ECOTOXICOLOGICAL IMPACTS OF ACID MINE DRAINAGE IN STREAMS OF INCREASING ORDER IN THE POWELL RIVER WATERSHED, VIRGINIA

by

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Abstract. The environmental impacts of acid mine drainage (AMD) at different levels of dilution were evaluated using transplanted Asian clams (*Corbicula fluminea*) and qualitative benthic macroinvertebrate community sampling. Three contiguous streams in the upper Powell River watershed were sampled/tested at sites upstream and downstream of AMD inputs. The first stream was considered small according to its relative flow (~0.1 relative units at downstream site), the second was medium sized (~0.4 relative units), and the third was large (~1.0 relative units). For the small stream, total invertebrate richness, Ephemeroptera-Plecoptera-Trichoptera (EPT) richness, and percent mayfly abundance were significantly lower downstream of AMD inputs compared to upstream sites. In the medium sized stream, only percent mayfly abundance was impaired downstream of AMD, while in the large stream, total richness and EPT richness decreased nominally downstream of AMD inputs. Multiple linear regression analysis suggested that water column conductivity was a good predictor of both total richness ($R^2 = 0.819$, $p = 0.0050$) and EPT richness ($R^2 = 0.836$, $p = 0.0039$), with increasing conductivity being associated with lower richness values. Clam survival decreased at the downstream site in the small stream, while clam growth was impaired at downstream sites in the medium and large streams. Water column pH was the best predictor of clam survival ($R^2 = 0.886$, $p = 0.0016$) while aluminum, conductivity, iron and flow described clam growth ($R^2 = 0.999$, $p = 0.0015$). These findings suggest that AMD inputs may be impacting streams beyond the zone of pH depression, even where flow was equal to approximately ten times the flow of the small, most severely impacted stream.

Additional Key Words: acid mine drainage, community impacts, population impacts

Introduction

Numerous studies have documented AMD and acid precipitation impacts on aquatic communities within the zone of pH depression. For example, zooplankton and phytoplankton communities have been observed to be less productive and less diverse under acidic conditions compared to communities in unpolluted waters (Havens and DeCosta 1987). Changes in plankton community structure include reductions in the number of taxa present and/or shifts from intolerant to tolerant species. Similar community impacts have been documented for benthic macroinvertebrates (Armitage 1980; Herricks and Cairns 1974; Rutherford and Mellow 1994; Smith and Frey 1971; Soucek et al. 2000a), and fish (Rutherford and Mellow 1994) exposed to AMD.

The combination of acid and high metal concentrations creates an extremely toxic environment for aquatic organisms. This point is illustrated by a previous study of the Puckett's Creek watershed in southwestern Virginia (Soucek et al. 2000a). Four sampling sites there with an average pH of 3.71 and average water column aluminum concentration of 30 mg/L supported an average of only five different kinds of organisms, while upstream reference sites with normal water quality supported an average of 18 different kinds of benthic organisms.

While numerous studies have documented the effects of AMD on benthic macroinvertebrate communities, much less work has focused on the environmental impacts of AMD beyond the zone of pH depression. Some researchers have found that Al is acutely toxic to fish in mixing zones (pH 4.8-6.5) below acidified tributaries (Rosseland et al. 1992, Henry, 1999), and metal inputs (copper and cadmium) from hard-rock mine drainages have

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been documented to cause acute sediment toxicity in neutral pH waters (Kemble et al. 1994). With the high concentrations of dissolved Al present in the acidified waters in the Puckett's Creek watershed (Soucek et al. 2000a), the potential for environmental impacts beyond the zone of pH depression was considered substantial.

The purpose of this study was to investigate environmental impacts of AMD after neutralization of acidic waters by receiving streams and further dilution downstream. This was accomplished using benthic macroinvertebrate data and *in situ* toxicity testing data for Asian clams (*Corbicula fluminea*) at neutral pH sites in three contiguous streams. The smallest one, a third-order stream, received input from an acidic tributary. The third-order stream then flowed into a fourth-order stream which eventually contributed to a fifth-order stream. Sites upstream and downstream of each of the three confluences were selected to determine how far downstream AMD impacts might extend. Our hypothesis was that population or community effects would be detectable at each level of AMD dilution, beyond where acute toxicity is detectable by standard bioassays.

