# BENTHIC MACROINVERTEBRATE ASSEMBLAGES AND SEDIMENT TOXICITY TESTING IN THE ELY CREEK WATERSHED RESTORATION PROJECT<sup>1</sup>

by

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<u>Abstract:</u> The Ely Creek watershed in Lee County, Virginia, contains an abundance of abandoned mined land (AML) seeps that contaminate the majority of the creek and its confluence into Big Stone Creek. Contaminated sediments had high concentrations of iron (~10,000 mg/kg), aluminum (~1,500 mg/kg), magnesium (~400 mg/kg) and manganese (~150 mg/kg). Copper and zinc generally ranged from 3 to 20 mg/kg. Benthic macroinvertebrates surveys at six of 20 sites sampled in the watershed yielded no macroinvertebrates, while eight others had total abundances of 1 to 9 organisms. Four reference sites contained  $\geq$  100 organisms and at least 14 different taxa. Laboratory, 10-day survival/impairment sediments tests with *Daphnia magna* did not support the field data. Mortality of 92 to 100% for *D. magna* occurred in samples collected from six sites. Daphnid reproduction was more sensitive than laboratory test organism survivorship; however, neither daphnid survivorship nor reproduction were good predictors of taxa richness. Laboratory test concerns included the use of a reference diluent water rather than site specific diluent water.

Additional Key Words: acid mine drainage, sediment bioassays, lab-to-field validation

#### Introduction

The United States Environmental Protection Agency (US EPA) has singled out acid mine drainage (AMD) from abandoned coal mines as being the number one water quality problem in Appalachia (Office of Surface Mining, 1995). Acid mine drainage water is produced when pyrite (iron-sulfide) rich coal and/or bedrock are exposed to oxygen and moisture by surface and underground mining operations. If produced in sufficient quantity, iron precipitates, heavy metals and sulfuric acid will contaminate surface and groundwater. With 557,650 abandoned mines throughout the US, the potential environmental impact is immense (US Water News, 1993).

Cherry et al. (1995) reconnaissanced 46 sampling in 13 streams of the Powell River watershed

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<sup>2</sup>David J. Soucek, Rebecca J. Currie and Henry A. Latimer, Research Assistants and Don Cherry, Professor, Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. G. Claire Trent, Virginia Department of Mined Land Reclamation, Big Stone Gap, VA 24219. in southwestern Virginia. The Ely Creek watershed was rated as the worst in the area and was thought to be the most impacted in the state. For example, pH measurements in areas of Ely Creek are consistently <3.0 throughout the year, and acidity continues through the confluence of the larger receiving Stone Creek. The preliminary survey in 1995 yielded zero benthic macroinvertebrates sampled at impacted Ely Creek stations. As a result, a bloassessment/ biomonitoring program designated as the Ely Creek Watershed Restoration Project was developed in conjunction with the Virginia Department of Mined Land Reclamation to provide background information identifying the current AMD environmental impact. Program objectives included water quality analysis, sediment metals analysis, habitat assessment, benthic macroinvertebrate sampling, in-situ toxicity testing with Asian clams (Corbicula fluminea), and laboratory sediment chronic toxicity testing with the water flea, Daphnia magna, and the midge, Chironomus tentans.

The usefulness of integrative environmental assessments, those which incorporate several measures of environmental quality, has been emphasized (Chapman et al., 1992). Measures that are commonly integrated include sediment toxicity tests, sediment chemistry, tissue chemistry, pathological studies, and endemic community structure studies. An assessment that includes sediment toxicity, sediment chemistry, and a measure of in-situ effects is said to involve the

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Sediment Quality Triad (SQT) (Chapman et al., 1992). The Ely Creek Watershed Restoration Project fulfills these criteria. The purpose of the present investigation is to compare community structure data collected in the field with laboratory sediment toxicity data and sediment chemistry data to determine the validity/realism of currently recommended sediment toxicity testing guidelines (ASTM, 1995; Nebeker et al., 1984), and to suggest possible biases in these testing methods.

