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INTEGRATIVE ASSESSMENT OF BENTHIC MACROINVERTEBRATE COMMUNITY IMPAIRMENT FROM METAL-CONTAMINATED WATERS IN TRIBUTARIES OF THE UPPER POWELL RIVER, VIRGINIA, USA

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Abstract—Benthic macroinvertebrate communities of the North Fork Powell River (NFP), southwest Virginia, USA, appear to be impacted by aluminum (Al) and iron (Fe) from acid mine drainage (AMD) beyond the zone of pH depression. As part of a watershed restoration project, we used integrative techniques, including water column, sediment, and in situ toxicity tests; sediment and water column chemistry; and habitat assessments, to detect AMD impacts. An analysis of variance, least significant difference post hoc test, and Spearman correlations were used to test the sensitivity of these integrative techniques to detect various (acidic or neutralized) levels of AMD input and to determine the mode of impairment (metal-contaminated sediments or water) to the benthic macroinvertebrate community. Benthic macroinvertebrate indices were the most sensitive endpoint to AMD inputs and were significantly correlated ($p \leq 0.05$) with water column metal concentrations in in situ and water column toxicity tests. Sediment chemistry and toxicity did not detect AMD impacts and were not significantly correlated with benthic macroinvertebrate indices. These results suggest that the primary mode of impairment to the benthic macroinvertebrate communities beyond the zone of pH depression were waterborne Al and Fe.

Keywords—Benthic macroinvertebrates Integrative bioassessment Mine drainage Aluminum Iron

INTRODUCTION

Acid mine drainage is evolved from reduced sulfur materials (e.g., pyrite or FeS_2 in the eastern United States) that have been oxidized on exposure to water and oxygen, a process often brought about through mining activities. The pyrite oxidation reactions produce sulfuric acid and ferric hydroxides and mobilize other trace metals depending on the surrounding mineralogy. These toxic acids and metals flow to surface waters, where the acid is eventually neutralized, causing metals to precipitate and coat streambeds with metal oxides, impairing habitat and adversely affecting water quality in over 13,000 mi of U.S. rivers [1]. The biotic effects associated with AMD impacted surface waters include acute impairment of benthic and fish communities as a result of low pH and elevated levels of dissolved heavy metals [2–5]. A decrease in benthic macroinvertebrate diversity and an increase of tolerant organisms are also often associated with heavy metal pollution in streams.

Throughout the North Fork Powell River, stream communities have experienced decades of impairment from drainage and sedimentation associated with mining activities and abandoned mined lands. In the main stem of the NFP, populations of unionid mussels have been extirpated, and reductions in populations of the common stonefly *Acroneuria* in one particular reach have been attributed to the chronic toxic effects of neutralized mine drainage [6,7]. Headwater streams, such as Ely and Puckett's Creeks, are direct recipients of AMD, rendering surface and sediment pore waters of these tributaries acutely toxic to cladocerans and transplanted Asian clams [8–11]. Further, benthic macroinvertebrate community indices were correlated with the acute toxicity testing endpoints in these Ely and Puckett's Creek studies.

Reconnaissance of other tributaries draining mined areas in the NFP watershed revealed impaired benthic communities in streams without acidic pH values and only slightly elevated water column metals concentrations near or below U.S. Environmental Protection Agency (U.S. EPA) water quality criteria. Recent investigations of heavy metal–laden stream sediments in the NFP and other watersheds have suggested that those metals may be bioavailable, may cause acute or chronic toxicity to standard test organisms, and may smother or cause physical abrasion of the resident infauna [10,12–14]. Many of these studies attributed sediment toxicity to sediment copper (Cu), cadmium (Cd), and lead (Pb); pore-water; or water column concentrations of these metals. Because Cu, Cd, and Pb are found in relatively low concentrations in the NFP river sediments, the potential for sediment toxicity may be less in these streams. The objective of the present study was to investigate the sensitivity of various assessment techniques to different levels of Al- and Fe-dominated AMD input as found in the tributaries of the NFP and further to determine the likely mode of impairment (exposure to metal-contaminated sediments or water) to the benthic macroinvertebrate communities at both acidic and circumneutral AMD–impacted tributaries.

MATERIALS AND METHODS

Sampling regime and station categorization

Samples were collected over a four-year period in Ely Creek (January 1997–March 1997), Puckett's Creek (October 1997–July 1998), and Reed's Creek (December 1999–November 2000). A total of 36 sampling stations were selected, 12 in each of the three subwatersheds. All stations are found in first- to third-order streams. To facilitate statistical comparisons be-

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tween different levels of AMD impact, each station was categorized according to the relative level of AMD input, mean pH, and position within the watershed. The reference station category (upstream) included stations categorized as upstream of all known AMD inputs. Stations continuously subjected to AMD input but that had median pH values >4.5 were categorized as neutralized AMD–impacted stations. A third station category (acidic AMD) consisted of stations continuously subjected to AMD input and having median pH values ≤ 4.5. No stations in Reed's Creek met the acidic station criteria. The limit for buffering capacity in natural surface waters that contain few solutes is approximately pH 4.5, a common condition found in many headwater streams of the NFP. This median pH criterion was selected to differentiate those stations that maintained a low median pH value naturally from those that were influenced by AMD.

