Introduction

An Ecotoxicological Evaluation of Active Coal Mining, Agricultural Sedimentation and Acid Mine Drainage in Three Tributaries of the Leading Creek Watershed, Meigs County, Ohio

On July 11, 1993 the Southern Ohio Coal Company's (SOCCO) Meigs mine #31 was flooded with approximately one billion gallons of acid mine drainage (AMD) from previously sealed and unused portions of the mine (Maloney, 1994; Consent Decree, 1995). These floodwaters were subsequently pumped from the mine and released into Parker Run, one of 61 tributaries which drain into Leading Creek. The entire Leading Creek watershed drains an area of 97,000 acres in Athens, Meigs and Gallia Counties, Ohio (Cherry et al, 1998).

The untreated and partially treated effluent was discharged at a rate of approximately 35,000 gallons per minute (gpm) for a period of 60 days. The dewatering process exceeded the limits set forth in SOCCO's National Pollutant Discharge Elimination System (NPDES) permit (Consent Decree, 1995). Birge et al (1995) reported that discharged waters were characterized by low pH (2.5 - 3.5), high conductivity (~4,000 – 8,000 μmhos/cm), and high concentrations of several metals including copper (Cu, ~25 – 75 μg/L), iron (Fe, ~50 – 70 mg/L), and zinc (Zn, ~110 – 140 μg/L). The dewatering of Meigs #31 mine decimated the aquatic communities in Parker Run as well as those in the lower half of the Leading Creek mainstem.

In an agreement with the United States Department of Justice (USDJ), United States Fish and Wildlife Service (USFWS), the United States Environmental Protection Agency (USEPA), and the Ohio Environmental Protection Agency (OEPA), the Leading Creek Improvement Plan (LCIP) was developed to assess the environmental impact to the watershed and to make recommendations for its recovery (Consent Decree, 1995).

As part of this improvement plan 27 sampling sites were established (17 tributary and 10 mainstem) to monitor the impacts in the watershed, evaluate the extent of ecological recovery and to establish a priority for selective tributary restoration. After extensive reconnaissance, three tributaries, Parker Run, Little Leading Creek and Thomas Fork, were determined to have a significant, negative environmental impact on the lower half of the Leading Creek mainstem. Together, these streams comprise most of the flow in the lower half of Leading Creek and greater than 95% during low flow conditions (Cherry et al, 1998).

Parker Run receives the majority of its flow directly from the Meigs #31 mine as active mine effluent, and is the greatest contributor in volume to Leading Creek. The mine effluent adds high concentrations of Na^+ (~900 mg/L), $SO_4^=$ (~2,000 mg/L), total dissolved solids (TDS, ~4,000 mg/L) and high conductivity (~6,000 μmhos/cm) to Parker Run. Little Leading Creek drains an area predominated by agriculture in its upper reaches, with limited abandoned mined land (AML) influence near the town of Rutland. These influences (agricultural and AML runoff) contribute substantial amounts of sediment to the stream and watershed. Thomas Fork, a large tributary in the lower watershed, is dominated by AMD. The stream typically has a low pH (~4.0 - 5.5) and high concentrations of metals (Al: ~10 mg/L and Fe: ~6 mg/L). These three uniquely different sources of environmental stress to aquatic communities (active coal mine effluent, agricultural and AML sedimentation, and AMD) form the basis for this research.

Following this introduction are three chapters comprising my thesis. Each of these chapters was written to stand alone and may be read independently of the other two. The first of these is a comparison of the ecotoxicological impacts of active coal mining, agricultural and AML sedimentation, and AMD on three tributaries of Leading Creek. The second chapter is a study of the recovery of the benthic macroinvertebrate communities in Parker Run from a catastrophic coal slurry spill that occurred in April 1997. The final chapter is a laboratory evaluation of the acutely toxic components of a synthetic coal mine effluent, similar in nature to that of the Meigs #31 mine effluent discharged into Parker Run.

Chapter 1.

An Ecotoxicological Evaluation of Active Coal Mine Effluent, Agricultural and Abandoned Mined Land Sedimentation, and Acid Mine Drainage in Three Tributaries of Leading Creek, Meigs County, Ohio.

Introduction

In the summer of 1993 the Meigs #31 deep coal mine in Meigs Co., Ohio was flooded with approximately one billion gallons of acid mine drainage (AMD) from previously sealed and unused portions of the mine. This partially treated effluent was subsequently pumped from the mine and released into Leading Creek, some of its tributaries and an adjacent watershed before entering the Ohio river (Cherry et al, 1998a). According to Birge et al (1995), the effluent was characterized by low pH (~2.5-3.5), high conductivity (~4,000 – 8,000 µmhos/cm) and high concentrations of several metals including copper (Cu, ~25 – 75 µg/L), iron (Fe, ~50 – 70 mg/L) and zinc (Zn, ~110 – 140 µg/L). As part of the Leading Creek Improvement Plan (LCIP) developed in conjunction with the United States Department of Justice, the United States Fish and Wildlife Service, The United States Environmental Protection Agency and The Ohio Environmental Protection Agency, an extensive watershed reconnaissance of the Leading Creek watershed was completed. Of 61 tributaries draining the 97,000-acre watershed, three were determined to have a disproportionately large, negative impact on the Leading Creek mainstem (Cherry, et al, 1998a). These streams, Parker Run, Little Leading Creek, and Thomas Fork, are impacted by the disparate environmental impacts of active coal mine effluent, agricultural and abandoned mined land (AML) sedimentation, and AMD, respectively.

Although the literature is replete with examples of the negative effects AMD, AML runoff, and associated mine spoils have on stream communities, little work has been done concerning the effects of active, treated coal mine effluent. Assessing the extent and mode of impact active mine effluent has on the receiving stream's benthic macroinvertebrate community was one of the primary goals of this investigation.

In 1990 the United States Environmental Protection Agency (USEPA) identified siltation as the most important source of river and stream pollution (USEPA, 1990). Indeed, the literature contains many examples of effects of sediment deposition on the benthic macroinvertebrate community (Bjornn, 1977; Dance and Hynes, 1980; McClelland and Brusven, 1980; Lemly, 1982; Whiting and Clifford, 1983; Lenat, 1984; Culp et al 1986; Graham, 1990). This study is unique however, in that it incorporates three different toxicity bioassays in isolating effects of sediment deposition from those of toxins often associated with both agricultural and AML sedimentation.

Herlihy, et al (1990), in their study of the extent of AMD, state that the water quality of approximately 10% of the streams in the Northern Appalachian region are negatively impacted by AMD. The authors further noted that AMD presents a serious problem to the water quality of streams in coal mining regions of the eastern United States.

It is important to note that active coal mining in the United States is regulated by the Surface Mining Control and Reclamation Act (SMCRA), which has greatly reduced the amount of AMD produced by active coal mining. Most of the current AMD impacts originate from waste piles and mines abandoned before the passing of SMCRA in 1977 (McElfish and Beier, 1990).

Pyrite (FeS_2) and other sulfide bearing ores commonly co-occur with coal and are exposed to oxidizing conditions during and after coal extraction. This oxidization follows the general formula (Stumm and Morgan, 1970):

$$4\ FeS_2 + 15\ O_2 + 14\ H_2O \rightarrow 4\ Fe(OH)_3 + 16\ H^+ + 8\ SO_4^=$$

AMD results when waters contaminated by products of this reaction reach the surface. Streams contaminated by AMD are generally characterized by low pH, high dissolved metal concentrations, and elevated conductivity (Mills, 1985).