Methods

Site Selection

Sampling sites upstream and downstream of acid mine drainage inputs were selected in three streams in a sub-watershed of the North Fork of the Powell River in Lee Co., Virginia (Table 1). Stations were placed into three groups, the Puckett Creek, Straight Creek and North Fork of the Powell River groups. The first two groups consisted of an upstream station, a downstream station, and one in the AMD-impacted tributary, while the North Fork group consisted only of an upstream and downstream station. Acid mine drainage inputs were present in Lick Branch, a tributary to Puckett Creek.

Chemical/Physical Analysis

Water samples from the seven stations were collected for analysis of selected water quality parameters in October 1997, January, February, May, and July 1998. Samples were brought to the laboratory, stored for 24 h at 4° C and measurements taken 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 $< \pm 0.05$ 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. In addition, water column samples were collected for metals analysis by Spectrum Laboratories, Coeburn, Virginia. Filtered (0.45 µm pore size) water samples were analyzed for aluminum (Al), and iron (Fe) by inductively coupled plasma spectrometry. Lower detection limits (mg/L) for metals were 0.001 and 0.002 for Al and Fe, respectively.

Table 1. Sampling sites in the Stone/Straight Creek sub-watershed of the North Fork of the Powell River. Note that station PC-4 is used in two different groups.

Site	Description
Puckett's Creek Group	
PC-8	Small, Upstream of AMD (Puckett's Creek)
LB-2	Acidic AMD Tributary (Lick Branch)
PC-4	Small, Downstream of AMD (Puckett's Creek)
Straight Creek Group	
SC-1	Medium, Upstream of AMD (Straight Creek)
PC-4	Neutral AMD Tributary (Puckett's Creek)
SW-19	Medium, Downstream of AMD (Straight Creek)
North Fork of Powell Group	
SW-20	Large, Upstream of AMD (North Fork of Powell)
SW-2	Large, Downstream of AMD (North Fork of Powell)

Physical analyses included measurements of discharge and habitat assessment according to US EPA Rapid Bioassessment Protocols (RBPs) (Plafkin et al. 1989). Discharge measurements were made with a Swoffer® flow meter. Habitat assessment of each station included nine parameters which described the ability of a stream to physically support aquatic life. Scores for each parameter were summed for each station, with higher total scores indicating better habitat.

Benthic Macroinvertebrate Sampling

Benthic macroinvertebrate surveys were conducted according to the US EPA RBPs (Plafkin et al. 1989). Riffle, run, pool and shoreline rooted areas were each 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 taxon richness (the number of different kinds of organisms), Ephemeroptera-Plecoptera-Trichoptera (EPT, the number of different kinds of organisms in the three most sensitive insect orders) richness, and percent Ephemeroptera abundance. Percent Ephemeroptera abundance is the number of mayflies (the most sensitive insect order) divided by the total number of organisms comprising a sample. Index values for replicate samples were combined to obtain mean index values per station (i.e. mean taxon richness, etc.).

In situ Clam Toxicity Testing

Asian clams 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. Clams were measured for width to the nearest 0.01 mm using Vernier calipers, and given a unique identification mark prior to placement into bags for testing. At the end of 31 days, clam bags were collected from each testing station and transported on ice to the laboratory. Clams were counted as dead or alive and measured for width. Clams found with valves separated, or that were easily opened were considered dead.

