski and Perry, 1993; Nelson and Roline, 1996; Roback and Richardson, 1969; Rutherford and Mellow, 1994; Smith and Frey, 1971; Winterbourn and McDiffet, 1996). In general, these studies showed that impacted sites supported fewer taxa than unimpacted sites with sensitive organisms often absent. Roback and Richardson (1969) observed the elimination of members of the Orders Odonata, Ephemeroptera, and Plecoptera under conditions of constant AMD input, while at sites with intermittent AMD, communities were diverse but somewhat depressed. Similar results were obtained in this study. Station groups receiving continuous acidic and neutral AMD (3 and 4, respectively) had significantly fewer EPT organisms and lower percent abundances of mayflies than the unimpacted station group, and group 2 (intermittent AMD) stations had slightly but not significantly decreased values for all community indices calculated as compared to group 1.

In this study, EPT richness and percent Ephemeroptera abundance were the two most sensitive composition metrics. EPT richness has been shown to be a good indicator of chemical degradation in southern Appalachian headwater streams (Wallace et al., 1996), and Smith and Voshell (1997) identified percent Ephemeroptera abundance as one of the ten best metrics (out of 69 analyzed) at providing discrimination between impacted and reference sites in Mid-Atlantic Highland streams. EPT richness also has been useful in demonstrating impacts of a neutral mine drainage with high metal concentrations, and recovery of aquatic communities in response to cessation of the drainage (Nelson and Roline, 1996).

Of the six toxicological endpoints used in this investigation, water column toxicity testing with *C. dubia* was the most sensitive, distinguishing between two levels of environmental impact. Significant correlations between *C. dubia* survival and benthic community indices (r-values ranging from 0.4939 to 0.8070) suggest that water column toxicity testing was an effective bioassessment technique for this AMD impacted watershed. Similar results were obtained in an investigation of an AMD impacted watershed in Colorado (Fucik et al., 1991). In that study, water column toxicity test results with *C. dubia*, brook trout (*Salvelinus fontinalis*), and fathead minnows

(*Pimephales promelas*) generally agreed with benthic macroinvertebrate community data in identifying two impacted stations out of the six sampled.

In situ toxicity testing with Asian clams was nearly as sensitive to AMD impacts as laboratory toxicity testing but did not appear in any of the MLRA models. Numerous studies have shown that Asian clams are an effective biomonitoring tool (Doherty and Cherry, 1988), and because of ease of execution and sensitivity, in situ testing should be considered for use in assessments of AMD impacted watersheds.

Sediment toxicity testing was not a sensitive bioassessment technique in terms of discrimination between impacted and unimpacted station groups. Similarly, *D. magna* was observed to be the least sensitive of four sediment test organisms exposed to metal contaminated sediments in the Clark Fork River, Montana (Kemble et al., 1994). *Chironomus tentans* was not used as a test organism in the Clark Fork study, but was insensitive to AMD inputs in the present study. While Kemble et al. (1994) observed correlations between high sediment metal concentrations contributed by mining activities and sediment toxicity to other test organisms, McCann (1993) found no evidence of sediment toxicity to juvenile mussels in 10-day tests with sediments collected from various sites within the Powell River watershed. Results obtained in the present study likely are in part attributable to increased solubility of most metals at stations with low mean pH values (the most severely impacted stations) which results in leaching of metals from sediments and/or prevention of adsorption of water column metals to sediments. Dissolved metals then should precipitate when pH levels downstream rise to the appropriate level for each particular metal (Snoeyink and Jenkins, 1980). However, chronic sediment toxicity was not observed for any samples collected from circum-neutral pH sites downstream of AMD point-sources (groups 4 and 5) where metals would be expected to accumulate in sediments.

These results suggest that metal deposition at circum-neutral stations was not a problem detectable by standard chronic sediment toxicity testing endpoints, and sediment metals data support this contention with no differences among groups for sediment Al, Cu, and Zn. In addition, sediment Fe concentrations were elevated only at group 3 stations. The trend with Fe likely is related to the fact that fully oxidized Fe can reach saturation in acid mine waters with respect to either ferrihydrite ($Fe(OH)_3{\cdot}XH_2O$) or the mineral jarosite ($KFe(SO)_4(OH)_6$), and precipitates of both of these minerals may be observed even in acidic streams (Nordstrom, 1982; Nordstrom et al., 1979). Sediment Fe was nominally lower at group 4 and 5 stations as compared to group 1 stations, and a similar trend was observed for sediment Mn.

Despite its lack of sensitivity, daphnid survival in sediment tests was well correlated with several community composition indices. In MLRA models, daphnid and chironomid survival in combination with other chemical and toxicological parameters explained 95 percent of the variation from station to station in EPT richness, and 96 percent of the variation in percent EPT abundance. Therefore, in this system, the combination of both water column and sediment toxicity data along with water column and sediment chemistry data were required to provide highly predictive models to explain changes in community structure in the Puckett's Creek watershed. The ETR system pooled this variable information into a composite value to characterize individual sampling stations. The approach was similar to the Sediment Quality Triad (Chapman et al., 1992), which has been used in a variety of polluted systems (Becker et al., 1990; Canfield et al., 1994; Long and Chapman, 1985), incorporating ecological, toxicological and chemical data. In this study, ETR scores differentiated between different types of AMD input with acidic AMD impacted stations having significantly lower ratings than both the neutral and intermittent AMD stations, which in turn had lower ratings than the unimpacted and receiving system groups. The approach used was specific to this watershed, but easily could be adjusted and used within and between adjacent watersheds in southwestern Virginia provided relatively unimpacted sites exist.

The present study was conducted in conjunction with Virginia Department of Mines, Minerals and Energy (VDMME), Division of Mined Land Reclamation efforts to survey the extent of AMD impact in the Powell River watershed. The long-term goal of their efforts is to reclaim abandoned mined lands (AML) and restore impacted streams. The ETR provided single number values that characterized the relative environmental impact at each station, simplifying prioritization of stations for remediation.

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Chapter 2.

In situ studies with Asian clams (*Corbicula fluminea*) detect acid mine drainage and nutrient inputs in low order streams

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Abstract - In situ Asian clam (*Corbicula fluminea* [Müller]) studies may effectively mirror resident community responses to both acute toxicants and nutrient inputs in low order streams. Clam survival and growth after 30 days in situ were compared to benthic macroinvertebrate community structural changes caused by acid mine drainage (AMD) and nutrient loading (measured as nitrate) in a small sub-watershed of the North Fork Powell River, Virginia, USA. Clam survival distinguished between two different levels of impact due to acidic, neutralized, and intermittent AMD inputs, was positively correlated with water column pH, and negatively correlated with conductivity and metal concentrations. Survival also was positively correlated with relative abundance of the Order Ephemeroptera, the most sensitive macroinvertebrate taxonomic group to AMD in this system. Clam growth was not related to AMD inputs, but was positively correlated with nitrate concentrations and the relative abundance of the collector-filterer functional feeding group. These results suggest that transplanted clam studies accurately reflect benthic macroinvertebrate community responses to multiple stressors from point- and non-point sources.

Introduction

Complex combinations of environmental factors regulate aquatic community structure and population distributions (Merrit and Cummins 1996). Lotic benthic macroinvertebrate communities in particular are structured by biotic factors such as competition and predation, and a number of abiotic factors, including climate, water quality, nutrient availability, stream order, and substrate composition. Studies of pollution impacts on aquatic communities are often inconclusive because a pollutant, such as an industrial effluent, may contain several interacting components. Some components may enhance an aspect of the community by providing a limited nutrient, while others have an inhibitory effect by causing toxicity (Wiederholm 1984). Ecosystems often receive inputs from multiple stressors, including both point- and non-point sources, further confounding our understanding of community responses to pollutants.

In situ toxicity tests have gained popularity as replacements for, or supplements to, standard bioassessment tools such as laboratory toxicity testing and benthic macroinvertebrate sampling. They are thought to provide more environmental realism than laboratory tests by incorporating continuous exposure over an extended period of time to the multiple stressors that regulate indigenous communities, and are less time and labour intensive than macroinvertebrate community sampling (Cherry 1996). The Asian clam (*Corbicula fluminea*) is particularly useful as a biomonitoring tool because of its availability, one to three-year life span, sedentary nature, sensitivity to different types of pollutants, and ability to accumulate organic pollutants and heavy metals (Doherty 1990; Doherty and Cherry 1988). Transplanted clams have been used widely to detect anthropogenic impacts in aquatic ecosystems, with endpoints ranging from survival and growth (Belanger 1991), to cellulolytic activity (Farris et al. 1988), valve movement (Allen et al. 1996), and DNA strand breakage (Black 1997).

While numerous studies have employed *Corbicula* as a biomonitoring tool, very few researchers (Farris et al. 1988) have compared transplanted Asian clam responses to those of benthic macroinvertebrate communities. The purpose of this study was to evaluate the correlation between transplanted Asian clam and indigenous community responses to acid mine drainage (AMD) and nutrient loading in first to third order streams. These objectives were accomplished by comparing the toxicological endpoints of clam survival and growth to benthic macroinvertebrate community indices, as community responses to both AMD and nutrient loading are well characterized (Hilsenhoff 1988; Kelly 1988; Wiederholm 1984). We hypothesized that (1) clam survival may be limited by acutely toxic AMD inputs in the upper portions of the watershed (first to third order streams), and (2) clam growth is likely to be related to trophic status in low order streams absent of AMD because they are filter-feeders. Changes in those endpoints should be related to shifts in relative abundance of ecologically different groups of benthic macroinvertebrates. Therefore, clam growth and survival endpoints in low order streams may provide different types of information to ecologists or risk assessors by responding in different ways to different types of pollutants.

Materials and methods

Sampling stations and groups

Puckett's Creek flows via Straight Creek and Stone Creek into the North Fork of the Powell River (NFP), which contributes to the Tennessee River drainage (Figure 1). Asian clam populations were well established in the Powell River before this study was conducted so potential introduction of an exotic species was not a concern. Fifteen sampling stations were selected in Puckett's Creek and its tributaries, including Lick Branch, which received input from several AMD discharges. In addition, four stations were selected in higher (fourth to sixth) order AMD influenced streams of the same sub-watershed of the North Fork of the Powell River. Stations were categorized based on level of AMD input as determined by location, stream order, and mean pH.

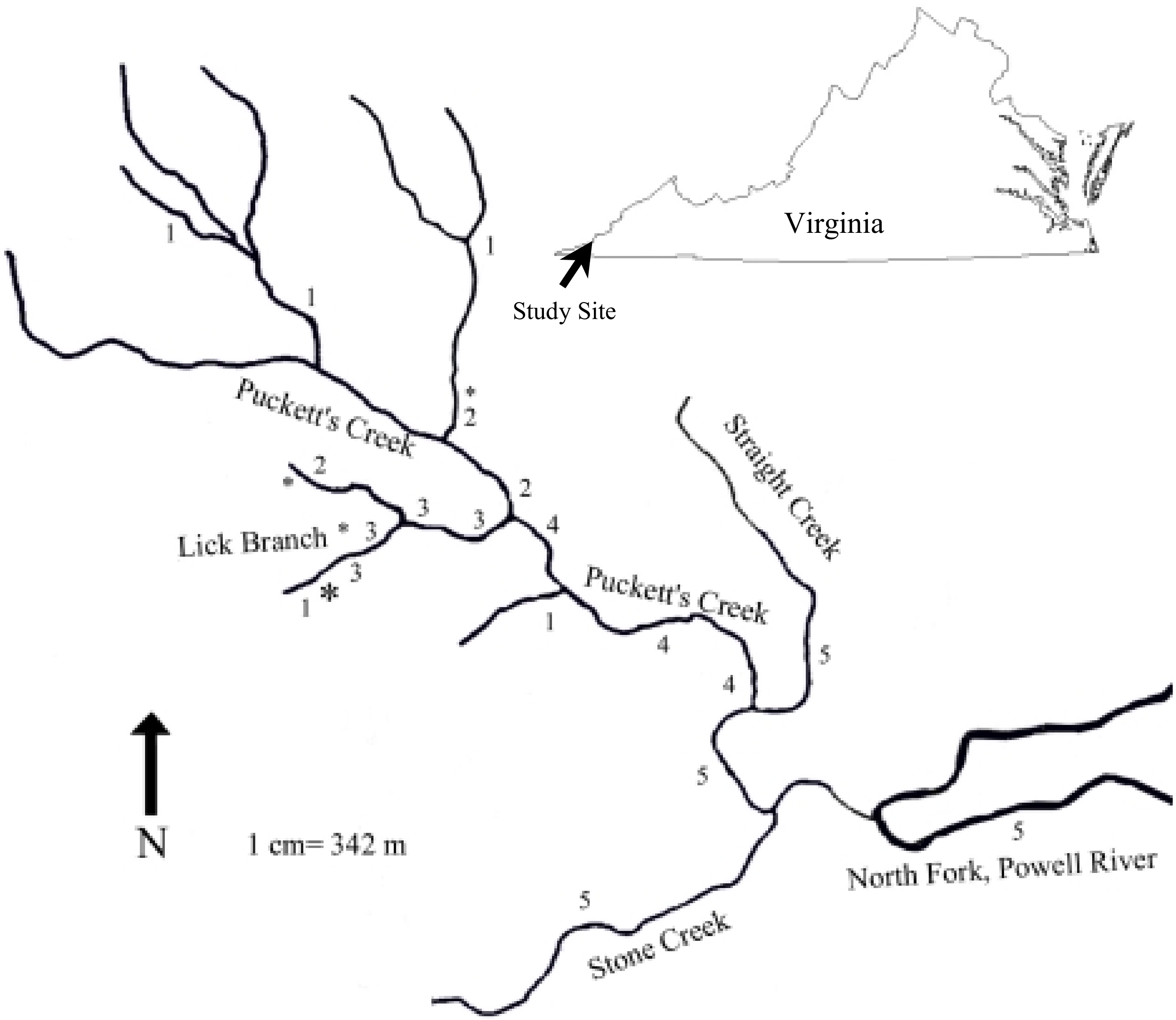

Figure 1. Diagram of the Puckett's Creek watershed showing locations of sampling stations by group and acid mine drainage discharges. 1 = upstream station, 2 = intermittent AMD stations, 3 = acidic AMD stations, 4 = neutralized AMD stations, and 5 = higher order stations. The large asterisk indicates a continuously flowing AMD discharge, while the small asterisks indicate intermittent discharges.

Categorization of stations facilitated determination of clam sensitivity to the different types of AMD impacts relative to the benthic macroinvertebrate communities by providing treatments to compare using analysis of variance (ANOVA). Five groups of stations were constructed as follows. The first group ($n = 5$) consisted of stations that were upstream of all known AMD inputs in Puckett's Creek and Lick Branch. Stream order for these five stations ranged from first to second. The second group ($n = 3$) consisted of stations ranging from first to third order and were subjected to intermittent AMD input. The intermittent designation was based upon a wide pH range over time (e.g., 3.17 – 6.30 for station LB-5), or being downstream of such a station but upstream of any continuous AMD input (Soucek et al. 2000, chapter 1). The third group ($n = 4$) consisted of those stations in Lick Branch (first to second order) continuously subjected to AMD input and having acidic mean pH values (≤ 4.50). The fourth group ($n = 3$) consisted of stations in the lower, third order section of Puckett's Creek below the confluence of Lick Branch; these stations were continuously subjected to AMD input but had circum-neutral mean pH values (6.11 – 7.42) because of dilution. Therefore, group 4 stations will be referred to as neutralized AMD stations. The fifth group ($n = 4$) consisted of stations in Straight Creek, Stone Creek and the NFP. While all group 5 stations were downstream of various AMD inputs, they were differentiated from group 4 as having a higher level of dilution, being located in fourth to sixth order streams.

One additional station (SC-2) was selected in a third order reach of the adjacent Straight Creek, but was not used in the primary analyses because it was located in a different creek upstream of AMD input. This station was suspected of receiving diluted domestic input (possibly septage) based upon observations and nutrient measurements, and therefore, was used as a case study of clam growth in low order streams where nutrient concentrations were elevated. Sampling at this station was conducted concurrently with, and was identical to that for the other stations in terms of types of samples collected and analyzed.

Water column chemistry

To characterize stations according to the degree of AMD input and nutrient levels, water samples were collected at each station for analysis of selected water quality parameters on four occasions from Fall 1997 to Summer 1998. Samples either were brought to the laboratory, stored for 24 h at 4 °C and measurements taken under laboratory conditions, or measurements were taken in the field. Sample pH was measured using an Accumet® (Fisher Scientific, Pittsburgh, PA, USA) pH meter equipped with an Accumet® gel-filled combination electrode (accuracy < ± 0.05 pH at 25 °C). Conductivity measurements were made using a Hach® (Hach, Loveland, CO, USA) conductivity/TDS meter. Dissolved oxygen, alkalinity and hardness data for the watershed are provided in Soucek et al. (2000, chapter 1). In addition, water column samples were prepared for metals analysis according to standard methods (U.S. EPA 1991). Samples were analyzed by inductively coupled plasma spectrometry for total aluminum (Al) and iron (Fe), which were previously determined to be the dominant metals in the system (Soucek et al. 2000, chapter 1). Lower detection limits ($mg \cdot L^{-1}$) were 0.001 and 0.002 for Al and Fe, respectively. Water samples also were analyzed for nitrate according to standard methods (U.S. EPA 1979).

Mean values for individual sampling stations were calculated for parameters of which more than one measurement was taken (i.e., pH and conductivity). Then, mean values for station groups 1 through 5 were compared by ANOVA. Student's T-test was used for post-hoc, pair-wise analysis at the $\alpha = 0.05$ significance level.

Benthic macroinvertebrate sampling

Benthic macroinvertebrate surveys were conducted according to the U.S. Environmental Protection Agency Rapid Bioassessment Protocols (RBP) (Plafkin et al. 1989). Riffle, run, pool and shoreline rooted areas were thoroughly sampled for 20 min per site using dip-nets with 800-µm mesh. Two replicate samples were collected per site and mean values for all indices were calculated for each site. All organisms in each sample were identified to the lowest practical taxonomic level (usually genus) using standard keys (Merritt and Cummins 1996; Pennak 1989). Chironomids were identified as either subfamily Tanypodinae or non-Tanypodinae.

For community analyses, taxa were placed into six categories: Ephemeroptera, Plecoptera, Trichoptera, Hydropsychidae, Chironomidae, and Other. The Other taxa category included the Megaloptera, Odonata, Coleoptera, non-chironomid-Diptera, and non-insects. The Trichoptera group included hydropsychid caddisflies, but hydropsychids were placed into an additional category because they may reach high densities in response to dilute organic inputs or mild eutrophication (Wiederholm 1984). The relative abundance of each taxonomic group was calculated for each station by dividing, for example, the number of mayflies by the total number of organisms. These values were compared within each station group by ANOVA and post-hoc tests as described previously to determine which type of organisms were dominant within each station group.

Each taxon identified also was assigned to a functional feeding group as described in Merritt and Cummins (1996). Feeding groups included collector-gatherers, shredders, collector-filterers, scrapers, and predators. Tanypod chironomids were considered predators, and non-tanypod chironomids were collector-gatherers. Similar analyses were conducted as with the taxonomic groups, i.e., mean relative abundances of feeding groups were compared within station groups.

To further test for the potential presence of dilute organic input at station SC-2, family-level-biotic index (FBI) values were calculated for the five upstream (group one) stations and for station SC-2 (Hilsenhoff 1988). The FBI value gives an indication of the level of organic pollution from sources such as agricultural runoff or septage based upon tolerance values of arthropod families found at the site, with higher scores indicating more organic pollution.

In situ clam toxicity testing

For in situ toxicity tests, Asian clams were collected from the New River near Ripplemead, Virginia, using clam rakes. Clams were held in Living Streams® (Toledo, OH) at the Ecosystem Simulation Laboratory, Virginia Tech, Blacksburg, VA, until use. Individual clams were given one of five distinctive marks and measured for width to the nearest 0.01 mm using Vernier calipers prior to placement into bags for testing. Testing procedures consisted of tying five mesh bags, each bag containing five clams, to stakes at each sampling station. Bags were 18 cm wide by 36 cm long with a mesh size of ~0.5 cm^2. At the end of thirty days, clam bags were collected from each testing station and transported on ice to the laboratory. Clams were counted as dead or alive; clams found with valves separated, or that were easily opened, were considered dead. Surviving clams were measured again for width, and growth was calculated by subtracting beginning from ending width. Mean clam survival and growth values for station groups 1 through 5 were compared using ANOVA and pair-wise analyses as described previously at the $\alpha = 0.05$ level.

Correlation analysis

To compare benthic macroinvertebrate sampling data with Asian clam-toxicity test data for the watershed, bivariate correlation analyses were conducted. Both clam survival and growth were compared to taxonomic and feeding group relative abundances. Values from all 19 stations were included in the analysis for clam survival, but stations where all clams died were excluded from the correlation analyses for clam growth. Additional correlation analyses were conducted between clam survival/growth and water chemistry parameters to determine if the clam endpoints were related to AMD inputs or to nutrient levels at a given station. Significance of correlation was determined at the $\alpha = 0.05$ level.

Results

Water chemistry

The most extreme values for the four AMD related parameters analyzed were observed in group three stations, where mean pH (3.71) was lowest, and conductivity (1216 µmhos·cm^{-1}), aluminum (30.7 mg·L^{-1}) and iron (17.09 mg·L^{-1}) were highest (Table 1). The intermittent nature of AMD input at the group two stations was demonstrated by relatively high standard deviations about the means for pH, conductivity, aluminum and iron. The group four stations had a circumneutral average pH (6.84), but elevated values for mean conductivity (559 µmhos·cm^{-1}) and aluminum (2.2 mg·L^{-1}). Mean water chemistry values for the group five stations were similar to those for the group one stations except for nominally elevated mean conductivity (469 µmhos·cm^{-1}), and a significantly higher mean nitrate concentration (0.40 mg·L^{-1}) than any of the lower order station groups (one through four).

Table 1. Mean (± standard deviation) [a] water chemistry values for station groups [b] in the Puckett's Creek watershed.

Station Group	pH mean	Conductivity (µmhos·cm^{-1})	Al (mg·L^{-1})	Fe (mg·L^{-1})	NO_3 (mg·L^{-1})
1	7.27 ± 0.48 A	187 ± 99 A	0.2 ± 0.1 A	0.04 ± 0.02 A	0.16 ± 0.09 B
2	6.63 ± 1.35 A	525 ± 180 B	9.2 ± 10.8 A	4.32 ± 4.60 A	0.13 ± 0.04 B
3	3.71 ± 0.69 B	1216 ± 365 C	30.7 ± 13.4 B	17.09 ± 19.12 A	0.19 ± 0.07 B
4	6.84 ± 0.66 A	559 ± 6 B	2.2 ± 1.9 A	0.09 ± 0.07 A	0.14 ± 0.04 B
5	7.76 ± 0.30 A	469 ± 187 AB	0.19 ± 0.2 A	0.08 ± 0.11 A	0.40 ± 0.18 A

[a] Means in a vertical series followed by the same letter are not significantly different (α = 0.05, ANOVA and Student's T-test).
[b] Group 1 = no AMD impact (n = 5), 2 = intermittent AMD (n = 3), 3 = acidic AMD (n = 4), 4 = neutral AMD (n = 3), 5 = higher order (n = 4).

Benthic macroinvertebrate communities

Benthic macroinvertebrate communities were sensitive to the various types of AMD input. In the group one stations, upstream of AMD input, Ephemeroptera and Plecoptera were the dominant taxa, making up 41.3% and 31.6%, respectively, of the mean total abundances (Table 2). The intermittent AMD stations (group two) had similar assemblages in terms of which taxa were dominant, but mayflies (Ephemeroptera) were slightly less abundant than stoneflies (Plecoptera), chironomid numbers decreased, and relative abundance of the "Other" group was not significantly different from that of the mayflies and stoneflies. In the acidic AMD stations, chironomids accounted for more than half of the organisms while mayflies, stoneflies and caddisflies made up less than 15 percent of the assemblages. The group four stations were dominated by the "Other" taxa group, and in the group five or higher order stations, no single group was dominant, as no significant differences were observed.

Table 2. Mean (± standard deviation) percent composition [a] by taxonomic and functional feeding group for the five station types in the Puckett's Creek watershed. AMD = acid mine drainage.

Taxonomic Group	Upstream Group 1 (n = 5)	Intermittent Group 2 (n= 3)	Acidic AMD Group 3 (n = 4)	Neutralized AMD Group 4 (n = 3)	Higher order Group 5 (n = 4)
Plecoptera	31.6 ± 22.5 A	41.5 ± 19.1 A	10.9 ± 15.7 BC	25.8 ± 19.9 AB	9.9 ± 3.3 A
Ephemeroptera	41.3 ± 18.2 A	25.4 ± 14.4 AB	2.2 ± 3.6 C	1.4 ± 2.4 C	19.6 ± 14.3 A
Trichoptera	9.6 ± 6.3 B	9.9 ± 9.8 BC	0.4 ± 0.7 C	20.9 ± 6.0 B	18.7 ± 8.4 A
Hydropsychidae	6.4 ± 6.2 B	6.2 ± 8.8 BC	0.4 ± 0.7 C	13.2 ± 6.4 BC	16.6 ± 9.9 A
Chironomidae	6.5 ± 9.6 B	2.1 ± 2.5 C	59.4 ± 28.7 A	6.7 ± 5.9 BC	17.3 ± 9.2 A
Other	11.0 ± 12.6 B	20.9 ± 13.6 ABC	27.0 ± 15.7 B	44.8 ± 13.7 A	34.9 ± 18.2 A

Feeding Group	Upstream Group 1 (n= 5)	Intermittent Group 2 (n = 3)	Acidic AMD Group 3 (n = 4)	Neutralized AMD Group 4 (n = 3)	Higher order Group 5 (n = 4)
Collector-Gath.	42.9 ± 18.0 A	28.3 ± 12.9 A	67.5 ± 27.4 A	7.4 ± 2.5 BC	37.0 ± 12.7 A
Scrapers	8.6 ± 3.9 B	6.7 ± 3.5 A	1.5 ± 1.8 B	4.0 ± 5.3 C	8.6 ± 6.5 C
Collector-Filterers	6.7 ± 6.4 B	7.0 ± 9.2 A	0.4 ± 0.8 B	15.4 ± 8.6 BC	23.2 ± 10.3 AB
Shredders	28.3 ± 22.5 A	40.0 ± 23.8 A	11.8 ± 17.0 B	26.8 ± 14.9 AB	11.7 ± 6.6 BC
Predators	8.5 ± 1.9 B	17.8 ± 10.6 A	18.6 ± 12.9 B	46.2 ± 16.4 A	19.3 ± 8.9 BC

[a] Means in a vertical series followed by the same letter are not significantly different ($\alpha = 0.05$, ANOVA and Student's T-test).

Changes also were observed in relative abundances of functional feeding groups in response to AMD. The collector-gatherer functional feeding group was dominant in all stations except for the neutralized AMD group (Table 2). In the group one stations, shredders were co-dominant, having a significantly higher relative abundance than the other three feeding groups. While shredders and collector-gatherers had high relative abundance values in the group two, intermittent AMD stations, their values were not significantly higher than the other groups. Collector-gatherers dominated the acidic AMD stations with a relative abundance of 67.5 percent. Predators were the dominant group at the neutralized AMD stations, making up almost half of the assemblages. In the higher order, group five stations, collector-gatherers and collector-filterers made up the greatest proportion of the macroinvertebrate assemblages, followed by predators, shredders and scrapers.