Water column and sediment chemistry

Water column chemistry was measured both in the field and laboratory. Field samples were stored at 4°C and analyzed within 24 h of collection. The pH was measured using either a Markson LabSales® (Wayne, NJ, USA) field pH meter with combination electrode or an Accumet® (Fisher Scientific, Pittsburgh, PA, USA) meter equipped with a gel-filled combination electrode. Conductivity was measured with a Hach® (Hach, Loveland, CO, USA) conductivity/total dissolved solids meter. Alkalinity and hardness were measured by titration [15]. Metals analyzed included total Al, Fe, and manganese (Mn). Copper, Cd, zinc (Zn), and Pb were measured in earlier studies but were not found above detection limits. Filtered water (0.45-μm pore size) samples were analyzed by inductively coupled plasma (ICP) spectrometry either by Spectrum Laboratories (Coeburn, VA, USA) or at the Virginia Tech Soil Testing Laboratory (Blacksburg, VA, USA). The lower detection limits for Al, Fe, and Mn were 0.06, 0.027, and 0.024 mg/L, respectively. When concentrations were below detection limits, one-half of that limit was used as the measured value for statistical analysis. Water quality parameters for each station were reported as median values.

Sediments were digested in 50% (v/v) nitric acid, 20% (v/v) hydrochloric acid (Fisher Scientific), with metals analysis following U.S. EPA protocol [16]. Total Al, Cu, Fe, Mn, and Zn were measured using ICP spectrometry. Sediments were collected with a polyurethane scoop from various points within a sampling station, placed in a freezer lock bag, and stored at 4°C. Each sample was homogenized, and 1-g samples were dried at 80°C for 24 h and weighed again to determine mean percentage water content. Mean values of replicate samples were used for statistical analysis.

In situ clam toxicity testing

Asian clams (*Corbicula fluminea*) [Müller] were collected from the New River near Ripplemead (VA, USA) using clam rakes. Clams were maintained in Living Streams® (Toledo, OH, USA) at the Virginia Tech Ecosystem Simulation Laboratory (Blacksburg, VA, USA) until needed for in situ toxicity testing. Five clams were placed into 18 × 36-cm-mesh (~0.5 cm^2) bags. At each sampling station, five bags were tied to a stake and left in the field for 30 d. After 30 d, clams were retrieved and transported to Virginia Tech, where mean survival was determined for each station. Clams were considered dead if they were found gaping, were easily opened, or failed to close when the visceral mass was touched with a blunt object.

Water column toxicity testing

Acute toxicity tests were preformed using *Ceriodaphnia dubia* cultured at Virginia Tech. Filtered culture/diluent water came from Sinking Creek Newport (VA, USA). Water quality parameters for Sinking Creek water were as follows: average pH 8.0 ± 0.1, conductivity 225 ± 5 μmho/cm, alkalinity 131 ± 9 mg/L $CaCO_3$, hardness 123 ± 4 mg/L $CaCO_3$, 1.6 μg Al/L, and 14.3 μg Fe/L; Cu and Zn were below detection. Organisms were fed 0.18 ml/30 ml test solution of a 1:1 (v/v) mixture containing *Selenastrum capricornutum* and yeast–cereal leaves–trout chow prior to testing. For toxicity tests, five organisms were placed into replicate 50-ml beakers (two replicates for Reed's Creek, four for Puckett's Creek, five for Ely Creek) containing site water. Tests were 48 h long, and temperature was maintained at 25 ± 1°C. Sinking Creek water was used as a control. Ely Creek stations were tested on one occasion for water column toxicity, Puckett's Creek three times, and Reed's Creek four times. For the purpose of statistical comparisons, mean survival for each test period was determined.

Sediment toxicity testing

Ten-day sediment toxicity tests were conducted within 14 d of sample collection using procedures outlined in Ingersoll et al. [17], Nebeker et al. [18], U.S. EPA protocols [19], and American Society for Testing and Materials (ASTM) [20] with modifications. Similarities in test procedures included the use of 5- to 6-d-old *Daphnia magna*, an ambient temperature of 25 ± 1°C, and overlying reference water collected from Sinking Creek; all controls met U.S. EPA and ASTM standards. Control sediments for Ely and Reed's Creeks were also collected from Sinking Creek; however, the control sediments for the Puckett's Creek investigation were formulated using a mixture of sand and potting soil (4:1, dry wt/dry wt). Overlying water was changed daily, and test organisms were fed 0.18 ml/30 ml of a 1:1 (v/v) mixture containing *Selenastrum capricornutum* and yeast–cereal leaves–trout chow daily. To minimize the effect of the different sediment test techniques used in these studies, mean survival and reproduction for each station were reported as percentage of mean control.

Sediment toxicity tests performed for the Ely and Puckett's Creek investigations utilized five replicated 1-L beakers filled with 200 ml of sediment and 800 ml of overlying water per station. Five *D. magna* were placed in each beaker. The sediment test chambers in the Reed's Creek investigation were 50-ml beakers each filled with 15 ml of site sediment overlaid with 35 ml of water and contained a single test organism. Eight replicates were used per station.