The goal of this investigation was to use an integrative ecotoxicological evaluation to determine factors responsible for reduced benthic macroinvertebrate community abundance and/or richness values observed in three tributaries of Leading Creek, Southeastern Ohio. Analysis performed included water quality monitoring, quantification of sediment deposition, water column toxicity testing with *Ceriodaphnia dubia*, chronic sediment bioassays using *Daphnia magna* and *Chironomus tentans*, *in situ Corbicula fluminea* (Asian clam) toxicity testing, periphyton analysis on artificial substrates and quantitative benthic macroinvertebrate community sampling.

Methods

Study Sites

Three tributaries (Parker Run, Little Leading Creek, and Thomas Fork) were selected for study based on their significant, negative environmental impact on the lower half of the Leading Creek mainstem. Together, these streams contribute the majority of the flow into the lower half of Leading Creek, greater than 95% during low flow conditions (Cherry et. al, 1998a). As a compliment to these three streams two additional reference stations were established, Symmes Creek in Wayne National Forest and an upstream segment of Parker Run that had remained unaffected by the coal mining activities.

Parker Run, a third order stream, located at km 26.27, in the middle of the Leading Creek watershed, has a total length of 8.33 km and drains an area of 4,715 acres (Cherry et. al., 1998a). This tributary flows through the Meigs #31 coal mine complex and is subjected to runoff and other discharges from the mine. However, the main impact on the stream is the actual mine effluent, released directly from settling ponds into the stream. This active coalmine effluent dominates the flow of Parker Run, increasing it by at least a factor of 10, and under low flow conditions constitutes its entire flow. Sampling in Parker Run was conducted at the Parker Run Rd bridge crossing.

Little Leading Creek, a fourth order creek, joins Leading Creek at creek km 14.19, has a drainage basin of 16,372 acres and a total length of 20.1 km, (Cherry et. al., 1998a). It drains an area predominated by agriculture in its upper reaches, with some AMD influence near the town of Rutland. This stream is heavily influenced by sedimentation from two different sources: agriculture and abandoned mined land runoff. All sampling in Little Leading Creek was conducted at the T-176 bridge crossing.

Thomas Fork a fourth order stream, which joins Leading Creek at creek km 2.61, has a total length of 11.6 km draining an area of 21,736 acres, (Cherry et. al, 1998a). The Thomas Fork watershed has seen extensive coalmining activity, resulting in numerous AMD inputs. These inputs negatively affect the water quality of Thomas Fork as well as contribute significant sedimentation to the stream. All sampling in Thomas Fork was conducted at the Rt. 124 bridge crossing.

Symmes Creek, located in Wayne National Forest, has a total length of 112.65 km and drains an area of 227,648 acres. This stream is noted as supporting an exceptional macroinvertebrate community and does not have any substantially diminished water quality or habitat (OEPA, 1991). This site was sampled as it passed through the Forest adjacent to Symmes Creek Road. The Parker Run reference station was sampled above the mine complex, adjacent to the mine parking lot. The stream at this point was considerably smaller than the lower Parker Run area which had the added mine effluent that made significant increases in flow.

Benthic Macroinvertebrate Sampling

Two replicate benthic macroinvertebrate community samples were taken during two sampling events at the Parker Run reference site, Parker Run, Little Leading Creek and Thomas Fork using a D-frame dip net with 800µm mesh. Wherever the habitat allowed, four different representative areas of the stream were sampled: riffle, pool, margin and run as per OEPA protocol for rapid bioassessment of benthic macroinvertebrates (Ohio EPA, 1989). Insects were identified according to the keys in Merritt and Cummins (1996), to genus level where possible. Other macroinvertebrates were identified according to the keys in Pennak (1989).

Periphyton

Measures of chlorophyll *a* were taken from periphyton allowed to colonize ceramic tiles, (4 replicate tiles with an area of 0.00235 m^2 each) for 10 days in each stream. The pigments were then extracted from the periphyton and analyzed spectrophotometrically using a Spectronic model 21D spectrometer (Milton Roy Co.) according to standard methods (APHA, et al, 1992).

Water Quality

The water quality at each site was monitored using the following parameters: pH, conductivity (µmhos/cm), dissolved oxygen (D.O., mg/l), total dissolved solids (TDS, mg/L, monitored only at Parker Run and the Parker Run reference), water-column metals (Na, Zn, K, Mg, Cu, Al, Mn, and Fe), total phosphorous (P) and other dissolved ions (Cl^-, $SO_4^=$, NO_2^- and NO_3^-).

Sediment Depth

Sediment depth in the stream channel was measured on two occasions. The first measure, in 1997, was a quantitative measurement using a 0.5-in. diameter steel rod forced into the sediment with three successive one-ft drops of a 5-lb weight. The mean length of rod pushed into the sediment at five sites on a cross section across each stream was recorded as the sediment depth. The second measurement of sediment depth was an absolute measure taken in 1998 with a 2” diameter wooden pole. The pole was forced through the sediment until gravel or clay streambed was reached. This measure was taken at three sites on a cross section of each stream and the mean depth was reported. The quantitative measure taken in 1997 used Symmes Creek as the reference and the absolute measure in 1998 used the Parker Run reference area.

Toxicity Testing

Forty-eight hr, static acute, water-column toxicity tests using <24 hr *Ceriodaphnia dubia* were performed, testing followed United States Environmental Protection Agency protocol (Weber, 1993). Water that was toxic at 100% concentration was diluted using a 0.5 dilution series (100, 50, 25, 12.5, 6.25% and control) with pristine local stream water as the diluent and control (Sinking Creek, Giles Co., VA).

Ten-day chronic impairment sediment toxicity tests using 5-day old *Daphnia magna* and 10-day old *Chironomus tentans* as the test organisms were performed 3 times (7/97,1/98, and 6/98). Symmes Creek was used as the reference site in the 7/97 test and the Parker Run reference was used in the second two tests. Five replicate 1-liter beakers per site were filled with 200 g unsieved site sediment and overlain with 800 ml of reference water (Sinking Creek, Giles Co., VA). Both test organisms, 5 *D. magna* and 10 *C. tentans*, were introduced into the same test beaker. These procedures were modifications of Nebeker et al (1984) and USEPA (1994).

Four *in situ* toxicity tests were conducted (6/97, 8/97, 6/98, and 8/98) using *Corbicula fluminea*, the Asian clam. Tests conducted in 1997 used Symmes Creek as a reference site, while those conducted in 1998 used the Parker Run reference site. Clams were collected using clam rakes from the New River near Ripplemead, VA. Clams used in testing had shell lengths between 9.0 – 12.0mm, measured with a Fowler & NSK Max-cal digital micrometer (0.0005”). After measurement, the clams were marked with a file and placed in plastic mesh bags, 5 clams per bag. Five bags were staked in the stream channel at each site, making a total of 25 clams per site. After 35 days $\pm$ one day, the clams were collected, re-measured and checked for mortality. These procedures were modifications of Cherry and Dobbs (1993).

Statistical Analysis

Statistical analysis, calculation of means and standard deviations was accomplished using version 3.2.1 of JMP IN© statistical software (Sall and Lehman, 1996). An alpha (α) level of 0.05 was used in all analysis.

Benthic Macroinvertebrates

Each sample taken (n = 4) was considered a replicate. One-way analysis of variance (ANOVA) and Tukey’s test were employed in determination of significant differences between sites. Kruskal-Wallis rank sum non-parametric analysis was employed in determining significant differences between sites with non-normally distributed data.