In situ clam toxicity testing

The clam survival endpoint was sensitive to AMD inputs, differentiating between two levels of environmental impact. Average survival of clams at the end of the thirty day test was highest in groups one and five (92.8% and 92.0%, respectively), while none of the clams placed in the group three, acidic AMD impacted stations survived (Table 3). As illustrated by standard deviations about means, survival in the stations with intermittent (group two) and neutralized AMD input (group four) was variable, with intermediate mean values of 54.6% and 36.0%, respectively. Groups one and five had significantly higher survival than group two, which in turn had significantly higher survival than those in group three stations.

Table 3. Mean (± standard deviation) survival and growth [a] in Asian clam in situ toxicity tests for station groups in the Puckett's Creek watershed. AMD = acid mine drainage

Station Group	Mean Clam survival (%)	Mean Clam growth (mm)
1) Upstream stations	92.8 ± 5.2 A	0.06 ± 0.25 A
2) Intermittent AMD	54.6 ± 47.7 B	-0.05 ± 0.04 A
3) Acidic AMD	0.0 ± 0 C	n/a
4) Neutralized AMD	36.0 ± 34.2 BC	-0.08 ± 0.03 A
5) Higher order	92.0 ± 5.6 A	0.37 ± 0.18 A

[a] Means in a vertical series followed by the same letter are not significantly different (α = 0.05, ANOVA and Student's T-test).

Clam growth was variable and less sensitive to AMD inputs, when excluding all sites where 100 percent mortality of clams was observed (Table 3). While station groups consisting of first to third order reaches (groups one, two and four) had substantially lower growth (-0.08 - 0.06 mm) than the higher order, group five stations (0.37 mm), differences were not significant due to high variability.

Correlation analysis

Clam survival data compared with benthic macroinvertebrate indices produced three significant correlations (Table 4). Clam survival was negatively correlated with the relative abundance of chironomids and positively correlated with relative abundance of mayflies (Ephemeroptera). The only functional feeding group that was correlated with clam survival was the scraper group, with a positive significant relationship.

Clam growth was negatively correlated with percent abundance of stoneflies (Plecoptera), but positively correlated with hydropsychid caddisflies and chironomids (Table 4). Growth also had a significant negative relationship with percent abundance of shredders, and a positive relationship with collector-filterers.

Table 4. Correlation coefficients (*r*)[a] between in situ clam toxicity test endpoints and taxonomic and functional feeding groups of benthic macroinvertebrates in the Puckett's Creek watershed.

Comparison	coefficient	p-value
Clam survival vs. Chironomidae	$r = -0.526$	$p = 0.0208$
Clam survival vs. Ephemeroptera	$r = +0.496$	$p = 0.0304$
Clam survival vs. Scrapers	$r = +0.469$	$p = 0.0428$
Clam growth vs. Plecoptera	$r = -0.675$	$p = 0.0113$
Clam growth vs. Hydropsychidae	$r = +0.650$	$p = 0.0161$
Clam growth vs. Chironomidae	$r = +0.819$	$p = 0.0006$
Clam growth vs. Shredders	$r = -0.663$	$p = 0.0135$
Clam growth vs. Collector-filterers	$r = +0.745$	$p = 0.0035$

[a] All significant relationships ($p \leq 0.05$) are shown.

Comparison of in situ toxicity test parameters with water chemistry data indicated that clam survival was significantly positively correlated with pH, and negatively correlated with conductivity, Al, and Fe concentrations in the water column (Table 5). Nitrate was not significantly correlated with clam survival; however, nitrate had a significantly positive relationship with clam growth.

Table 5. Correlation coefficients (r) [a] between in situ clam toxicity test endpoints water quality data for the Puckett's Creek watershed.

Clam Survival vs.	coefficient	p-value	
pH	$r = + 0.732$	$p = 0.0008$	*
Conductivity	$r = - 0.763$	$p = 0.0004$	*
Al	$r = - 0.653$	$p = 0.0045$	*
Fe	$r = - 0.535$	$p = 0.0270$	*
NO_3	$r = + 0.337$	$p = 0.1865$	
Clam Growth vs.	**coefficient**	**p-value**	
pH	$r = + 0.472$	$p = 0.1423$	
Conductivity	$r = + 0.205$	$p = 0.5453$	
Al	$r = - 0.197$	$p = 0.5617$	
Fe	$r = + 0.173$	$p = 0.6106$	
NO_3	$r = + 0.647$	$p = 0.0313$	*

[a] * indicates a significant relationship ($p \leq 0.05$).

Clam responses to nutrient loading

Comparing nutrient levels and their effects in the absence of AMD, the upstream, group one stations in Puckett's Creek had a mean nitrate concentration of 0.16 ± 0.09 $mg \cdot L^{-1}$, while SC-2, a similar type of station in the adjacent Straight Creek watershed, had 0.56 mg $NO_3 \cdot L^{-1}$. Mean clam growth was substantially higher at SC-2 (1.1 mm) compared to the mean value for the group one stations (0.062 ± 0.25 mm). Likewise, percent abundance of collector-filterers and FBI score were substantially higher in SC-2 (79.2%, and 3.86, respectively) compared to the means for the Puckett's Creek group one stations (6.7 ± 6.4%, and 2.54 ± 0.48, respectively).

Discussion

These results suggest that Asian clams may be used to detect two different types of pollution in headwater streams: acute toxicants and dilute nutrient inputs. In addition, in situ clam tests appear to accurately reflect benthic macroinvertebrate responses to these types of pollutants. Clam survival was sensitive to various levels of AMD inputs, and was correlated with dominance of the most AMD sensitive taxonomic groups (i.e., mayflies). Conversely, clam growth was correlated with dominance of benthic macroinvertebrate groups (i.e., hydropsychids and chironomids) that have been observed to thrive downstream of mild organic or nutrient inputs.

Clam survival effectively distinguished between different levels of environmental impact due to acidic, neutralized and intermittent AMD inputs in this small watershed. While significant mortality of transplanted clams has been observed in response to organochlorine contamination (Hayward et al. 1996) and sewage treatment plant effluents (Belanger 1991), most transplant-studies with Asian clams have investigated sublethal responses to pollutants such as DNA strand breakage (Black 1997), enzyme activity (Farris et al. 1988), bioaccumulation (e.g., Andrès et al. 1999; Gunther et al. 1999), and valve movement (Allen et al. 1996). These types of responses have been sensitive to low levels of pollution, but they may be too time-intensive for a preliminary bioassessment of a whole watershed. In this study, the clam survival endpoint was sensitive to two different levels of environmental impact, and these survival responses were correlated with AMD related water chemistry parameters such as pH, conductivity, and metal concentrations.

The benthic macroinvertebrate community responses to AMD in Puckett's Creek were similar to those observed in other AMD impacted watersheds. For example, mayfly numbers often are depressed by mine drainages (e.g., Merret et al. 1991; Nelson and Roline 1996; Roback and Richardson 1969). In Puckett's Creek, they were the most sensitive taxonomic group to AMD inputs, dominating the assemblages upstream of AMD inputs, but comprising less than 2.5 percent of the assemblages in the acidic and neutralized AMD stations. Their relative abundance also was low in the stations with intermittent AMD inputs. Chironomid relative abundance also responded to AMD impacts as has been observed in other studies (e.g., Armitage 1980; Roback and Richardson 1969; Rutherford and Mellow 1994), increasing in relative abundance in the acidic AMD stations compared to unimpacted sites. These findings suggest that this watershed was a good system to use in a comparison of transplanted Asian clam responses to AMD with those of the resident community.

To our knowledge, one other study has compared transplanted Asian clam responses to indigenous community responses (Farris et al. 1988). In that study, clam cellulolytic activity was related to presence of suspended particles with bound metals, while invertebrate communities were most severely impacted where dissolved metals were highest. Clam and macroinvertebrate community endpoints were not compared statistically. In the present study, clam survival was positively correlated with relative abundance of Ephemeroptera, and negatively correlated with Chironomidae. These were the most and least sensitive taxonomic groups, respectively, to various levels of AMD input. Thus, clam survival in transplant tests appears to mirror benthic macroinvertebrate community responses to AMD in this watershed.

Clam growth could not be determined at the most severely impacted sites (group three) because all clams died, and at stations where clams survived, growth was minimal except in the fourth to sixth order stations (group five). Furthermore, clams decreased in size in the group two and four stations, as has been observed in response to low levels of copper, zinc, and chrysotile asbestos (Belanger et al. 1986a,b; Belanger et al. 1990), but those means were not significantly different from the mean for the upstream stations due to variability within groups.

Although clam growth was not statistically sensitive to AMD inputs and was not significantly correlated with AMD related chemistry parameters, it was positively correlated with nitrate concentrations, suggesting that the nutrient loads explained variations in clam growth in this headwater system. Growth was greatest in the larger, group five stations, which had the highest nitrate concentrations and the greatest flow, being fourth to sixth order streams. Clam growth was negatively correlated with relative abundance of the Plecoptera taxonomic group and the shredder functional feeding group, and positively correlated with relative abundance of hydropsychids, chironomids, and collector-filterers, providing further evidence of this nutrient-level connection with clam growth.

As filter-feeders, the success of Asian clams in terms of building biomass is dependent upon suspended and/or dissolved carbon as a food source. Coarse particulate organic matter (CPOM) is generally the major carbon source in a small system of first to third order streams. Thus, shredders, which feed upon CPOM, should be one of the dominant functional feeding groups in low-order streams (Vannote et al. 1980), and they had the second highest relative abundance in the group one stations of this study. Collector-filterers generally are not as abundant as shredders in headwater streams because of the relative lack of fine particulate or dissolved organic matter. Therefore, the poor clam growth in the group one stations is not unusual; however, these results have other bioassessment implications.

Based on these data, one should expect poor growth of Asian clams at stations upstream of acutely toxic inputs in headwater systems, as was observed for the group one stations, unless some artificial nutrient source is available. Both hydropsychids (collector-filterers) and chironomids (collector-gatherers) have been observed to reach high densities in response to dilute eutrophication or organic input (Wiederholm 1984). Clam growth in this watershed was significantly positively correlated with the relative abundance of both hydropsychids and chironomids. Therefore, robust growth of clams in a first to third order stream may also be an indicator of dilute anthropogenic organic or nutrient inputs (i.e., dilute septage or agricultural runoff).

To test this hypothesis, we conducted the comparison of clam growth and macroinvertebrate community indices for the Puckett's Creek group one stations versus the values for station SC-2, which was suspected of receiving elevated nutrient inputs. The nitrate concentration at SC-2 was 3.5 fold higher than the mean for the Puckett's Creek stations, and family-level biotic index (FBI) scores and percent collector-filterer values responded accordingly with 1.5 and 11.8 fold increases, respectively. An FBI score of 2.54 (the average for Puckett's Creek group one stations) corresponds to an 'excellent' rating with 'organic pollution unlikely', while a score of 3.86 (SC-2) indicates ‘possible slight organic pollution’ (Hilsenhoff 1988). Likewise, clam growth was about 18 fold greater in SC-2 than the mean for the Puckett’s Creek upstream stations. Thus, clam growth in transplant toxicity tests appears to be related to nutrient levels, and accurately reflects benthic macroinvertebrate responses to nutrient loading.

While clam survival and growth may provide much information about a small watershed, the tests are simple to conduct and cost little in terms of time and materials. Disadvantages of transplant studies with Asian clams include potential vandalism of test containers, predation upon test organisms, and potential release of propagules into previously uncolonized areas (Cherry 1996). However, these data suggest that transplanted clams may be a useful tool for preliminary reconnaissance of small headwater systems that Asian clams have already colonized.

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Chapter 3.

Relative acute toxicity of acid mine drainage water column and sediments to *Daphnia magna* in the Puckett's Creek watershed, Virginia, USA.

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Abstract - Acid mine drainage (AMD) is produced when pyrite (FeS_2) is oxidized upon exposure to oxygen and water to form ferric hydroxides and sulfuric acid. If produced in sufficient quantity, iron precipitate, heavy metals (depending on soil mineralogy) and sulfuric acid may contaminate surface and groundwater. A previous study of an AMD impacted watershed (Puckett's Creek, Powell River drainage, southwestern Virginia, USA) conducted by these researchers indicated that both water column and sediment toxicity were significantly correlated with benthic macroinvertebrate community impacts. Sites that had toxic water or sediment samples had significantly reduced macroinvertebrate taxon richness. The present study was designed to investigate the relative acute toxicity of acid mine drainage (AMD) water column and sediments to a single test organism (*Daphnia magna*), and to determine which abiotic factors were the best indicators of toxicity in this system. Nine sampling stations were selected based on proximity to major AMD inputs in the watershed. In 48-hour exposures, sediment samples from three stations were acutely toxic to *D. magna*, causing 64 to 100% mortality, while water samples from five stations caused 100% mortality of test organisms. Forty-eight-hour LC50 values ranged from 35 to 63% for sediment samples and 27 to 69% for water column samples. Sediment iron concentration and several water chemistry parameters were the best predictors of sediment toxicity, and water column pH was the best predictor of water toxicity. Based on these correlations and on the fact that toxic sediments had high percent water content, water chemistry appears to be a more important adverse influence in this system than sediment chemistry.

Introduction

The United States Environmental Protection Agency (US EPA) has singled out acid mine drainage (AMD) from abandoned coal mines as being the greatest water quality problem in the Appalachian region of the USA (Office of Surface Mining 1995). Acid mine drainage is produced when pyrite (FeS_2) rich coal and/or bedrock are exposed to oxygen and water, primarily due to anthropogenic activities. Through a number of reactions (some catalyzed by iron oxidizing bacteria), pyrite is oxidized, eventually to form ferric hydroxides and sulfuric acid. If produced in sufficient quantity, iron precipitate, heavy metals (depending on soil mineralogy) and sulfuric acid will contaminate surface and groundwater.

Acidic pH and trace heavy metals are each known to adversely affect benthic macroinvertebrate communities (Bell and Nebeker 1969; Clements *et al*. 1989; Haines 1981; Winner *et al*. 1980). In addition, several investigators have examined the effects of AMD on benthic macroinvertebrates (Armitage 1980; Cherry *et al*. 1995; Cherry *et al*. 1997; Herricks and Cairns 1974; Rutherford and Mellow 1994; Short *et al*. 1990; Smith and Frey 1971; Winterbourn and McDiffet 1996), and fish (Rutherford and Mellow 1994). These field studies generally revealed reduced diversity and abundance in impacted areas relative to unimpacted sites and/or species shifts from intolerant to tolerant taxa. Relative to the number of field studies of AMD impacts on biota, few investigators have examined toxicity of acid mine drainage water column and/or sediments in the laboratory. Vinyard (1996) observed chronic water column toxicity to *Ceriodaphnia dubia* with acidic samples collected from an actively mined area, and Fucik and colleagues (1991) found water samples collected from an AMD impacted watershed in Colorado to be acutely toxic to *C. dubia* and two fish species (*Salvelinus fontinalis* and *Pimephales promelas*).

Previously, an integrative assessment was conducted at 21 sampling stations in Puckett's Creek, a small watershed in southwestern Virginia, Powell River drainage, to determine environmental impacts of several AMD inputs (Cherry *et al*. 1998; Soucek *et al.* in press, chapter 1). In that study, the assessment techniques were successful in pinpointing severely impacted stations, which were characterized chemically by acidic pH (2.98-3.32), high conductivity (1456-1600 μmhos/cm), high water column concentrations of aluminum (Al) and iron (Fe) (35-46 and 18-43 mg/L, respectively), and high concentrations of Fe in sediments (53-174 g/kg). In addition, significant correlation was observed between laboratory toxicity testing results and benthic macroinvertebrate community data. That is, water column and sediment samples were significantly more toxic at stations with less rich benthic macroinvertebrate communities and vice versa. This large system included a number of stations that were not influenced by AMD inputs, but were potentially subjected to urban and/or domestic influences (Cherry *et al*. 1998; Soucek *et al.* in press, chapter 1).

The purpose of the present study was to focus on the area of the Puckett's Creek watershed (nine stations) most severely impacted by AMD to examine the relative acute toxicity of water column and sediments to a single test organism: the cladoceran, *Daphnia magna*. This test organism was chosen because of its use of both the water column and sediment portions of the aquatic environment and because approved protocols are available for toxicity testing in both media. As an epifaunal zooplankter, *D. magna* often is observed to skim the sediment surface, and therefore is exposed to both water-soluble contaminants and particle-bound contaminants on the sediment surface through ingestion (ASTM 1995). The second objective of this study was to determine which abiotic endpoints were correlated with acute water column and sediment toxicity within this system, and further, to evaluate the usefulness of sediment toxicity testing in AMD impacted watersheds.

Materials and methods

Study site

This study employed sediment and water column samples collected from the Puckett's Creek mainstem and Lick Branch, an AMD impacted tributary to Puckett's Creek, of the Powell River watershed, Lee County, Virginia, USA. Both water column and sediment samples were collected from nine sampling stations within Lick Branch and Puckett's Creek (Table 1 and Fig. 1). One station was a reference (LB-4), and the remaining eight were AMD impacted stations at various distances from point sources. The impacted sampling stations were selected to represent different levels of potential heavy metal contamination and acidity.

Water chemistry analysis

Water samples returned to the laboratory were stored for 24 hours at 4° C and analyzed under laboratory conditions. The pH was measured using an Accumet® (Fisher Scientific, Pittsburgh, PA, USA) pH meter equipped with an Accumet® gel-filled combination electrode (accuracy < ± 0.05 pH at 25 °C). A Yellow Springs (RDP, Dayton, OH, USA) model 54A meter was used to measure dissolved oxygen in the field. Conductivity measurements were made using a Hach® (Hach, Loveland, CO, USA) conductivity/TDS meter.

Metals Analysis

Water and sediment samples from each station were prepared according to US EPA (1991) methods for analysis for selected metals by the Virginia Tech soil testing laboratory via inductively coupled plasma spectrometry (ICP). Total recoverable metals analyzed included iron (Fe), aluminum (Al), manganese (Mn) and zinc (Zn). These metals were selected based on previous data (Cherry *et al*. 1998; Soucek *et al.* in press, chapter 1). Lower detection limits (mg/L) for metals were 0.001, 0.002, 0.002, 0.001, 0.007, and 0.002 for Al, Cu, Fe, Mg, Mn, and Zn, respectively. Correlation analysis then was conducted between all of the water chemistry and metals parameters using the JMP IN ® computer program (Sall and Lehman 1996) with significant relationships determined at the $\alpha = 0.05$ level.

Table 1. Sampling stations in Lick Branch and the mainstem of Puckett's Creek. * indicates station severely impacted by AMD.

Station	Station Type	Description
LB-5	Intermittent Impact	Left fork of Lick Branch near a minor acidic seep
LB-4	Reference	Right fork of Lick Branch above AMD inputs
LB-3*	AMD Impact	First AMD point source in right fork of Lick Branch prior to entrance into main creek body
PS-9*	AMD Impact	Second AMD point source in right fork of Lick Branch at point of entrance into main creek body
LB-2*	AMD Impact	Confluence of left and right forks of Lick Branch
LB-1*	AMD Impact	Lower section of Lick Branch mainstem prior to confluence with Puckett's Creek
PC-4	Impact/Recovery	Puckett's Creek just below Lick Branch confluence
PC-2	Impact/Recovery	Puckett's Creek ~0.5 mile downstream of Lick Branch
PC-1	Impact/Recovery	Puckett's Creek ~1 mile downstream of Lick Branch

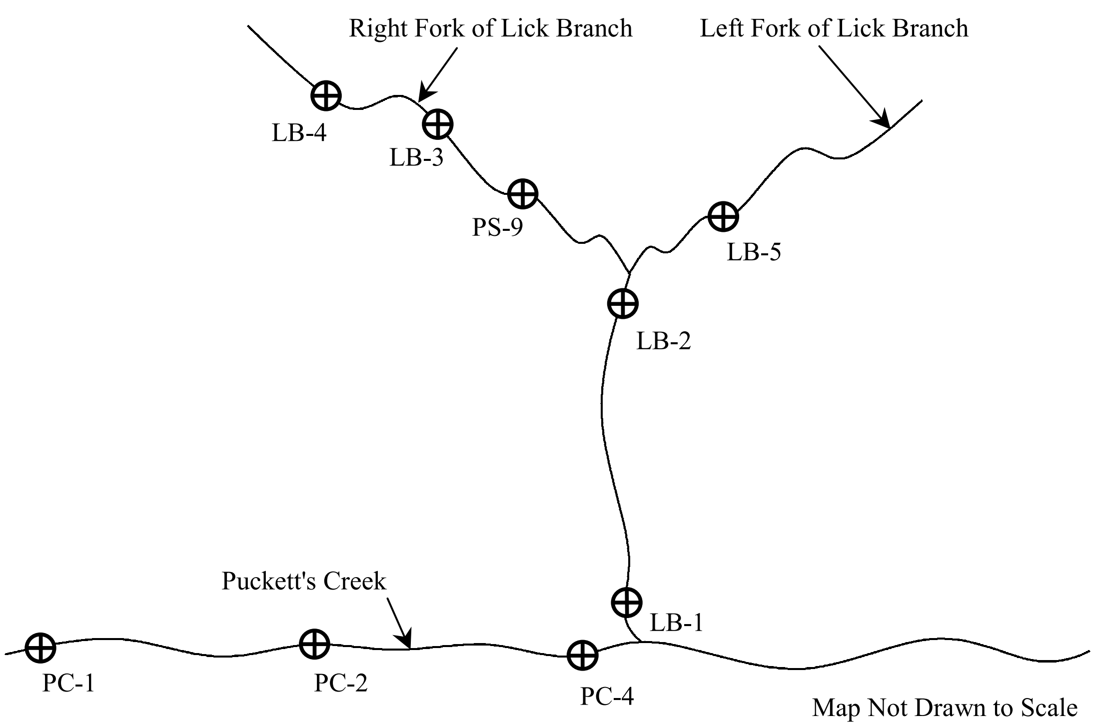

Figure 1. Diagram showing relative locations of Lick Branch and Puckett's Creek sampling stations. Acid mine drainage point sources enter the system at stations LB-3 and PS-9, while LB-5 has intermittent acidic input.

Culturing of Test Organisms

Test organisms were cultured in filtered water from a non-toxic reference stream near Newport, Virginia (Sinking Creek). Prior to testing, organisms were fed a diet of *Selenastrum capricornutum* and a Yeast-Cereal Leaves-Trout Chow (YCT) mixture at a rate of 0.18 ml each per 30-ml water, daily. Analysis of the culture water indicated that metal concentrations were low to undetectable (1.6 µg Al/L, 14.3 µg Fe/L, and copper (Cu) and Zn were below detection limits). Average pH, conductivity, alkalinity, and hardness for culture/diluent water was 8.01 ± 0.10, 225 ± 5.48 µmhos/cm, 131 ± 9.45 mg/L as $CaCO_3$, and 122.6 ± 4.16 mg/L as $CaCO_3$, respectively.

Water Column Toxicity

Range finding tests using 100 percent strength water samples were conducted to determine stations at which water column samples are acutely toxic. Five, 5-day-old *Daphnia magna* were placed into each of four replicate 50-ml beakers containing test solutions (four beakers per station). For stations at which greater than 25 percent mortality occurred at the end of 48 hours, full 48-hour acute toxicity tests were performed. In these tests, a 50 percent dilution series was utilized (100, 50, 25, 12.5 and 6.25 percent strength of sample), and tests were conducted according to standard methods for whole effluent toxicity testing (US EPA 1993). Water from Sinking Creek was used as diluent and control water. Forty-eight-hour LC50 values for acute tests were calculated using the Toxstat ® statistical software (Gulley 1993). In addition, bivariate correlation analyses were conducted between percent survival data and water and sediment chemistry data using the JMP IN ® computer program (Sall and Lehman 1996) to determine which abiotic parameters were correlated with water toxicity.

Sediment Toxicity

Sediment toxicity tests were performed for each sampling station using standard methods for 5-day-old *D. magna* (US EPA 1994; ASTM 1995); however, tests were conducted for 48 hours rather than seven days for comparison with water column tests. For stations in which mortality was observed within 48 hours, acute 48-hour sediment toxicity tests were performed with serial dilutions of sediments. A formulated sediment (80 percent sand and 20 percent potting soil) shown to provide consistently acceptable survivability and reproduction results (Soucek, unpublished data) according to US EPA (1994) and ASTM (1995) standards was used as a control sediment to make dilutions of 50, 25, 12.5 and 6.25 percent. This procedure allowed calculation of LC50 values for sediments using the Toxstat ® statistical software (Gulley 1993). As for water column tests, bivariate correlation analyses were conducted between percent survival data and water and sediment chemistry data using the JMP IN ® computer program (Sall and Lehman 1996) to determine which abiotic parameters were correlated with sediment toxicity. Sediment samples also were analyzed for percent water by weighing out 1-gram samples (three replicates per station), drying for 24 hours at 280ºC, and then re-weighing samples to obtain the difference.

Ferric Hydroxide Toxicity

To evaluate potential toxicity of iron precipitates in the laboratory, ferric hydroxide ($Fe(OH)_3$), or ferrihydrite ($Fe(OH)_3 \cdot XH_2O$) precipitate was prepared by adding ferric sulfate ($Fe_2(SO_4)_3$) to deionized water and neutralizing the solution with sodium carbonate (Na_2CO_3). The precipitate was allowed to settle, and the overlying water was decanted and replaced several times with deionized water. When the conductivity of the overlying water was less than 100 μmhos/cm the precipitate was centrifuged and the overlying water discarded. Aliquots of the semi-solid preparation then were placed into four replicate 50-ml beakers and covered with the same reference water used in other toxicity tests. When the precipitate had settled, test organisms (5-day old *D. magna*) were added to four replicate beakers (five daphnids per beaker), and mortality was evaluated after 48 hours.

Results

The water and sediment chemistry of samples collected from the nine stations (Table 2) for the present study was similar to that observed in the previous, whole watershed study (Cherry *et al*. 1998; Soucek *et al*. in press, chapter 1). The stations nearest the acid mine drainage point-sources had pH values ranging from 3.29 to 3.77, and conductivity ranging from 1070 to 1720 μmhos/cm. Water column Fe (0.77 to 6.56 mg/L) and Al (10.60 to 53.01 mg/L) concentrations also were highest at stations closest to point-sources as were sediment Fe concentrations (36.46 to 91.55 g/kg). None of the other sediment metals followed any discernable trend. Percent water of sediment samples did not follow a particular trend but was high at stations LB-3 and PS-9 (72.2 and 57.7%, respectively), and at PC-2 and PC-1 (69.8 and 69.1%, respectively).

Water column samples collected from five stations (LB-5, LB-3, PS-9, LB-2, and LB-1) were acutely toxic to *D. magna*, killing all test organisms within 48-hours (Table 3). LC50 values generated for those stations ranged from 27.08% (LB-3) to 69.43% (LB-1). Water samples from the remaining four stations were not acutely toxic to *D. magna*.

Table 2. Water chemistry and metals analysis for nine sampling stations.
* indicates station severely impacted by acid mine drainage. Cond = conductivity, Sed. = sediment, H2O = water column.