### Methods

### Water Quality

Field measurements of selected water quality parameters were made on March 5, 1997. The pH was measured using a Markson Field, pH meter. A YSI 50B dissolved oxygen meter was used to measure DO. Conductivity measurements were made using a Hach portable conductivity meter.

### **Benthic Macroinvertebrates**

Benthic macroinvertebrate surveys were conducted at 20 in-stream stations in the Ely Creek watershed and surrounding streams (Table 1). The sampling was done according to the US Environmental Protection Agency (US EPA/444 (4-89-001) Rapid Bioassessment Protocols (RBP), which also are cited as Plafkin et al. (1989). Riffle, pool and shore-line rooted areas were thoroughly sampled by dip netting. The RBP tier III approach to genus level identification was undertaken.

### Habitat Assessment

A habitat assessment of each station included nine parameters. These were: 1) bottom substrate/available cover, 2) embeddedness, 3) velocity/depth, 4) channel alteration, 5) bottom scouring and deposition, 6) pool/riffle-run/bend ratio, 7) bank stability, 8) bank vegetative stability, and 9) streamside cover. A rating of 0-10 to 0-20 was developed for each parameter, and the higher the score, the more pristine the station. The habitat assessment approach was the same as that outlined in the US EPA Rapid Bioassessment Protocols (Plafkin et al. 1989). This assessment was important to determine if shoreline degradation from outside sources (i.e., erosion, misuse of farmland) is instrumental in impairing in-stream habitat or if water-column stress originating from AML input is the problem.

## Sediment Toxicity Testing and Metals Analysis

Sediment collected from the 20 sampling stations was returned to the laboratory for chronic impairment testing of the water flea, Daphnia magna. Samples were collected with a polyurethane dipper, placed in plastic freezer bags and stored on ice at 4°C. A separate set of samples was collected for analysis of heavy and soft metals at Spectrum Laboratories, Elements analyzed included Coeburn, Virginia. aluminum, cadmium, chromium, copper, iron, manganese, nickel, and zinc. Toxicity tests were performed according to guidelines as described by Nebeker et al. (1984) and the American Society for Testing and Materials (1995) with slight modifications. Test containers were one liter beakers with 200 g sediment and 800 ml reference diluent. The D. magna test, which extends for 10 days, evaluates organism survival and reproductive impairment.

## Statistical Analysis

Regression analysis was performed to determine the correlation between field collected benthic macroinvertebrate data (taxa richness), laboratory sediment toxicity data (*D. magna* survival and reproduction), pH and sediment concentrations of four metals (zinc, iron, copper and aluminum). Further, the correlation between taxa richness and habitat assessment score was determined. Significance of correlation was determined at the 95 percent confidence level using SAS (SAS Institute Inc., 1990). Differences in mortality and reproduction among treatments in sediment toxicity tests were analyzed at the 95 percent confidence level using Toxstat<sup>®</sup> 3.3 (Gulley, 1993).

## **Results**

### Water Quality

The best indicator of poor water quality was observed in acidic pH measurements of 3.06 at station SW-8 (Table 2). Other stations with low pH (e.g. 3.11-4.24) included SW-9 and SW-10. Reference stations SW-1, SW-2, SW-14 and SW-16 had neutral pH measurements of 6.39 to 6.45. Dissolved oxygen was above saturation at all stations. Conductivity followed a trend similar to pH; as pH became more acidic, conductivity increased. Station SW-8, which had the lowest pH, had the highest conductivity (1,600  $\mu$ mhos/cm). For most stations, conductivity ranged from 110 to 350  $\mu$ mhos/cm.