Acute Water-Column Toxicity Testing

Percentage survival of test organisms in the 100% sample concentration (4 replicates, 5 organisms per replicate) and 48-hr LC_{50} values were determined for each 48-hr acute water column test performed. LC_{50} values were calculated using the Toxstat® statistical package (Gulley 1993). In calculating significant differences among sites for mean survival in 100% concentrations, each test was considered a replicate. For determining differences among sites for 48-hr LC_{50} data, only those tests generating an LC_{50} were used. One-way ANOVA, with Kruskal-Wallis rank sum tests used for non-parametric data, and Tukey’s test were used to determine significant differences among sites.

Sediment and in situ Toxicity Testing

Each beaker of a sediment test was used as a replicate (5 beakers per test), means were calculated using all replicates (15) from all tests (3). Each bag of five clams was considered a replicate (5 bags per site) and means were calculated using all replicates (20) from all In Situ toxicity tests (4). Significant differences in percentage survival of test organisms, *D. magna*, *C. tentans*, and *C. fluminea*, were determined using one-way ANOVA, with Kruskal-Wallis rank sum analysis used for non-parametric data, and Tukey's test. Differences in *D. magna* reproduction, *C. tentans* and *C. fluminea* growth were determined in the same manner, except that replicates with 100% mortality of the test organism in question were excluded from analysis.

<u>Results</u>

<u>Benthic Macroinvertebrates</u>

The mean total abundance of benthic macroinvertebrates sampled in Parker Run, Little Leading Creek and Thomas Fork was shown to be significantly ($\alpha = 0.05$) lower than that measured at the reference station (Table 1). Significant differences also were seen for mean taxon richness values between communities in Little Leading Creek and Thomas Fork and those of Parker Run and the reference station. Although there was a lower mean taxon richness value at Parker Run (18.25), it was not significantly lower than that observed at the reference (24.3).

A similar trend was demonstrated for mean abundance of Ephemeroptera, Trichoptera and Plecoptera (EPT), the three most sensitive orders of aquatic insects (Table 2). Again, each of the three streams had significantly lower mean EPT abundance values than the reference. The percentage of the total abundance comprised of EPT organisms was significantly higher at the reference (60.0%) than both Little Leading Creek (8.1%) and Thomas Fork (4.6), values at Parker Run (26.9) were not significantly different than any other site. Mean EPT richness values were not significantly different between any of the three streams, nor was there a significant difference between Parker Run (5.5) and the reference site (9.0). The reference site did, however, have a significantly greater mean EPT richness value than that observed in Little Leading Creek (2.8) or Thomas Fork (0.3).

Table 1. Measures of mean total abundance and mean taxon richness from benthic macroinvertebrate sampling events in 1998. Values followed by the same designation are not significantly different ($\alpha = 0.05$, n = 4).

	Total Abundance			Taxa Richness		
Site	Mean	SD	Designation	Mean	SD	Designation
Parker Run Ref.	431.30	90.40	A	24.30	4.03	A
Parker Run	104.50	47.59	B	18.25	2.99	A
Little Leading Creek	74.30	64.64	B	9.50	3.87	B
Thomas Fork	8.00	3.46	B	3.50	1.00	B

Table 2. Measures of mean EPT abundance, mean EPT abundance as % of total abundance and mean EPT richness from benthic macroinvertebrate sampling events in 1998. Values followed by the same designation are not significantly different ($\alpha = 0.05$, n = 4).

	EPT Abundance			EPT as % of Total Abundance			EPT Richness		
Site	Mean	SD	Designation	Mean	SD	Designation	Mean	SD	Designation
Parker Run Ref.	272.00	166.23	A	60.0%	25.70%	A	9.00	2.94	A
Parker Run	26.25	20.21	B	26.9%	20.45%	A,B	5.50	3.79	A,B
Little Leading Creek	3.80	1.89	B	8.1%	5.24%	B	2.80	1.71	B
Thomas Fork	0.50	1.00	B	4.6%	9.10%	B	0.30	0.50	B

Mean abundance of mayflies (order Ephemeroptera) were significantly depressed in all three streams (0.5 – 3.3) relative to the mean value in the Parker Run reference site (59.0). The same trend was observed for the percentage of the total community composed of mayflies. Although the differences were not significant, percent mayfly abundance at the Parker Run reference site (15.0%) was substantially higher than in any of the three streams (2.3% - 4.5%) (Table 3).

Table 3. Mean mayfly (Ephemeroptera) abundance and mean mayfly abundance as % of total abundance from benthic macroinvertebrate sampling events in 1998. Values followed by the same designation are not significantly different ($\alpha = 0.05$, n = 4).

Site	Mean mayfly Abundance	SD	Designation	% mayfly Abundance	SD	Designation
Parker Run Ref.	59.0	43.95	A	15.0%	12.94%	A
Parker Run	3.3	0.96	B	3.8%	2.50%	A
Little Leading Creek	1.3	0.96	B	2.3%	2.63%	A
Thomas Fork	0.5	1.00	B	4.5%	9.00%	A

Mean chironomid abundance at Parker Run reference (56.3), Parker Run (39.5) and Little Leading Creek (57.8) were all similar to one another. Although mean abundance at Thomas Fork was substantially lower (4.5) than any other site, the differences among the sites were insignificant. Mean percentage of the total benthic macroinvertebrate community made up of chironomids was substantially higher than the reference site (14.2%) at both Little Leading Creek (56.6%) and Thomas Fork (51.8%), although no significant differences were observed (Table 4).

Table 4. Mean abundance and percentage of the total abundance of benthic macroinvertebrates consisting of insects in the order Chironomidae. Values followed by the same designation are not significantly different ($\alpha = 0.05$, n = 4).

Site	Mean Chironomid Abundance	SD	Designation	Mean % Chironomidae	SD	Designation
Parker Run Ref.	56.3	23.54	A	14.2%	8.10%	A
Parker Run	39.5	35.63	A	33.6%	17.80%	A
Little Leading Creek	57.6	64.30	A	56.6%	37.10%	A
Thomas Fork	4.5	3.00	A	51.8%	15.50%	A

Periphyton

Periphyton data, reported as chlorophyll *a* (mg/m^2) sampled from several sites in the Leading Creek watershed and one site in Federal Creek, a nearby reference stream, demonstrated relatively oligotrophic conditions in the three study streams (Table 5). A headwater stream with observed high degrees of algal growth had 1.345 mg/m^2 chlorophyll *a*, which contributed substantially to heavy growth observed in the first Leading Creek Station (0.757 mg/m^2). This is in sharp contrast to the low levels of chlorophyll *a* observed in each of the three study streams (0.034 – 0.051 mg/m^2), associated Leading Creek stations receiving waters from these streams (0.013 – 0.132 mg/m^2), and the Federal Creek reference (0.166 mg/m^2).

Table 5. Mean (4 replicates per site) measures of periphyton as chlorophyll *a* (mg/m^2) sampled from several sites in the Leading Creek watershed and Federal Creek, a reference stream in the ecoregion, 1996.