Station	pH	Cond. (μmhos/cm)	H2O Fe (mg/L)	H2O Al (mg/L)	Sed. Fe (g/kg)	Sed. Al (g/kg)	Sed. Mn (g/kg)	Sed. Zn (mg/kg)	Sed. % H2O
LB-5	5.43	230	4.75	0.02	25.35	2.08	0.082	13.78	31.8
LB-4	7.20	300	0.08	0.03	6.42	1.83	1.435	10.35	26.9
LB-3*	3.30	1720	6.38	53.01	91.55	1.22	0.015	13.44	72.2
PS-9*	3.29	1160	6.56	20.14	90.39	1.84	0.067	15.72	57.7
LB-2*	3.34	1060	5.78	16.67	43.46	1.95	0.094	16.52	31.7
LB-1*	3.77	1070	0.77	10.60	36.46	5.00	0.118	28.87	35.3
PC-4	6.08	650	0.43	0.02	19.78	4.03	0.247	50.12	54.3
PC-2	7.39	510	0.02	0.08	11.92	8.81	1.221	119.32	69.8
PC-1	7.44	480	0.04	0.12	6.68	5.99	0.389	120.98	69.1

Table 3. Percent survival of *D. magna* and LC50 values for acid mine drainage water column and sediment acute (48-hour) toxicity tests. * indicates station severely impacted by acid mine drainage.

Station	Water Column % Survival	Water Column LC50 (%)	Sediment % Survival	Sediment LC50 (%)
LB-5	0	69.01	100	>100
LB-4	80	>100	96	>100
LB-3*	0	27.08	0	35.39
PS-9*	0	33.18	0	63.93
LB-2*	0	43.52	36	55.07
LB-1*	0	69.43	96	>100
PC-4	70	>100	100	>100
PC-2	96	>100	96	>100
PC-1	100	>100	96	>100

Sediment samples from three stations (LB-3, PS-9, and LB-2) were acutely toxic to *D. magna,* with survival ranging from zero (LB-3 and PS-9) to 36% (LB-2) after 48 hours (Table 3). The other stations had 96 to 100% survival. Calculated LC50 values for sediment samples were similar to water column LC50 values, ranging from 35.39% (LB-3) to 63.93% (PS-9). Two stations that had toxic water column samples but non-toxic sediment samples were LB-5 and LB-1. LB-1 is approximately 1-mile downstream of the major AMD point source, while LB-5, in the opposite fork of Lick Branch as the major point sources, has proven to be an intermittent AMD seep, periodically causing water column toxicity (Cherry *et al*. 1998; Soucek *et al*. in press, chapter 1).

Sediment and water column toxicity test data then were compared to concurrent abiotic data using correlation analysis. The only sediment metal that significantly correlated with sediment test survival was Fe ($r = -0.95$) (Table 4); however, four water chemistry parameters (pH, conductivity, water column Fe and Al) significantly correlated with sediment test survival. Water column toxicity test survival was most strongly correlated with pH ($r = +0.92$). Both water column and sediment Fe concentrations were significantly negatively correlated with water column survival; however, water column Al was not. In addition, sediment Mn and Zn concentrations were significantly correlated with water column survival, but those correlations were positive, suggesting that the more Mn and Zn in the sediments, the less toxic the overlying water.

Correlation analysis between abiotic parameters revealed several significant relationships (Table 5). For example, water column pH was significantly negatively correlated with conductivity, water column Fe and Al, and sediment Fe concentrations with r-values ranging from -0.66 (Al) to -0.85 (sediment Fe). This suggested that the higher the pH value at a given station, the lower the sediment Fe concentration, conductivity, and so on. Alternatively, sediment Mn and Zn were significantly positively correlated with water column pH. Additional significant relationships are shown in Table 5.

Finally, in the toxicity test with pure ferric hydroxide precipitate as bottom substrate, all of the test organisms died within 48-hours. Analysis of overlying water by atomic absorption spectrometry indicated that dissolved Fe concentrations in the water column were below detection limits (0.002 mg/L).

Table 4. Correlation analysis of *D. magna* sediment and water column 48-hour toxicity test survival with concurrent abiotic data for the nine sampling stations. Only significant correlations ($p<0.05$) are shown.

Parameter (vs. Sed. Survival)	r-value	Parameter (vs. H2O Survival)	r-value
pH	+0.67	pH	+0.92
conductivity	-0.79	H20 Fe Conc.	-0.82
Sed. Fe Conc.	-0.95	Sed. Fe Conc.	-0.74
H2O Al Conc.	-0.93	Sed. Mn Conc.	+0.74
H2O Fe Conc.	-0.77	Sed. Zn Conc.	+0.69

Table 5. Correlation coefficients (r) for water chemistry and metals data from nine sampling stations. Cond = conductivity, Sed. = sediment, H2O = water column. Only significant correlations ($p<0.05$) are shown. Note that a negative sign before an r-value indicates a negative correlation, while no sign indicates a positive correlation.

Station	Cond.	H2O Fe	H2O Al	Sed. Fe	Sed. Al	Sed. Mn	Sed. Zn
pH	-0.82	-0.81	-0.66	-0.85	-	0.75	0.68
Cond.	-	-	0.87	0.87	-	-	-
H2O Fe	-	-	0.73	0.84	0.70	-	-
H2O Al	-	-	-	0.86	-	-	-
Sed. Fe	-	-	-	-	-	-	-
Sed. Al	-	-	-	-	-	-	0.90

Discussion

Overall, low pH, high conductvity, high water column metals concentrations, and high sediment Fe concentrations were correlated with low survival of daphnids in both water column and sediment toxicity tests. While others have observed correlations between high sediment metal concentrations contributed by hard-rock mining activities and sediment toxicity (Kemble *et al*. 1994), McCann (1993) found no evidence of sediment toxicity to juvenile mussels in 10-day tests with sediments collected from various sites within the coal-mining-influenced Powell River watershed, southwestern Virginia, USA. A potential explanation for this disparity is that the hard-rock mining sediments had high concentrations of cadmium and copper (up to 31.3 and 6971 mg/kg, respectively) than those previously observed in Powell River watershed station sediments (undetectable for cadmium and up to 14.7 mg/kg for copper, Kemble *et al*. 1994; Cherry et al., 1998). McCann (1993) did not analyze sediments for metal concentrations. In addition to those studies, our previous work in an AMD impacted watershed (Ely Creek) adjacent to Puckett’s Creek suggested that sediment metals concentrations in that system were not correlated with sediment toxicity (Soucek *et al*. 1998). Again, in this study sediment toxicity did not correlate with concentrations of any other sediment metals (Al, Mn and Zn). These results likely are in part attributable to increased solubility of most metals at low pH values which results in leaching of metals from sediments and/or prevention of adsorption of water column metals to sediments. Dissolved metals then precipitate when pH levels downstream rise to the appropriate level for each particular metal (Snoeyink and Jenkins 1980). However, in the Puckett's Creek whole watershed study, chronic sediment toxicity was not observed for any samples collected from circum-neutral pH sites (averaging 6.11 to 8.01) downstream of AMD point-sources where metals would be expected to accumulate in sediments (Cherry *et al*. 1998; Soucek *et al*. in press, chapter 1). These results suggest that metal deposition at these sites was not a problem detectable by standard chronic sediment toxicity assessment parameters.

While most sediment metal concentrations did not correlate with sediment toxicity, sediment Fe concentrations correlated with both sediment and water column toxicity. The correlation between sediment Fe and water column toxicity probably is not a causal relationship. This trend likely is related to the fact that fully oxidized Fe can reach saturation in acid mine waters with respect to either ferric hydroxides ($Fe(OH)_3$) or the mineral jarosite ($KFe(SO)_4(OH)_6$), and precipitates of both of these minerals may be observed even in acidic streams (Nordstrom 1982; Nordstrom *et al*. 1979). In addition, sites in this study nearest the AMD point-sources had a high percentage of water (31 to 72%) in their sediment samples, and some dissolved Fe may have been measured in the sediment Fe analysis concurrently with precipitated Fe. As a result, sites nearest AMD point-sources had the highest sediment Fe concentrations, and there was a strong negative correlation in this study between sediment Fe concentration and water column pH ($r = -0.85$). This, coupled with the fact that pH was highly correlated with water column toxicity ($r = 0.92$), could explain the relationship between water toxicity and sediment iron concentrations. A similar relationship was observed for sediment Mn and Zn as both were positively correlated with water column toxicity test survival, and both also were significantly positively correlated with pH.

Conversely, Fe in sediments may or may not have a causal role in sediment toxicity. Others have suggested that ferric precipitates from AMD may have a smothering effect on biota (Hynes 1960; Lackey 1938) and that they may even cause physical abrasion (Eddlemon and Tolbert 1983). To evaluate these claims in the laboratory, we conducted the toxicity test with pure ferric hydroxide as bottom substrate. While all test organisms died within 48 hours, no dissolved Fe was detected in the overlying water. This preliminary experiment supports the hypothesis that Fe precipitates in sediments may impart some sort of toxicity to aquatic biota, either through ingestion or a smothering effect.

In this reduced nine station system, several water column chemistry parameters (pH, conductivity, metals) were good predictors of both sediment and water column toxicity. The high percentages of water (with acidic pH and elevated metals) in sediment samples probably account for the correlation of water chemistry parameters with sediment toxicity. These results suggest that water column chemistry has a more important adverse influence on this system than sediment chemistry. McCann (1993) observed similar results in the Powell River watershed. When using dechlorinated tap water for overlying water in sediment tests, no toxicity was observed for sediments collected downstream of a coal processing plant; however, when site specific overlying water was used in tests, toxicity was observed in samples from downstream sites after ten days. Her results suggest that water-borne metals had a greater influence on aquatic biota than sediment bound metals in an area influenced by mining activities. Further evidence of the importance of water chemistry in the present study is provided by the fact that no sediment toxicity occurred at sites where there was no water column toxicity. In addition, two sites (LB-5 and LB-1) that had water column toxicity but relatively high LC50 values (69.01 and 69.43%, respectively) did not have sediment toxicity. Compared to the other AMD impacted stations, these had higher pH values (5.43 and 3.77, respectively), lower water column Al concentrations (0.02 and 10.6 mg/L, respectively), and only 31.8 to 35.3 % water in sediments. These two stations also had lower sediment Fe concentrations than those with acutely toxic sediments. As discussed previously, precipitated Fe may impart some sort of physical impairment on test organisms, which may act in combination with dissolved metals and acid present in sediment pore water.

A final point to be made from these data is that the scale of a project may be important when deciding whether or not to use sediment toxicity testing as a bioassessment tool for AMD impacted watersheds. Bioassays are most effective when they accurately determine environmental impacts of pollutants in a cost and time efficient manner, but in some cases, several types of bioassays may be required to accurately describe a system. Soucek et al (in press, chapter 1) concluded that because of the diversity of inputs to the 21 sampling stations, both water column and sediment toxicity testing were required to adequately describe the environmental status of the whole watershed. Both water column and sediment toxicity test survival were significantly correlated with benthic macroinvertebrate taxon richness at the 21 sampling sites; however, using the same data but including only the nine stations used in the present study, only water column toxicity correlated with taxon richness (Cherry *et al*. 1998; Soucek *et al*. in press, chapter 1). Given those results and the fact that this study suggests that sediment toxicity is closely related to water chemistry in the reduced system, water column toxicity testing alone may be a sufficient bioassessment tool in a small-scale study consisting of stations in which acute water column toxicity occurs and the only major adverse impact is AMD.

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THEME II.

Impacts of Acid Mine Drainage Beyond the Zone of pH Depression

Chapter 4.

Aluminum dominated acute toxicity in neutral waters downstream of an acid mine drainage discharge

Abstract - Acid mine drainage is traditionally considered to impact aquatic ecosystems by acidification, metal precipitation smothering stream substrates, and sediment toxicity in association with trace metals. We conducted Whole Effluent Toxicity (WET) tests with both field collected and laboratory synthesized AMD samples to determine the mechanism of reduced benthic macroinvertebrate community diversity in neutral (pH >7.0) waters downstream of an acidified tributary. Our results indicate that Al and Fe in transition from acidic waters to neutralizing receiving streams can cause acute toxicity to standard invertebrate test organisms at neutral pH. Aluminum in the process of precipitation was determined to be the cause of acute toxicity in the field for up to a mile downstream of the AMD influenced tributary, and was the likely cause of reduced community diversity at those sites. While Fe singly may cause acute toxicity in this type of a system, it appears to reduce the toxicity of combinations of other metals such as Al, Cu, and Zn.

Introduction

A number of investigators have studied the toxic interactions of reduced pH and increased metal concentrations in aquatic systems, a scenario produced in the field by acid precipitation and acid mine drainage (AMD). Acid mine drainage occurs when minerals containing reduced forms of sulfur (pyrites, sulfides) are oxidized upon exposure to water and oxygen. The oxidation process results in production of strong acids, which mobilize acid soluble metals, including iron (Fe), aluminum (Al), copper (Cu), zinc (Zn), and others depending on the mineralogy of the disturbed area. The mixture of acid and dissolved metals creates a severe environment for aquatic organisms. Numerous studies have documented the impacts of AMD upon aquatic communities in acidified stream reaches (e.g., Armitage 1980; Kelly 1988; Roback and Richardson 1969); these impacts include reduced taxon richness and abundance, and/or a shift from pollution sensitive to pollution tolerant species.

While the pH-related ecosystem impacts of AMD are well documented, AMD can also cause impairment to aquatic organisms beyond where surface waters are acidified. Sediment toxicity may occur where trace metal concentrations are high (Kemble et al. 1994). Ferric hydroxide precipitate (a product of pyrite oxidation) is thought to cause ecosystem impairment by smothering available habitat and reducing periphyton productivity (McKnight and Feder 1984; Scullion and Edwards 1980). Solid ferric hydroxide also has been shown to cause acute toxicity to *Daphnia magna* in the absence of dissolved Fe (Soucek et al. 2000a, chapter 3). Furthermore, Al has been observed to cause acute toxicity to fish in mixing zones (pH 4.8-6.5) below acidic tributaries (Henry et al. 1999; Rosseland et al. 1992), and to alter snail behavior through ingestion (Campbell et al. 2000; Elangoven et al. 1997). These findings suggest that AMD is a complex pollutant that can impair aquatic ecosystems in a variety of ways, and that further research should focus upon effects beyond the zone of pH depression.

The purpose of the present study was to investigate the cause of significant benthic macroinvertebrate community impairment within the circumneutral sites downstream of Lick Branch, an acidified tributary to Puckett's Creek of the Powell River watershed, southwestern Virginia, USA. At these sites, several community indices, including total abundance, Ephemeroptera-Plecoptera-Trichoptera (EPT) richness, and percent Ephemeroptera abundance, were significantly lower than at stations upstream of AMD inputs or stations further downstream with additional dilution (Soucek et al. 2000b, chapter 1). These community impacts extended approximately one mile downstream of the confluence of the acidified tributary, but despite the observed precipitation of metals at these sites, sediment tests with standard test organisms did not detect toxicity. Therefore we hypothesized that impairment was due to water column toxicity, and we conducted laboratory tests with both field collected water samples and synthesized AMD effluents to determine the mechanism of toxicity. The cladoceran *Ceriodaphnia dubia* was chosen as the test organism, as responses of this organism to AMD samples were previously observed to correlate significantly with several benthic macroinvertebrate community indices for this watershed (Soucek et al. 2000b, chapter 1).

Materials and methods

Experimental design

Investigation of the toxic mechanisms in the circumneutral pH sites began by collecting water samples from the discharge source in Lick Branch (designated as station LB-3) and from three points in the neutralizing receiving stream, Puckett's Creek. Acute toxicity tests were conducted with these samples using *C. dubia* to obtain 48-hour LC50 values and/or percent survival in full strength samples. Then, metals analyses were conducted on the samples to determine Al, Cu, Fe, and Zn concentrations in addition to pH and conductivity values.

Next, a number of artificial AMD effluents were formulated in the laboratory containing acid alone or acid plus one to four metals in various combinations at pH, conductivity and metal concentrations similar to those measured for the point source. These artificial effluents were used as starting solutions in Whole Effluent Toxicity (WET) tests with a 50% dilution series mimicking downstream dilution. Then, mean LC50 values were calculated for each artificial effluent, and metal concentrations at LC50 values were calculated based on measured metal concentrations in starting solutions. Comparison of metal concentrations at LC50 values for artificial effluents with those in field collected samples from the LB-3 discharge and the three downstream stations allowed determination of which metals had the largest toxic role in the neutral receiving stream. Then, Marking's Additive Index values (Marking 1977) were calculated to determine if metal combinations acted synergistically or antagonistically. In addition, the hypothesis that precipitation of Al and Fe was involved in the mechanism of AMD toxicity was tested by conducting time-delayed toxicity assays.

Water chemistry of field collected AMD samples

Water samples were collected from the LB-3 discharge in Lick Branch, a tributary to Puckett's Creek, Powell River drainage, southwestern Virginia, USA, and from three stations in Puckett's Creek downstream of Lick Branch. The three Puckett's Creek stations were immediately downstream (station PC-4), 0.5 miles downstream (PC-2), and one mile downstream (PC-1) of Lick Branch. Samples were stored at 4° C until analyzed.

Water quality parameters including pH, conductivity, and dissolved oxygen were measured for each sample, and alkalinity, acidity and hardness were measured where appropriate. The pH was measured using an Accumet® (Fisher Scientific, Pittsburgh, PA, USA) pH meter equipped with an Accumet® gel-filled combination electrode (accuracy < ± 0.05 pH at 25 °C). A Yellow Springs (RDP, Dayton, OH, USA) model 54A meter was used to measure dissolved oxygen. Conductivity measurements were made using a Hach® (Hach, Loveland, CO, USA) conductivity/TDS meter. Alkalinity, acidity, and hardness were measured by titration as described in APHA et al. (1995). In addition, water column samples were analyzed for total Al, Cu, Fe, and Zn concentrations. Cu and Zn were previously determined to be the most concentrated trace metals in the discharge (Soucek unpublished data). Samples were acidified, filtered (0.45 μm pore size) and then analyzed by inductively coupled plasma spectrometry. Lower detection limits ($mg \cdot L^{-1}$) for metals were 0.001 (Al), 0.0005 to 0.0018 (Cu), 0.002 (Fe), and 0.002 (Zn).

Culturing of test organisms

Ceriodaphnia dubia were used in toxicity tests and were cultured in filtered water from a non-toxic reference stream near Newport, Virginia (Sinking Creek). Analysis of the culture water indicated that metal concentrations were low to undetectable (2 $\mu g \cdot L^{-1}$ Al, 14 $\mu g \cdot L^{-1}$ Fe, and Cu and Zn were below detection limits). Average pH, conductivity, alkalinity, and hardness for culture water was 8.01 ± 0.10, 225 ± 5 $\mu mhos \cdot cm^{-1}$, 131 ± 9 $mg \cdot L^{-1}$ as $CaCO_3$, and 122 ± 4 $mg \cdot L^{-1}$ as $CaCO_3$, respectively. Prior to testing, organisms were fed a diet of *Selenastrum capricornutum* and a Yeast-Cereal Leaves-Trout Chow (YCT) mixture at a rate of 0.18 ml each per 30-ml water, daily.

Water column toxicity testing with field collected samples

Seven replicate whole effluent toxicity tests were conducted with the LB-3 discharge effluent sample according to U.S. EPA (1993) methods. U.S. EPA (1993) moderately hard synthetic freshwater was used to dilute the effluent (50% dilution series) and as a control. The lowest concentration used was 0.78%. Five organisms were placed into each of four replicate 50-ml beakers per concentration for 48 hours. Tests were conducted at 25 °C. At the end of the test period, the number surviving per concentration was recorded, and LC50 values were calculated using the Spearman-Karber method.

For the three downstream stations (PC-1, PC-2, and PC-4), only undiluted samples were tested. Samples from these sites were collected, analyzed for water chemistry parameters as described previously, and tested for toxicity on two separate occasions: under moderate and low flow conditions. Again, four replicate beakers were used per site, with five organisms per 50-ml beaker. Percent survival for each station was recorded after 48 hours.

Synthetic AMD solutions

After water chemistry data for the LB-3 discharge were collected, synthetic AMD solutions were made to simulate the field-collected sample. The first synthetic AMD effluent, containing all four measured metals, was designated as S-LB-3. Metal salts (Al, Cu, Fe^{3+} and Zn sulfates) were added in appropriate concentrations to moderately hard synthetic freshwater acidified to a pH of approximately 2.7 with sulfuric acid. With metal salts added to the acidic solution to ensure complete dissolution, the pH, conductivity and acidity then were adjusted to appropriate levels using sodium bicarbonate and sulfuric acid.

Nine other synthetic solutions also were developed to investigate individual and interacting effects of metal combinations and acid. They contained zero to three of the four metals at concentrations equal to those present in the LB-3 sample, and had the same pH, conductivity and acidity. In addition to four solutions containing only one of the four metals, combinations of Al-Fe, Cu-Zn, Al-Cu-Zn, Cu-Zn-Fe, and acid only were synthesized. These solutions served as starting points for WET tests, diluted in a 50% series with moderately hard synthetic freshwater and conducted as described previously for the field collected sample. Three to four WET tests were conducted with each starting solution to allow statistical comparison. The starting solutions then were analyzed for appropriate metal concentrations as described previously to confirm nominal concentrations. Mean LC50 values for synthesized solutions and the LB-3 sample were compared by analysis of variance (ANOVA) using JMP IN ® software (Sall and Lehman 1996) to determine how well the synthesized solutions simulated the actual effluent. Student's *t* test was used for post-hoc, pair-wise analysis at the $\alpha = 0.05$ level.

Synergism/antagonism in metal mixtures

To investigate possible synergistic and/or antagonistic toxic effects of combinations of two, three and four AMD metals, Marking's Additive Index (MAI; Marking 1977) values were calculated using the following formula:

$$(\mathrm{A}m/\mathrm{A}i) + (\mathrm{B}m/\mathrm{B}i) + (\mathrm{C}m/\mathrm{C}i) + (\mathrm{D}m/\mathrm{D}i) = \mathrm{S} \qquad (1)$$

where A, B, C, and D are different metals, *i* is the LC50 concentration of the metal individually, and *m* is the LC50 concentration of the metal in the given mixture. The index value is made symmetric about zero as follows:

$$\text{if } \mathrm{S} \geq 1.0,\ \mathrm{MAI} = -\mathrm{S} + 1.0; \qquad (2)$$

$$\text{if } \mathrm{S} \leq 1.0,\ \mathrm{MAI} = (1/\mathrm{S}) - 1.0. \qquad (3)$$

If the MAI value is positive, the mixture is synergistic, while negative values indicate antagonism. Statistical significance then can be determined by inserting mean 95% confidence limits into the MAI equations. The lower limits of the individual concentrations (A*i*, B*i,* etc.) and the upper limits of the mixture concentrations (A*m,* B*m,* etc.) are used to determine lower limits of the index. Likewise, the upper limits of the individual concentrations and the lower limits of the mixture concentrations are used to calculate the upper limits of the index. This method provides the greatest deviation from the LC50 additive index. If upper and lower confidence limit MAI values do not overlap zero, the LC50 MAI value is considered different from zero (Marking 1977).

Precipitation experiments

Others have suggested that the mechanism of Al toxicity to fish is the process of hydroxide precipitation, forming polymers on fish gills upon neutralization (Neville and Campbell 1988; Exley et al. 1996; Poléo 1995). Therefore, we conducted additional assays with both Fe and Al individually to test the hypothesis that precipitating metals played a role in the toxic mechanism of AMD in our system. We did this by placing test organisms into a neutralized solution containing Al or Fe beginning to precipitate immediately after mixing, and at a given time period post-mixing. We proposed that if the difference in organism survival in these different treatments was significantly greater than that explained by increased organism fitness upon additional time spent feeding and growing, it may be concluded that the precipitation process is contributing to toxicity of the solution. That is, if organisms survive better in a solution after precipitation has occurred for a time, the precipitation process may be the mechanism of toxicity.

Starting solutions were prepared as described previously with each metal at its appropriate concentration (as measured in LB-3) and at pH 2.9. Then, solutions with metal concentrations equal to the 48-hour LC50 values (as determined in this study) were prepared by mixing starting solutions with appropriate amounts of Moderately Hard Synthetic water (U.S. EPA 1993), causing neutralization of the solution. Each solution was poured into two sets of five beakers concurrently. Test organisms (*C. dubia*, five per beaker) then were placed into the beakers at time zero (immediately after mixing), and 8 hours after mixing (five replicates each). The eight-hour time period was determined in preliminary experiments to be the point at which nominally increased survival was observed in tests with aluminum (Soucek unpublished data). The pH was measured in each set of beakers upon addition of test organisms and metal concentrations were measured as described previously. Percent survival was determined for each solution 48 hours after organisms were added.

To ensure that changes in mortality over time were not due to increased age/strength of test organisms, the same tests were conducted with sodium chloride (2.5 $g \cdot L^{-1}$ NaCl). This salt should remain dissolved indefinitely under the test conditions used; thus, any decrease in mortality during the salt tests should be due to increased fitness of test organisms. ANOVA and Student's *t* test as a pair-wise post-hoc analysis were used to determine significant differences ($\alpha = 0.05$) between the Al, Fe, and NaCl treatments in average increase in survival for organisms added at 8 hours post-mixing.

Results

Chemistry and toxicity of field collected samples

Water chemistry parameters for the field-collected LB-3 sample were as follows: pH = 2.9, conductivity = 2000 μmhos·cm^{-1}, acidity = 625 mg·L^{-1} as $CaCO_3$, total Al = 77.2 mg·L^{-1}, total Fe = 24.26 mg·L^{-1}, total Cu = 88.6 μg·L^{-1}, and total Zn = 1.251 mg·L^{-1}. Hardness was not determined due to interference in the titration method. Dissolved oxygen concentrations in all field-collected samples were at saturation. All synthesized AMD solutions were made using these water quality parameters as target values (Table 1). The LB-3 discharge was extremely toxic with a mean (n = 7) 48-hour LC50 value for *C. dubia* of 1.76 ± 1.06%.

Table 1. Summary of nominal water chemistry parameters* for synthetic acid mine drainage starting solutions for Whole Effluent Toxicity tests.

Solution	pH	Al (mg·L^{-1})	Cu (mg·L^{-1})	Fe (mg·L^{-1})	Zn (mg·L^{-1})
1) Al	2.90	77	0	0	0
2) Cu	2.90	0	0.089	0	0
3) Fe	2.90	0	0	24	0
4) Zn	2.90	0	0	0	1.25
5) Cu & Zn	2.90	0	0.089	0	1.25
6) Al & Fe	2.90	77	0	24	0
7) Al, Cu & Zn	2.90	77	0.089	0	1.25
8) Cu, Zn, & Fe	2.90	0	0.089	24	1.25
9) S-LB-3	2.90	77	0.089	24	1.25
10) Acid	2.90	0	0	0	0

*Conductivity for each solution was approximately 2,000 μmhos·cm^{-1}, and acidity was 500 to 600 mg·L^{-1} as $CaCO_3$. Metal concentrations are based on those from the LB-3 effluent sample.

The three downstream stations had low metal concentrations and near neutral pH values under moderate flow (Table 2). Except for Al at all three stations, metals were below acute water quality criteria (WQC) limits for protection of aquatic life (U.S. EPA 1986). Copper concentrations were below detection limits (0.0018 mg·L^{-1}) at the three downstream sites. Alkalinity and hardness values were 42, 32, 40, and 110, 96, 104 $mg \cdot L^{-1}$ as $CaCO_3$ for PC-4, PC-2, and PC-1, respectively. In undiluted samples from these stations, all organisms survived the 48-hour tests.