Benthic macroinvertebrate sampling

Benthic macroinvertebrate surveys followed U.S. EPA Rapid Bioassessment Protocol [21]. Two composite samples were collected at each station from riffle, run, pool, and shoreline rooted areas using dip nets of 800-μm mesh. Organisms were identified to lowest practical taxonomic level (usually genus) from standard taxonomic keys [22]. The community indices calculated included total taxon richness, ephemeroptera–plecoptera–trichoptera (EPT) richness, Ephemeroptera richness, Plecoptera richness, Trichoptera richness, EPT − (Hydropsychidae), EPT − (Leuctridae), and EPT − (Hydropsychidae

Table 1. Mean ± (standard deviation) of water chemical parameters at each station category in Ely, Puckett's, and Reed's Creeks (VA, USA)[a]

Water chemistry	Station category	Ely Creek (n = 12)	Puckett's Creek (n = 12)	Reed's Creek (n = 12)
Al in H_2O (mg/L)	Upstream	0.09 ± 0.01 B	0.18 ± 0.14 C	0.18 ± 0.05 A
	Neutralized AMD[b]	2.28 ± 1.16 A	2.44 ± 0.49 B	0.31 ± 0.17 A
	Acidic AMD	5.22 ± 4.48 A	29.34 ± 21.95 A	NA[c]
Fe in H_2O (mg/L)	Upstream	0.26 ± 0.07 B	0.34 ± 0.22 C	0.42 ± 0.36 A
	Neutralized AMD	6.80 ± 5.29 A	2.46 ± 0.77 B	1.09 ± 0.83 A
	Acidic AMD	7.29 ± 6.34 A	18.54 ± 18.42 A	NA
Mn in H_2O (mg/L)	Upstream	0.02 ± 0.00 B	0.5 ± 0.5 B	0.15 ± 0.19 A
	Neutralized AMD	0.94 ± 0.22 A	1.11 ± 1.38 A	0.35 ± 0.22 A
	Acidic AMD	1.68 ± 1.72 A	2.98 ± 1.42 A	NA
Conductivity (μS)	Upstream	107 ± 47 B	195 ± 113 B	179 ± 40 B
	Neutralized AMD	373 ± 53 A	541 ± 123 AB	284 ± 95 A
	Acidic AMD	418 ± 127 A	980 ± 654 A	NA
pH	Upstream	7.27 ± 0.11 A	7.39 ± 0.73 A	6.95 ± 0.18 A
	Neutralized AMD	5.81 ± 0.42 B	7.21 ± 0.25 AB	6.81 ± 0.24 A
	Acidic AMD	3.62 ± 0.57 C	3.61 ± 0.68 B	NA

[a] Means followed by the same uppercase letter are not significantly different; least significant difference $p < 0.05$.
[b] AMD = acid mine drainage.
[c] NA = none available.

+ Leuctridae). Community indices were calculated for each composite sample and were combined to obtain a mean for each station.

Habitat assessment

Habitat assessments in Ely and Puckett's Creeks were performed using U.S. EPA Rapid Bioassessment Protocol [21]. Nine parameters were measured, including bottom substrate/available cover, embeddedness, velocity/depth, channel alteration, bottom scouring and deposition, pool/rifle-run/bend ratio, bank stability, bank vegetative stability, and streamside cover. In Reed's Creek, habitat assessments were performed using the revised U.S. EPA Rapid Bioassessment Protocol [23]. Ten parameters were measured: epifaunal substrate/available cover, embeddedness, velocity/depth regime, sediment deposition, channel flow status, channel alteration, frequency of riffles (or bends), bank stability, vegetative protection, and riparian vegetation zone width. Ratings ranging from 0 to 10 or 0 to 20 (depending on the parameter) were used to distinguish physical integrity of the sampling station and its availability of niches for aquatic life. Two independent researchers conducted habitat assessments at each station simultaneously. In all cases, habitat assessment scores were reported as percentage of reference and were reported as means.

Statistical analysis

To analyze the differences between station categories (upstream of AMD impacts, acidic AMD impacts, and neutralized AMD impacts), means and medians for each data type (pH or sediment Fe [mg/kg]) from each station were pooled and averaged for all stations within a category for a given subwatershed. As these pooled means did not meet the primary assumptions of normality and homogeneity of variance, all data were rank transformed, and mean station category ranks were compared by a one-way analysis of variance and least significant difference post hoc test, using Statistical Analysis System® software [24]. For example, median pH was reported for each station (n = 36). The median pH values were then rank transformed and pooled into the three station categories (upstream of AMD impacts and acidic or neutralized AMD–impacted stations). These three pooled mean pH values for upstream of impact, acidic AMD–impacted, and neutralized AMD–impacted stations were then compared by a one-way analysis of variance and least significant difference.

To characterize the relationships between the integrative data at different pH regimes, stations were segregated into two subsets: acidic AMD–impacted stations and neutralized AMD–impacted stations. Integrative data from the upstream station group were added to both subsets, creating data sets of upstream and acidic AMD–impacted stations as well as an upstream with neutralized AMD–impacted stations data set. Because these two data sets did not meet the assumptions of normality and homogeneity of variance, Spearman correlation analysis was used to compare the different types of assessment endpoints using Statistical Analysis System software. To minimize the risk of an increasing type I error with multiple comparisons, a Bonferroni adjustment ($p = \alpha/\sqrt{n}$, $\alpha = 0.05$) was used to adjust the p value for each correlation matrix (Tables 5 to 8).