Site	Chlorophyll *a* (mg/m^2)	SD
Federal Creek - Ref. Stream	0.166	0.064
Head-water Tributary #1	1.345	1.277
First L.C. Station #1	0.757	0.949
Parker Run	0.034	0.426
L.C. Below Parker Run	0.060	0.068
Little Leading Creek	0.051	0.094
L.C. Below Little Leading C.	0.013	0.021
Thomas Fork	0.051	0.102
L.C. Below Thomas Fork	0.132	0.106

Water Quality

Fifteen parameters were measured (Tables 6 and 7), and the four most affected by AMD were pH, manganese, aluminum and iron concentration (Figure 1). Mean pH in Thomas Fork was 5.42 and measurements as low as 4.09 were recorded during the low dilution summer months (Table 6). Concentrations of the metals manganese (Mn, 2.43 mg/L) and iron (Fe, 5.74 mg/L) were substantially higher than concentrations measured at either reference station (Mn = 0.37 mg/L, Fe = 0.83 mg/L at Symmes Ck. Ref. while Fe = 0.04 mg/L at Parker Run Ref.). Aluminum levels measured in Thomas Fork were an order of magnitude higher than concentrations observed in the Parker Run Reference area (9.785 mg/L vs. 0.024 mg/L).

Active coal mine effluent had a dramatic influence on mean levels of four parameters measured in Parker Run, including conductivity (5,009 µmhos/cm), sodium (Na^+, 910.53 mg/L), sulfate ($SO_4^{=}$, 1,945.9 mg/L) and chloride (Cl^-, 348.7 mg/L). They were a full order of magnitude higher than those observed in any other stream site (Figure 2). Additionally, total dissolved solids (TDS) was much higher in Parker Run than in the Parker Run reference area (3,470.23 vs. 355.50 mg/L).

All water quality parameters, except for Fe (3.17 mg/L vs. 0.83 mg/L at Symmes Ck. Ref. and 0.04 mg/L at Parker Run Ref.), monitored at Little Leading Creek were similar to levels observed in the reference streams. Little Leading Creek did not appear to have any substantial water quality problems. Dissolved oxygen was at or near saturation at each sampling event and was not reported.

Sediment Deposition

Extensive sediment deposition was apparent in both Little Leading Creek and Thomas Fork (Figure 3). Mean sediment depths in Little Leading Creek varied from a quantitatively measured 21.5-in. to an absolute depth of 32.7-in, while those in Thomas Fork varied from 35.3-in. to 60.0-in., respectively. These massive amounts of sediment in the stream channel resulted in 100% embeddedness of cobble substrates and a homogeneous streambed.

Table 6. Summary of non-metal water chemistry data collected for each stream.

Site	Parameter	Mean Value	SD	Range
Symmes Creek Ref.	pH	7.07	0.350	6.49 - 7.42
Parker Run Ref.		7.23	0.510	6.70 - 7.71
Parker Run		8.05	0.260	7.52 - 8.38
Little Leading Creek		7.35	0.240	6.76 - 7.71
Thomas Fork		5.42	1.070	4.09 - 7.34
Symmes Creek Ref.	TDS (mg/L)	NA	NA	NA
Parker Run Ref.		355.50	120.3	244.4 – 483.3
Parker Run		3470.23	1926.8	1245.5 – 4602.2
Little Leading Creek		NA	NA	NA
Thomas Fork		NA	NA	NA
Symmes Creek Ref.	Conductivity (µmhos/cm)	247	29.6	214 - 295
Parker Run Ref.		457	194.0	330 - 680
Parker Run		5009	1374.0	870 - 7000
Little Leading Creek		656	1037.0	208 - 4395
Thomas Fork		833	891.4	210 - 4000
Symmes Creek Ref.	NO_3^{-}-$NO_2^{=}$ (mg/L)	0.14	0.114	0.05 - 0.30
Parker Run Ref.		NA	NA	NA
Parker Run		0.34	0.084	0.05 - 0.30
Little Leading Creek		0.34	0.172	0.17 - 0.83
Thomas Fork		0.24	0.104	0.09 - 0.41
Symmes Creek Ref.	P (mg/L)	0.02	0.010	0.01 - 0.05
Parker Run Ref.		NA	NA	NA
Parker Run		0.01	0.000	0.01 - 0.01
Little Leading Creek		0.10	0.260	0.01 - 1.05
Thomas Fork		0.03	0.030	0.01 - 0.08
Symmes Creek Ref.	$SO_4^{=}$ (mg/L)	65.4	5.03	59.0 - 70.0
Parker Run Ref.		112.0	90.63	44.6 - 215.0
Parker Run		1945.9	473.03	478.0 - 2470.0
Little Leading Creek		121.1	27.00	53.0 - 157.0
Thomas Fork		320.5	112.20	157.0 - 582.0
Symmes Creek Ref.	Cl^{-} (mg/L)	6.2	1.92	4.0 - 9.0
Parker Run Ref.		17.0	12.17	8.7 - 31.0
Parker Run		348.7	92.74	74.0 - 456.0
Little Leading Creek		9.2	4.00	4.0 - 16.0
Thomas Fork		24.5	9.08	8.0 - 42.0

Table 7. Summary of water column metals data collected for each stream.

Site	Parameter	Mean Value	SD	Range
Symmes Creek Ref.	Na (mg/L)	5.83	0.830	5.16 - 7.00
Parker Run Ref.		32.93	27.850	16.83 - 65.09
Parker Run		910.53	287.290	225.00 - 1360.00
Little Leading Creek		12.51	3.820	6.95 - 21.10
Thomas Fork		25.51	8.200	12.30 - 44.20
Symmes Creek Ref.	Mg (mg/L)	8.8	1.16	7.3 – 10.3
Parker Run Ref.		NA	NA	NA
Parker Run		38.0	7.22	14.2 - 44.4
Little Leading Creek		10.7	1.32	8.2 - 12.3
Thomas Fork		22.5	7.20	12.0 - 39.6
Symmes Creek Ref.	K (mg/L)	2.78	1.26	2.0 - 5.0
Parker Run Ref.		NA	NA	NA
Parker Run		12.58	2.72	4.4 - 15.5
Little Leading Creek		2.43	1.05	1.5 - 5.2
Thomas Fork		2.58	0.85	1.5 - 4.6
Symmes Creek Ref.	Al (mg/L)	NA	NA	NA
Parker Run Ref.		0.024	NA	BDL - 0.024
Parker Run		0.610	0.760	BDL - 1.154
Little Leading Creek		0.024	0.024	BDL - 0.024
Thomas Fork		9.785	6.211	3.085 - 15.350
Symmes Creek Ref.	Mn (mg/L)	0.37	0.099	0.22 - 0.47
Parker Run Ref.		0.05	NA	0.05
Parker Run		0.47	0.222	0.1 - 0.78
Little Leading Creek		0.37	0.179	0.2 - 0.97
Thomas Fork		2.43	0.917	1.05 - 4.52
Symmes Creek Ref.	Fe (mg/L)	0.83	0.230	0.55 - 1.16
Parker Run Ref.		0.04	NA	0.04
Parker Run		0.43	0.370	0.10 - 1.54
Little Leading Creek		3.17	9.710	0.12 - 38.2
Thomas Fork		5.74	5.196	0.59 - 18.00
Symmes Creek Ref.	Cu (mg/L)	0.002	0.001	0.001 - 0.004
Parker Run Ref.		0.010	0.010	BDL - 0.014
Parker Run		0.002	0.002	0.001 - 0.010
Little Leading Creek		0.003	0.007	0.001 - 0.030
Thomas Fork		0.009	0.008	0.002 - 0.030
Symmes Creek Ref.	Zn (mg/L)	0.007	0.002	0.005 - 0.012
Parker Run Ref.		NA	NA	BDL
Parker Run		0.008	0.009	0.004 - 0.040
Little Leading Creek		0.016	0.023	0.004 - 0.093
Thomas Fork		0.160	0.099	0.041 - 0.383