Table 2. Water column metals ($mg \cdot L^{-1}$), pH and percent survival of *C. dubia* in full strength samples from an AMD discharge (LB-3), and from three stations downstream of the AMD impacted tributary collected under moderate and low flow conditions. AMD = acid mine drainage

Station	Al	Fe	Cu	Zn	pH	% Survival
			Moderate Flow			
LB-3	77.2	24.26	0.088	1.251	2.9	0
PC-4	1.619	0.4818	<0.0018	0.0277	7.3	100
PC-2	1.110	0.1989	<0.0018	0.0264	7.5	100
PC-1	0.913	0.2089	<0.0018	0.0315	7.7	100
			Low Flow			
Station	Al	Fe	Cu	Zn	pH	% Survival
LB-3	60.50	24.39	<0.0005	0.987	3.0	0
PC-4	2.095	1.444	<0.0005	0.0168	7.2	20
PC-2	2.653	1.504	<0.0005	0.0258	7.5	20
PC-1	2.512	1.469	<0.0005	0.0207	7.7	25

A second set of samples was collected from the field stations under low flow conditions. Concentrations of Al and Zn were slightly lower in the LB-3 sample compared to the previous sample, the Fe concentration increased slightly, and Cu was below detection limits (Table 2). The pH (3.0) of the sample was slightly higher than that of the previous sample. With lower flow conditions in the receiving system, Al and Fe concentrations were higher (2.095 $mg \cdot L^{-1}$ - 2.653 $mg \cdot L^{-1}$ and 1.444 $mg \cdot L^{-1}$ - 1.504 $mg \cdot L^{-1}$, respectively) in the three downstream stations than in the previous samples, while Zn concentrations were similar. Again, pH values at the stations were near neutral, ranging from 7.2 (PC-4) to 7.7 (PC-1). Alkalinity values were similar to those of the previous samples (48 $mg \cdot L^{-1}$, 40 $mg \cdot L^{-1}$, and 40 $mg \cdot L^{-1}$ as $CaCO_3$ for PC-4, PC-2 and PC-1, respectively), but hardness values were not obtained due to interference in the titration method. With increased Al and Fe concentrations, toxicity of the undiluted samples increased, as only 20%, 20%, and 25% survival were observed in the PC-4, PC-2, and PC-1 samples, respectively, after 48-hours.

Synthetic AMD solutions

For the moderately hard synthetic water used as the base in synthesized AMD solutions and diluent in toxicity tests, the mean pH, conductivity, alkalinity, and hardness were 8.15, 300 μmhos$\cdot$cm^{-1}, 70 mg$\cdot$L^{-1} as $CaCO_3$, and 89 mg$\cdot$L^{-1} as $CaCO_3$, respectively.

Comparing mean LC50 values, several synthesized solutions had similar effects to those of the LB-3 sample (Table 3). The S-LB-3 solution, containing all four metals at pH 2.90, and the solutions containing Al-Cu-Zn, and Al-Fe at pH 2.90, most closely simulated the actual effluent with LC50 values of 2.81%, 2.15%, and 2.82%, respectively, as compared to 1.76% for LB-3. While S-LB-3, Al-Cu-Zn, and Al-Fe simulated the actual discharge, the mean LC50 for Al alone at pH 2.9 (3.825 %) was not significantly different from that of LB-3. The other six starting solutions had mean LC50 values that were significantly higher than that of LB-3, ranging from 4.79% (Fe) to 34.95% (acid alone).

The pH values at LC50 concentrations for tests with metals all were circumneutral, ranging from 7.4 for Zn to 7.8 for the LB-3 sample (Table 3). The pH values of test concentrations nearest LC50 values were taken as estimates of pH at LC50 concentrations except for in the case of the acid alone solution. The pH at the LC50 concentration was not estimated for the acid alone solution because the LC50 concentration was centered between two high percent dilutions (25% and 50%), and there was a large difference between the mean pH values of the two concentrations.

Table 3. Mean LC50 values (as percent of starting solution) and mean pH values at LC50 concentrations for field collected and laboratory-synthesized solutions[a] with various metals.

Starting solution	n[b]	Mean LC50 (%) ± SD	Sig. [b]	pH @ LC50
LB-3 (Al, Cu, Fe, Zn, pH 2.9)	7	1.76 ± 1.06		7.8 ± 0.2
Al, pH 2.9	4	3.83 ± 2.03		7.6 ± 0.2
Cu, pH 2.9	3	7.76 ± 0.80	*	7.6 ± 0.
Fe, pH 2.9	3	4.79 ± 1.16	*	7.6 ± 0.1
Zn, pH 2.9	3	27.63 ± 6.16	*	7.4 ± 0.1
Cu & Zn, pH 2.9	3	6.43 ± 0.12	*	7.6 ± 0.1
Al & Fe, pH 2.9	3	2.83 ± 1.09		7.6 ± 0.1
Al, Cu & Zn, pH 2.9	4	2.15 ± 0.79		7.7 ± 0.1
Cu, Zn, & Fe, pH 2.9	4	7.77 ± 3.06	*	7.7 ± 0.1
S-LB-3[c] (Al, Cu, Zn, Fe, pH 2.9)	4	2.81 ± 0.46		7.7 ± 0.1
Acid, pH 2.9	3	34.95 ± 0.70	*	N/A

[a]Starting solutions had similar pH and metal concentrations to those in the field collected sample: pH – 2.9, Al – 77 mg·L^{-1}, Fe - 25 mg·L^{-1}, Cu – 89 µ g·L^{-1}, Zn – 1.2 mg·L^{-1}.
[b]n = number of tests conducted, Sig.* = statistically different from LB-3 LC50.
[c]S-LB-3 = synthesized LB-3, which contained all four metals.

Based on metals analysis of starting solutions, metal concentrations at LC50 values were calculated. For the LB-3 and S-LB-3, and Al-Cu-Zn solutions, Fe (if present), Cu and Zn were below WQC limits, while Al was above the limits at the LC50 concentration (Table 4). For tests with individual metals, Al, Zn and Fe LC50 concentrations were above WQC limits, but Fe, at 1.17 mg·L^{-1}, was close to the limit of 1.0 mg·L^{-1}. Copper has a hardness-based WQC, which at a value of 89 (the mean hardness for diluent water and near the hardness of the field receiving system) is 0.016 mg·L^{-1}. In tests with copper at pH 2.9 as a starting solution, the LC50 concentration was 0.0068 mg·L^{-1}, lower than the WQC limit.

Table 4. Mean concentrations of respective metals and 95% confidence limits (in parentheses) at LC50 values for field collected (LB-3) and laboratory synthesized solutions.

Solution	Al ($mg \cdot L^{-1}$)	Fe ($mg \cdot L^{-1}$)	Cu ($\mu g \cdot L^{-1}$)	Zn ($\mu g \cdot L^{-1}$)
LB-3	1.36 (1.05, 1.78)	0.43 (0.33, 0.56)	1.5 (1.2, 2.0)	22.1 (16.9, 28.8)
S-LB-3[a]	1.84 (1.52, 2.23)	0.57 (0.47, 0.69)	1.9 (1.6, 2.3)	54.6 (45, 66.1)
Al-Cu-Zn	1.32 (1.11, 1.59)	-	1.4 (1.1, 1.6)	40.3 (33.8, 48.4)
Cu-Zn-Fe	-	1.57 (1.26, 1.95)	5.3 (4.3, 6.6)	134.7 (108.2, 167.9)
Al-Fe	2.15 (1.80, 2.57)	0.69 (0.58, 8.32)	-	-
Cu-Zn	-	-	3.8 (3.5, 4.1)	107.8 (99.0, 117.4)
Al	2.88 (2.31, 3.59)	-	-	-
Fe	-	1.16 (0.95, 1.48)	-	-
Cu	-	-	6.9 (6.1, 7.9)	-
Zn	-	-	-	353.9 (300.4, 417.5)
WQC[b]	0.750	1.0	16.0	288

[a]S-LB-3 = synthesized LB-3, which contained all four metals.
[b]WQC = water quality criterion value.

Table 5. Marking's Additive Index (MAI) values[a] for synthetic AMD solutions containing two or more metals. AMD = acid mine drainage

Solution	MAI	(95% C. L.)	Sig. [b]
Cu-Zn	0.169	(-0.063, 0.470)	
Al-Fe	-0.341	(-0.988, 0.119)	
Al-Cu-Zn	0.290	(-0.112, 0.889)	
Cu-Zn-Fe	-1.502	(-2.694, -0.655)	*
Al-Cu-Zn-Fe	-0.560	(-1.289, -0.051)	*
LB-3	-0.123	(-0.783, 0.413)	

[a]A positive (MAI) value indicates synergism, while a negative value indicates antagonism. 95% confidence limits (C.L.) that do not overlap zero indicate MAI value is significantly different from zero.
[b] Sig. = statistically different from zero.

Synergism/antagonism in metal mixtures

Calculation of MAI indicated that solutions of Cu-Zn-Fe, and Al-Cu-Zn-Fe were less toxic than the sum of their components (antagonistic) with values of –1.502 and -0.560, respectively (Table 5). Confidence limits did not overlap zero so the values are considered significantly different from zero. The remaining four combinations had MAI values ranging from –0.341 (Al-Fe) to 0.290 (Al-Cu-Zn), but confidence intervals overlapped zero for these solutions. Therefore, these metal combinations were considered additive under these testing conditions (i.e., not more toxic than the sum of the individual metals).

Precipitation experiments

In the precipitation experiments, test organisms placed in neutralized Al solutions 8 hours after mixing had an average increase in percent survival of 53.0 ± 16.7% compared to those placed into the solution immediately after mixing (Fig. 1). This increase in percent survival was significantly higher ($p<0.05$) than the difference in survival of organisms added at different times to the NaCl solution (17.3 ± 9.2%), which as explained previously, should be a measure of increased organism fitness after 8 hours of feeding. The average difference in percent survival between organisms placed into the neutralized Fe immediately, and 8 hours after mixing was 30.0 ± 10.1%. This value was not significantly different from the value for the salt solution ($p>0.05$). The average pH values (± SD) for the Al and Fe solutions immediately after mixing were 6.6 ± 0.3 and 6.9 ± 0.4, respectively, and 8 hours later they were 7.4 ± 0.1 and 7.5 ± 0.2, respectively. The average pH for the NaCl solution was 7.9 ± 0.2.

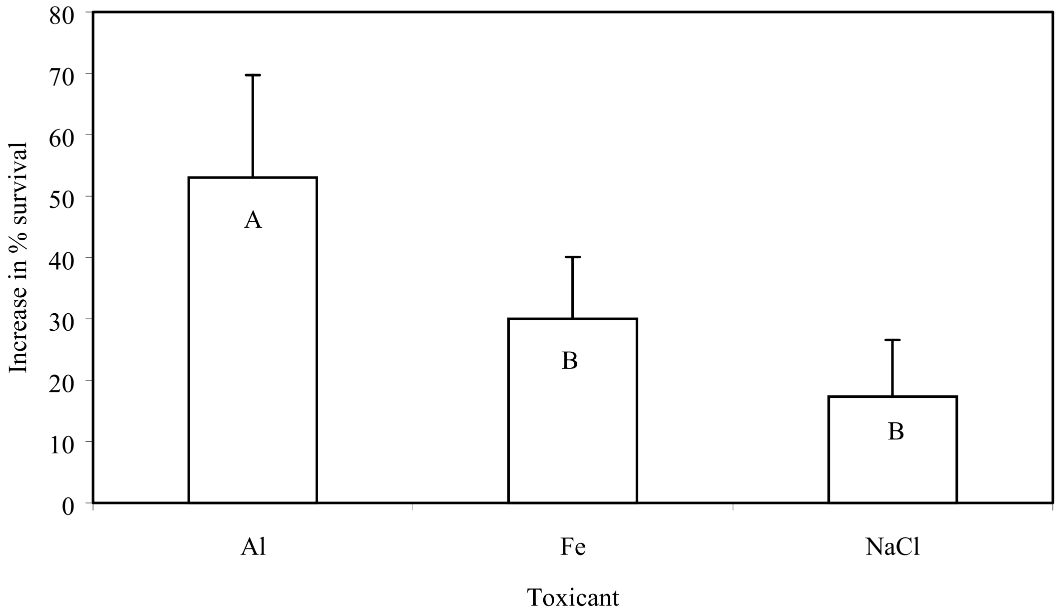

Figure 1. Average (± SD) difference in percent survival of test organisms placed into various solutions immediately after mixing (neutralization) and 8 hours after mixing. Metal concentrations were equal to average 48-hour LC50 values as calculated in this study. The sodium concentration was 2.5 $g \cdot L^{-1}$. Different capital letters indicated significantly different means ($p<0.05$)

Discussion

Acid mine drainage is traditionally considered to impact aquatic ecosystems by acidification in combination with dissolved metals, Fe^{3+} and Al hydroxide precipitation covering the stream substrate, and sediment toxicity due to metals like Cu and cadmium. Our tests with both field samples and laboratory solutions indicate that Al is the primary source of toxicity in the circumneutral waters (pH>7.0) of Puckett's Creek downstream of an acidified tributary, and is the probable cause of reduced benthic macroinvertebrate diversity. In the first set of water samples collected from downstream stations PC-4, PC-2, and PC-1, all test organisms survived, but on the second collection period, samples from all three downstream stations caused acute toxicity to *C. dubia*. While Zn concentrations were lower at all stations in the second set of samples than in the first and Cu was below the detection limit, the Fe concentration was similar to that in the first sample, and Al concentrations were higher in the second set of samples. This result suggests that Cu and Zn are minor toxic factors in this particular system, and that Al and Fe are more important. Laboratory tests with ten different synthesized solutions indicated that mixtures not containing Al had significantly higher mean LC50 values than the mean for the field collected AMD sample (LB-3), while none of the solutions containing Al had significantly different LC50s than the LB-3 sample. These data suggest that Al was a more significant contributor of toxicity than Fe in this system.

While Al is the third most common element in the Earth's crust, its low solubility at circum-neutral pH values generally prevents it from being present as a dissolved species at high concentrations in most oxygenated surface waters. However, acidic conditions resulting from acid precipitation or AMD cause dissolution of Al from common minerals. As a result, a number of studies have examined the combined impacts of acid and Al upon fish, invertebrates, and plants (e.g, Burton and Allan 1986; Engleman and McDiffet 1996; Havas 1985), but relatively few researchers have documented toxicity due to Al at circumneutral pH values. These have consisted of laboratory studies (Havas 1985), studies of alum ($Al_2(SO_4)_3$) treatment (Doke et al. 1995; Smeltzer et al. 1999) and mixing zone studies with fish (Henry et al. 1999; Rosseland et al. 1992). Recent work (Campbell et al. 2000; Elangoven et al. 1997) also indicates that snails bioaccumulate Al, which significantly alters their behavior, by grazing upon extracellular mucopolysaccharides that have bound polyhydroxy-Al at neutral pH. Our results provide evidence that Al is contributing to decreased benthic macroinvertebrate diversity and abundance in neutral waters (pH>7.0) for up to one mile downstream of an acidified tributary.

Several authors have suggested that the mode of Al toxicity to fish is transformation of Al^{3+} to polymers/colloids by hydrolysis with increased pH. Fish gills then act as a nucleating surface onto which the polymers precipitate, leading to suffocation (Exley et al. 1996; Neville and Campbell 1988; Poléo 1995). In plants however, the tridecameric species $[AlO_4Al_{12}(OH)_{24}(H_2O)_{12}]^{7+}$, hereafter referred to as $[Al_{13}]^{7+}$, has been experimentally shown to be at least ten times more toxic than the Al^{3+} species (Parker et al. 1989). While speculation has been made that $[Al_{13}]^{7+}$ may be the toxic form to fish, a direct comparison of $[Al_{13}]^{7+}$ and Al^{3+} toxicity to experimental fish suggested that this was not the case (Exley et al. 1994).

Aluminum was measured as total Al in our experiments so it is unknown which species is the cause of low benthic macroinvertebrate community diversity in Puckett's Creek below its acidic tributary, Lick Branch. As waters from Lick Branch (pH ~4.5 at the furthest downstream point, Soucek et al. 2000a, chapter 3) enter Puckett's Creek, they are immediately neutralized to a pH of greater than 7.0. This rapid increase in pH provides the conditions necessary to form $[Al_{13}]^{7+}$ (Parker and Bertsch 1992) suggesting that this species may be responsible for the observed community impacts. Our time-delayed toxicity tests support the suggestion that $[Al_{13}]^{7+}$ is the toxic agent because concentrations of this species decrease in solution over time (Parker et al. 1989), accounting for the reduced toxicity we observed. However, these experiments also support the theory that the toxic mechanism of Al to invertebrates may be similar to that found by others for fish because allowing Al to precipitate for eight hours significantly reduced the toxicity of the solution.

The fact that toxicity occurred up to one mile downstream of Lick Branch may be attributable to the kinetics of the Al polymerization reaction. Lydersen et al. (1991) found that when acidic waters containing Al^{3+} are neutralized, some solid $Al(OH)_3$ begins to form instantaneously, but at five minutes post-neutralization, polymerization/precipitation was proceeding at a rate such that the time needed for half of the dissolved Al to precipitate was approximately 10 minutes. After the first five minutes the rate of polymerization was much slower and both rates varied depending on the neutralization pH and temperature. Based upon our velocity measurements (Soucek unpublished data), the travel time from where the acidic tributary enters Puckett's Creek to the last site at which toxicity was observed (PC-1) is approximately fifteen minutes. This observation indicates that some amount of Al may still be in the process of polymerization at the site one mile downstream of the Lick Branch confluence, contributing to the observed toxicity. Likewise, the tridecameric species is meta-stable and substantial concentrations of this species potentially would remain in solution fifteen minutes after formation (Parker et al. 1989). Further research should be conducted to determine to most toxic Al species to invertebrates at neutral pH.

Our data suggest that Fe can also cause acute toxicity in circumneutral waters downstream of acidified tributaries. While 7-day LC50 values for ferrous iron ranging from 0.38 to 1.62 mg Fe(II)/L have been documented for *C. dubia* in neutralized mine effluents (Milam and Farris 1998), a 48-hour LC50 value of 1.17 mg total Fe/L was obtained in the present study. Field collected samples were only analyzed for total Fe, and while ferrous iron may have been present in the acidic effluent from LB-3, the simulated AMD solutions were made with ferric iron (as $Fe_2(SO_4)_3$). Regardless of the oxidation state of the dissolved Fe species, acute toxicity was observed in neutral waters for both field collected and laboratory prepared solutions at total Fe concentrations just above WQC limits. While the field-collected samples also had high Al concentrations, which were probably responsible for the observed toxicity in the field, our results obtained with simulated Fe solutions suggest that acidified solutions containing only ferric iron may cause toxicity in downstream neutral receiving systems. The time-delayed experiment suggests that ferric iron may have a different mode of toxicity than Al after neutralization as the increase in survival of organisms placed in the solutions eight hours after mixing was not significantly greater than that due to increased organism hardiness alone.

Iron may have another role in the metal mixtures in Puckett's Creek. Our results indicate that two of the synthetic solutions containing Fe (Cu-Zn-Fe and Al-Cu- Zn-Fe) were less toxic than the sum of their parts, or the combinations of metals were antagonistic according to their MAI values. The other solution containing Fe in combination with another metal (Al-Fe) had a nominally negative MAI value (suggesting antagonism), but the confidence interval was not significant. These results suggest that, in the field situation, Fe may be decreasing the toxicity of metal combinations, perhaps by co-precipitating other metals such as Cu, Zn and Al.

Despite the fact that the first sample collected from PC-4 contained Al, Fe, Cu and Zn at concentrations similar to the toxic concentration as predicted by the LB-3 and S-LB-3 laboratory tests, toxicity was not observed in this sample. These results suggest that WET testing with AMD effluents may not adequately describe in-field impacts; however, the effluents were diluted with U.S. EPA (1993) moderately hard synthetic freshwater containing only deionized water and four salts (KCl, $NaHCO_3$, $MgSO_4$, and $CaSO_4 \cdot 2H_2O$). The relative lack of complexing agents in the synthetic water probably artificially overestimates metal toxicity in laboratory testing. More complexing agents are likely to occur in natural waters such as those of Puckett's Creek, and Al and Cu both are known to form both organic and inorganic complexes, reducing their toxicity (Burton and Allan 1986; McCahon and Pascoe 1989).

Mechanisms of AMD toxicity are of interest because large sums are being expended to remediate AMD-impacted waters throughout the United States each year by federal agencies (including the Office of Surface Mining and the U.S. Environmental Protection Agency), state agencies in AMD-impacted areas, and local watershed associations. Improved understanding of AMD toxicity mechanisms can allow water-quality renovation expenditures to be prioritized and allocated more effectively. Our results suggest that Al and Fe in transition from acidic waters to neutralizing receiving streams cause acute toxicity to standard invertebrate test organisms at pH values greater than 7.0. Aluminum appears to be the cause of benthic macroinvertebrate community impairment in Puckett's Creek for up to a mile downstream of the AMD influenced tributary, Lick Branch. Further experiments supported the position that the mechanism of Al toxicity to invertebrates is the process of precipitation, as has been found to be the case for fish. While Fe singly may cause acute toxicity in this type of a system, it appears to reduce the toxicity of combinations of other metals such as Al, Cu, and Zn. The implication of this research is that agencies hoping to remediate AMD impacts must broaden their scope beyond examination of toxic effects within the zone of pH depression.

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Chapter 5

Individual and community level impacts of mine drainage and urban inputs in the Powell River, Virginia, USA.

Abstract- The Clinch-Powell River system is among the most biodiverse ecosystems in the world and has been identified as a conservation priority of national importance; however, the system has experienced biodiversity decline, especially in freshwater mussel populations. To investigate potential factors contributing to these declines, we compared the effects of metal loading from acid mine drainage (AMD) impacted tributaries of the Powell system to those produced by inputs from three urban centers using both individual (growth of Asian clams, *Corbicula fluminea*) and community level (benthic macroinvertebrate community richness and balance) endpoints. Urban runoff had a greater adverse impact than AMD inputs, and benthic macroinvertebrate community impairment observed downstream of urban areas (i.e., reduced diversity and richness) was corroborated by reduced clam growth. Conversely, the AMD impacted tributaries, as a group, appeared to have little impact; however, one downstream station had extreme values for a number of biotic and abiotic parameters, warranting a more detailed investigation. The use of two levels of biological organization provided confidence in attributing community impairment to contaminants rather than to other factors that may confound studies aimed only at higher levels of organization. These findings suggest that while past declines in mussel populations may have been due to coal mining and associated activities, urban inputs presently appear to have a greater adverse impact on the aquatic biota of the upper Powell River system.

Introduction

The Clinch-Powell River system, extending from northeastern Tennessee into Virginia's Scott and Lee Counties northward to headwater areas in Wise and Tazewell Counties, is among the most biodiverse ecosystems in the world (Chaplin et al., 2000). Thirty species federally listed as threatened or endangered occur there; most of the listed species are aquatic. In response to observed biodiversity declines, The Nature Conservancy, an international non-profit organization that seeks to preserve biodiversity by protecting land and water habitat, has identified the Clinch-Powell River system as a conservation priority of national importance (Chaplin et al., 2000). The decline in Powell River mussel communities in particular has largely been attributed to coal mining activity (Ahlstedt and Tuberville, 1997; McCann, 1993; Neves 1991; Wolcott, 1990). However, other factors including urban runoff and sedimentation/siltation may have contributed to the population declines, and could play a role in preventing recolonization of these areas by freshwater mussels.

Acid mine drainage (AMD), a result of oxidation of minerals containing reduced forms of sulfur (pyrites, sulfides) upon exposure to water and oxygen, is an environmental problem associated with abandoned mined lands (AML). The ecosystem impacts of AMD within acidified surface waters are well documented (e.g., Armitage 1980; Kelly 1988; Roback and Richardson 1969), but considerably less work has been done to characterize the effects of AMD on aquatic biota beyond the zone of pH depression. In the upper Powell River watershed, we have observed benthic macroinvertebrate community impairment due to acute aluminum (Al) toxicity in neutral pH waters (Soucek et al., in review, chapter 4), and the potential for both direct and indirect effects from ferric hydroxide precipitation (Soucek et al., 2000a, chapter 3) in addition to acidification impacts (Soucek et al., 2000b, chapter 1). These effects were observed primarily in first to third order tributaries of the North Fork of the Powell. There are at least 10 AMD impacted tributaries in the upper Powell watershed, but the effects of these tributary level inputs upon the mainstem of the Powell and its North Fork are not well characterized.

While several studies have been directed at the impacts of active mining and AML on the upper Powell River ecosystem, the potential for runoff from urban areas to impair aquatic communities within the watershed has received minimal attention. Diffuse urban runoff is one of the leading causes of surface water impairment in the United States (Tucker and Burton, 1999), introducing a variety of potential toxicants to receiving systems such as metals, salts, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, ammonia, suspended solids, and others. These inputs have been observed to impair both fish and invertebrate communities (e.g., Magaud et al., 1997; Mulliss et al., 1996; Tucker and Burton, 1999). Four urbanized areas potentially impact the aquatic biota of the upper Powell watershed: Norton, Appalachia, Big Stone Gap, and Pennington Gap, Virginia.

Through use of both individual and community level endpoints, we intended to compare the effects of metal loading from AMD impacted tributaries on the Powell River biota to those produced by inputs from Appalachia, Big Stone Gap, and Pennington Gap. The individual-level endpoint was Asian clam (*Corbicula fluminea*) growth during *in situ* toxicity tests, and the community-level endpoints were various gross measures of benthic macroinvertebrate community diversity and sensitivity. We did not intend to use Asian clams as surrogates to assess freshwater mussel responses to pollutants; rather, they were used as experimental animals which would respond to contaminant inputs rather than to habitat variability, predation, or competition. Thus, by using multiple levels of biological organization we hoped to simultaneously isolate variables that confound population, community, and ecosystem level studies, while maintaining the ecological relevance lacking in studies of lower organizational levels (Clements, 1997; Clements, 2000).

Materials and methods

Sampling stations

This study was conducted for two consecutive summers (1999 and 2000) in the main bodies of the upper Powell River and its North Fork in Lee and Wise Counties, Virginia. Sampling stations were placed into one of four different station categories including: (1) upstream and (2) downstream of AMD impacted tributaries, and (3) upstream and (4) downstream of urban areas, with each of the four categories consisting of three stations (Table 1, Fig. 1). The stations in the two AMD categories were upstream and downstream of the three most severely impacted tributaries of similar discharge in the upper Powell watershed. The AMD tributaries were, for the most part, rural, forested areas without much urbanization. One of these, the Stone/Straight Creek sub-watershed, contains a small town (St. Charles). While a source of septage input from home pipes, the major stressor in this sub-watershed is AMD (Cherry et al., 2000; Schmidt et al., in review; Soucek et al., 2000b, chapter 1).

Sampling stations were selected based discharge similarities to minimize benthic macroinvertebrate community variations simply due to stream size and carbon source (Vannote et al., 1980). This was tested by comparing mean discharge, measured using a Flo-Mate® model 2000 portable flow meter (Marsh-McBirney, Inc.), and benthic macroinvertebrate community index values (determined as described below) for sites upstream of AMD tributaries and upstream of urban areas (see below for details on statistical analyses).

Table 1. Sampling stations used to compare the relative impacts of acid mine drainage (AMD) and urban runoff upon the upper Powell River and its North Fork.