RESULTS

Water chemical and physical parameters

Mean water column metals concentrations and conductivity at upstream stations were significantly lower than neutralized or acidic AMD–impacted stations in Ely and Puckett's Creeks (Table 1). In Reed's Creek, only mean conductivity was found to distinguish upstream from neutralized AMD–impacted stations. In general, acidic stations in Ely and Puckett's Creeks averaged higher water column metals concentrations and conductivity than neutralized stations. However, in Puckett's Creek, significant differences were observed between all three station categories for mean concentrations of Al and Fe. Similarly, in Ely Creek, significant differences were observed between all three station categories for mean pH (7.27, upstream; 5.81, neutralized; and 3.62 acidic AMD–impacted stations), while in Puckett's Creek, only between upstream (7.39) and acidic (3.61) stations were mean pH significantly different. No differences were found in Reed's Creek. Few significant differences in sediment metals concentrations were observed between station categories (Table 2). Mean habitat scores at upstream stations were generally higher than at either acidic or neutralized AMD stations. The only significant differences observed for mean habitat score were in Ely Creek.

Table 2. Mean ± (standard deviation) of sediment chemical and physical parameters at each station category in Ely, Puckett's, and Reed's Creeks (VA, USA)[a]

Sediment chemistry	Station category	Ely Creek (n = 12)	Puckett's Creek (n = 12)	Reed's Creek (n = 12)
Sediment Al (mg/kg)	Upstream	1,623 ± 533 A	5,428 ± 954 A	3,802 ± 836 A
	Neutralized AMD[b]	1,480 ± 354 A	5,300 ± 826 A	4,592 ± 757 A
	Acidic AMD	1,664 ± 638 A	4,686 ± 2,772 A	NA[c]
Sediment Cu	Upstream	3.04 ± 1.96 A	8.56 ± 3.33 A	9.98 ± 12.17 A
	Neutralized AMD	2.78 ± 1.24 A	8.03 ± 1.80 A	7.84 ± 2.15 A
	Acidic AMD	1.38 ± 1.6 A	8.97 ± 5.01 A	NA
Sediment Fe (mg/kg)	Upstream	4,398 ± 1,744 A	26,860 ± 12,318 B	18,240 ± 1,798 A
	Neutralized AMD	5,071 ± 3,726 A	24,400 ± 1,609 B	26,655 ± 9,709 A
	Acidic AMD	10,134 ± 7,162 A	86,050 ± 58,897 A	NA
Sediment Mn (mg/kg)	Upstream	107.0 ± 43.9 A	980.6 ± 679.1 A	1,004 ± 675 A
	Neutralized AMD	126.9 ± 163.4 A	779.7 ± 119.9 A	1,235 ± 1,317 A
	Acidic AMD	33.7 ± 24.1 A	111.3 ± 97.5 B	NA
Sediment Zn (mg/kg)	Upstream	14.12 ± 3.72 A	45.20 ± 11.84 A	49.87 ± 16.29 B
	Neutralized AMD	16.35 ± 5.22 A	80.57 ± 7.87 A	79.79 ± 19.13 A
	Acidic AMD	7.67 ± 3.79 A	45.03 ± 31.86 A	NA
Habitat score[d]	Upstream	96.9 ± 2.2 A	91.9 ± 10.2 A	77.8 ± 7.9 A
	Neutralized AMD	71.1 ± 7.3 B	85.7 ± 7.9 A	70.5 ± 18.1 A
	Acidic AMD	62.3 ± 11.5 B	66.3 ± 26.2 A	NA

[a] Means followed by the same uppercase letter are not significantly different; least significant difference $p < 0.05$.
[b] AMD = acid mine drainage.
[c] NA = none available.
[d] Percentage of reference.

Toxicological parameters

In Ely Creek, 48-h *C. dubia* and 30-d in situ Asian clam toxicity tests had significantly greater survival at upstream stations than at acidic AMD–impacted stations (Table 3). *Ceriodaphnia* and Asian clam survival at neutralized AMD–impacted stations were not different from upstream stations or acidic AMD–impacted stations.

In Puckett's Creek, *C. dubia* survival was significantly greater at upstream stations as compared to neutralized AMD–impacted or acidic AMD–impacted stations (Table 3). However, at neutralized AMD–impacted stations, *C. dubia* survival was significantly greater than that at acidic AMD–impacted stations. Asian clam survival was significantly greater at upstream stations than at either neutralized or acidic AMD–impacted stations. No differences in Asian clam survival were observed between neutralized or acidic AMD–impacted stations.

In Reed's Creek, no significant differences in *C. dubia*, *D. magna*, and Asian clam survival or *D. magna* reproduction were observed (Table 3). Survivorship for each station category was high, ranging from 80 to 100% survival among the three test organisms.

Benthic macroinvertebrate parameters

In Ely Creek, all eight benthic macroinvertebrate indices had significantly lower values at acidic and neutralized AMD–impacted stations than upstream stations (Table 4). However, no differences were found in any benthic macroinvertebrate indices between acidic and neutralized AMD–impacted stations. In Puckett's Creek, all eight benthic macroinvertebrate indices statistically differentiated between upstream and AMD-impacted stations (Table 4). In Reed's Creek, all metrics except taxon richness and Ephemeroptera richness were sensitive to AMD-impacted station categories (Table 4).