Statistical Analysis

Upstream, downstream and tributary values for benthic macroinvertebrate indices and *in situ* clam survival and growth were compared within the groups of stations: the Puckett Creek group (PC-8, LB-2, PC-4), the Straight Creek group (SC-1, PC-4, SW-19), and the North Fork of the Powell River stations (SW-20, SW-2). Note that station PC-4 was used in two different analyses. Analysis of variance (ANOVA) and Student's T-test (as a post-hoc pairwise analysis) were used to test statistical significance at the $\alpha = 0.05$ level with JMP-IN® software (Sall and Lehman 1996). In ANOVA, five replicates were used per station for clam data, and two replicates per station were used for benthic macroinvertebrate community indices. In addition, multiple linear regression analyses (MLRA) were conducted with benthic macroinvertebrate community indices, and toxicity test parameters as dependent variables, and chemical and physical parameters as independent variables using JMP-IN® software. A step-wise selection process was used with a significance level of $p \leq 0.05$ required for variable entry into the model with mean values for each station included in the analyses (n=7). This was done to investigate whether physical or chemical parameters were more important in shaping aquatic communities in a system consisting of several levels of dilution of toxic AMD inputs.

Results

Physical/chemical parameters

Flow measurements at the sampling stations indicated that, in most cases, the tributaries increased the discharge of their respective receiving streams (Table 2). The exception was the Straight Creek group, where the downstream site (SW-19) apparently had slightly lower flow than the upstream site. This may be due to error in the flow measurement. Despite this discrepancy, the flow data indicated that Straight Creek was about four times as large as its tributary, Puckett's Creek, and that the North Fork of the Powell River had ten-fold greater flow compared to Puckett's Creek.

Habitat Assessment Scores (HAS) varied overall from 54 at LB-2 to 100 at SW-2 (Table 2). In Straight Creek, HAS decreased slightly downstream of AMD inputs, while, in Puckett's Creek and the North Fork of the Powell, habitat scores increased slightly downstream of the AMD tributary compared to upstream. In general, AMD inputs had no substantial effect upon the physical habitat of these streams.

In two of the three station groups (Puckett's Creek and Straight Creek), pH decreased downstream of the AMD input (Table 2). The pH in the North Fork of the Powell River increased slightly at the downstream site. Despite the pH decreases in the two smaller streams, all values still were above 6.0, and within US EPA Water Quality Criteria (WQC) limits for protection of aquatic life. In Puckett's Creek, the pH decreased from 7.75 to 6.11 below the AMD tributary. The pH decrease in Straight Creek was less, probably due to its greater flow. Conductivity values increased downstream of AMD input in Puckett's Creek (from 200 µmhos/cm upstream to 564 µmhos/cm downstream) and the North Fork of the Powell (from 266 to 362 µmhos/cm), but decreased downstream of the AMD input in the Straight Creek group (Table 2). Dissolved oxygen was always at or near saturation for all of the stations.

In all cases, both Fe and Al either increased or stayed the same downstream of AMD inputs. The largest increase occurred in Puckett's Creek, where the downstream concentrations for Fe and Al were 0.171 and 3.98 mg/L, respectively compared to 0.026 and 0.14, upstream. Metal concentrations also increased substantially in the North Fork of the Powell, as did Al in Straight Creek downstream of the AMD input. Iron did not increase in Straight Creek downstream of Puckett's Creek.

Benthic Macroinvertebrate Sampling

Substantial decreases in benthic macroinvertebrate index values were observed for all station groups (Table 3). The downstream station in Puckett's Creek had significantly ($p < 0.05$) lower taxon richness (12.5), EPT richness (5.5), and % Ephemeroptera abundance (4.3), compared to the upstream station (22.5, 15, and 44, respectively). For the medium sized stream (Straight Creek), the only significant difference between upstream and downstream index values was for % Ephemeroptera abundance, with an upstream value of 17.5% compared to the downstream value of 7%. For the largest stream, no significant differences between upstream and downstream values were observed, although taxon richness and EPT richness were nominally lower downstream.

In situ Toxicity Testing

In the smallest stream (Puckett's Creek), clam survival was significantly lower downstream of the AMD tributaries; in fact, all of the clams survived upstream while all died downstream. In the larger two streams, there was no statistical difference between upstream and downstream clam survival values. Conversely, in the streams where clams had substantial survival (Straight Creek and the North Fork), clam growth was significantly lower downstream of AMD inputs.

Table 2. Physical and chemical data for the three station groups.