Table 1.	In-stream, surface water (	SW) monitoring locations.
Station	Station type	Description
SW-1	Reference	Stone Crk above Ely Crk
SW-2	Impact/Recovery	N. Fork Powell River @ Stone Face Rock
SW-3	Impact/Recovery	Ely Crk downstream of RT 712
SW-4	Impact/Recovery	Ely Crk, point nearest mouth of Ely Crk
SW-5	Impact/Recovery	un-named tributary to Ely Crk along RT 712
SW-6	Impact/Recovery	Bean Crk, above confluence with Ely Crk
SW-7	Impact/Recovery	Ely Crk, below confluence with Goose Crk
SW-8	Impact/Recovery	Goose Crk, discharge of acid bog
SW-9	Impact/Recovery	Bean Crk, head of impact area
SW-10	Reference	Bean Crk, upstream of impact area
SW-11	Impact/Recovery	Ely Crk, upstream of Goose Crk
SW-12	Impact/Recovery	Ely Crk, head of impact area
SW-13	Reference	un-named tributary to Ely Crk, upstream of impact
SW-14	Reference	un-named tributary to Goose Crk, upstream of impact
SW-15	Reference	Ely Crk, upstream of impact area
SW-16	Reference	Goose Crk, upstream of impact area
SW-17	Impact/Recovery	Stone Crk, below Ely Crk along RT 713
SW-18	Impact/Recovery	Stone Crk, upstream of Straight Crk confluence
SW-19	Impact/Recovery	Straight Crk, upstream of Stone Crk confluence
SW-20	Reference	N. Fork of Powell River, upstream of Straight Crk

Table 2. Water che	mistry data collected from	m the Elv Creek wa	tershed under h	uigh flow	
conditions,	March 5, 1997.	II the Lay wreak the	CISHOU MINUT I	теп пом	1
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Site	Temperature	Conductivity	ΒH	DO	1
	(°C)	(µmhos/cm)	F	(mg/L)	-
SW-1	16.1	50	6.39	10.8	1
SW-2	17.1	200	6.41	10.5	1
SW-3	15.7	270	5.84	10.4	ł
SW-4	17.8	280	7.13	10.5	!
SW-5	16.6	320	5.85	10.6	,
SW-6	18.2	260	6.06	10.2	,
SW-7	17.0	350	6.31	10.5	ļ
SW-8	16.1	1600	3.06	10.4	ļ
SW-9	18.0	450	4.24	10.6	,
SW-10	17.8	710	3.11	10.5	,
SW-11	17.0	350	6.82	10.6	ļ
SW-12	16.5	130	7.79	10.6	ļ
SW-13	16.9	110	7.74	10.5	ļ
SW-14	18.4	140	6,42	10.5	
SW-15	15.9	90	8.02	10.5	
SW-16	16.9	110	6.45	10.8	ł
SW-17	17.1	170	7.15	10.7	
SW-18	16. <b>9</b>	150	6.39	10.8	
SW-19	15.0	300	7.30	10.5	
SW-20	15.7	160	7.10	10.5	

# **Benthic Macroinvertebrates**

Of the 20 stations sampled, only six (SW-1, SW-2, SW-14, SW-15, SW-16, and SW-20) had more than 10 different invertebrate taxa (Table 3). Twelve stations had four or fewer taxa, and six stations (SW-4, SW-6, SW-7, SW-8, SW-12 and SW-19) had zero organisms. Hence, 60 percent of

the sampling stations were environmentally stressed. Of the six stations with zero organisms, all but one (SW-19) were associated with abandoned mined land seeps. Station SW-19 was stressed from an active mine coal slurry release. Overall, the benthic macroinvertebrate data were reliable indicators of good versus environmentally impacted sites.

Table 3. Benthic for the E	ble 3. Benthic macroinvertebrate taxa richness and habitat assessment scores (HAS) for the Ely Creek watershed sampling stations.					
Site	Richness	HAS	Site	Richness	HAS	
SW-1	14	114	SW-11	4	73	
SW-2	15	106	SW-12	0	72	
SW-3	3	89	SW-13	3	88	
SW-4	0	88	SW-14	16	110	
SW-5	3	82	SW-15	10	108	
SW-6	0	77	SW-16	13	110	
SW-7	0	69	SW-17	8	82	
SW-8	0	56	SW-18	7	82	
SW-9	1	77	SW-19	0	96	
SW-10	1	80	SW-20	18	112	