Table 3. Mean ± (standard deviation) for toxicological parameters at each station category in Ely, Puckett's, and Reed's Creeks (VA, USA)[a]

Toxicological parameters	Station category	Ely Creek (n = 12)	Puckett's Creek (n = 12)	Reed's Creek (n = 12)
Ceriodaphnia dubia water column survival	Upstream	98.8 ± 2.5 A	86.2 ± 11.1 A	99.4 ± 1.3 A
	Neutralized AMD[b]	40.0 ± 54.8 AB	45.3 ± 32.7 B	90.7 ± 12.9 A
	Acidic AMD	0 ± 0 B	0 ± 0 C	NA[c]
Asian clam in situ survival	Upstream	80.0 ± 40.0 A	92.8 ± 5.22 A	80.0 ± 32.0 A
	Neutralized AMD	45.0 ± 44.7 AB	36.0 ± 34.2 B	83.0 ± 29.1 A
	Acidic AMD	0 ± 0 B	0 ± 0 B	NA
Daphnia magna sediment reproduction[d]	Upstream	75.2 ± 92.0 A	67.8 ± 14.8 A	87.8 ± 25.8 A
	Neutralized AMD[d]	292.8 ± 170.2 A	92.7 ± 21.6 A	59.5 ± 16.8 A
	Acidic AMD	77.0 ± 133.4 A	49.0 ± 58.1 A	NA
Daphnia magna sediment survival[d]	Upstream	35.0 ± 52.0 A	67.0 ± 12.2 A	103.6 ± 13.7 A
	Neutralized AMD	80.0 ± 24.5 A	84.7 ± 2.1 A	96.4 ± 18.3 A
	Acidic AMD	46.7 ± 50.3 A	53.0 ± 61.2 A	NA

[a] Means followed by the same uppercase letter are not significantly different; least significant difference $p < 0.05$.
[b] AMD = acid mine drainage.
[c] NA = none available.
[d] Percentage of reference.

Table 4. Mean ± (standard deviation) for ecological parameters at station categories in Ely, Puckett's, and Reed's Creeks (VA, USA)[a]

Ecological parameters	Station type	Ely Creek (n = 12)	Puckett's Creek (n = 12)	Reed's Creek (n = 12)
Taxon richness	Upstream	13.0 ± 2.9 A	18.5 ± 5.6 A	19.5 ± 6.2 A
	Neutralized AMD[b]	1.2 ± 1.6 B	9.3 ± 2.5 B	11.9 ± 4.4 A
	Acidic AMD	0.7 ± 0.6 B	4.6 ± 1.5 C	NA[c]
EPT richness[d]	Upstream	2.8 ± 1.5 A	4.8 ± 1.8 A	2.0 ± 1.9 A
	Neutralized AMD	0.2 ± 0.4 B	0.3 ± 0.6 B	0.7 ± 1.0 A
	Acidic AMD	0 ± 0 B	0.4 ± 0.5 B	NA
Ephemeroptera richness	Upstream	2.8 ± 0.5 A	5.3 ± 0.8 A	3.1 ± 1.0 A
	Neutralized AMD	0.2 ± 0.4 B	1.5 ± 0.5 B	1.4 ± 0.6 B
	Acidic AMD	0 ± 0 B	0.5 ± 0.7 B	NA
Plecoptera richness	Upstream	2.8 ± 0.5 A	2.7 ± 1.7 A	2.4 ± 0.3 A
	Neutralized AMD	0.0 ± 0.0 B	2.5 ± 1.0 A	1.4 ± 1.0 B
	Acidic AMD	0.3 ± 0.6 B	0.1 ± 0.3 B	NA
Trichoptera richness	Upstream	8.3 ± 1.3 A	12.8 ± 3.8 A	7.5 ± 25 A
	Neutralized AMD	0.4 ± 0.9 B	4.3 ± 1.0 B	3.5 ± 2.2 B
	Acidic AMD	0.3 ± 0.6 B	1.0 ± 0.7 C	NA
EPT richness − Hydrop. richness[e]	Upstream	7.5 ± 1.0 A	11.6 ± 3.1 A	6.6 ± 2.3 A
	Neutralized AMD	0.4 ± 0.9 B	3.0 ± 0.5 B	2.9 ± 1.9 B
	Acidic AMD	0.3 ± 0.6 B	0.9 ± 0.8 C	NA
EPT richness − Leuctr. richness[f]	Upstream	8.3 ± 1.3 A	11.8 ± 3.8 A	6.5 ± 2.5 A
	Neutralized AMD	0.4 ± 0.9 B	3.7 ± 1.3 B	2.6 ± 2.1 B
	Acidic AMD	0.3 ± 0.6 B	0.8 ± 0.5 C	NA
EPT richness − (Hydrop. richness + Leuctr. richness)	Upstream	7.5 ± 1.0 A	10.6 ± 3.1 A	5.6 ± 2.3 A
	Neutralized AMD	0.4 ± 0.9 B	2.3 ± 0.8 B	2.1 ± 1.8 B
	Acidic AMD	0.3 ± 0.6 B	0.3 ± 0.6 C	NA

[a] Means followed by the same uppercase letter are not significantly different; least significant difference $p < 0.05$.
[b] AMD = acid mine drainage.
[c] NA = none available.
[d] EPT = Ephemeroptera + Plecoptera + Trichoptera.
[e] Hydrop. = Hydropsychidae.
[f] Leuctr. = Leuctridae.