Site	Relative Flow (ft/sec)	Habitat Score	pH	Conductivity (µmhos/cm)	Fe (mg/L)	Al (mg/L)
Puckett's Creek						
1) PC-8	0.01	72	7.75	200	0.026	0.14
2) LB-2	0.017	54	4.07	918	2.68	25.5
3) PC-4	0.103	76	6.11	564	0.172	3.98
Straight Creek						
4) SC-1	0.448	82	8.01	624	0.02	0.26
3) PC-4	0.103	76	6.11	564	0.172	3.98
5) SW-19	0.393	75	7.73	496	0.02	0.42
North Fork						
6) SW-20	0.835	92	7.52	266	0.24	0.09
7) SW-2	1.0	100	7.65	362	0.45	0.19

Table 3. Benthic macroinvertebrate community indices and *in situ* toxicity test data for the three station groups. Treatments in each section with different letters are significantly different from each other (α=0.05, Student's T-test). Comparison made only within sections.

Site	Taxon Richness	EPT Richness	% Eph. Abundance	Clam Survival (%)	Clam Growth (mm)
Puckett's Creek					
1) PC-8	22.5 a	15 a	44 a	100 a	0.50 a
2) LB-2	6.5 b	1.5 b	12 b	0 b	0 b
3) PC-4	12.5 b	5.5 b	4.3 b	0 b	0 b
Straight Creek					
4) SC-1	11 a	6.5 a	15.9 a	92 a	0.56 a
3) PC-4	12.5 a	5.5 a	4.3 b	0 b	0
5) SW-19	12.5 a	5.5 a	7 b	88 a	0.34 b
North Fork					
6) SW-20	24.0 a	15.5 a	39.2 a	100 a	0.56 a
7) SW-2	13.5 a	8.5 a	40.3 a	100 a	0.45 b

Table 4. Prediction equations for ecological and toxicological parameters from Multiple Linear Regression Analysis. Only models in which all variables were significant at the α=0.05 level were chosen (n=7).

Taxon Richness = 26.13 – 0.023(conductivity)	R^2 = 0.8197, p = 0.0050
EPT Richness = 17.83 – 0.019(conductivity)	R^2 = 0.8365, p = 0.0039
Clam Survival = 1.188 – 1.02 (Al in water)	R^2 = 0.8860, p = 0.0016
Clam Growth = -0.19 – 0.52 (Al) + 0.0008(cond) – 0.08 (flow) + 0.06(Fe)	R^2 = 0.9990, p = 0.0015

Multiple Linear Regression Analyses

Multiple linear regression analyses were conducted to determine which physical and/or chemical factors had the greatest influence on the ecological and toxicological parameters (Table 4). For three of the five dependent variables tested, regression equations included only one significantly correlated independent variable. None of the physical or chemical variables were significantly correlated with % Ephemeroptera abundance, and four different variables were correlated with clam growth.

For taxon richness and EPT richness, conductivity explained 81.9 and 83.6 percent of the variation from site to site (R^2 values of 0.819 and 0.836), respectively, while variations in clam survival were best explained by water column aluminum concentrations (R^2 values of 0.886). For clam growth, the four independent variables included Al, conductivity, iron and flow, combining for a total R^2 value of 0.999. Clam growth was the only parameter in which a physical parameter (flow) explained some of its variation from site to site.

Discussion

Benthic macroinvertebrate communities are useful as biological indicators of water quality because they are generally non-motile, have life cycles of one or more years, and are composed of species of varying levels of tolerance to different forms of pollution (Cairns and Dickson 1971). Responses of benthic macroinvertebrate communities to acid mine drainage inputs generally consist of reduction in taxon richness and species diversity, and often, complete elimination of the most sensitive insect species, especially mayflies (Roback and Richardson 1969; Armitage 1980). In the present study, the only station that had an acidic mean pH value (LB-2) supported about one third the number of different kinds of organisms that the upstream reference station (PC-8) supported. It had one-tenth the number of different kinds of the three most sensitive organisms, and had substantially fewer mayflies per total population sampled. These observations are in agreement with findings of other researchers.