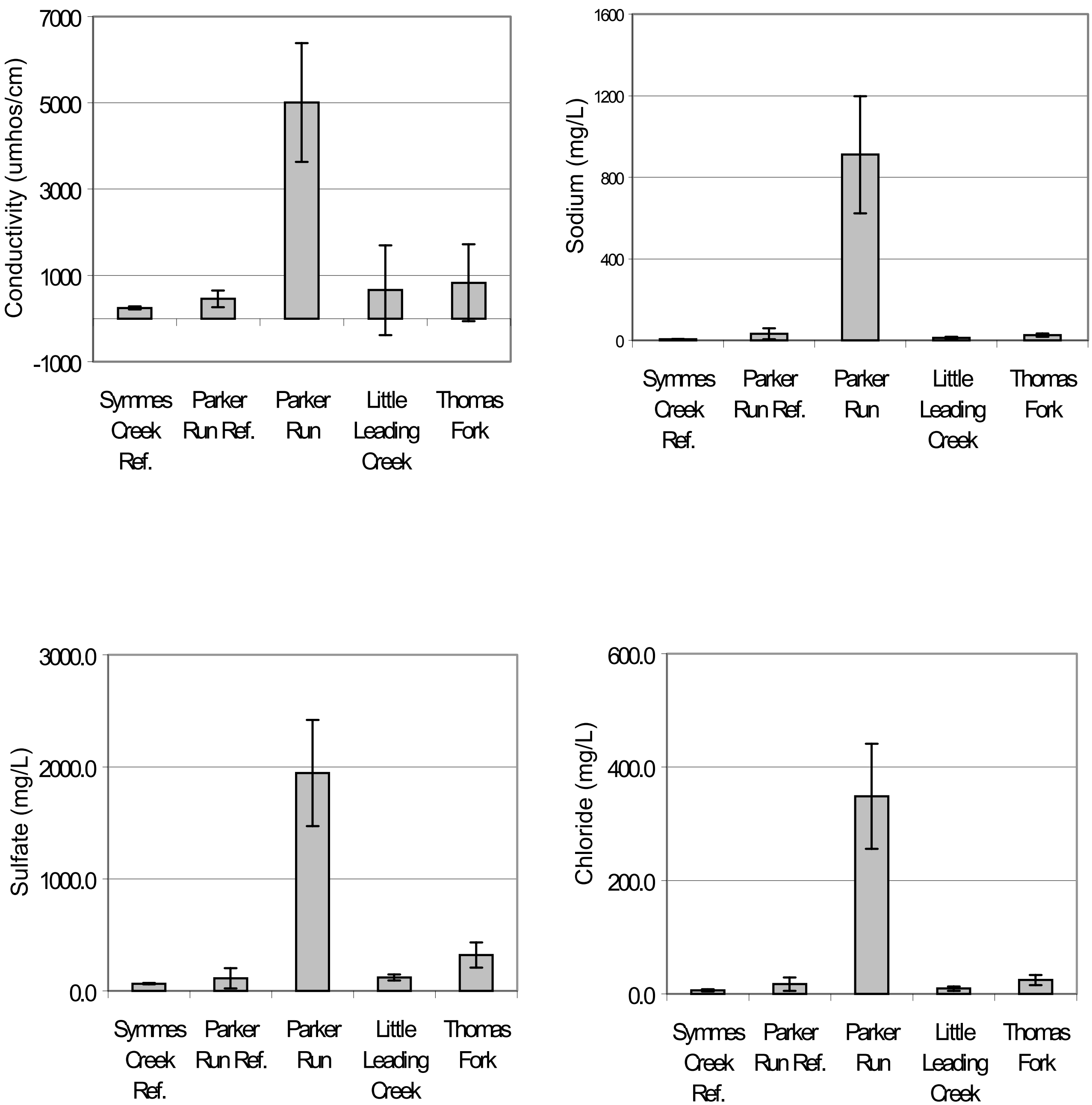
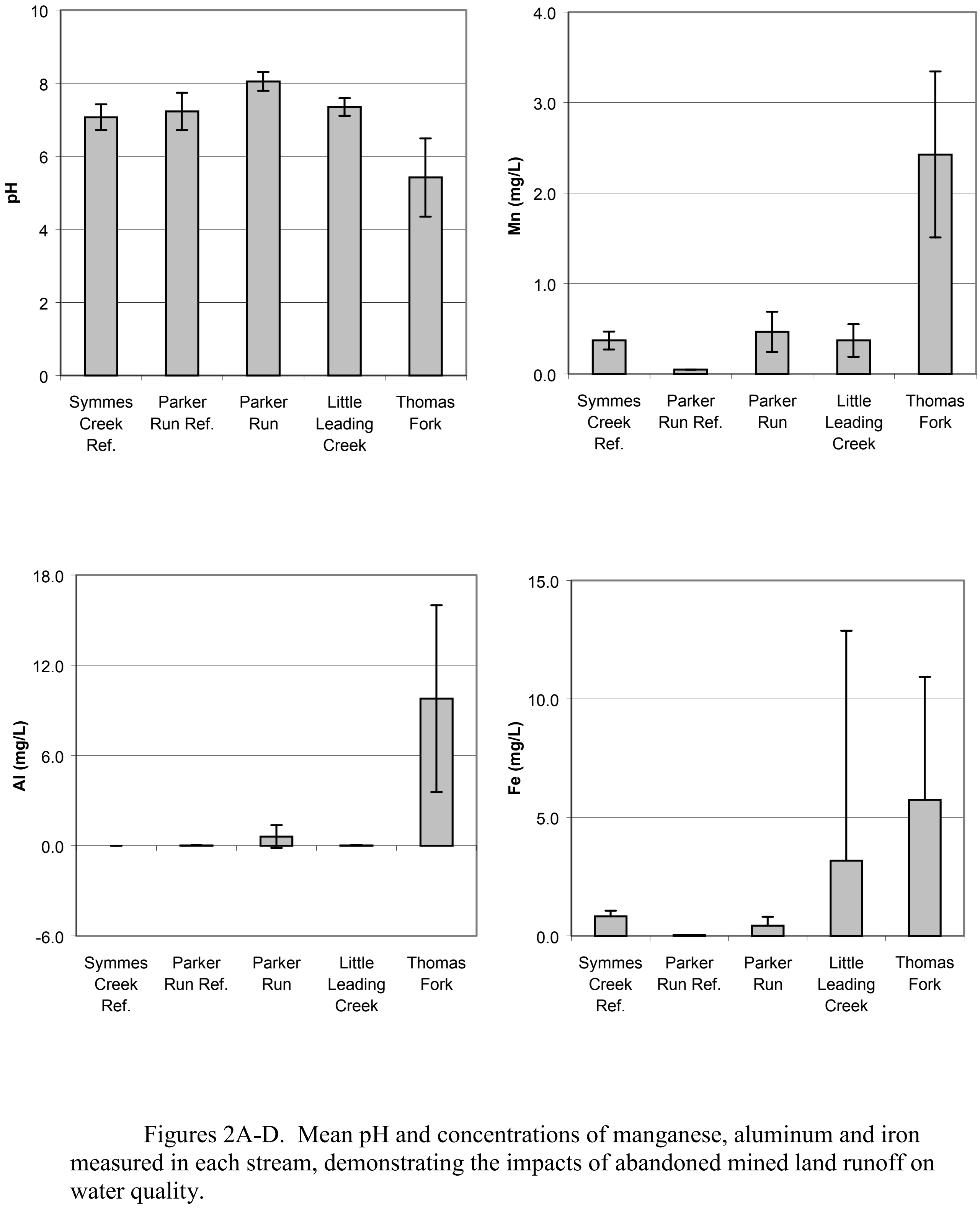


Figure 1A-D. Mean levels of conductivity, and concentrations of sodium, sulfate and chloride, demonstrating the impact of active coal mine effluent on water quality.