Upstream and acidic AMD impacted stations correlation analysis

Correlations between water column Al, Fe, Mn, conductivity, pH, and the benthic macroinvertebrate indices resulted in many significant associations, with coefficients ranging from −0.54 (Trichoptera richness vs conductivity) to 0.89 (EPT richness vs pH) (Table 5). In particular, median pH and water column Fe concentrations were significantly correlated with all eight benthic macroinvertebrate indices. However, few significant correlations were observed between sediment chemistry and the benthic macroinvertebrate indices except for sediment Mn.

Significant correlations occurred between all water chemistry parameters and both *C. dubia* (ranging from −0.72 to −0.90) and Asian clam survival (−0.69 to −0.89) at upstream and acidic AMD–impacted stations (Table 6). *Daphnia* survival and reproduction were not correlated with either water or sediment chemistry. However, *C. dubia* survival and Asian clam survival (r = 0.93, $p < 0.0001$), as well as *Daphnia* reproduction and survival (r = 0.82, p = 0.0001), were significantly correlated with each other.

Upstream and neutralized AMD impacted stations correlation analysis

Most correlations between water column parameters and the benthic macroinvertebrate indices at upstream and neutralized AMD–impacted stations were significant, ranging from −0.54 (conductivity vs EPT richness and Trichoptera richness) to 0.89 (pH vs EPT richness) (Table 7). No significant correlations were found between sediment chemistry parameters and the benthic macroinvertebrate indices. Habitat assessment score was significantly correlated with six of the eight benthic macroinvertebrate indices excluding taxon richness (r = 0.35, p = 0.06) and Trichoptera richness (r = 0.49, p = 0.02).

Correlations between *C. dubia* and Asian clam survival were significantly correlated with water column Al (−0.54, −0.54, respectively) and Fe (−0.55, −0.64, respectively) concentrations at upstream and neutralized AMD–impacted stations (Table 8). *Ceriodaphnia* and Asian clam survival were correlated with each other (r = 0.58, p = 0.001). However, no other significant correlations were found between the toxicological and chemical/physical parameters at upstream and neutralized AMD–impacted stations.

DISCUSSION

The results of this study had three specific trends: Only benthic macroinvertebrate diversity indices consistently differentiated upstream reference stations from AMD-impacted stations; impaired habitat, elevated metals in the water column, and depressed pH levels downstream of AMD inputs were associated with reduced benthic macroinvertebrate diversity and high mortality to Asian clams and *C. dubia*; and considerable variability was observed in sediment metal concentrations and toxicity among station categories, which were not generally correlated with benthic macroinvertebrate richness, in situ toxicity, water column toxicity, or water column chemistry. These results suggest that benthic macroinvertebrate communities are more sensitive to AMD impacts than were the toxicity testing endpoints used in this study. However, through the use of water column and sediment toxicity tests, it was possible to elucidate that the primary mode of impairment to the benthic macroinvertebrate communities beyond

Table 5. Correlation coefficients between ecological parameters and chemical and physical data at upstream and acidic mine drainage–impacted stations ($n = 16$)

Chemical and physical vs ecological parameters	Al in H_2O	Fe in H_2O	Mn in H_2O	Conductivity	pH	Sediment Al	Sediment Cu	Sediment Fe	Sediment Mn	Sediment Zn	Habitat[a]
Taxon rich.[b]	−0.64	−0.72[c]	−0.64	−0.54	0.88[c]	0.22	0.18	−0.03	0.71[c]	0.41	0.48
EPT rich.[d]	−0.66	−0.74[c]	−0.69[c]	−0.62	0.89[c]	0.29	0.21	−0.10	0.76[c]	0.40	0.51
Ephemeroptera rich.	−0.61	−0.70[c]	−0.66	−0.65	0.83[c]	0.27	0.19	−0.04	0.74[c]	0.39	0.39
Plecoptera rich.	−0.63	−0.74[c]	−0.73[c]	−0.64	0.81[c]	0.42	0.29	−0.10	0.85[c]	0.50	0.59
Trichoptera rich.	−0.76[c]	−0.78[c]	−0.69[c]	−0.54	0.82[c]	−0.15	−0.16	−0.35	0.38	0.06	0.36
EPT rich. − Hydrop. rich.[e]	−0.66	−0.74[c]	−0.68[c]	−0.63	0.87[c]	0.30	0.20	−0.12	0.75[c]	0.39	0.50
EPT rich. − Leuctridae rich.[f]	−0.65	−0.74[c]	−0.68[c]	−0.60	0.88[c]	0.23	0.15	−0.12	0.72[c]	0.35	0.48
EPT rich. − (Hydrop. rich. + Leuctr. rich.)	−0.66	−0.75[c]	−0.68[c]	−0.63	0.87[c]	0.28	0.18	−0.15	0.74[c]	0.36	0.50

[a] Percentage of reference.
[b] rich. = richness.
[c] Significant correlation at the $p < 0.005$ level.
[d] EPT = Ephemeroptera + Plecoptera + Trichoptera.
[e] Hydrop. = Hydropsychidae.
[f] Leuctr. = Leuctridae.

the zone of pH depression was metal-contaminated waters, not metal-contaminated sediments.