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Table 4. Analysis of e	eight elen	nents tak	en from	sediment s	amples	used in se	diment		······································
toxicity tests.	Concen	trations i	n mg/Kg	ŗ.	<u>-</u>				
Site	Mn	Cd	Cu	Fe	Ni	Al	Cr	Zn	
SW-1	106.4	0.592	1.529	5944.9	2.956	971.0	2.425	13.625	
SW-2	231.5	0.878	4.778	13132.7	6.640	1478.9	3.758	27.029	
SW-3	70.8	0.346	3.938	6310.6	3.819	1842.4	2.292	19.797	
SW-4	79.2	0,568	4.314	10858,8	4.357	1786.2	3.884	20,343	
SW-5	416.7	0.091	1.905	1124.3	6.496	1062.3	0.611	20.255	
SW-6	26.3 ·	0.333	1.856	3229.6	1.254	1167.1	1.844	9.807	
SW-7	41.6	0.225	1.864	3829.4	2:587	1543.2	1.299	11.528	
SW-8	8.1	1.268	-0.481	18392.3	0.792	928.9	1.143	3.308	
SW-9	37.0	0.263	2,000	5619.8	3.816	1993.6	5,031	10.138	
SW-10	55.9	0.459	2.622	6930.2	2.722	2069.9	2.776	9.564	
SW-11	32.5	0.188	2.286	5657.6	2.220	1799.8	1.582	10.862	
SW-12	32.2	0.479	1.410	6896,8	2.639	2000.9	3,183	10.081	
SW-13	37.7	0.196	1.758	3137.9	1.686	1048.0	1.333	7.563	
SW-14	83.7	0.182	2.738	2961.4	2.737	1887.3	2.115	9.240	
SW-15	69.1	0.150	2.001	2816.6	2.254	1441.2	1.594	15.557	
SW-16	168.6	0.308	5.884	5868.5	3.8 <b>95</b>	2192.6	3.097	18.042	
SW-17	71.4	0.270	3.035	4014.6	4.189	1563.3	4.423	14.600	
SW-18	359.7	0.987	2.966	14510.6	9.908	1985.8	7.223	29.710	
SW-19	291.2	0.488	4.475	8605.1	6.516	2350.2	3.389	21.909	
SW-20	195.8	0.456	3.448	6956.0	5.790	1540.2	2.849	20.427	

## Habitat Assessment

A habitat assessment of the stations was conducted to determine if surrounding habitat (i.e., bank contour) and in-stream habitat conditions were limiting the success of the benthic fauna (Table 3). The reference station (SW-1) had the highest score (114) followed by reference stations SW-14 and SW-16 (110). The lowest score was 56 (SW-8) followed by 69 (SW-7). Within the AML influenced stations, sediment embeddedness was evident. Most stations had a similar degree of flow/velocity. Station SW-8 was the only one classified as non-supporting due to the high degree of bank erosion and embeddedness in the channel. Ten of the 20 stations listed had only a partially supportive score ( $\leq 82$ ) or less relative to the reference site.

### Sediment Metals Analysis

At all 20 stations, 8 metals or elements were analyzed (Table 4). Sediment concentrations were highest for iron (1,124-14,510 mg/Kg), followed by aluminum (971-2,350 mg/Kg), magnesium (126-647 mg/Kg), and manganese (8-360 mg/Kg). Trace heavy metals were low, e.g. copper (1.4-5.9 mg/Kg), cadmium (0.09-1.3 mg/Kg), and zinc (3.3-29.7 mg/Kg). Iron was highest at station SW-8 (14,510 mg/Kg) but strangely, Cu, Al, Mg, Mn, Ni and Zn concentrations were lowest at this station. No one station stood out as being predominantly high for most elements.

# Sediment Toxicity

Daphnia magna was a sensitive test organism to toxic sediment conditions in the Ely Creek watershed (Table 5). At three sites (SW-8, SW-14 and SW-15), all daphnids died in the 10-day test, and at one site (SW-8) they died within one hour. Percent survival was significantly lower than the reference site at stations SW-4, SW-19, SW-7, SW-8, SW-10, SW-12, SW-13, SW-14, SW-15 and SW-16. Reproductive impairment also was significantly low at most of the stations where daphnid survival was affected. In general, most of the abandoned mined land influenced sites had toxic sediment.