AMD tributaries	
Station	Location
Above Cox Creek	North Fork
Below Cox Creek	North Fork
Above Reed/Jones Creeks	North Fork
Below Reed/Jones Creeks	North Fork
Above Stone/Straight Creeks	North Fork
Below Stone/Straight Creeks	North Fork
Urban Areas	
Station	Location
Above Appalachia	Powell
Below Appalachia	Powell
Above Big Stone Gap	Powell
Below Big Stone Gap	Powell
Above Pennington Gap	North Fork
Below Pennington Gap	North Fork

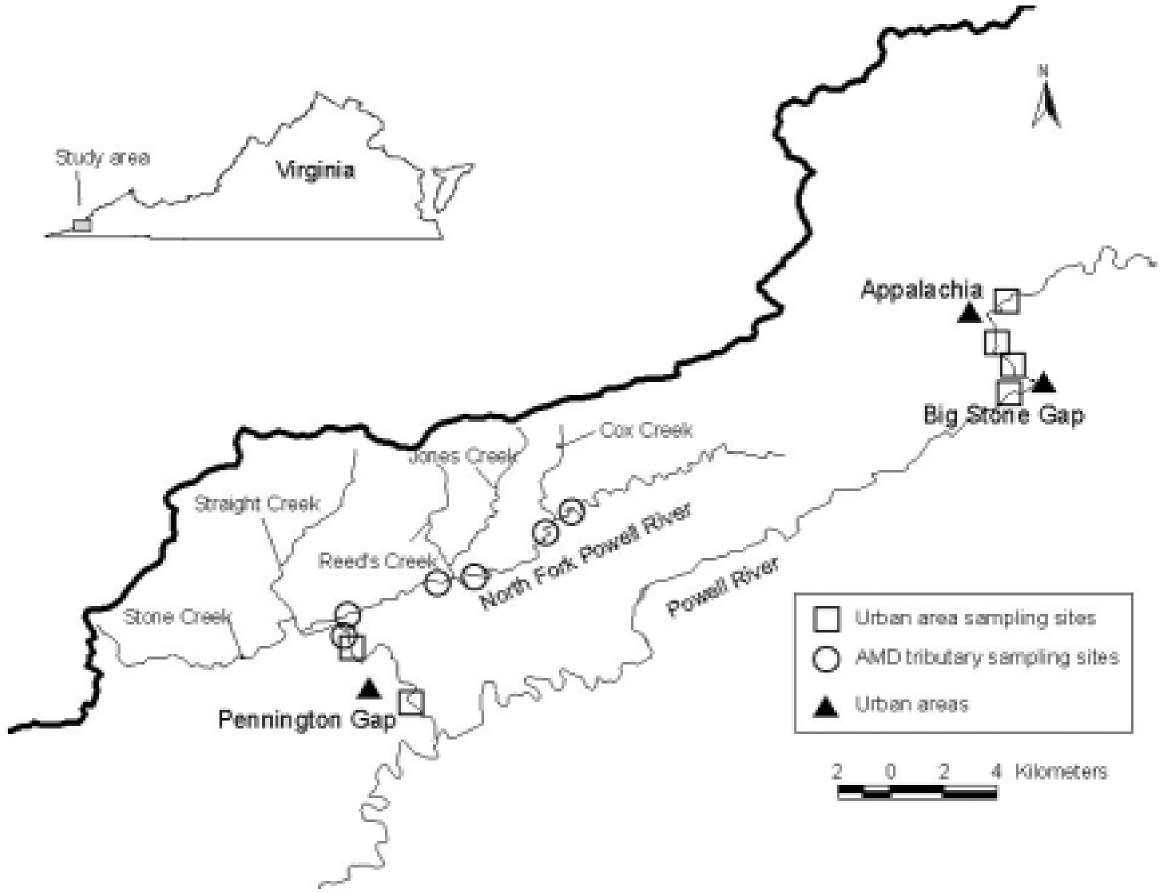

Figure 1. Map of the study area indicating sampling stations.

Experimental design

Data were analyzed in two different ways to determine the relative impact of the two pollutant types on the main river system. Rather than examining impacts on a station-by-station basis, we used upstream and downstream stations as replicates in overall means to obtain an analysis of consistent effects. Thus, the mean values (n = 3) for abiotic and biotic parameters for the stations upstream of urban areas were compared to means for the three stations downstream of urban areas. The same was true for the AMD tributary stations. We utilized pair-wise analysis (t-tests) with the assumption that enough distance existed between tributaries/urban areas to allow system recovery before receiving input from the next pollution source, thereby making observations independent.

Since all six AMD tributary stations and four of the urban area sites were located consecutively along a longitudinal gradient in the North Fork of the Powell and the Powell mainstem, respectively (Fig. 1), it was also necessary to determine if observations at a given station were actually dependent upon impactors located upstream. To this end, all measured parameters were included as dependent variables in correlation analyses using site number as the independent variable. Site number increased moving down the longitudinal gradients; for example, the station upstream of Cox Creek (an AMD impacted tributary) was designated as station number one, downstream of Cox Creek was number two, upstream of Reed/Jones Creek was number three, etc. The four urban area sites in the Powell mainstem were numbered in the same fashion. Therefore, if a significant correlation was observed, e.g., if richness decreased moving from the furthest station upstream to the furthest downstream, it could be determined that the trend was associated with additional AMD and or/urban area impacts. This trend would be likely to prevent the observation of significant differences in pair-wise analyses between upstream and downstream stations, but still suggest that the pollutant impacted the river system.

Further bivariate correlation analyses were conducted to compare biotic parameters to chemistry data, and then data for benthic macroinvertebrate sampling to water Asian clam growth in the watershed. Values from the AMD tributary and urban area stations were included in separate analyses, i.e., chemistry data for AMD tributary sites were compared to biotic data for AMD tributary sites, etc.

Water column chemistry

Water samples were collected at each station for analysis of selected water quality parameters at the beginning and end of in situ clam toxicity tests each year. Chemistry parameters were aimed more at AMD related measurements because urban runoff introduces numerous different chemical types to receiving waters. The urban areas were treated more as a “Black-Box” source of toxicity to aquatic biota.

Samples were either brought to the laboratory, stored for 24 hours at 4° C with measurements taken under laboratory conditions, or measurements were taken in the field. Sample pH was measured using an Accumet® (Fisher Scientific, Pittsburgh, PA, USA) pH meter equipped with an Accumet® gel-filled combination electrode (accuracy < ± 0.05 pH at 25 °C). Conductivity measurements were made using a Hach® (Hach, Loveland, CO, USA) conductivity. Dissolved oxygen, alkalinity and hardness were analyzed according to standard methods (APHA et al., 1995).

In addition, water column samples were prepared for metals analysis according to standard methods (U.S. EPA 1991). Samples were analyzed by inductively coupled plasma spectrometry for total aluminum (Al), iron (Fe), copper (Cu) and zinc (Zn), which were previously determined to be the dominant four metals in the system (Soucek et al. 2000b, chapter 1). Lower detection limits (mg/L) were 0.001, 0.002, 0.0017 and 0.0005 for Al, Fe, Cu and Zn, respectively. For all water chemistry parameters, mean values for each station were calculated using the data from both years, and then a mean value for each category (i.e., upstream and downstream of AMD tributaries and urban areas) was calculated.

Benthic macroinvertebrate sampling

Benthic macroinvertebrate surveys were conducted according to the U.S. Environmental Protection Agency Rapid Bioassessment Protocols (RBP) (Barbour et al. 1999). Riffle, run, pool and shoreline rooted areas were sampled for 5 minutes per site using dip-nets with 800-µm mesh. Two replicate samples were collected per site and mean values for all indices were calculated for each site. All organisms in each sample were identified to the lowest practical taxonomic level (usually genus) using standard keys (Merritt and Cummins 1996; Pennak 1989).

A number of benthic macroinvertebrate community indices were calculated including total richness, Shannon-Wiener diversity (*H*), Ephemeroptera-Plecoptera-Trichoptera (EPT) richness, Family Level Biotic Index (FBI), and percent collector-filterers. Richness was calculated as the total number of different taxa, and EPT richness was the number of taxa in the orders Ephemeroptera, Plecoptera, and Trichoptera. FBI was calculated as described by Hilsenhoff (1988) with higher values indicating a more tolerant assemblage, and percent Collector Filterers was the number of organisms in the functional feeding group divided by the total number of organisms sampled. The Shannon-Wiener index, which integrates richness and evenness into a measure of general diversity, was calculated according to formula (1):

$$H = -\sum_{k=1}^{S} p_k \log p_k \qquad (1)$$

where S = the number of taxa, and p_k = proportion of individuals in taxa *k.* As with water chemistry data, mean values over the two-year sampling period for individual stations were calculated for all parameters, then overall means were obtained for the four station categories.

In situ clam toxicity testing

For *in situ* toxicity tests, Asian clams were collected from the New River near Ripplemead, Virginia, using clam rakes. Clams were held in Living Streams® (Toledo, OH) at the Ecosystem Simulation Laboratory, Virginia Tech, Blacksburg, VA, until use. Individual clams were given one of five distinctive marks and measured for width to the nearest 0.01 mm using Vernier calipers prior to placement into bags for testing. Testing procedures consisted of tying five mesh bags, each bag containing five clams, to stakes at each sampling station. Bags were 18-cm wide by 36-cm long with a mesh size of ~0.5 cm^2. At the end of 60 days, clam bags were collected from each testing station and transported on ice to the laboratory. Clams were counted as dead or alive; clams found with valves separated, or that were easily opened, were considered dead. Surviving clams were measured again for width, and growth was calculated by subtracting beginning from ending width. Clam survival and growth values for both years were averaged for each station and then mean values were calculated for the four station categories.

Statistical analysis

Upstream and downstream means were compared separately for AMD tributaries and urban areas using either Student's t-test or the Mann-Whitney U test, depending on whether data were normal or non-normal, respectively. Significance was determined at the $\alpha = 0.05$ level, and data were tested for normality using a Shapiro-Wilk W test. Mean flow and benthic macroinvertebrate community index values for stations upstream of AMD tributaries were compared to those upstream of urban areas in the same manner. For correlation analyses, significance of correlation was determined at the $\alpha = 0.05$ level. All statistical analyses were conducted using JMP-IN® software (Sall and Lehman, 1996).

Results

Comparison of upstream stations

The stations upstream of urban areas were similar to those upstream of AMD impacted tributaries in terms of size and macroinvertebrate community diversity (Table 2). While the average discharge of the urban stations was nominally higher than that of the AMD stations, the difference was not significant ($p > 0.05$). The average benthic macroinvertebrate community index values for the two station types were similar, with richness values of ~19 to 20, EPT richness of ~8 to 10, and FBI values of ~3.6 to 4.0. There was a significant difference between the two station types in Shannon-Wiener diversity, but this difference was a function of the small variance in both groups. Ecologically, the difference between the values of 0.97 and 0.95 is insignificant. Mean percent collector-filterer values were ~35% for the AMD stations and ~44% for the urban areas.

Table 2. Comparison[a] of flow and benthic macroinvertebrate indices at sites upstream of urban areas and acid mine drainage (AMD) impacted tributaries.				
	Urban (n = 3)		AMD (n = 3)	
Flow (cfs)	54.75 ± 24.23	A	21.92 ± 5.46	A
pH (s.u.)	8.0 ± 0.2	A	7.6 ± 0.2	A
Conductivity (µmhos/cm)	646 ± 130	A	366 ± 5	B
H	0.97 ± 0.01	A	0.95 ± 0.01	B
Richness	19.25 ± 1.25	A	20.58 ± 1.76	A
EPT Richness	8.00 ± 1.09	A	10.42 ± 2.32	A
FBI	4.09 ± 0.46	A	3.62 ± 0.07	A
%Coll-Filt	44.50 ± 19.06	A	35.17 ± 7.57	A

[a] means in a horizontal series followed by the same capital letter are not significantly different ($\alpha = 0.05$). Yearly values for chemistry endpoints are means for two sampling occasions.

There was a nominal difference between the upstream groups in average pH, and a significant difference in conductivity, with the average for the urban area stations being higher than that of the AMD tributary stations (646 and 366 µmhos/cm). Because of the difference in conductivity at upstream stations, comparisons were not made between stations upstream and downstream of each pollutant type, and conductivity and pH values were not included in correlation analyses. However, differences in upstream and downstream metal concentrations were compared for each pollutant type.

Water chemistry

No significant differences were observed in metal concentrations for stations upstream of urban inputs when compared to those for downstream stations (Table 3). Aluminum concentrations were virtually the same (44 and 43 µg/L) for the two groups, and Fe decreased nominally (from 145 to 117 µg/L) downstream of urban areas. Measured concentrations of Cu and Zn were below detection limits on all occasions. Mean alkalinity and hardness values for all urban area sites were 142 ± 43 mg/L as $CaCO_3$ and 235 ± 78 mg/L as $CaCO_3$, respectively. Differences between upstream and downstream sights were insignificant. Dissolved oxygen concentrations were at saturation on all sampling occasions.

Table 3. Average values[a] for water chemistry parameters at sites upstream and downstream of three urban areas and three acid mine drainage (AMD)-impacted tributaries.

Urban Runoff	Upstream (n = 3)		Downstream (n = 3)	
Al (μg/L)	44 ± 9	A	43 ± 5	A
Fe (μg/L)	145 ± 60	A	117 ± 19	A
AMD Tributaries	**Upstream (n = 3)**		**Downstream (n = 3)**	
Al (μg/L)	18 ± 4	A	31 ± 17	A
Fe (μg/L)	92 ± 27	A	166 ± 130	A

[a] means in a horizontal series followed by the same capital letter are not significantly different ($\alpha = 0.05$). Values shown are site means for two sampling seasons in which samples were collected at the beginning and end of *in situ* clam tests.

Larger nominal differences in mean metal concentrations were observed between stations upstream and downstream of AMD impacted tributaries (18 and 31μg Al/L, and 92 and 166 μg Fe/L for upstream and downstream stations, respectively), but again, the differences were not significant ($p > 0.05$, Table 3). As with the urban area stations, Cu and Zn were below detection limits on all sampling occasions. Mean alkalinity and hardness values for all AMD tributary sites were 60 ± 8 mg/L as $CaCO_3$ and 160 ± 7 mg/L as $CaCO_3$, respectively. Again, differences between upstream and downstream sites were insignificant, and dissolved oxygen concentrations were at saturation on all sampling occasions.

Benthic macroinvertebrate sampling

Comparing mean community index values for the stations upstream and downstream of urban areas, the communities at the downstream stations appeared to be impaired (Table 4). Average Shannon-Wiener diversity was significantly higher upstream of urban runoff (0.97) than downstream (0.80). Total richness (the number of types of organisms), and EPT richness were substantially higher upstream of urban inputs compared to downstream stations, and while not significant at the $\alpha = 0.05$ level, the difference in total richness was significant at the $\alpha = 0.06$ level. The mean FBI values for both groups were similar (4.09 upstream and 3.95 downstream), as were values for percent collector-filterers (44.5% upstream and 40.6 % downstream).

Table 4. Average values[a] for benthic macroinvertebrate indices at sites upstream and downstream of three urban areas and three acid mine drainage (AMD)-impacted tributaries.

Urban Runoff	Upstream (n = 3)		Downstream (n = 3)	
Shannon-Wiener	0.97 ± 0.01	A	0.80 ± 0.09	B
Richness	19.35 ± 1.25	A	16.08 ± 1.77	A
EPT Richness	8.00 ± 1.09	A	6.17 ± 1.26	A
FBI	4.09 ± 0.46	A	3.95 ± 0.09	A
%Coll-Filt	44.50 ± 19.06	A	40.67 ± 18.46	A
AMD Tributaries	**Upstream (n = 3)**		**Downstream (n = 3)**	
Shannon-Wiener	0.95 ± 0.01	A	1.00 ± 0.03	B
Richness	20.58 ± 1.76	A	20.92 ± 1.91	A
EPT Richness	10.42 ± 2.32	A	10.08 ± 2.98	A
FBI	3.62 ± 0.01	A	3.99 ± 0.45	A
%Coll-Filt	35.17 ± 7.57	A	39.83 ± 13.19	A

[a] means in a horizontal series followed by the same capital letter are not significantly different ($\alpha = 0.05$). Values shown are site means for two sampling seasons.
FBI = Family-Level Biotic Index, EPT = Ephemeroptera-Plecoptera-Trichoptera, Coll-Filt = Collector-Filterers.

In the case of the sites upstream and downstream of AMD impacted tributaries, the differences in community indices were not as substantial (Table 4). While there was a significant difference between the groups in Shannon-Wiener diversity, the higher mean value occurred at the stations downstream of the AMD tributaries (1.00 downstream and 0.95 upstream). Mean upstream and downstream values were similar for total richness (20.58 and 20.92), EPT richness (10.42 and 10.08), and percent collector-filterers (35.17 and 39.83). The mean FBI value was higher downstream of AMD tributaries, suggesting a more pollution-tolerant assemblage, but the difference was not significant.

In situ clam toxicity testing

Clam survival at all sites during both years was greater than 90% so these data will not be discussed further. Mean growth of clams placed upstream of urban areas (1.40 ± 0.28 mm) was significantly higher than that of clams placed downstream (0.84 ± 0.15 mm) (Figure 2.). A different response was observed in the AMD tributary sites (Figure 3). Clams grew more on the average downstream of AMD impacted tributaries (0.82 ± 0.55 mm) as compared to those placed upstream of AMD inputs (0.59 ± 0.06), but this difference was not significant.

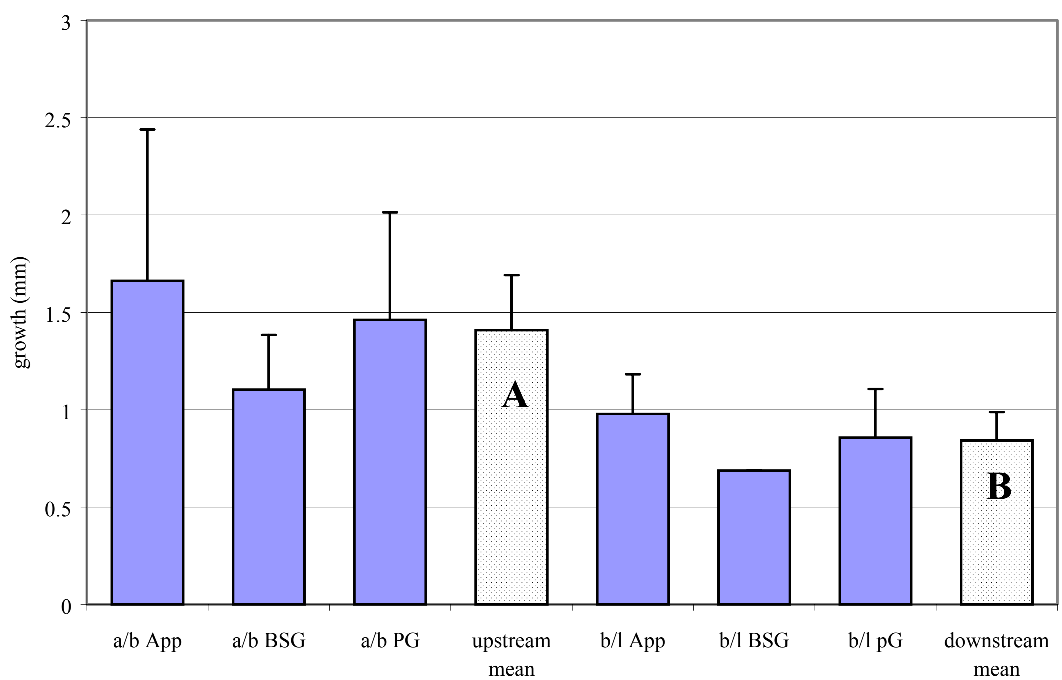

Figure 2. Two-year average clam growth values for individual urban area sites, and means for upstream and downstream groups. Error bars = standard deviation. Means with different upper-case letters are significantly different ($p < 0.05$). a/b = above, b/l = below, App = Appalachia, BSG = Big Stone Gap, PG = Pennington Gap.

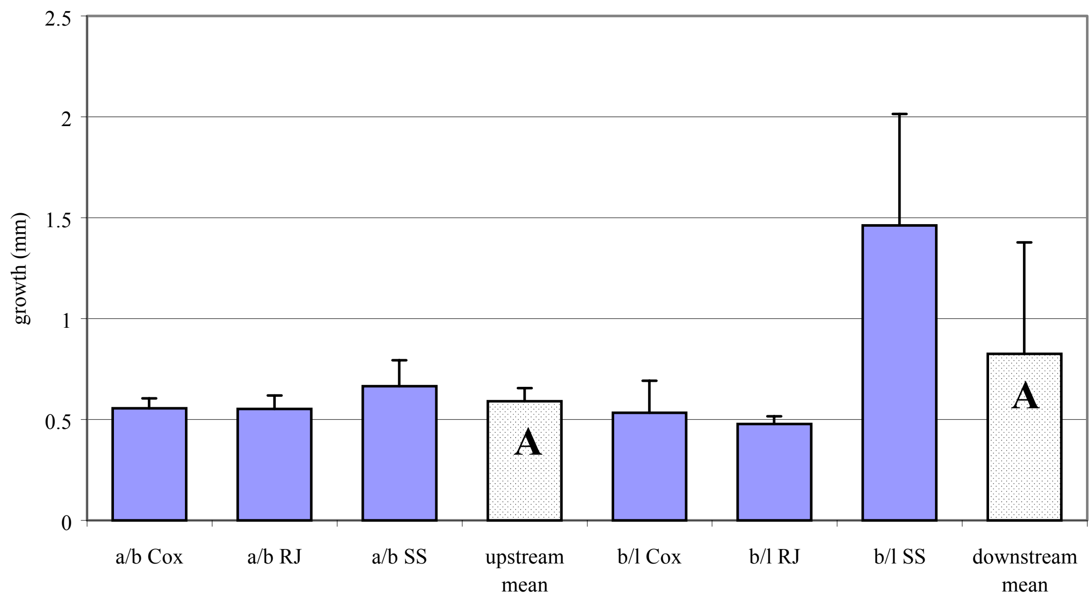

Figure 3. Two-year average clam growth values for individual AMD tributaries sites, and means for upstream and downstream groups. Error bars = standard deviation. Means with different upper-case letters are significantly different ($p < 0.05$). a/b = above, b/l = below, Cox = Cox Creek, RJ = Reed/Jones Creek, SS = Stone/Straight Creek.

Correlation analysis

Only one significant correlation was observed in the correlation analysis of all measurements versus site number for the AMD tributary stations (n = 6). Site number was positively correlated with percent collector-filterers ($r = 0.891$, $p = 0.017$). However, two parameters were significantly correlated with site number at the $\alpha \leq 0.07$ level. Water column Al was positively correlated with site number ($r = 0.776$, $p = 0.069$), and EPT richness was negatively correlated with site number ($r = - 0.802$, $p = 0.055$). However, an outlier (statistically determined as a data point that was greater than two standard deviations from the mean of the values for the other sites) was observed in all three of these scatter plots. The outliers were suspected of having a large influence on the correlation coefficient because of the relatively small sample sizes. When outliers were removed from the analyses, the correlations for water column Al and EPT richness were no longer significant and that for percent collector-filterers was significant at the $\alpha = 0.066$ level. For the four consecutive urban area sites, no significant correlations were observed.

Comparing chemical parameters with benthic macroinvertebrate community indices and clam growth at urban areas sites (n = 6), only one significant correlation was observed. Percent collector-filterer values were positively correlated with water column Al concentration ($r = 0.932$, p = 0.007). Three significant correlations were observed for the AMD tributary sites (n = 6). Percent collector-filterer values were positively correlated with water column Al ($r = 0.875$, p = 0.002), and FBI values were positively correlated with water column conductivity ($r = 0.917$, p = 0.01) and Al ($r = 0.943$, p = 0.005). Again, outliers were observed in these data. When they were removed from the scatter plot for each of the above-mentioned analyses, the correlation coefficients dropped substantially and the relationships were no longer significant ($r = 0.326$, p = 0.591; $r = 0.515$, p = 0.374; $r = 0.689$, p = 0.196, respectively).

When comparing clam growth data to benthic macroinvertebrate community indices at urban area sites, growth was positively correlated with Shannon-Wiener diversity ($r = 0.826$, p = 0.043) (Figure 4). No other significant correlations were observed between clam growth and the various community indices for the urban area sites. Two significant relationships were observed for the AMD tributary sites. Clam growth was positively correlated with FBI ($r = 0.853$, p = 0.031) and percent collector-filterers ($r = 0.893$, p = 0.016); however, outliers were observed again for these relationships and when removed, the relationships were no longer significant.

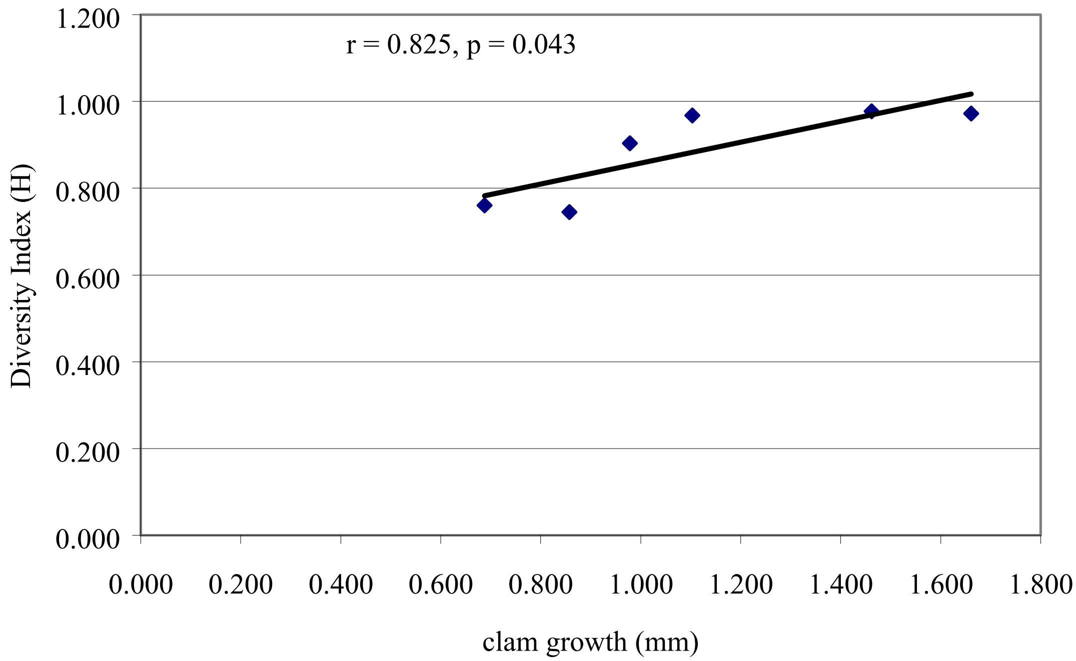

Figure 4. Correlation between Shannon-Wiener diversity index and clam growth at sites upstream and downstream of urban areas.

The presence of outliers for a number of measurements at one station in the AMD tributary-group warrants a more detailed review of the tributary involved. The outlier measurements occurred at the station downstream of Stone/Straight Creek (Table 5). Examining two-year averages moving from upstream to downstream, clam growth increased substantially downstream of the tributary (0.665 to 1.462 mm), as did the value for percent collector-filterers (40.5 to 55.0%). Taxonomic richness and Shannon-Wiener diversity increased slightly downstream. Conversely, EPT richness decreased nominally (from 8.50 to 6.75) and FBI increased substantially (from 3.67 to 4.45) downstream of Stone/Straight Creeks. Furthermore, average values for three chemical measurements (conductivity, Al, and Fe) were substantially higher downstream of the tributary than values measured upstream.

Table 5. Two-year averages [a] for biotic and abiotic measurements upstream and downstream of Stone/Straight Creek, an acid mine drainage (AMD) impacted tributary to the North Fork of the Powell River.