The observed benthic macroinvertebrate responses to AMD and heavy metal contamination were similar to those found in other studies [25–27]. Even in Reed's Creek, where in general both water and sediment chemistry were not significantly different below AMD inputs as compared to upstream, a reduction in the diversity of Ephemeroptera, Plecoptera, and Trichoptera was observed. However, in Reed's Creek, 48-h acute water column toxicity tests using *C. dubia*, 10-d chronic sediment toxicity tests using *D. magna*, and 30-d in situ Asian clam survival detected no differences between upstream and downstream stations. Cladocerans have been found to be very sensitive to heavy metal contamination, even in neutral waters, and in some cases determined to be more sensitive than some genera of the order Ephemeroptera [10,28–32]. Asian clam in situ toxicity has been determined to be sensitive to AMD inputs, and test endpoints are found to be predictive of the resident benthic macroinvertebrate communities [11]. These data reinforce the importance of using multiple toxicity techniques in biological assessments, as no single-most-sensitive species or the small battery of single-species toxicity test organisms utilized in this investigation revealed environmental effects that were observed at higher levels of biological organization in Reed's Creek [33,34].

Further evidence that batteries of toxicity tests and field studies are required to conduct watershed evaluations lies in the need to connect laboratory with field data through in situ experiments. In situ tests utilizing Asian clams are useful, as they bridge the gap between laboratory and field evaluations, and the clams' role as sediment residing filter feeders exposes them to both water column and sediment-bound toxicants [35]. However, in situ test organisms are exposed to unpredictable field conditions that go relatively unmonitored. These uncertainties can be manifested as increased mortality resultant from predation, dry weather periods, or vandalism, all conditions of little interest to those researchers quantifying the affects of AMD to aquatic communities. Synchronizing in situ Asian clam experiments with laboratory-controlled sediment and water column toxicity tests, as was the case in this investigation, can help diagnose false positives as a result of uncertain field conditions while also quantifying both sediment and water column exposures.

The combination of in situ and laboratory toxicity tests used in this investigation also helped to emphasize that, regardless of water column pH and sediment metal contaminant concen-

Table 6. Correlation coefficients between select toxicological parameters and chemical and physical data at acidic mine drainage–impacted stations ($n = 16$)

Toxicological vs chemical and physical parameters	*Ceriodaphnia dubia* water column survival	Asian clam in situ survival	*Daphnia magna* sediment reproduction[a]	*Daphnia magna* sediment survival[a]
Al in H_2O	−0.90[b]	−0.85[b]	−0.22	−0.03
Fe in H_2O	−0.78[b]	−0.89[b]	−0.21	−0.2
Mn in H_2O	−0.74[b]	−0.77[b]	−0.20	−0.1
Conductivity	−0.72[b]	−0.69[b]	−0.31	−0.25
pH	0.84[b]	0.84[b]	0.31	0.19
Sediment Al	−0.10	0.03	0.37	0.47
Sediment Cu	−0.17	−0.00	0.17	0.30
Sediment Fe	−0.51	−0.40	−0.10	0.11
Sediment Mn	0.45	0.56	0.43	0.44
Sediment Zn	−0.05	0.12	0.31	0.43
Habitat[c]	0.67[b]	0.64[b]	0.48	0.31

[a] Percentage of control or reference.
[b] Significant correlation at the $p < 0.008$ level.
[c] Percentage of reference.

Table 7. Correlation coefficients between select ecological parameters and chemical and physical data at upstream and neutral mine drainage–impacted station ($n = 29$)

Chemical and physical vs ecological parameters	Al in H_2O	Fe in H_2O	Mn in H_2O	Conductivity	pH	Sediment Al	Sediment Cu	Sediment Fe	Sediment Mn	Sediment Zn	Habitat[a]
Taxon rich.[b]	−0.54[c]	−0.54[c]	−0.59[c]	−0.50	0.53[c]	0.24	0.20	0.31	0.37	0.10	0.35
EPT rich.	−0.59[c]	−0.65[c]	−0.80[c]	−0.61[c]	0.73[c]	0.21	0.12	0.11	0.24	−0.09	0.63
Ephemeroptera rich.	−0.55[c]	−0.55[c]	−0.69[c]	−0.57[c]	0.49	0.11	0.02	0.03	0.11	−0.21	0.54
Plecoptera rich.	−0.59[c]	−0.69[c]	−0.78[c]	−0.66[c]	0.66[c]	0.27	0.23	0.13	0.32	−0.06	0.63
Trichoptera rich.	−0.49	−0.50	−0.66[c]	−0.39	0.80[c]	0.16	0.05	0.17	0.22	0.08	0.49
EPT rich. − Hydrop. rich.[d]	−0.61[c]	−0.65[c]	−0.82[c]	−065[c]	0.69[c]	0.18	0.12	0.08	0.22	−0.13	0.63
EPT rich. − Leuctridae rich.[e]	−0.60[c]	−0.64[c]	−0.80[c]	−0.61[c]	0.74[c]	0.19	0.09	0.08	0.20	−0.14	0.65
EPT rich. − (Hydrop. rich. + Leuctr. rich.)	−0.63[c]	−0.64[c]	−0.82[c]	−0.65[c]	0.68[c]	0.15	0.07	0.06	0.19	−0.16	0.65

[a] Percentage of reference.
[b] rich. = richness.
[c] Significant correlation at the $p < 0.005$ level.
[d] Hydrop. = Hydropsychidae.
[e] Leuctr. = Leuctridae.