Figures 2A-D. Mean pH and concentrations of manganese, aluminum and iron measured in each stream, demonstrating the impacts of abandoned mined land runoff on water quality.

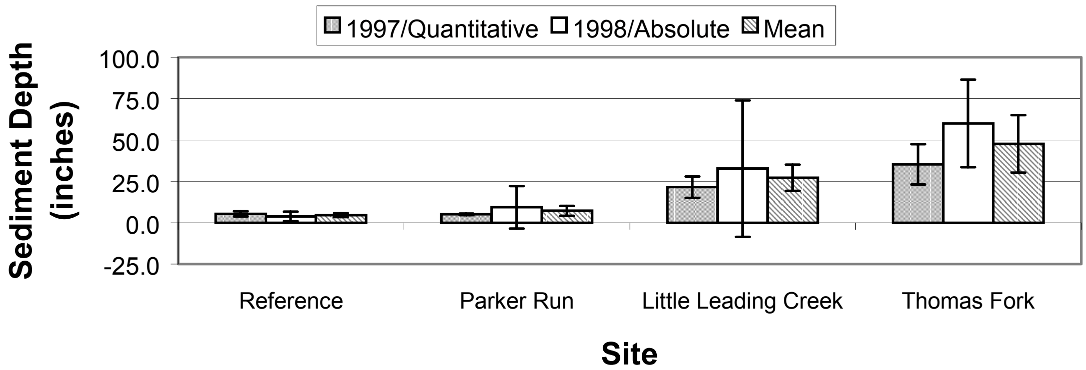


Figure 3. Sediment depths (inches) in each stream and a reference measured quantitatively in 1997 and as an absolute measurement in 1998.

Toxicity Testing

Water column acute toxicity was observed in both Parker Run and Thomas Fork (Table 8). In all six 48-hr acute toxicity tests conducted with *C. dubia*, samples of Thomas Fork water resulted in 100% mortality of test organisms in the 100% sample concentration and a mean LC_{50} of 30.33%. Parker Run had a more periodic acute toxicity with a mean of 61.5% survival in the 100% sample concentration and an LC_{50} range of 66.1 - >100%. No acute water column toxicity was demonstrated in Little Leading Creek.

Table 8. Summary of acute *Ceriodaphnia dubia* 48-hr water-column toxicity data. Mean percent mortality values followed by the same designation are not significantly different ($\alpha = 0.05$).

Site	n	Mean Survival in 100% Conc.	SD	Designation	Mean 48-hr LC_{50} (when generated)	SD	48-hr LC_{50} Range
Parker Run	10	61.5%	31.2%	A	81%	13.9%	66.1 - >100%
Little Leading Creek	4	97.5%	5.0%	B	>100%	0.0%	>100%
Thomas Fork	6	0.0%	0.0%	C	30.33%	23.2%	8.84 - 65.97%

No significant differences were detected between any of the sites for mean *D. magna* survival and reproduction or *C. tentans* survival and growth in the 10-day chronic sediment bioassays (Tables 9 and 10). *D. magna* survival varied from a high of 88% at the reference site to a low of 60% at Parker Run, while mean reproduction was highest at Thomas Fork (74.08) and lowest at Little Leading Creek (52.79). Mean survival of *C. tentans* followed the same trend observed with *D. magna*, as the reference had the highest mean survival (73%) and Parker Run the lowest (57%). Similar results were seen for C. tentans growth, organisms placed in the reference sediment had a mean growth of 3.92 mg while those placed in Parker Run sediment exhibited the lowest (3.36 mg) growth.

Table 9. Summary of laboratory 10-day chronic sediment toxicity tests using *Daphnia magna.* Values followed by the same designation are not significantly different ($\alpha = 0.05$, n = 3).

Site	% Survival	SD	Designation	Mean Neonate Production	SD	Designation
Reference	88%	19.7%	A	66.53	31.94	A
Parker Run	60%	44.7%	A	67.00	45.51	A
Little Leading Creek	64%	32.2%	A	52.79	47.49	A
Thomas Fork	75%	37.4%	A	74.08	42.10	A

Table 10. Summary of laboratory 10-day chronic sediment toxicity tests using *Chironomus tentans.* Values followed by the same designation are not significantly different ($\alpha = 0.05$, n = 3).

Site	% Survival	SD	Designation	Mean Growth (mg)	SD	Designation
Reference	73%	17.9%	A	3.92	0.72	A
Parker Run	57%	31.8%	A	3.36	0.72	A
Little Leading Creek	71%	16.4%	A	3.84	1.23	A
Thomas Fork	61%	20.7%	A	3.77	1.38	A

No significant differences in *C. fluminea* survival or growth were observed between Little Leading Creek and the reference stations (Table 11). Mean survival for clams placed in Parker Run was significantly lower than those at the reference stations; however, surviving clams did not grow significantly less than those at the reference stations. All clams placed in Thomas Fork died during each test period.

Table 11. Mean survival and growth (mm) data from 35-day *Corbicula fluminea in situ* toxicity tests. Values followed by the same designation are not significantly different ($\alpha = 0.05$, n = 4).

Site	% Survival	SD	Designation	Mean Growth (mm)	SD	Designation
Reference	93%	11.7%	A	0.81	0.60	A
Parker Run	47%	40.1%	B	0.74	0.61	A
Little Leading Creek	98%	6.2%	A	0.61	0.58	A
Thomas Fork	0%	0.0%	C	0.0	0.00	B

Discussion

Benthic Macroinvertebrates

Each of the three streams in this study had significantly impaired benthic macroinvertebrate communities, relative to total abundance and EPT abundance levels observed in the Parker Run reference area. Additionally, Little Leading Creek and Thomas Fork each had significant reductions in community richness values, EPT abundance as percent of total abundance, and substantial increases in percent chironomid abundance. All three streams had significantly lower mean mayfly abundance relative to the reference, although mayfly abundance as a percent of total abundance was not significantly different among the sites. The impaired benthic macroinvertebrate communities found in each of these three streams is evidence of the negative impacts active coal mine effluent, agricultural and AML sedimentation, and AMD have on stream ecosystems.

Numerous studies have presented similar evidence of the destructive effects these and similar sources have on stream benthic macroinvertebrate communities. Scullion and Edwards (1980) observed 80-90% reductions in benthic macroinvertebrate abundance in a Welsh river below an area of combined AMD and active coal mining relative to a reference area. The literature concerning effects of sedimentation was thoroughly reviewed by Waters (1995) and will be discussed in greater detail below. As reviewed by Kelly (1988), the AMD literature contains many examples of AMD influenced streams with high acidity having decreased benthic macroinvertebrate species richness values, relative to uninfluenced reaches. These are similar to results seen in several AMD influenced watersheds in Southwestern Virginia, USA. Streams in the Puckett's Creek and Ely Creek watersheds, Powell River drainage, were observed to have significantly lower benthic macroinvertebrate abundance and richness values in AMD influenced reaches relative to upstream reference areas (Cherry et al 1998b; Cherry and Currie, 1997).