In addition to the overt effects produced at the acidic site, the circum-neutral (pH = 6.11) site in Puckett's Creek below the acidic tributary had significantly reduced taxon richness, EPT richness, and % Ephemeroptera abundance compared to the upstream site in the same stream. Rutherford and Mellow (1994) have documented similar effects in borderline circumneutral waters below AMD inputs. They observed decreased taxon richness and total abundance at two stations (average pH values of 6.2 and 5.9) downstream of a pH 3.5 site. The present study further documents decreased percent abundance of mayflies upon addition of AMD inputs to a fourth order stream having an average pH of 7.73. In addition, nominal decreases in taxon richness and EPT richness were observed downstream of AMD inputs in the fifth order stream.

Asian clams and other bivalves have long been used for biomonitoring of anthropogenic inputs to stream ecosystems (Doherty and Cherry 1988). Asian clams are efficient bioaccumulators of metals (Cherry et al. 1980). In this study, clams were unable to survive in the third order stream below AMD inputs, and had significantly decreased growth below AMD inputs in both the fourth-order stream and the fifth-order stream. Metal concentrations were diluted in both of those streams, reducing concentrations to below WQC limits for protection of aquatic life, but as stated previously, Asian clams are efficient accumulators of metals. Increased tissue metal concentrations have been found by others to cause a variety of adverse effects in aquatic biota including sterility, decreased metabolic rates, and increased frequency of oral deformities (Lemly 1985; Rowe et al. 1998; Rowe et al. 1996). In this study, three AMD related water chemistry parameters explained more than 99% of the variation from station to station in clam growth. These data suggest that further research should be conducted to determine the relationship between AMD inputs at varying levels of dilution and clam growth.

While habitat assessment scores increased slightly in two of the streams below AMD-impacted tributaries, physical parameters were not correlated with community or organismal level impacts with the exception of clam growth, where it was the third variable to enter the regression model. Because clams are filter-feeders, flow volume should have an impact on their growth rates. However, chemistry rather than increased flow or poor habitat appears to be largely responsible for most of the impacts observed. It should be noted that other AMD inputs are present in the general vicinity of these streams. A small watershed (Ely Creek), which is adjacent to the Puckett's Creek watershed, also contributes AMD to the North Fork of the Powell River in this general area, and similar trends in community indices have been observed there (Cherry and Currie 1997). The present study does not imply that all of the impacts in the fifth order stream are due to input from Lick Branch (station LB-2). The data only suggest that mining-related inputs may affect aquatic biota at concentrations well below WQC limits for protection of aquatic life. In addition, the present study does not present direct evidence for a causal relationship between AMD inputs and reduced macroinvertebrate communities in the fifth order stream; however, the MLRA equations suggest that the variation from station to station in invertebrate community index values is related to conductivity, which has been shown in the past to be a good indicator of AMD and active mining impacts (Soucek et al. 2000 a,b; Latimer 1999). On the organismal level, clam survival and growth also were explained largely by chemical rather than physical data. Despite limits of this study, the data suggest that impacts on aquatic biota may be observed far beyond the zone of pH depression below AMD inputs, possibly due to long-term uptake of metals into biological tissues.

Conclusion/Future Research

This preliminary analysis of historical data documents community and organismal level impacts downstream of AMD inputs in third, fourth and fifth order streams, despite the fact that the largest stream had ten times the flow of the original AMD-receiving stream (Puckett's Creek). Correlation analysis indicated that these impacts were not related to changes in habitat or flow but were explained best by chemical data that have been shown in the past to be related to mining activities. However, to further solidify these trends, more rigorous research is warranted. All of the AMD inputs in the sub-watershed should be incorporated into the investigation and more replication of sampling will provide more robust statistics. In addition, laboratory or mesocosm studies should be conducted to determine if there is a causal relationship between AMD derived chemistry and community/organismal impacts at points far beyond where acute toxicity is expected. We can conclude, however, that AMD potentially has much more far reaching impacts than those observed within the zone of pH depression.

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