	Upstream of Stone/Straight Creek			Downstream of Stone/Straight Creek		
	1999	2000	mean	1999	2000	mean
Clam growth (mm)	0.58	0.75	0.66	1.85	1.07	1.46
Shannon-Wiener	0.95	0.94	0.95	0.89	1.07	0.97
Richness	19.50	18.00	18.75	18.00	20.50	19.25
EPT Richness	9.00	8.00	8.50	7.00	6.50	6.75
FBI	3.33	4.01	3.67	4.32	4.58	4.45
% Collector-filterers	40.00	41.00	40.50	51.00	59.00	55.00
Conductivity (μmhos/cm)	435	304	369	575	366	470
Al (μg/L)	5	41	23	45	56	51
Fe (μg/L)	103	71	87	137	53	95

[a] Yearly values for chemistry endpoints are means for two sampling occasions. FBI = Family-Level Biotic Index, EPT = Ephemeroptera-Plecoptera-Trichoptera.

Discussion

These data indicate that, at the level of resolution permitted by the experimental design, urban runoff had a greater impact upon the biota of the Powell River and its North Fork than inputs from AMD impacted tributaries. Benthic macroinvertebrate community impairment observed downstream of urban areas (i.e., reduced diversity and richness) was corroborated by the experimental responses of individuals, with clam growth being reduced where communities were less diverse. Conversely, pair-wise analysis showed that the AMD impacted tributaries, as a group, appeared to have little impact; however, correlation analysis indicated one downstream station had extreme values for a number of parameters, warranting a more detailed investigation.

The comparison of the upstream stations for both the urban areas and AMD tributary sites indicated that both systems were of comparable size, and, more importantly, had benthic macroinvertebrate assemblages of similar diversity, richness, and sensitive taxa composition. Therefore, we were able to effectively compare the relative impacts of the two pollutant types upon similar systems.

As expected, inputs from the urban areas did not substantially change any of the measured chemistry parameters. This was because the parameters were intended to be indicative of AMD impacts, not those brought on by urban runoff such as increased levels of pesticides, turbidity, organics, and ammonia (Magaud et al., 1997; Mulliss et al., 1996; Tucker and Burton, 1999; Wenning et al., 1994). In addition, samples for chemistry analysis were collected only at the beginning and end of the *in situ* toxicity tests, and therefore, did not reflect high flow-conditions when urban runoff would have its greatest impact. However, since the clams were left in place for 60 days and invertebrate communities are a measure of cumulative impacts (Mason, 1991), we feel that we were able to obtain valuable data regarding the impacts of urban inputs on aquatic biota of the Powell River.

The pair-wise analysis of stations upstream and downstream of AMD impacted tributaries indicated that the tributaries did not significantly affect the chemistry of the mainstem of the river as one might expect. However, correlation analysis indicated that Al concentrations in the water column increased moving downstream in the North Fork of the Powell. This significant correlation was largely due to the site downstream of Stone/Straight Creek, which contributed substantial levels of Al and Fe, and increased the average conductivity of the receiving system by nearly 100 µmhos/cm. Levels of Al and Fe at this downstream station were below Water Quality Criterion (WQC) limits for protection of aquatic life (U.S. EPA, 1999), but these and other metals may have lower level effects on aquatic biota. For example, recent work (Campbell et al. 2000; Elangoven et al. 1997) indicated that snails bioaccumulate Al, which significantly alters their behavior, by grazing upon extracellular mucopolysaccharides that have bound polyhydroxy-Al at neutral pH. While Cu and Zn were not found at measurable concentrations in the water column at this site, they have been measured in high concentrations in AMD seeps found in a tributary to Straight Creek (Soucek et al., in review, chapter 4), and may be accumulating in sediments in the larger system. These metals also are known to accumulate in biological tissues (reviewed in Goodyear and McNeill, 1999) and may cause long-term impacts not measured in this study.

Benthic macroinvertebrate community data indicate that there may be multiple sources of impact from the Stone/Straight Creek tributary, previously determined to be severely impacted by AMD (Cherry et al., 2001; Schmidt et al., in review; Soucek et al., 2000b, chapter 1). While total taxonomic richness and Shannon-Wiener diversity were similar at downstream and upstream sites, the two-year average for EPT richness was nominally lower at the downstream site. Others have found EPT richness to be a sensitive indicator of chemical degradation in Appalachian streams (Wallace et al., 1996), and in a study conducted in a tributary of the Powell watershed, EPT richness was sensitive to various types of AMD inputs (Soucek et al., 2000b, chapter 1). Conversely, the combination of increased percent collector-filterers and FBI values suggest an increased level of organic pollution at the downstream site (Hilsenhoff 1988; Wiederholm, 1984). This finding is supported by the observation of increased clam growth, which has been found to mirror community responses to nutrient loading in low order streams (Soucek et al., 2001, chapter 2). While AMD has been found to be the major stressor within this tributary, septage inputs from home pipes have been observed at various sites throughout the sub-watershed (Soucek, personal observation). Therefore, more detailed studies must be conducted to determine which input is having the greater impact on this stretch of the North Fork of the Powell.

While AMD inputs may be impacting the larger receiving system downstream of one tributary in the North Fork of the Powell, as evidenced by reduced EPT richness, the three urban areas had more severe and consistent negative impacts. Correlation analysis results indicated the existence of ample recovery distance between the two cities in the mainstem of the Powell (Appalachia and Big Stone Gap), thereby allowing meaningful pair-wise analysis (which also included the sites in the North Fork, upstream and downstream of Pennington Gap). Significant and/or substantial decreases in average

Shannon-Wiener diversity, total richness, and EPT richness were observed moving from upstream to downstream stations. Other researchers have observed similar changes in benthic communities due to urban runoff (e.g., Lenat and Crawford, 1994; Seager and Abrahams, 1990).

The urban input-induced community level impairment observed in this study was supported by the experimental study with transplanted Asian clams. *In situ* toxicity studies with a number of organisms, including amphipods, isopods, midges, mussels and others have been successfully used to assess urban runoff impacts (e.g., Day et al., 1990; Mulliss et al., 1996; Tucker and Burton, 1999). *In situ* studies are valuable because they are more environmentally realistic than short-term laboratory toxicity tests, and allow for isolation of variables that may confound observational studies of indigenous communities. Asian clams are sensitive to and/or accumulate a variety of pollutant types potentially found in urban runoff, including pesticides, sewage, metals, and other organics (Belanger, 1991; Doherty and Cherry, 1988; Hayward et al., 1996; Pereira et al., 1996). Chemicals including Cu, Zn, and chrysotile asbestos have been shown to reduce their growth (Belanger et al., 1986a,b; Belanger et al., 1990); therefore, reduced clam growth is considered a valid response to chemical pollutants. In the present study, clam growth was significantly lower downstream of the urban areas. Because clams grew well downstream of Stone/Straight Creek, which may be influenced by dilute septage input, perhaps metals or other organics such as PAHs and pesticides are responsible for the observed impacts below urban areas.

Not only was clam growth reduced below urban areas in the present study, but it was positively correlated with Shannon-Wiener diversity values for the urban area sites, suggesting that where clams grew the most, communities were the most diverse. Others have recognized that by assessing environmental impacts using multiple levels of biological organization (i.e., individual and community level endpoints) stronger inferences may be made regarding causality of observed impairment to aquatic ecosystems (Adams et al., 1992; Clements, 2000; Clements and Kiffney, 1994). For example, changing tissue concentrations of free amino acids (FEE) in freshwater mussels have been observed to be indicative of exposure to urban runoff (Day et al., 1990); however, the environmental relevance of this molecular-level response is unknown. Conversely, it is difficult to attribute observed benthic macroinvertebrate community impairment to contaminants because of potential influences of competition, predation, habitat availability, and other factors. In this study, we attempted to eliminate these potential confounding variables by conducting controlled experiments with caged Asian clams. The correlation between decreased clam growth and community impairment below urban areas provides strong evidence that pollutants were responsible for decreased richness and diversity; however, further studies should be conducted to definitively demonstrate impairment causation.

In conclusion, this study provides evidence that, while metal loading from individual AMD impacted tributaries may have low level impacts upon their receiving systems, various unknown inputs from urban areas have a severe and consistent impact upon the aquatic communities of the Powell River and its North Fork. These urban inputs may also have had an impact upon freshwater mussel populations and could limit natural and anthropogenic recolonization efforts. Further research should be conducted to determine which specific chemicals are responsible for the toxic effects observed, and if the toxicity is episodic or continuous. In addition, research should be conducted to investigate potential chronic effects of metal inputs from the Stone/Straight Creek tributary into the North Fork of the Powell.

Acknowledgement

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Chapter 6

Reduced common stonefly (Plecoptera) numbers downstream of a mine drainage impacted tributary to the North Fork Powell River, VA.

Abstract – A study was conducted to quantify impairment to invertebrate predator populations, particularly to common stoneflies (*Acroneuria*), downstream of an acid mine drainage-impacted tributary to the North Fork of the Powell River, southwestern Virginia. Predatory insects comprised 9.0 ± 1.3% of the total abundance at the three stations upstream of the impacted tributary, but were significantly reduced ($p = 0.0039$) downstream (3.9 ± 0.6%). Predatory stonefly numbers followed the same trend with the upstream average ($n = 3$) of 2.5 ± 0.2 being significantly higher ($p = 0.0001$) than the downstream average of 0.1 ± 0.2%. Despite the fact that stream order can play a role in structuring benthic macroinvertebrate communities their re-occurrence further downstream of the study area indicated that this reduction was not associated with the size or discharge of the river. Using correlation analysis, we evaluated the relationship between predatory stonefly numbers throughout this reach and metal concentrations in water, sediment, and biological tissues (invertebrate predators and primary consumers), and found that the strongest association was with water column aluminum (Al) concentrations. Over the course of the two-year study, Al concentrations in the water explained nearly 93% of the variation in predatory stonefly numbers. While this correlation exists, it may not indicate a causal relationship, and experiments should be conducted to determine the long-term toxicity of Al to predatory stoneflies.

Introduction

Acid mine drainage (AMD), a result of oxidation of minerals containing reduced forms of sulfur (pyrites, sulfides) upon exposure to water and oxygen, is an environmental problem associated with abandoned mined lands (AML). The oxidation process results in production of strong acids, which mobilize acid soluble metals, including iron (Fe), aluminum (Al), copper (Cu), zinc (Zn), and others depending on the mineralogy of the disturbed area. The mixture of acid and dissolved metals creates a toxic environment for aquatic organisms. Numerous studies have documented the impacts of AMD upon aquatic communities in acidified stream reaches (e.g., Armitage 1980; Kelly 1988; Roback and Richardson 1969); these impacts include reduced taxonomic richness and abundance, and/or a shift from pollution sensitive to pollution tolerant species.

Beyond the zone of pH depression, a variety of community impacts have been documented. Sediment toxicity may occur where trace metal concentrations are high (Kemble et al. 1994). In hard-rock mine impacted areas, bioaccumulation of metals such as Cu, cadmium (Cd), and Zn has been associated with benthic macroinvertebrate impairment (Besser et al., 2001; Kiffney and Clements, 1993; Saiki et al., 1995). Ferric hydroxide precipitate (a product of pyrite oxidation) is thought to cause ecosystem impairment by smothering available habitat and reducing periphyton productivity (McKnight and Feder 1984; Scullion and Edwards 1980). Solid ferric hydroxide also has been shown to cause acute toxicity to *Daphnia magna* in the absence of dissolved Fe (Soucek et al. 2000a, chapter 3). Furthermore, Al has been observed to cause acute toxicity to fish and invertebrates in circumneutral pH waters below acidic tributaries (Henry et al. 1999; Rosseland et al. 1992; Soucek et al., 2001a, chapter 4), and to alter snail behavior through ingestion (Campbell et al. 2000; Elangoven et al. 1997). These findings indicate that AMD is a complex pollutant that can impair aquatic ecosystems in a variety of ways.

The Clinch-Powell River system of northeastern Tennessee and southwestern Virginia has experienced a decline in biodiversity (Chaplin et al., 2000). The decline in Powell River unionid mussel communities in particular has largely been attributed to coal waste and mining-associated metal loading (Ahlstedt and Tuberville, 1997; McCann, 1993; Neves 1991). There are at least 10 AMD impacted tributaries in the upper Powell watershed, but the effects of these tributary level inputs upon the biota of the mainstem of the Powell and its North Fork (NFP) are not well characterized. The most severely impacted sub-watershed within the NFP is Stone/Straight Creek, due to two highly impacted tributaries, Ely and Puckett's Creeks (Cherry et al., 2001; Soucek et al., 2000b, chapter 1). Community diversity and richness are not significantly impaired downstream of Stone/Straight Creek relative to upstream levels (Soucek et al., 2001b, chapter 5); however, during the course of a two-year study, we observed a reduction in common stonefly (*Acroneuria*) numbers below of this tributary. The purpose of this study was to quantify the impairment to invertebrate predators in general and particularly to predatory stoneflies. We further attempted to determine if the observed impairment was associated with water column or sediment metal concentrations or bioaccumulation of metals in tissues.

Materials and methods

Sampling stations

This study was conducted during the summers of 1999 and 2000. Six sampling stations were selected in the North Fork of the Powell River (NFP), Lee County, VA, U.S.A. (Fig. 1). Three stations were located upstream of a tributary severely impacted by AMD, Stone/Straight Creek, at NFP km-10 (Cherry et al., 2001; Soucek et al., 2000b, chapter 1). The remaining three were downstream of the tributary with the last one located just upstream of the NFP confluence with the Powell River mainstem (NFP km-0). To determine if the size of the river was a factor in reduced predator numbers, a seventh site was sampled for benthic macroinvertebrates in the second year of the study. This site was at Fletcher Ford, a freshwater mussel preserve ~57 km downstream of the NFP/Powell River confluence.

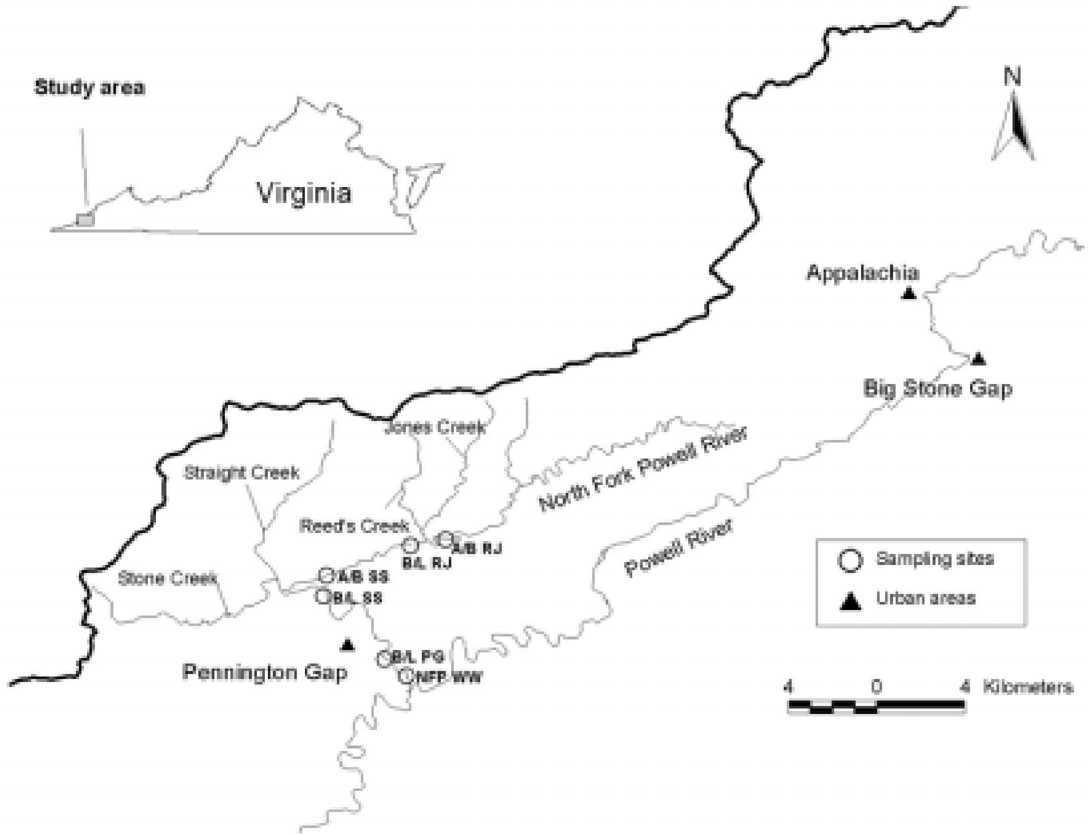

Figure 1. Map of study area indicating sampling stations. A/B = above; B/L = below; RJ = Reed/Jones Creek; SS = Stone/Straight Creek; PG = Pennington Gap; NFP WW = North Fork Powell at Woodway.

Benthic macroinvertebrate sampling

Benthic macroinvertebrate surveys were conducted according to the U.S. Environmental Protection Agency Rapid Bioassessment Protocols (RBP) (Barbour et al. 1999). Riffle, run, pool and shoreline rooted areas were sampled for 5 minutes per site using dip-nets with 800-µm mesh. Two replicate samples were collected per site and mean values for all indices were calculated for each site. All organisms in each sample were identified to the lowest practical taxonomic level (usually genus) using standard keys (Merritt and Cummins 1996; Pennak 1989). Chironomids were identified as either subfamily Tanypodinae or non-Tanypodinae. Each taxon identified was assigned to a functional feeding group as described in Merritt and Cummins (1996). Feeding groups included collectors, shredders, scrapers, and predators. Tanypod chironomids were considered predators, and non-tanypod chironomids were collectors. Percent abundance of each functional feeding group was calculated (e.g., number of predators divided by the number of organisms in a sample = % predators), as was percent common stonefly abundance.

Mean values for percent predator abundance and percent common stonefly abundance for the three stations upstream of Stone/Straight Creek were compared to those at the three downstream stations using Student's T-test in the JMP-IN® software package (Sall and Lehman, 1996).

Water column and sediment chemistry/metals

Water samples were collected at each station for analysis of selected water quality parameters on two separate occasions per year. Samples were brought to the laboratory and stored for 24 hours at 4° C before measurements were taken under laboratory conditions. Sample pH was measured using an Accumet® (Fisher Scientific, Pittsburgh, PA, USA) pH meter equipped with an Accumet® gel-filled combination electrode (accuracy $< \pm 0.05$ pH at 25 °C). Conductivity measurements were made using a Hach® (Hach, Loveland, CO, USA) conductivity/TDS meter. Dissolved oxygen (measured in the field), alkalinity and hardness were analyzed according to standard methods (APHA et al., 1995).

Sediment samples were collected using a polyurethane scoop, placed in freezer bags and stored at 4 °C until analysis for metals. Sediment and water column samples were prepared for metals analysis according to standard methods (U.S. EPA, 1991). Samples were analyzed at the Virginia Tech Soil Testing Lab via inductively coupled plasma (ICP) spectrometry for total recoverable aluminum (Al), iron (Fe), copper (Cu) and zinc (Zn), which were previously determined to be the dominant four metals in the system (Soucek et al., 2000b, chapter 1). Lower detection limits (μg/L) were 1.0, 2.0, 1.7 and 0.5 for Al, Fe, Cu and Zn, respectively.

Bioaccumulation study

To determine if bioaccumulation and/or biomagnification of metals from insects at the primary consumer level to predatory insects was associated with the decline in predator numbers, an additional set of benthic macroinvertebrate samples was collected in 2000 for measurement of metal concentrations. Available habitats were sampled qualitatively using a D-frame net, and organisms were sorted and identified in the field according to functional feeding groups (Merrit and Cummins, 1996). Predators and primary consumers (including shredders, scrapers, and collectors) were placed in separate vials and chilled but not frozen for 24 hours to allow organisms to clear their gut contents. Samples then were frozen and prepared for metals analysis according to standard methods (U.S. EPA, 1991). Tissues were analyzed for Al, Fe, Cu, and Zn at the Virginia Tech ICP Lab. Lower detection limits (μg/L) were again 1.0, 2.0, 1.7 and 0.5 for Al, Fe, Cu, and Zn, respectively.

Using average metal concentrations for predators and primary consumers at the six sites, bioaccumulation factors (BF) were calculated for the four analyzed metals. For example, the average concentration of Cu in predatory insects for the six sampling stations was divided by the average concentration in primary consumers at the six sites. The same process was used for the other three metals, and the mean concentrations ($n = 6$) for predators and primary consumers were compared statistically with Student's t-test using JMP-IN® software (Sall and Lehman, 1996). Thus, if for a given metal $BF > 1$ and the mean values were significantly different, biomagnification occurred. If $BF < 1$ and means were significantly different, trophic dilution occurred. Simple transfer ($BF = 1$) occurred if mean concentrations in predators and primary consumers were not significantly different (Newman, 1995).

Multiple linear regression analysis

To statistically evaluate factors associated with declines in predatory stonefly numbers downstream of the Stone/Straight Creek tributary, stepwise Multiple Linear

Regression Analysis (MLRA) was conducted using JMP-IN® software (Sall and Lehman, 1996). Percent abundance of predatory stoneflies in 2000 was used as the dependent variable, and metal concentrations in sediment, water column, predatory insects, and primary consumers were the independent variables. This process selected the parameters that best described variation from site to site in predatory stonefly numbers with overall model significance determined at the $\alpha = 0.05$ level. Only variables that individually contributed significantly to the overall model ($\alpha = 0.05$) were included.

Results

Benthic macroinvertebrate sampling

Generally, two-year average values for percent abundance of shredders, scrapers, and collectors (Fig. 2) followed trends as would be predicted by the River Continuum Concept (RCC, Vannote et al., 1980). Shredders decreased in relative abundance from ~25% at the furthest upstream station to ~5% just upstream of the confluence with the Powell mainstem. Conversely, there was a general trend of increasing relative abundance of scrapers from the furthest upstream site (~22%) to the bottom one (~47%). Percent abundance of collectors was variable from site to site, ranging from ~43% to 71%, but decreasing again to ~43% at the furthest downstream site.

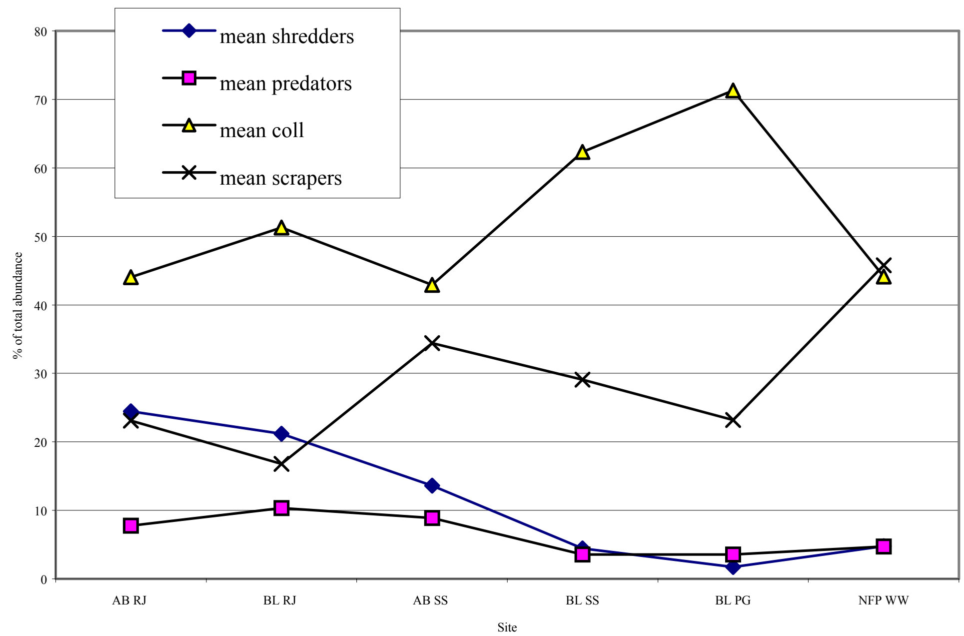

Figure 2. Two-year mean values for benthic macroinvertebrate functional feeding groups at six sites moving from upstream to downstream in the North Fork of the Powell River.

Two-year average values for percent abundance of predatory insects and common stoneflies dropped substantially beginning at the station downstream of Stone/Straight Creek (Fig. 3). Comparing averages for the three stations upstream of Stone/Straight to those for the downstream stations, predatory insects comprised an average of 9.0 ± 1.3% of the total abundance at the upstream stations, but were significantly reduced ($p = 0.0039$) downstream (3.9 ± 0.6%) (Fig. 4). Common stonefly numbers followed the same trend with the upstream average ($n = 3$) of 2.5 ± 0.2 being significantly higher ($p = 0.0001$) than the downstream average of 0.1 ± 0.2%. In the 2000 sampling season, no predatory stoneflies were found in the three downstream stations; however, at Fletcher Ford, ~57 km downstream of the NFP/Powell mainstem confluence, percent predator abundance was 10.10% and predatory stoneflies comprised 1.36% of the total abundance.

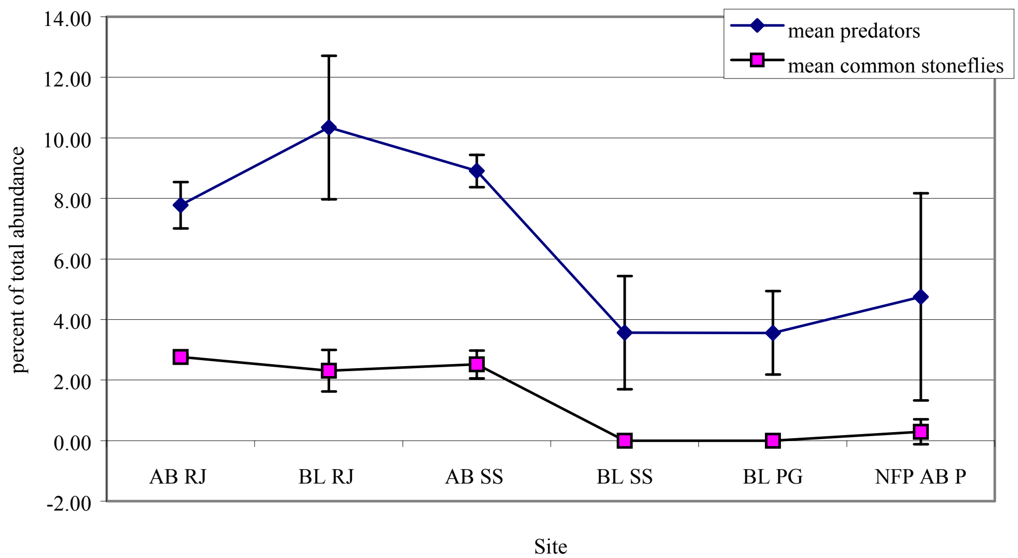

Figure 3. Two-year mean values for all predators and common stoneflies (Plecoptera) at six sites moving from upstream to downstream in the North Fork of the Powell River.

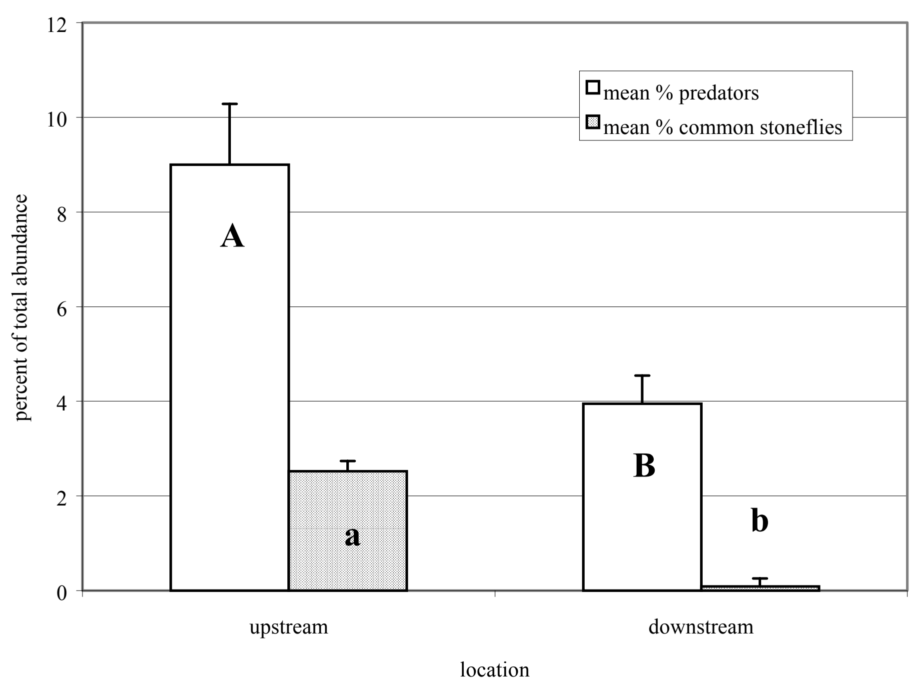

Figure 4. Comparison of predator and common stonefly numbers upstream and downstream of Stone/Straight Creek.