Periphyton

Measures of periphyton as chlorophyll *a* (mg/m^2) were used to compare periphyton levels seen in a eutrophic tributary of Leading Creek (Headwater Tributary 1, 1.345 mg/m^2) to those seen in a reference station (Federal Creek, 0.166 mg/m^2) and the three study streams (0.034 – 0.051 mg/m^2). Data collected from all sites fall well below a proposed nuisance level of 100-150-mg/m^2 chlorophyll *a* suggested by Welsh et al (1988). A key difference in methodology is that Welsh et al (1988) scraped existing periphyton from rocks in the study streams, while a measure of 10-day periphyton colonization was used in the current study. These data are most useful in that they demonstrated relatively low periphyton levels in Little Leading Creek and the Leading Creek station below Little Leading Creek, an observation in direct contrast with observations of eutrophication in Headwater Tributary 1. Thus, agricultural eutrophication, which may have been anticipated, was not seen in Little Leading Creek, the stream heavily influenced by agricultural runoff.

Water Quality

Elevated conductivity levels (5,009 µmhos/cm) observed in Parker Run are the result of high concentrations of sodium, sulfate, chloride and to a lesser degree magnesium and potassium. The extreme concentrations of these salts all contribute to the questionable water quality conditions observed in Parker Run. Of the major ions present in the stream, there is only an enforceable USEPA water quality criterion for the protection of aquatic life (WQC) for chloride (USEPA 1986). Although none of these ions have been shown to be acutely toxic at the concentrations measured in Parker Run, their sum total concentration may be. However, it has been shown that the toxicity of a solution characterized by high TDS and conductivity may not be solely attributable to a single toxic component, but rather to its sum osmotic effects (Meyer et al 1985; Boelter et al 1992; Ingersoll et al 1992; Jop and Askew 1994; and Dickersonet al 1996).

Agricultural and abandoned mined land runoff appeared to have no substantial, negative impact on the water quality in Little Leading Creek. Concentrations of nitrite/nitrate were similar to those seen in reference streams while phosphorous concentrations were elevated, suggesting some nutrient addition to Little Leading Creek. Iron concentrations were also elevated, most likely from upstream AMD inputs. However at the pH (7.35) of Little Leading Creek, observed concentrations of iron (3.17 mg/L) are not bioavailable and do not contribute to water column toxicity (Grundl and Delwiche 1993).

The introduction of AMD into the lotic environment has been documented to impair benthic macroinvertebrate and fish communities, primarily resulting from elevated metal concentrations and depressed pH (Roback and Richardson 1969; Scullion and Edwards 1980; Ramesy and Brannon 1988; Skinner and Arnold 1990; Vinyard 1996). Thomas Fork, which receives AMD inputs, had the most severely impacted water quality with a mean pH of 5.42 and high concentrations of several metals, the most significant of which was Al (9.785 mg/L). These pH and Al values are similar to those reported by Cherry and Currie (1997) and Cherry et al (1998b) at AMD impacted sites in the Ely Creek (pH ~3.1 – 6.9, Al ~ 0.21 – 9.56 mg/L) and Puckett's Creek (pH ~3.0 – 4.5, Al ~ 4.0 – 46.9 mg/L) watersheds, where acute *C. dubia* water column toxicity, reduced benthic macroinvertebrate abundance and richness values were observed.

Depressed pH values have been demonstrated to cause reductions in stream benthic macroinvertebrate abundance values (Kratz et al 1994). However, the high concentrations of Al are likely the primary cause of the decimated benthic macroinvertebrate community observed in Thomas Fork. At the pH values commonly measured in Thomas Fork (4.1 ~ 5.4) Al is highly bioavailable and toxic (Driscoll et al 1980; Helliwell et al 1983). The mayfly, *Baetis rhodani*, had only a 1.4 fold increase in its drift rate in response to pH 4.3, while an increase of 8.4 times was observed when 347 µg/L Al was experimentally added to a stream acidified to a pH of 4.3 (Ormerod et al 1987). Weatherley et al (1988), reported 270 µg/L Al at pH 4.9 to cause a 60% decrease in benthic invertebrate abundance. Additionally, the toxicity of Al has been measured for several freshwater invertebrates in the laboratory. A <16-hr LC_{50} to *C. dubia* of 1,900 μg/L at pH 7.4 was determined by McCauley et al (1986), while McCahon et al (1989) measured 20% mortality of *Rhyacophila* and *Dioncras* species at 890 μg/L Al at pH 4.7. Based on these previous studies, the consistently low pH (<5.5) and Al concentrations of nearly 10 mg/L in Thomas Fork create a highly toxic and inhospitable environment for most benthic macroinvertebrates.

Two streams in the study (Little Leading Creek and Thomas Fork) exceeded, on at least one occasion, the 47 μg/L provided as a maximum 24 hr average Zn concentration for the protection of aquatic life (USEPA 1981). Concentrations of Zn in Little Leading Creek surpassed this level in 7 of 15 samples analyzed and Thomas Fork exceeded the level in all but one sample (14 of 15); however, these are discrete data points, not 24-hr mean values. The USEPA Water Quality Criterion (WQC) for the Protection of Aquatic Life sets forth guidelines for levels of Zn that should never be exceeded and are hardness (mg/L as $CaCO_3$) dependent (USEPA 1981). Since interference from metal cations made hardness determination difficult in Thomas Fork (APHA et al 1992) hardness values from other streams in the study (ranging from ~150 – 200 mg/L as CaCO3 in Little Leading Creek and Parker Run Reference, respectfully), were used as approximates of conditions in Thomas Fork. These hardness values yield a WQC of ~450 – 570 μg Zn/L. Using these data, the WQC for the Protection of Aquatic Life for Zn was not exceeded in any of the study sites (highest value of 383μg/L recorded at Thomas Fork). Further, a *D. magna* LC_{50} value of 525 μg/L (hardness of 105 as mg $CaCO_3$/L) reported by Chapman et al (manuscript) is considerably higher than the highest value recorded at any site. Based on these data, it is likely that Zn contributed to water column toxicity in Thomas Fork and may have adverse affects on the benthic community in Little Leading Creek.

Concentrations of Cu were also elevated, relative to one or both reference sites, in at least one analysis in each stream. Similar to Zn, the USEPA WQC for the Protection of Aquatic Life for Cu is hardness (mg/L as CaCO3) dependent (USEPA 1985). Using hardness values from Little Leading Creek and Parker Run reference as a guide the maximum 4-day average Cu concentration should not exceed 17 – 21 μg/L more than once every three years and the one-hour average concentration should not exceed 26 – 34 μg/L more than once every three years. Both Little Leading Creek and Thomas Fork violated the 4-day maximum concentration (1 out of 15 Little Leading Creek samples and 3 out of 15 in Thomas Fork) with a maximum-recorded concentration of 30 μg/L, measured once in each stream. If discrete data points may be considered one-hour average values, both Little Leading Creek (1/15 samples) and Thomas Fork (1/15 samples) also exceeded the one-hour maximum concentration set by the WQC. Copper concentrations in excess of either the four-day or one-hour average values were not recorded in Parker Run. Additionally, two *D. magna* LC_{50} values (30 and 38 μg/L Cu at hardness values of 105 and 106 mg/L as $CaCO_3$ respectively) reported by Chapman et al (manuscript) were equal to or in excess of the maximum concentration recorded in either stream and a value measured in hard water (69 μg/L at a hardness of 207 mg/L as $CaCO_3$) was much greater. As with Zn, it is likely that Cu contributed to acute water column toxicity in Thomas Fork and reached levels in Little Leading Creek that may have negatively affected the stream biota.