### **Regression Analysis**

Taxa richness in the Ely Creek watershed was highly correlated (p=0.0001) with habitat assessment score; that is, as the HAS increased, taxa richness increased (Fig. 1). None of the other SQT measures, including sediment toxicity (survival and reproduction) and sediment metal concentrations, were correlated with taxa richness at the 95 percent confidence level (Figs. 1 and 2). Surprisingly, pH measurements were poorly correlated (p=0.2267) with taxa richness (Fig. 1). Zinc was the only metal that was significantly correlated with daphnid survival (p=0.0106) and neonate production (p=0.0060) in sediment toxicity tests (Figs. 3 and 4); however, correlations in both cases were positive.

Station	% Survival	Mean Neonate Production	Station	%Survival	Mean Neonate Production
SW-1	88	54.44	SW-11	24*	19.8*
SW-2	96	102.2	SW-12	8*	3.6*
SW-3	76	71.6	SW-13	1 <b>2*</b>	4.6*
SW-4	44*	57.6	SW-14	0*	0*
SW-5	88	115.4	SW-15	0*	0*
SW-6	68	56.0	SW-16	24*	9.0*
SW-7	44*	55.8*	SW-17	72	79.8
SW-8	0*	0*	SW-18	76	74.6
SW-9	80	61.0	SW-19	32*	11.4*
SW-10	32*	0*	SW-20	72	60.8

#### Discussion

Benthic macroinvertebrate surveys yielding zero organisms at six of 20 sampling stations and less than five taxa at more than half of the stations indicate that the environmental impact in the Ely Creek watershed is severe. Sediment toxicity data for the watershed support this assessment as samples from 11 stations were significantly toxic relative to reference sites. Acidic pH and elevated metal concentrations individually are known to have adverse effects on invertebrate communities (Bell and Nebeker, 1969; Clements et al., 1988; Clements et al., 1989; Haines, 1981; Katz, 1969; Lechtleitner et al., 1985; Winner et al., 1980), and several authors have observed decreased diversity in streams affected by acid mine drainage (Armitage, 1980; Herricks and Cairns, 1974; Smith and Frey, 1971). Based on these reports, it was hypothesized that sites with high metal concentrations and low pH levels also would have lower richness of benthic macroinvertebrate taxa and be more toxic in laboratory tests. The hypothesis was not supported by the data. Metal concentrations and pH were not correlated with taxa richness, and only zinc was significantly correlated with daphnid survival and reproduction, with the relationship being positive. Further, daphnid survival and reproductive impairment were very poor predictors of taxa richness. The only good predictor of taxa richness in the watershed was habitat assessment score, suggesting that habitat loss due to sedimentation is a more important factor limiting endemic invertebrate communities.

It has been suggested that field validation of laboratory toxicity tests should include a demonstration that indigenous communities suffer effects similar to those observed for laboratory test organisms (Cairns, 1986). Although data from this study show that the sediment is toxic, they do not fulfill this requirement. Sediment samples from stations that had the lowest taxa richness were not necessarily the most toxic. Chapman (1995) proposed the idea that validity of sediment bioassays is not dependent on correlation with in-situ ecological changes; however, with a watershed like Ely Creek in which the worst areas must be singled out for reclamation, choosing stations would be difficult based on the data available. If proactive or worst-case toxicity testing is to be employed, making validation impractical (Chapman, 1995), sediment bioassay methodology should be improved first. For example, the use of site specific diluent water could make a substantial difference in the outcome of chronic sediment toxicity tests with AMD contaminated sediments. It has been shown that pH levels of 4.6 and lower are lethal to the daphnid, *Ceriodaphnia dubia* (Belanger and Cherry, 1990), and at pH 5.5 and below, *Daphnia magna* does not reproduce (Parent and Cheetham, 1980). With several Ely Creek stations having pH values less than 5.0, making sediment bioassays more realistic by using site specific diluent water potentially could produce significantly different results from those observed using reference diluent water.

In conclusion, all three components of the Sediment Quality Triad suggest that the Ely Creek watershed is severely environmentally impacted. Despite this finding, environmental descision making may be difficult. The complexity of the AMD contaminated environment resulted in a lack of correlation between the three SQT components, and the need for improved sediment bioassay methodology is evident.

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