Water column and sediment chemistry/metals

In general, water column Al concentrations were higher throughout the system in 1999 than in the 2000 sampling season, with a general trend of increased concentrations of Al at the station below Stone/Straight Creek in both years (Table 1). The two-year average values for the three stations upstream of Stone/Straight ranged from 15 to 24 μg Al/L, while the average value for the station below Stone/Straight was 51 μg Al/L, decreasing to 48 and 33 μg Al/L moving further downstream. The trend for Fe concentrations was not as distinct (Table 1). Concentrations of Cu and Zn in the water column were always below detection limits (1.7 and 0.5 μg/L, respectively). Average values for pH, conductivity, alkalinity, and hardness were relatively consistent throughout the system with average values of 7.9 ± 0.1 S.U., 486 ± 56 μmhos/cm, 75 ± 22 mg/L as $CaCO_3$, and 165 ± 5 mg/L as $CaCO_3$, respectively. Dissolved oxygen was always at saturation.

Table 1. Water column metal concentrations for two years at sites within the North Fork of the Powell River. Yearly values are averages for two sampling occasions, and mean values are averages for both years. A/B = above; B/L = below; RJ = Reed/Jones Creek; SS = Stone/Straight Creek; PG = Pennington Gap; NFP WW = North Fork Powell at Woodway.

	Al (μg/L)			Fe (μg/L)		
	1999	2000	mean	1999	2000	mean
A/B RJ	30	1	15	41	93	67
B/L RJ	47	1	24	79	93	86
A/B SS	41	5	23	71	103	87
B/L SS	56	45	51	53	137	95
B/L PG	62	35	48	65	124	95
NFP WW	57	9	33	44	90	67

Sediment metals concentrations were variable and did not follow a distinct trend; Al and Cu concentrations were lower downstream of Stone/Straight Creek than those at the upstream station (Table 2). Concentrations of Fe in sediments were virtually the same at the two sites while Zn increased nominally at the downstream site.

Table 2. Metal concentrations in sediments at sites within the North Fork of the Powell River. A/B = above; B/L = below; RJ = Reed/Jones Creek; SS = Stone/Straight Creek; PG = Pennington Gap; NFP WW = North Fork Powell at Woodway.

	Sediment Metals			
	Al (g/kg)	Fe (g/kg)	Cu (mg/kg)	Zn (mg/kg)
A/B RJ	19.07	105.43	6.09	330.32
B/L RJ	49.73	261.19	6.43	343.78
A/B SS	28.27	135.90	17.58	360.45
B/L SS	25.18	136.83	11.90	391.15
B/L PG	30.54	235.24	5.26	349.36
NFP WW	31.54	281.61	10.70	365.51

Bioaccumulation study

No general trend of increased tissue metal concentrations was observed downstream of Stone/Straight Creek; in fact, concentrations of all four metals were lower in predators downstream of the tributary than at the station just upstream of Stone/Straight Creek (Table 3). Comparing average tissue metal concentrations at the six stations, the general trend was that predators had lower concentrations than primary consumers (Fig. 5). Concentrations in primary consumers were significantly higher ($p < 0.05$) than in predators for Al (24.6 and 11.6 g/kg, respectively), Fe (39.7 and 20.3 g/kg, respectively) and Zn (329.3 and 182.2 mg/kg, respectively). The average BF values of 0.48, 0.52, and 0.55 for Al, Fe, and Zn, respectively, indicated "trophic dilution". Concentrations of Cu were not significantly different ($p = 0.1277$) between predators (26.6 mg/kg) and primary consumers (18.8 mg/kg) indicating that the BF values of 1.44 was not significantly different from 1.0, and that "simple transfer" occurred.

Table 3. Metal concentrations in predatory insect- and potential prey insect tissues at sites within the North Fork of the Powell River. A/B = above; B/L = below; RJ = Reed/Jones Creek; SS = Stone/Straight Creek; PG = Pennington Gap; NFP WW = North Fork Powell at Woodway.

	Predatory Insects			
	Al (g/kg)	Fe (g/kg)	Cu (mg/kg)	Zn (mg/kg)
A/B RJ	9.01	14.35	23.32	239.74
B/L RJ	7.20	14.41	20.99	189.15
A/B SS	18.22	34.71	22.13	229.07
B/L SS	9.19	16.35	18.06	196.70
B/L PG	14.18	27.82	25.11	158.00
NFP WW	14.92	20.07	47.86	141.43
	Primary consumers			
	Al (g/kg)	Fe (g/kg)	Cu (mg/kg)	Zn (mg/kg)
A/B RJ	26.89	42.97	10.81	305.41
B/L RJ	25.73	41.36	24.09	459.09
A/B SS	24.10	41.45	18.25	282.30
B/L SS	22.41	36.08	19.41	389.71
B/L PG	24.21	39.44	15.87	255.08
NFP WW	25.36	42.30	21.13	369.43

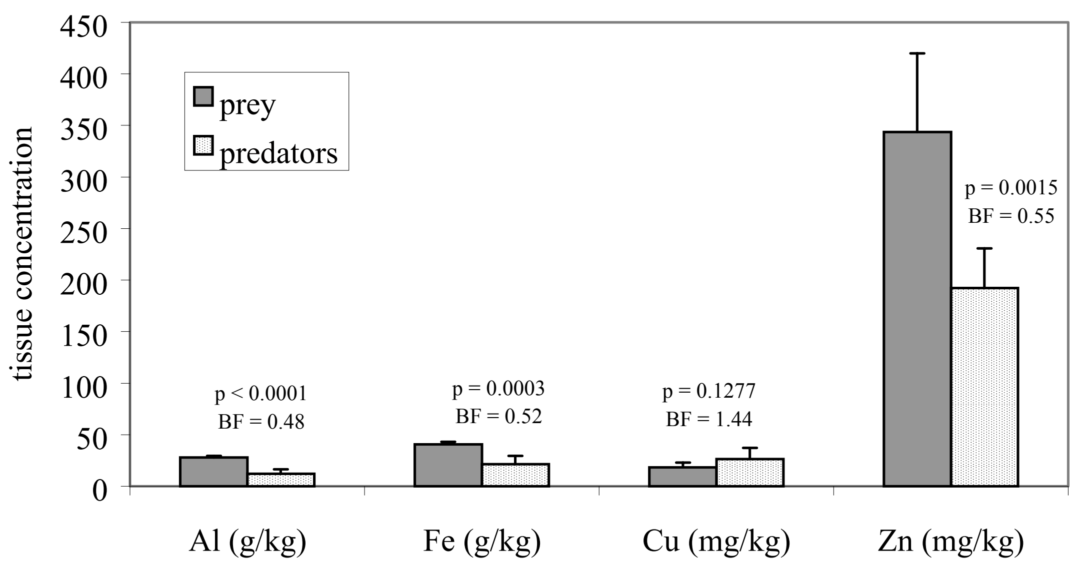

Figure 5. Tissue concentrations of four metals in benthic macroinvertebrates of two trophic levels. Values shown are means (± SD) for the six sampling sites in the North Fork of the Powell River.

Multiple linear regression analysis

MLRA was used to determine which factors (i.e., water column exposure to metals, or bioaccumulation) were associated with variations in common stonefly numbers. The model that best described the dependent variable consisted of four independent variables, with water column Al alone describing nearly 95% of the variability (Table 4). Other independent variables that entered the stepwise model included Al in primary consumers, Zn in predators, and Zn in primary consumers. The first two variables had negative coefficients, suggesting that the higher the Al in the water column and primary consumer tissues, the lower the abundance of common stoneflies. The other two variables had positive coefficients suggesting positive correlations.

Table 4. Prediction equation for 2000 predatory stonefly percent abundance in the North Fork of the Powell River based upon multiple linear regression analysis (n = 6). Overall model and all contributing independent variables are significant at $p < 0.05$.

Dependent variable	Intercept	Independent variables	cumulative R^2
% predatory stoneflies =	5.81	-0.0754 (water column Al)	0.9447
		-0.0001 (Al in prey insects)	0.9863
		+0.0069 (Zn in predatory insects)	0.9953
		+0.0014 (Zn in prey insects)	1.0000

Because of the high correlation between water column Al and percent predatory stoneflies during the year-2000 season, we conducted bivariate correlation analysis between the two-year averages for both parameters, and obtained a correlation coefficient (r) of -0.928 (p = 0.0076) (Fig. 6).

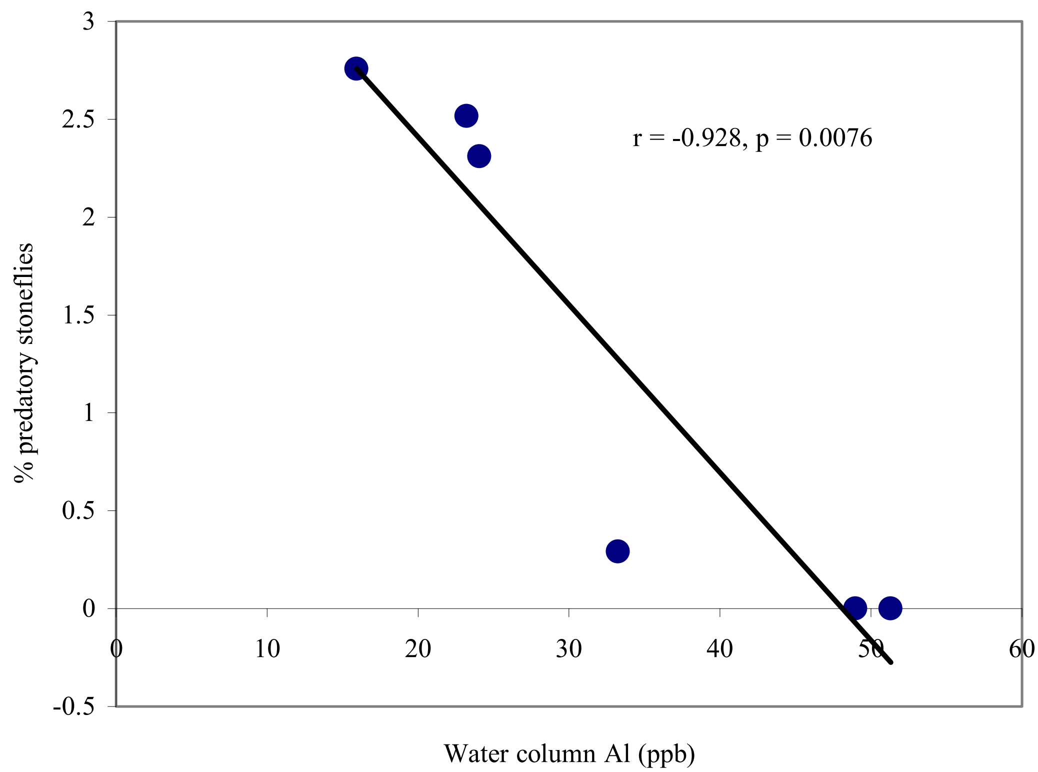

Figure 6. Correlation between two-year average values for water column Al concentration and percent abundance of common stoneflies at six sites moving from upstream to downstream in the North Fork of the Powell River.

Discussion

These data indicate that numbers of predatory invertebrates in general and particularly common stoneflies (*Acroneuria*) were significantly reduced downstream of the AMD impacted tributary to the NFP, Stone/Straight Creek. In addition, trends in predatory stonefly numbers over the course of the two-year study were strongly associated with Al concentrations in the water column.

While percent abundance of other functional-feeding groups changed moving from the furthest upstream to the furthest downstream station, the changes could largely be explained by the RCC (Vannote et al., 1980). For example, percent abundance of shredders decreased moving downstream. This is expected because the amount of coarse particulate organic matter (CPOM), upon which shredders feed, decreases with increasing stream size. Conversely, scrapers increased in percent abundance, a phenomenon associated with increased amounts of primary production (periphyton) with increasing stream size. Relative abundance of collectors was variable, with the largest value occurring at the site below the city of Pennington Gap, potentially due to dilute sewage inputs (Soucek personal observation).

While relative abundance of predators should remain fairly consistent with increasing stream size (Vannote et al., 1980), their numbers dropped significantly below Stone/Straight Creek, and common stoneflies were only found at one site below Stone/Straight Creek on one occasion during the two-year study. Stoneflies are known to be sensitive to chemical degradation in Appalachian streams, especially metal inputs, as part of the EPT (Ephemeroptera-Plecoptera-Trichoptera) index (Soucek et al., 2000b, chapter 1; Wallace et al., 1996). Density of predatory stoneflies (*Acroneuria*) has been observed to be reduced by overflow from fly-ash settling ponds (Specht et al., 1984), and experimental studies have demonstrated the sensitivity of stoneflies to extreme acidic and alkaline pH (Lechleitner et al., 1985) and various pesticides (Breneman and Pontasch, 1993; Harrahy et al., 1994). While some families of stoneflies are tolerant to acidic conditions (Kobuszewski and Perry, 1993), they are generally sensitive to pollutant inputs.

According to our MLRA results, the factor most strongly associated with variation in predatory stonefly numbers from site to site during the 2000 sampling season was total Al in the water column, which explained nearly 95% of the variability. Further support for the relationship is provided by the high correlation coefficient (-0.928) for the two-year averages of the parameters. While Al is generally not thought to be a toxic influence in neutral pH surface waters because of its low solubility, studies suggesting otherwise are accumulating. These have consisted of laboratory studies with the standard toxicity testing organism, *Daphnia magna* (Havas 1985), studies of alum ($Al_2(SO_4)_3$) treatment (Doke et al. 1995; Smeltzer et al. 1999) and mixing zone studies with fish

(Henry et al. 1999; Rosseland et al. 1992). Recent work (Campbell et al. 2000; Elangoven et al. 1997) also indicates that snails bioaccumulate Al (which significantly alters their behavior) by grazing upon extracellular mucopolysaccharides that have bound polyhydroxy-Al at neutral pH. Furthermore, Al may be contributing to decreased benthic macroinvertebrate diversity and abundance in neutral waters (pH>7.0) for up to one mile downstream of an acidified tributary in the NFP watershed (Soucek et al., 2001, chapter 4).

The toxic species of Al to invertebrates is not known. Several authors have suggested that the mode of Al toxicity to fish is transformation of Al^{3+} to polymers/colloids by hydrolysis with increased pH. Fish gills then act as a nucleating surface onto which the polymers precipitate, leading to suffocation (Exley et al. 1996; Neville and Campbell 1988; Poléo 1995). In plants however, the meta-stable tridecameric species $[AlO_4Al_{12}(OH)_{24}(H_2O)_{12}]^{7+}$, hereafter referred to as $[Al_{13}]^{7+}$, has been experimentally shown to be at least ten times more toxic than the Al^{3+} species (Parker et al. 1989). While speculation has been made that $[Al_{13}]^{7+}$ may be the toxic form to fish, a direct comparison of $[Al_{13}]^{7+}$ and Al^{3+} toxicity to experimental fish suggested that this was not the case (Exley et al. 1994). As waters from Lick Branch (pH ~4.5 at the furthest downstream point, Soucek et al. 2000a, chapter 3) enter Puckett's Creek, they are immediately neutralized to a pH of greater than 7.0. This rapid increase in pH provides the conditions necessary to form $[Al_{13}]^{7+}$ (Parker and Bertsch 1992) suggesting that this species, which slowly decreases in concentration over time, may be responsible for the observed impacts; however, further experiments should be conducted to verify this speculation.

The acute water quality criterion value (Criteria Maximum Concentration) for Al is 750 μg/L, and in the calculation of that value, a predatory stonefly (*Acroneuria*) was included with an LC50 value of 22,600 μg/L (U.S. EPA, 1988). This LC50 value is orders of magnitude higher than the mean concentrations of Al found downstream of Stone/Straight Creek of ~50 μg/L. However, 50 μg/L is close to the Criterion

Continuous Concentration (CCC) for Al (the estimate of the highest concentration to which aquatic communities can be exposed to indefinitely without unacceptable effects) which is 87 µg/L at pH 6.5 to 9.0 (U.S. EPA, 1999). Individual measurements included as means in this study were as high as 89.9 µg/L, indicating that at times, Al downstream of Stone/Straight Creek exceeds CCC values. Chronic continuous exposure to these concentrations of Al may be toxic to predatory stoneflies, but further long-term studies should be conducted to determine the chronic toxicity of various Al species to these organisms.

The results of the resident invertebrate tissue metals analysis and the MLRA suggested that bioaccumulation was only a minor factor in determining predatory stonefly abundances. In fact metal concentrations in predators were lower downstream of the impacted tributary than they were upstream. Further, mean tissue concentrations in predators were lower than those found in primary consumers at the six stations, as has been observed in other studies (Smock, 1983). While common stoneflies have been observed to accumulate Al in their chloride cells under acidic conditions (Guerold et al., 1995), there is no evidence that invertebrates biomagnify Al (reviewed in Gensemer and Playle, 1999). Some have suggested that Zn increases in concentration at higher invertebrate trophic levels (Timmermans et al., 1989); however, most studies indicate that Cu and Zn are not biomagnified in predators (Goodyear and McNeil, 1999). Our results support this contention, and our BF values for Cu (1.44) and Zn (0.55) are similar to mean values of 1.2 (range of 1.0 to 1.9) for Cu and 0.31 (range of 0.15 to 0.63) for Zn found by Besser et al. (2001). Thus, while accumulation of mining associated metals in tissues has been documented to impair benthic macroinvertebrate communities and food webs (Beltman et al., 1999; Besser et al., 2001; Saiki et al, 1995), bioaccumulation does not appear to be strongly associated with the reduction in common stonefly numbers downstream of Stone/Straight Creek.

In conclusion, we have observed the virtual elimination of common stoneflies downstream of an AMD impacted tributary in the North Fork of the Powell River. Despite the fact that stream order can play a role in structuring benthic macroinvertebrate communities (Vannote et al., 1980), their occurrence further downstream at Fletcher Ford indicates that this disappearance is not associated with the size or discharge of the river. We evaluated the relationship between predatory stonefly numbers throughout this reach and metal concentrations in water, sediment, and biological tissues (invertebrate predators and primary consumers), and found that the strongest association was with water column Al concentrations. While this correlation exists, it does not indicate a causal relationship, and experiments should be conducted to determine the long-term toxicity of Al to common stoneflies.

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Appendix

Taxon List for Powell River and NFP Stations 1999/2000

	AB Cox	BL Cox	AB RJ	BL RJ	AB SS	BL SS	BL PG	NFP AB P	BL Black	AB Pig	BL Pig	BL BSG
Oligochaeta	0	0	0	1	1	0	0	2	2	0	0	0
Asellidae	0	0	0	0	0	0	0	0	0	0	2	0
Cambaridae	0	0	1	1	1	1	1	1	1	1	1	1
Acroneuria	5	7	7	2	5	3	0	0	0	0	0	0
Chloroperlidae	0	0	0	1	0	0	0	0	0	0	0	0
Taeniopteryx	90	70	45	49	36	10	2	1	14	2	1	0
Leuctra	4	11	1	3	3	2	0	0	13	0	0	0
Ephemera	0	0	0	3	0	0	0	0	0	0	0	0
Caenis	1	0	1	2	0	0	0	1	0	0	0	1
Baetisca	0	0	0	0	0	0	0	1	0	0	0	0
Ephemerella	1	2	0	1	0	0	0	0	0	0	0	0
Eurylophella	2	0	0	7	1	0	0	0	0	0	0	0
Baetis	2	2	4	8	7	3	0	2	7	30	19	3
Stenonema	1	1	21	18	17	15	11	11	5	5	9	13
Stenacron	0	0	0	0	0	0	0	19	0	0	0	0
Epeorus	0	0	0	0	0	0	0	0	0	0	0	0
Isonychia	27	23	62	67	42	45	97	10	29	32	27	58
Cordulegaster	0	0	0	0	0	0	0	0	0	0	0	0
Gomphus	0	0	0	1	1	1	0	0	0	0	0	0
Stylogomphus	2	1	3	2	2	1	0	0	1	0	1	0
Hagenius	0	0	0	0	0	1	0	0	0	0	0	0
Macromia	0	0	0	0	0	1	0	0	0	0	0	0
Boyeria	0	0	0	0	0	0	0	0	0	0	0	0
Argia	0	0	1	1	0	2	0	1	0	0	0	0
Sialis	1	0	0	0	0	0	0	0	0	0	0	0
Corydalus	6	5	6	3	5	3	5	2	0	9	6	3
Nigronia	10	6	1	1	1	1	0	0	1	0	0	1
Hydroptilidae	0	0	0	0	0	0	0	0	0	0	1	0
Hydropsyche	7	6	12	19	12	22	6	6	29	7	5	5
Cheumatopsyche	12	16	17	22	12	42	5	4	50	7	9	13
Ceratopsyche	13	8	0	0	0	0	0	0	0	3	1	6
Pycnopsyche	0	0	0	0	0	0	0	2	0	0	0	0
Goera	0	0	0	0	0	0	0	1	0	0	0	0
Chimarra	8	7	6	4	13	2	0	0	0	0	1	0
Wormaldia	0	0	0	0	0	0	0	0	0	0	0	0
Rhyacophila	2	2	0	2	0	2	0	0	2	1	2	0
Mystacides	0	0	0	0	0	0	0	0	0	0	0	0
Polycentropus	2	1	0	0	0	0	0	0	0	0	1	0
Oecetis	0	0	0	0	0	0	0	0	0	0	0	0
Pyralidae	0	0	0	0	0	0	0	0	0	0	0	0
Gyrinidae	0	0	0	0	0	0	0	1	0	0	0	0
Dytiscidae	0	0	0	0	0	0	0	0	0	0	0	0
Hydrophilidae	0	0	0	0	0	0	0	0	0	0	0	0

Taxon List for Powell River and NFP Stations 1999/2000, cont'd												
	AB Cox	BL Cox	AB RJ	BL RJ	AB SS	BL SS	BL PG	NFP AB P	BL Black	AB Pig	BL Pig	BL BSG
Psephenus	3	2	8	5	3	3	1	2	2	7	7	4
Ectopria	0	0	0	0	0	0	0	0	0	0	1	0
Elmidae	37	21	48	24	29	37	33	24	23	51	28	93
Tipula	4	2	2	35	3	9	2	4	2	1	0	1
Antocha	0	0	0	0	0	2	0	0	0	3	6	1
Simuliidae	2	6	3	17	3	3	11	2	4	1	1	1
Tanypodinae	0	0	0	0	0	0	1	0	0	0	1	0
Chironominae	8	7	2	11	2	15	23	20	3	8	8	7
Atherix	2	2	2	3	3	4	2	1	12	1	1	2
Empididae	0	1	0	0	0	0	0	0	0	0	0	0
Physidae	0	0	0	0	0	0	0	0	0	0	0	0
Planorbidae	0	0	0	0	0	0	0	0	0	0	0	0
Ancylidae	0	0	1	7	0	2	2	4	2	0	0	4
Leptoxis	0	0	0	1	0	0	1	5	0	0	0	0
Pleurocera	0	0	2	4	1	2	1	9	0	31	62	1
Corbicula	0	0	0	8	0	2	1	3	2	1	1	2

Taxon List for Puckett's Creek Sampling Sites 1997-1999												
	PC-9	PC-8	PC-7	PC-6	PC-5	PC-4	PC-3	PC-2	PC-1	LB-5	LB-4	LB-3
Heptageniidae/Epeorus	35	18	5	1	1	0	0	0	0	0	0	0
Heptageniidae/Stenonema	2	1	3	0	0	0	0	0	0	0	2	0
Ameletidae/Ameletus	13	1	8	2	0	0	3	0	0	3	23	0
Ephemerellidae/Ephemerella	108	33	37	7	0	0	1	0	0	3	26	0
Ephemerellidae/Eurylophella	0	4	5	1	3	1	0	0	0	0	1	0
Ephemerellidae/Drunella	0	0	1	0	0	0	0	0	0	0	0	0
Caenidae/Caenis	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae/Baetis	8	2	73	1	0	0	4	0	0	37	11	1
Leptophlebiidae/Paraleptophlebia	0	2	9	0	0	0	0	0	0	0	2	0
Ephemeridae/Ephemera	0	1	0	0	0	0	0	0	0	0	0	0
Isonychiidae/Isonychia	0	0	0	0	0	0	0	0	0	0	0	0
Leptophlebiidae/Leptophlebia	0	0	0	0	0	0	0	0	0	0	0	0
Rhyacophilidae/Rhyacophila	3	0	0	0	0	1	0	1	1	2	0	0
Limnephilidae/Pycnopsyche	1	1	0	0	0	1	0	0	0	6	0	0
Lepidostomatidae/Lepidostoma	1	1	2	1	1	0	0	1	0	0	18	0
Polycentropodidae/Polycentropus	2	0	0	0	0	0	0	1	0	0	0	0
Hydropsychidae/Diplectrona	38	16	3	0	3	3	0	0	0	1	12	1
Hydropsychidae/Hydropsyche	3	2	1	1	3	2	0	1	1	0	0	0
Uenoidae/Neophylax	2	1	0	0	1	1	1	0	0	0	0	0
Glossosomatidae/Agapetus	0	0	3	0	0	0	0	0	0	0	0	0
Philopotamidae/Dolophilodes	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae/Triaenodes	0	0	0	0	0	0	0	0	0	0	0	0
Leuctridae/Leuctra	38	4	56	3	11	1	11	1	1	91	16	0
Peltoperlidae/Peltoperla	2	0	4	0	2	1	20	3	4	11	8	0
Nemouridae/Amphinemura	17	1	8	0	0	0	0	0	0	2	15	0
Perlodidae/Isoperla	9	2	1	5	1	0	2	0	0	2	11	0
Perlodidae/Yugus	5	2	12	0	0	0	1	0	0	1	6	0
Perlodidae/Remenus	0	4	8	1	0	0	0	0	0	2	0	0
Chloroperlidae/Haploperla	2	0	0	0	0	0	2	0	0	2	1	0
Perlidae/Acroneuria	0	0	1	0	0	0	0	0	0	1	0	0
Perlidae/Perlesta	0	0	0	0	0	0	0	0	0	0	0	0
Perlidae/Paragnetina	0	0	0	0	0	0	0	0	0	0	0	0
Sialidae/Sialis	0	0	0	0	0	1	0	0	0	0	0	1
Corydalidae/Nigronia	0	1	0	0	5	4	0	1	0	0	0	0
Corydalidae/Corydalus	0	0	0	0	0	0	0	0	0	0	0	0
Psephenidae/Ectopria	1	1	1	0	1	0	0	0	0	0	0	0
Psephenidae/Psephenus	0	2	0	0	2	0	0	1	0	0	0	0
Elmidae	1	2	0	0	1	0	0	1	0	2	2	1
Hydrophilidae	1	0	0	0	0	0	0	0	1	0	2	0
Dytiscidae	0	0	0	0	0	0	0	1	0	0	0	0
Carabidae	0	0	0	0	0	0	0	0	0	0	0	0
Cordulegastridae/Cordulegaster	0	1	3	1	2	1	0	0	1	1	5	0