trations, ecological effects at the community level were consistently correlated with water chemistry and habitat availability. In a study of the AMD impacts in Puckett's Creek, Soucek et al. observed acute water column toxicity to *C. dubia*, with a 50% lethal concentration (LC50) <2% mine effluent as a result of high water column concentrations of Al and Fe in association with low pH [9]. However, Al toxicity to *C. dubia* persisted even in neutral waters a mile downstream of this mine effluent input into the stream, resultant from the unusual precipitation kinetics of Al [32]. Under acidic to slightly acidic conditions, it is thought that Al^{3+} causes ionoregulatory stress to invertebrates; however, under rapid neutralization events (mixing zones or interflow/surface water interface), high concentrations of dissolved Al^{3+} can polymerize as the tridecameric species $(AlO_4Al_{12}(OH)_{24}(H_2O)_{12})^{7+}$ and precipitate on gill structures, causing respiratory stress to invertebrates in neutral waters downstream [36,37]. Also, an investigation of metal-contaminated sediments in Puckett's Creek found that a manufactured amorphous solid iron oxyhydroxide (FeOOH), precipitated from a neutral solution of iron sulfate $(Fe_2SO_4)^3$, in the absence of dissolved Fe^{3+}, caused toxicity to *D. magna*, presumably through either physical abrasion to respiratory structures or ingestion [10].

In the present study, more evidence supporting the idea that Al and Fe can cause persistent toxicity in neutral waters was generated. The benthic communities in Reed's Creek, a subwatershed without acidic AMD stations, were impaired downstream of neutralized AMD inputs. Aluminum and Fe in the water column were the only chemical/physical parameters significantly correlated with *C. dubia* and Asian clam survival at upstream and neutralized AMD–impacted stations. These data indicate that at neutral pH, a nominal increase in water column Al and Fe, as found in Reed's Creek, can create persistent water column toxicity, impairing benthic communities downstream of mining activities. Further, the precipitation of Al and Fe can limit habitat availability by filling crevices with flocculants, much like sedimentation, as evidenced by the positive correlation coefficients between habitat score and the benthic macroinvertebrate diversity.

Sediment toxicity tests were insensitive to AMD inputs in this investigation. In fact, to our knowledge, no other investigation has attributed toxicity to invertebrates to elevated concentrations of Al or Fe in stream sediments. Most investigations of mining-related sediment toxicity have concentrated on Cu, Zn, Pb, and Cd [36]. The variability found in the sediment metals of this investigation contributes greatly to the fact that few significant differences were found between station category sediment chemistry. In addition, Fe oxyhydroxide (FeOOH) is thought to sorb other potentially toxic metals, especially in oxic sediments found in high-gradient headwater streams [38]. Other studies investigating the toxicity or bioavailability of metal-contaminated sediments have found that

Table 8. Correlation coefficients between toxicological parameters and chemical and physical data at upstream and neutral mine drainage–impacted stations ($n = 29$)

Toxicological vs chemical and physical parameters	*Ceriodaphnia dubia* water column survival	Asian clam in situ survival	*Daphnia magna* sediment reproduction[a]	*Daphnia magna* sediment survival[a]
Al in H_2O	−0.54[b]	−0.54[b]	0.35	0.21
Fe in H_2O	−0.55[b]	−0.64[b]	0.24	0.08
Mn in H_2O	−0.26	−0.39	0.32	0.20
Conductivity	−0.45	−0.40	0.18	0.05
pH	0.14	0.25	−0.22	−0.30
Sediment Al	−0.24	−0.10	−0.28	−0.07
Sediment Cu	−0.22	0.02	−0.10	0.12
Sediment Fe	−0.14	0.05	−0.31	0.06
Sediment Mn	0.08	0.14	−0.12	0.20
Sediment Zn	−0.09	0.03	−0.24	0.24
Habitat[a]	0.17	0.22	0.01	−0.27

[a] Percentage of control.
[b] Significant correlation at the $p < 0.008$ level.

water column or pore-water concentrations of heavy metals are more predictive of benthic macroinvertebrate community structure than are whole-sediment metals concentrations [10,12,29,38,39]. In Puckett's Creek, Soucek et al. found significant correlations between sediment Fe concentrations and sediment toxicity, which was also correlated with acidic pH. Sediments in that study were found to have high percentage water content, and it was suggested that pore-water concentrations of free Fe ions in association with acidic pH were the likely source of toxicity [10]. In addition, Schmidt et al. [40] found that sediment toxicity tests utilizing *D. magna* or *Chironomus tentans*, a sediment-dwelling dipteran, were not predictive of benthic macroinvertebrate community responses to Al- and Fe-dominated AMD seepage or sediments.

In conclusion, integrative assessments of Al- and Fe-dominated AMD-impacted watersheds should focus on benthic macroinvertebrate community structure, water column metals, and in situ and water column toxicity tests. Benthic macroinvertebrate indices were found more sensitive to Al- and Fe-dominated AMD than all other bioassessment techniques utilized in the present study, regardless of pH. Also, the use of multiple toxicity tests, both in situ and laboratory, can elucidate the mode of toxicity, focusing future research efforts at the causative agents of toxicity. These data support past investigations that suggest that Al and Fe can be persistent toxicants in neutral waters. Also, Al- and Fe-contaminated sediments in steep headwater streams may not be toxic to *D. magna* or benthic macroinvertebrates; however, through sedimentation, precipitates may impair habitat availability to aquatic communities.

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