Taxon List for Puckett's Creek Sampling Sites 1997-1999, cont'd												
	PC-9	PC-8	PC-7	PC-6	PC-5	PC-4	PC-3	PC-2	PC-1	LB-5	LB-4	LB-3
Aeshnidae/Boyeria	0	1	0	0	0	1	0	0	0	0	0	0
Gomphidae/Lanthus	0	0	0	0	1	1	0	0	0	0	0	0
Calopterygidae/Calopteryx	0	0	0	0	0	1	0	0	0	0	0	0
Chironomidae	3	30	3	0	2	1	2	2	1	3	5	24
Dixidae	0	0	1	0	0	0	0	0	0	0	0	0
Tipulidae/Antocha	3	0	1	0	0	0	0	0	0	1	60	0
Tipulidae/Hexatoma	2	0	0	0	0	0	0	0	1	0	2	1
Tipulidae/Tipula	2	1	0	1	0	2	0	1	1	1	0	0
Simuliidae	0	0	1	0	0	0	0	0	0	1	0	0
Tabanidae	0	0	0	0	0	0	0	0	0	0	1	0
Stratiomyidae	0	0	0	0	0	0	0	0	0	0	4	2
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0	0	7
Athericidae/Atherix	0	0	0	0	0	0	0	0	0	0	0	0
Pyralidae	0	0	0	0	0	0	0	0	0	0	1	0
Oligochaeta	2	3	0	1	2	1	1	0	0	0	2	0
Isopoda	0	0	0	5	0	4	2	1	0	1	9	1
Cambaridae	2	1	4	0	1	1	1	1	1	5	1	0
Collembola	1	0	0	0	0	0	0	0	1	1	3	1
Physidae	0	2	0	0	0	0	0	0	0	0	1	0
Planorbidae	0	1	0	0	0	0	0	0	0	0	0	0
Pleuroceridae	0	0	0	0	0	0	0	0	0	0	0	0

Taxon List for Puckett's Creek Sampling Sites 1997-1999									
	PS-9	LB-2	LB-1	SC-2	SC-1	SW-18	SW-19	SW-20	SW-2
Heptageniidae/Epeorus	0	0	0	0	2	0	2	1	1
Heptageniidae/Stenonema	0	0	0	0	0	0	0	5	2
Ameletidae/Ameletus	0	1	0	1	0	1	0	1	0
Ephemerellidae/Ephemerella	0	0	0	0	1	1	0	2	0
Ephemerellidae/Eurylophella	0	0	0	0	0	0	1	9	1
Ephemerellidae/Drunella	0	0	0	0	0	0	0	0	0
Caenidae/Caenis	0	0	0	0	1	0	0	1	0
Baetidae/Baetis	0	2	0	1	6	1	1	16	7
Leptophlebiidae/Paraleptophlebia	0	0	0	0	0	1	0	0	1
Ephemeridae/Ephemera	0	0	0	0	0	0	0	0	0
Isonychiidae/Isonychia	0	0	0	0	0	0	0	5	3
Leptophlebiidae/Leptophlebia	0	0	0	0	0	0	0	1	0
Rhyacophilidae/Rhyacophila	0	0	0	0	0	0	0	0	0
Limnephilidae/Pycnopsyche	0	0	0	0	0	0	0	0	0
Lepidostomatidae/Lepidostoma	0	0	0	0	0	0	1	1	0
Polycentropodidae/Polycentropus	0	0	0	0	0	1	0	0	0
Hydropsychidae/Diplectrona	0	0	0	0	0	2	0	0	1
Hydropsychidae/Hydropsyche	0	0	0	61	16	0	5	7	4

Taxon List for Puckett's Creek Sampling Sites 1997-1999												
	PS-9	LB-2	LB-1	SC-2	SC-1	SW-18	SW-19	SW-20	SW-2			
Uenoidae/Neophylax	0	0	0	0	0	0	0	0	0			
Glossosomatidae/Agapetus	0	0	0	0	0	0	0	0	0			
Philopotamidae/Dolophilodes	0	0	0	0	0	0	0	1	0			
Leptoceridae/Triaenodes	0	0	0	0	0	0	0	1	0			
Leuctridae/Leuctra	0	4	6	1	1	2	0	4	1			
Peltoperlidae/Peltoperla	0	0	1	2	1	0	2	3	0			
Nemouridae/Amphinemura	0	0	0	0	2	0	2	4	1			
Perlodidae/Isoperla	0	0	0	0	0	0	1	2	0			
Perlodidae/Yugus	0	0	0	0	0	0	0	1	1			
Perlodidae/Remenus	0	0	0	0	0	0	0	0	0			
Chloroperlidae/Haploperla	0	0	1	0	0	0	0	0	0			
Perlidae/Acroneuria	0	0	0	0	0	1	0	5	0			
Perlidae/Perlesta	0	0	0	0	0	0	0	8	1			
Perlidae/Paragnetina	0	0	0	0	0	0	0	1	0			
Sialidae/Sialis	1	0	0	0	0	0	1	0	0			
Corydalidae/Nigronia	0	0	0	1	0	0	0	2	0			
Corydalidae/Corydalus	0	0	0	2	3	0	3	1	1			
Psephenidae/Ectopria	0	0	0	0	0	0	0	0	0			
Psephenidae/Psephenus	0	0	0	2	0	1	2	5	1			
Elmidae	0	1	0	0	1	1	1	4	0			
Hydrophilidae	0	1	0	0	0	0	0	0	0			
Dytiscidae	0	0	1	0	0	0	0	0	0			
Carabidae	0	0	1	0	0	0	0	0	0			
Cordulegastridae/Cordulegaster	0	0	0	0	0	0	0	0	0			
Aeshnidae/Boyeria	0	0	0	0	0	0	0	2	0			
Gomphidae/Lanthus	0	0	0	0	1	1	1	3	1			
Calopterygidae/Calopteryx	0	0	0	0	0	1	0	0	0			
Chironomidae	34	11	2	7	16	3	3	6	5			
Dixidae	0	1	1	0	0	0	0	0	0			
Tipulidae/Antocha	0	0	0	1	0	0	1	0	0			
Tipulidae/Hexatoma	0	0	0	0	0	0	0	0	0			
Tipulidae/Tipula	0	0	0	1	0	0	0	1	0			
Simuliidae	0	0	0	0	0	0	0	0	1			
Tabanidae	0	0	0	0	0	0	0	0	0			
Stratiomyidae	0	0	1	0	0	1	0	0	0			
Ceratopogonidae	0	1	0	0	0	1	0	0	1			
Athericidae/Atherix	0	0	0	0	1	0	0	1	0			
Pyralidae	0	0	0	0	0	0	0	0	0			
Oligochaeta	0	1	0	0	4	4	13	2	5			
Isopoda	1	0	2	1	0	0	0	0	0			
Cambaridae	0	0	0	1	0	1	1	2	1			
Collembola	0	1	0	0	0	0	0	0	0			
Planorbidae	0	0	0	1	0	0	0	0	0			
Pleuroceridae	0	0	0	0	0	0	0	1	0			

David John Soucek **Curriculum Vitae**

May 2, 2001

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Date and Place of Birth: 31 October 1970, Euclid, Ohio.

Education:

Aug. 1997 - Present: Enrolled as a Doctoral student in Ecotoxicology under Dr. D.S. Cherry. Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA. Expected graduation date: May 2001. Dissertation research involves integrative bioassessment of acid mine drainage impacts upon the upper Powell River watershed, southwestern Virginia, with an emphasis on assessment methodologies and organismal/community level impacts beyond the zone of pH depression.

August 1997: Master of Science in Zoology under Dr. G.P. Noblet, Clemson University, Clemson, SC. ("Copper toxicity to the endoparasitic *Posthodiplostomum minimum* relative to snail and fish intermediate hosts and effects of copper on host-parasite interactions").

May 1993: Bachelor of Arts in Zoology, Miami University, Oxford, OH.

Experience:

August 1997 – December 1997, May 1999 - Present: Research Assistant - Virginia Polytechnic Institute and State University, Blacksburg, VA.

August 1997 – May 1999: Teaching Assistant - Virginia Polytechnic Institute and State University, Blacksburg, VA.

August 1994 - August 1997: Teaching Assistant - Department of Biological Sciences, Clemson University, Clemson, SC.

August 1995 - August 1997: Personalized Assistance Laboratory Attendant, Biology Program, Clemson University, Clemson, SC.

Memberships:

Society of Environmental Toxicology and Chemistry
American Fisheries Society
American Society for Surface Mining and Reclamation

Awards:

Outstanding Graduate Teaching Assistant, Clemson University, Spring 1997. Presented by the Clemson University Board of Visitors.

Outstanding Graduate Teaching Assistant for the Department of Biological Sciences, Spring 1997, Presented by the Clemson University Department of Biological Sciences.

Manuscript Reviewer:
Archives of Environmental Contamination and Toxicology
Bulletin of Environmental Contamination and Toxicology
Environmental Pollution
Environmental Toxicology and Chemistry

Invited Lecturer:
Invited by the Wellington School, Columbus, OH, to speak to 6th grade science students about benthic macroinvertebrates, bioassessment, and acid mine drainage. October 1, 1999.
Invited by Dr. Jon Cawley of Roanoke College, Salem, VA, to speak to his Environmental Science class (upperclassmen) about ecosystem impacts of acid mine drainage. October 4, 2000.

Laboratory Courses Taught:
Introductory Biology (Fall '94, Spring '95, Fall '97, Clemson University, Virginia Tech)
Introductory Biology for Biology Majors (Fall '98, Virginia Tech)
Medical and Veterinary Parasitology (Fall '95, '96, Clemson University),
Protozoology (Spring '96, Clemson University),
Developmental Biology (Spring '97, Clemson University),
Ecology (Summer '96, Clemson University)
Ornithology (Spring, '99, Virginia Tech)

Journal Articles:
Soucek, D.J. and Noblet, G.P. 1998. Copper toxicity to the endoparasitic trematode *Posthodiplostomum minimum* relative to physid snail and bluegill intermediate hosts. Environmental Toxicology and Chemistry 17(12):2512-2516.

Soucek, D.J., Cherry, D.S., and Trent, G.C. 2000. Relative Acute Toxicity of Acid-Mine Drainage Water Column and Sediments to *Daphnia magna* in the Puckett's Creek Watershed, VA. Archives of Environmental Contamination and Toxicology 38:305-310.

Soucek, D.J., Cherry, D.S., Currie, R.J., Latimer, H.A., and Trent G.C. 2000. Laboratory to field validation in an integrative assessment of an acid mine drainage impacted watershed. Environmental Toxicology and Chemistry 19(4):1036-1043.

Cherry, D.S., Currie, R.J., Soucek, D.J., Latimer, H.A. and Trent, G.C. 2001. An Integrative Assessment of a Watershed Impacted by Abandoned Mined Land Discharges. Environmental Pollution 111(3):377-388.

Soucek, D.J., Schmidt, T.S., Cherry, D.S. 2001. In Situ Studies with Asian Clams (*Corbicula fluminea*) Detect Acid Mine Drainage and Nutrient Inputs in Low Order Streams. Canadian Journal of Fisheries and Aquatic Sciences 58(3):602-608.

Soucek, D.J., Cherry, D.S., and Zipper, C.E. Accepted with Revisions. Aluminum Dominated Toxicity in Neutral Waters Below an Acid Mine Drainage Discharge. Canadian Journal of Fisheries and Aquatic Sciences.

Journal Articles (continued):

Schmidt, T.S., Soucek, D.J., Cherry, D.S. In Review. Modification of an Ecotoxicological Rating to Bioassess Small Acid Mine Drainage Impacted Watersheds Exclusive of Benthic Macroinvertebrate Analysis. Environmental Toxicology and Chemistry.

Soucek, D.J., Schmidt, T.S., Cherry, D.S., and Zipper, C.E. In Review. Individual and Community Level Impacts of Urban Runoff and Mine Drainage in the Upper Powell River Watershed, Virginia. Journal of Aquatic Ecosystem Stress and Recovery.

Journal Articles in Preparation:

Cherry, D.S., Currie, R.J., Soucek, D.J., Van Hassel, J.H. An Ecotoxicological Rating (ETR) system to evaluate point and nonpoint source impacts on a watershed scale. Environmental Pollution. 4 tables.

Cherry, D.S., Van Hassel, J.H., Farris, J.L., Soucek, D.J. Site-Specific Derivation of the Acute Copper Criteria for the Clinch River, Virginia. Environmental Toxicology and Chemistry. 5 tables.

Soucek, D.J., Denson, B.C., Schmidt, T.S., Cherry, D.S., Zipper, C.E. Reduced predatory invertebrate numbers downstream of a mine drainage impacted tributary to the North Fork of the Powell River, VA. Archives of Environmental Contamination and Toxicology. 4 figures, 2 tables.

Peer-Reviewed Conference Proceedings:

Soucek, D.J., Currie, R.J., Cherry, D.S., Latimer, H.A. and Trent, G.C. 1998. Benthic Macroinvertebrate Assemblages and Sediment Toxicity Testing in the Ely Creek Watershed Restoration Project. Proceedings of the American Society for Surface Mining and Reclamation. St Louis, MO. May 16-21, 1998.

Soucek, D.J., Cherry, D.S., Currie, R.J, Latimer, H.A. and Trent, G.C. 2000. Ecotoxicological Impacts of Acid Mine Drainage in Streams of Increasing Order in the Powell River Watershed, Virginia. In Daniels, W.L., and Richardson, S.G (Eds) Proceedings of the American Society for Surface Mining and Reclamation, Tampa, FL, June 11-15, 2000.

Schmidt, T.S., Soucek, D.J., Cherry, D.S., Currie, R.J, Latimer, H.A. and Trent, G.C. 2000. Integrative Bioassessment Techniques to Predict Ecotoxicological Impairment of Acid Mine Drainage. In Daniels, W.L., and Richardson, S.G (Eds) Proceedings of the American Society for Surface Mining and Reclamation, Tampa, FL, June 11-15, 2000.

Non-Refereed Publications:

Soucek, D.J., Cherry, D.S., and Zipper, C.E. 2000. Influence of Acid Mine Drainage from Abandoned Mines on Aquatic Biota in the Powell River System. Powell River Project Research and Education Program Report.

Invited Reports:

Cherry, D.S., Currie, R.J., and Soucek, D.S. 2000. Review of the Global Adverse Environmental Impacts to Ground Water and Aquatic Ecosystems from Coal Combustion Wastes: Final Report. Prepared for the U.S. Environmental Protection Agency on behalf of the Hoosier Environmental Council and Citizens Coal Council, Indianapolis, IN 46202.

Grants:

Cherry, D.S., Zipper, C.E, and Soucek D.J. 1999. Influence of Acid Mine Drainage from Abandoned Mines on Unionid Mussels in the Powell River Receiving System. Funded by the Powell River Project. Amount Requested: $24,800. Amount Received: $23,000.

Cherry, D.S., Zipper, C.E, Currie, R.J., Soucek, D.S., and Latimer, H.A. 1999. Streamlined Feasibility Study for Ecosystem Restoration: Interim Feasibility report for Ely and Puckett's Creeks in the Powell River Watershed, VA. Funded by U.S. Army Corps of Engineers and Virginia Department of Mines Minerals and Energy. Amount Received: $8,500.

Cherry, D.S., Soucek, D.J., Schmidt, T.S., Currie, R.J. 2000. Continuation of the Integrative Bioassessment of the Straight, Reeds, Jones, and Cox Sub-Basins Impacted by Abandoned Mined Land and Acid Mine Discharges in the Powell River Watershed. Submitted February 2000 to David Miller and Associates, Inc, Vienna, VA, and Virginia Department of Mines Minerals and Energy, Division of Mined Land Reclamation. Amount Received: $59,478.

Cherry, D.S., Zipper, C.E, and Soucek D.J. 2000. Influence of Acid Mine Drainage from Abandoned Mines on Unionid Mussels in the Powell River Receiving System: Request for continued support. Funded by the Powell River Project. Amount Requested: $24,800, Amount Received: $20,000.

Presentations/Published Abstracts:

Soucek, D.J. and Noblet, G.P. Effects of copper contamination on recruitment of *Posthodiplostomum minimum* (Trematoda) by bluegill sunfish (*Lepomis macrochirus*). Platform presentation at a joint meeting of the Association of Southeastern Biologists and the Southeastern Society of Parasitologists, Furman University, Greenville, South Carolina, April 1997.

Soucek, D.J., Currie, R.J., Cherry, D.S., Latimer, H.A. and Trent, G.C. 1998. Benthic Macroinvertebrate Assemblages and Sediment Toxicity Testing in the Ely Creek Watershed Restoration Project. Poster presented at the 1998 National Meeting of the American Society of Surface Mining and Reclamation, St. Louis, MO, May 16-21, 1998.

Soucek, D.J., Cherry, D.S., and Trent, G.C. 1998. Relative Toxicity of Acid-Mine Drainage Water Column and Sediments to *Daphnia magna*. Poster presented at the 1998 National Meeting of the Society of Environmental Toxicology and Chemistry, Charlotte, NC, November 14-18, 1998.

Presentations/Published Abstracts (continued):

Soucek, D.J., Cherry, D.S., Currie, R.J, Latimer, H.A. and Trent, G.C. 1999. Laboratory to Field Validation in an Integrative Assessment of an Acid Mine Drainage Impacted Watershed. Platform presentation at the 1999 National Meeting of the Society of Environmental Toxicology and Chemistry, Philadelphia, PA. November 15-18, 1999.

Soucek, D.J., Cherry, D.S., Currie, R.J, Latimer, H.A. and Trent, G.C. 2000. Ecotoxicological Impacts of Acid Mine Drainage in Streams of Increasing Order in the Powell River Watershed, Virginia. Platform presentation made at the 17th National Meeting of the American Society for Surface Mining and Reclamation, June 11-15, 2000, Tampa FL.

Schmidt, T.S., Soucek, D.J., Cherry, D.S., Currie, R.J, Latimer, H.A. and Trent, G.C. 2000. Integrative Bioassessment Techniques to Predict Ecotoxicological Impairment of Acid Mine Drainage. Poster presented at the 17th National Meeting of the American Society for Surface Mining and Reclamation, June 11-15, 2000, Tampa, FL.

Soucek, D.J, Schmidt, T.S., and Cherry, D.S. 2000. Transplanted Asian clams (*Corbicula fluminea*) as dual-purpose bioassessment tools in low order streams. Poster presented at the 21st Annual National Meeting of the Society of Environmental Toxicology and Chemistry. November 12-16, 2000, Nashville, TN.

Schmidt, T.S., Soucek, D.J., Cherry, D.S., Currie, R.J, Latimer, H.A. and Trent, G.C. 2000. A modified ecotoxicological rating to delineate acid mine drainage impacted sub-watersheds. Platform presentation at the 21st Annual National Meeting of the Society of Environmental Toxicology and Chemistry. November 12-16, 2000, Nashville, TN.

Soucek, D.J. 2001. Impacts of acid mine drainage on the benthic macroinvertebrate communities of the Powell River watershed. Virginia Tech Chapter of the Wildlife Society, Blacksburg, VA 24061. February 7, 2001.

Technical Reports:

Cherry, D.S., Soucek, D.J., Currie, R.J., and Latimer, H.A. 1998. Benthic macroinvertebrate assemblages, habitat assessment, laboratory chronic and in-situ sediment toxicity testing in the Puckett's Creek Watershed Restoration Project: Preliminary Report. Submitted to Virginia Division of Mined Land Reclamation, Big Stone Gap, VA.

Cherry, D.S., Soucek, D.J., Currie, R.J., and Latimer, H.A. 1998. Benthic macroinvertebrate assemblages, habitat assessment, laboratory chronic and in-situ sediment toxicity testing in the Puckett's Creek Watershed Restoration Project: Second Report. Submitted to Virginia Division of Mined Land Reclamation, Big Stone Gap, VA.

Cherry, D.S., Soucek, D.J., Currie, R.J., and Latimer, H.A. 1998. Benthic macroinvertebrate assemblages, habitat assessment, laboratory chronic and in-situ sediment toxicity testing in the Puckett's Creek Watershed Restoration Project: Final Report. Submitted to Virginia Division of Mined Land Reclamation, Big Stone Gap, VA.

Cherry, D.S., Currie, R.J., Latimer, H.A. and Soucek, D.J. 1998. Revisions recommended for the draft NPDES permit of Dominion Semiconductor in Manassas, Virginia. Submitted to Dominion Semiconductor, Manassas, VA.

Technical Reports (continued):

Cherry, D.S., Currie, R.J., Latimer, H.A. and Soucek, D.J. 1998. Annual chronic biomonitoring results of a water flea (*Ceriodaphnia dubia*) and fathead minnow (*Pimephales promelas*) to Pine Bluff Mill Effluent, International Paper Company, Pine Bluff, Arkansas.

Cherry, D.S., Currie, R.J., Latimer, H.A. and Soucek, D.J. 1998. Evaluation of Physically Induced Impairment Potential of Whole Effluent Toxicity on Bioassay Organisms – North Hudson Sewerage Authority at the River Road and Adams Street Waste Water Treatment Plants. Prepared for CH2M Hill, Herndon, VA.

Cherry, D.S., Currie, R.J., Latimer, H.A. and Soucek, D.J. 1998. Chronic Water Column and Sediment Toxicity Evaluation of Selected Sites, Independence Township, Beaver County, PA. Submitted to Aquatic Systems Corporation, Pittsburgh, PA.

Cherry, D.S., Currie, R.J., Latimer, H.A. and Soucek, D.J. 1998. Chronic Effluent Toxicity Test Report to BrushWellman, Inc. Submitted to BrushWellman, Inc., Shoemakersville, PA.

Cherry, D.S., Currie, R.J., and Soucek, D.J. 1999. Quarterly chronic biomonitoring results of a water flea (*Ceriodaphnia dubia*) and fathead minnow (*Pimephales promelas*) to Pine Bluff Mill Effluent, International Paper Company, Pine Bluff, Arkansas.

Cherry, D.S., Currie, R.J., and Soucek, D.J. 1999. Chronic Effluent Toxicity Test Report to BrushWellman, Inc. Submitted to BrushWellman, Inc., Shoemakersville, PA.

Cherry, D.S., and Soucek, D.J. 1999. Toxicity Evaluation of Two Abandoned Mined Land Seeps in the Puckett's Creek Watershed. Two Reports Submitted to David Miller and Associates, Inc., Vienna VA.

Cherry, D.S., and Soucek, D.J. 1999. Predictive Capability of the Ecotoxicological Rating System for Six AML/AMD Reclamation Sites in the Ely and Puckett's Creek Watersheds. Submitted to David Miller and Associates, Inc., Vienna VA.

Cherry, D.S., and Soucek, D.J. 1999. Prediction of Reclamation Success in Ely and Puckett's Creeks with Ecotoxicological Ratings Based on Projected Water Quality Parameters. Submitted to U.S. Army Corps of Engineers, Nashville District.

Cherry, D.S., Currie, R.J., and Soucek, D.J. 2000. Chronic Effluent Toxicity Test Report to BrushWellman, Inc. Submitted to BrushWellman, Inc., Shoemakersville, PA.

Cherry, D.S., Currie, R.J., Soucek, D.J., Schmidt, T.S., Hull, M.S., Kennedy, A.J. 2000. Toxicity Test Laboratory Performance Evaluation (DMR-QA Study 20) for Acute and/or Chronic Tests. US EPA Round Robin Test Evaluations.

Cherry, D.S., Currie, R.J., Soucek, D.J., Schmidt, T.S., Hull, M.S., Kennedy, A.J. 2000. Sediment Toxicity Testing of Stream and River Sites Near American Electric Power (AEP) Facilities in Ohio, West Virginia, Virginia, Kentucky, and Indiana. Submitted to AEP, Columbus, Ohio.

Technical Reports (continued):

Cherry, D.S., Currie, R.J., Soucek, D.J., Schmidt, T.S., Hull, M.S., Kennedy, A.J. 2000. Sediment Toxicity Testing of Stream and River Sites Near American Electric Power (AEP) Facilities in Ohio, West Virginia, Virginia, Kentucky, and Indiana. Group 2: Conesville, Muskingum River and Picway Plants. Submitted to AEP, Columbus, Ohio.

Cherry, D.S., Currie, R.J., Soucek, D.J., Schmidt, T.S., Hull, M.S., Kennedy, A.J. 2000. Sediment Toxicity Testing of Stream and River Sites Near American Electric Power (AEP) Facilities in Ohio, West Virginia, Virginia, Kentucky, and Indiana. Group 3: Cardinal and Mitchell Plants. Submitted to AEP, Columbus, Ohio.

Cherry, D.S., Currie, R.J., Hull, M.S., Soucek, D.J., Schmidt, T.S., Kennedy, A.J. 2000. Sediment Toxicity Testing of Stream and River Sites Near American Electric Power (AEP) Facilities in Ohio, West Virginia, Virginia, Kentucky, and Indiana. Group 4: Glen Lyn and Clinch River Plants. Submitted to AEP, Columbus, Ohio.

Cherry, D.S., Schmidt, T.S., Soucek, D.J., 2000. Preliminary Review of the Integrative Biological Assessment of Sites in Five Sub-watersheds, and in the North Fork Powell River. In, North Fork of the Powell River Ecosystem Restoration 2nd Interim Feasibility Study. Design Conference October 23- 27, 2000, Big Stone Gap, Virginia.

Cherry, D.S., Soucek, D.J., Schmidt, T.S. 2001. Metal accumulation in sediment, Asian clams (*Corbicula fluminea*), and periphyton at selected freshwater mussel preserves in the Clinch and Powell Rivers, Virginia. Report to: The Nature Conservancy, Clinch Valley Program, Abingdon, VA. Virginia/Tennessee Field Offices.

Cherry, D.S., Soucek, D.J., Hull., M.S. 2001. Chronic 21-day Renvewal Toxicity Responses with *Daphnia magna* to a Biofungicide from Sybron Chemicals, Inc. Submitted to Sybron Chemicals, Inc., 111 Kesler Mill Road, Salem, VA 24153. March 28, 2001.

Laboratory and Field Skills:

Benthic macroinvertebrate collection and identification

Standard water chemistry analysis and stream physical habitat characterization

Conduction of acute and chronic water column and sediment toxicity tests using *Ceriodaphnia, Pimephales*, *Lepomis, Mysidopsis, Menedia, Daphnia, Chironomus, and Hyalella*

Maintenance of cultures of *Ceriodaphnia dubia*, *Daphnia magna*, *Daphnia pulex,* and *Chironomus tentans*

In situ toxicity testing using *Corbicula fluminea*

Preparation of water, sediment, periphyton and animal tissue samples for metals analysis

Maintenance of parasitic life-cycles of *Posthodiplostomum minimum* and *Hymenolepis diminuta* in the laboratory

Preparation of permanent microslides of parasitic protozoans and metazoans

References:

Dr. D.S. Cherry, Department of Biology, 2006 Derring Hall, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. (540) 231-6766

Dr. C.E. Zipper, Department of Crop and Soil Environmental Sciences, 363 Smyth Hall, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. (540) 231-9782

Dr. J.R. Voshell, Department of Entomology, 302 Price Hall, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. (540) 231-5707

Dr. G.P. Noblet, Clemson University, Department of Biological Sciences, Clemson, SC, 29634. (864) 656-3589