The final metal observed in concentrations above reference station background levels was Mn. Few data exist in the literature regarding the toxicity of Mn to aquatic organisms and there is no USEPA ambient WQC. Stubblefield et al (1997) reported an IC25 (25% inhibition concentration, based on both survival and body weight in a 62-day chronic test) for brown trout (*Salmo trutta*) larvae of 5.59 mg/L Mn at a hardness of 150 mg/L as $CaCO_3$. The authors also present LC_{50} data (adjusted to hardness of 50 mg/L as $CaCO_3$) for *C. dubia* (12.70 mg/L Mn), *Pimephales promelas* (7.96 mg/L Mn), and others. These data support the assertion by the USEPA (1986) that because Mn concentrations in aquatic systems generally fall below 1.0 mg/L, Mn is not a substantial problem in most systems. The only stream in the study in which concentrations of Mn above 1.0 mg/L were measured was Thomas Fork (mean value of 2.43 mg/L Mn). These data suggest that Mn may have had a slight contribution to toxicity in Thomas Fork (a peak value of 4.52 mg/L Mn was recorded), but probably did not have substantial effects on any other stream in the study.

Sediment Deposition

Extensive sediment deposition has been implicated in reducing both benthic macroinvertebrate abundance and richness, particularly the more sensitive EPT organisms (McClelland and Brusven 1980). Several researchers have observed sedimentation to cause increases in chironomid abundance while decreasing abundance of EPT and other taxa (Dance and Hynes 1980; Whiting and Clifford 1983; Lenat 1984), a community response similar to that seen in Little Leading Creek of this study.

McClelland and Brusven (1980) demonstrated that as the degree of cobble embeddedness increases, crucial interstitial pore space habitat becomes unavailable to non-burrowing insect populations. This habitat displacement through substrate embeddedness has been implicated in decreased insect abundance (Bjornn 1977). The dramatic sediment deposition observed in Little Leading Creek and Thomas Fork has homogenized the streambed at both sites and displaced the benthos from their preferred habitat.

Another effect of sedimentation is to scour or cover periphyton, thereby making it less available or appealing to insects as a food source (Graham 1990). Culp et al (1986) demonstrated that saltating sediments, those transported by bouncing along the sediment-water interface rather than in suspension, can decrease the total abundance of benthic macroinvertebrates by more than 50%. Both of these studies determined catastrophic drift to be the primary mode of decreased abundance. More direct, lethal effects of sedimentation were discussed by Lemly (1982) in a study that observed inorganic sediments adhering to insect bodies allowing bacterial colonization, which eventually smothered the insects.

Water Column Toxicity

Parker Run was shown to have intermittent acute water column toxicity, with 48-hr *C. dubia* LC_{50} values ranging from 66.1% to >100% of the sample. Cherry et al

(1998b) observed a significant correlation between benthic macroinvertebrate richness and acute water column toxicity to *D. magna*. These data suggest that the periodic acute toxicity observed in Parker Run may negatively impact the benthic macroinvertebrate community.

Acute water column toxicity observed in Thomas Fork (mean LC_{50} of 30.33% and 100% mortality of *C. dubia* in each 100% sample) is typical of a stream heavily impacted by AMD. Fucik et al (1991) observed AMD to cause acute toxicity to *C. dubia*, brook trout (*Salvelinus fontinalis*) and the fathead minnow (*Pimephales promelas*). Furthermore, the authors noted a correlation between water column toxicity and impairment of the benthic macroinvertebrate communities. Vinyard (1996) observed water column toxicity to *C. dubia* as well as significant decreases in periphyton primary production in a stream affected by AMD.

Sediment Toxicity

Relative to the two reference sites, sediment toxicity was not observed in any of the three impacted streams. There is a question of whether the overlying reference water used in testing could have alleviated some of the toxic influences that sediments may exhibit in the field. The reference water may have affected sediment toxicity in Parker Run, as the water chemistry differed substantially from that of the reference stream (Sinking Creek, Giles Co, VA). The waters in Sinking Creek have a hardness of approximately 130 mg/L as $CaCO_3$ and a circumneutral pH (~8.0). The hardness in Parker Run is generally in excess of 500 mg/L as $CaCO_3$ and pH values are similar to those in Sinking Creek (mean of 8.05). It is unlikely that the reference water masked any sediment toxicity in Parker Run. The water quality of the reference water was similar in characteristic to Little Leading Creek and probably did not substantially alter bioavailability of metals sequestered in the sediments. Thomas Fork sediment, overlain with acidic water *in situ*, may have been rendered less toxic by the neutral overlaying reference waters, as most metals are more soluble, thus more bioavailable and toxic, under acidic conditions (Snoeyink and Jenkins 1980). Because the water column in Thomas Fork is highly toxic (mean 48-hr *C. dubia* LC_{50} of 30.33% of field sample), it is unlikely that any undetected sediment toxicity significantly affected the benthic macroinvertebrate community in this stream. Sediment toxicity was therefore ruled out as a causative factor impacting the benthic macroinvertebrate community in any of the streams.

In Situ Toxicity

The Asian clam, *C. fluminea*, has been used extensively as a biological monitor, particularly in detecting non-point source pollution (Cherry 1996; Doherty 1990). Soucek et al (1999) observed *C. fluminea in situ* bioassays to be highly sensitive estimators of toxic AMD influences in a watershed in southwestern Virginia, USA. The *in situ* testing conducted in this study proved to be the most sensitive instrument in detecting toxic conditions in each stream. Significantly depressed survival of clams placed in Parker Run demonstrated chronic toxicity of the water column, which was periodically detected in 48-hr acute water column bioassays using *C. dubia*. There were no differences detected between survival and growth of clams placed in Little Leading Creek and survival or growth of those placed at the reference site. Finally, *in situ* survival data supported the *C. dubia* laboratory acute toxicity data from Thomas Fork, all test organisms, both *C. dubia* and *C. fluminea*, experienced 100% mortality during each test, further demonstrating the consistent, acute toxicity of Thomas Fork.

Sensitivity of Methods Used

Significant differences among sites for *C. fluminea* survival in 35-day *in situ* bioassays proved most useful in determining the nature and severity of the environmental impact on each stream. Benthic macroinvertebrate sampling was the next most useful tool utilized in this study, secondary to the *in situ* clam bioassays in that they are considerably more difficult to execute and evaluate and provide a lesser degree of differentiation between streams. Following these in order of utility were *C. dubia* 48-hr acute water column toxicity bioassays, water quality analysis, sediment depth profiling, periphyton artificial substrate colonization studies, and ranked last were 10-day chronic sediment bioassays. These results are somewhat different than those reported by Soucek et al (1999) in a similar evaluation of streams in the Puckett's Creek watershed, southwestern Virginia, which is heavily impacted by AMD. In the Puckett's Creek watershed, *C. dubia* water column acute toxicity testing was the most useful bioassessment technique used, followed by *in situ C. fluminea* bioassays. These discrepancies are most likely due to the disparate impacts that contribute varying degrees of water column toxicity to the streams in the current study as compared to the AMD generated, acute water column toxicity reported at multiple sites in the Puckett's Creek investigation. These differences also point out the importance of using multiple techniques (abiotic, bioassay, and biotic) in the evaluation of environmental impacts on a stream or watershed.

Conclusions

Of the three influences analyzed, the acute water-column toxicity associated with AMD had the most detrimental impact on the benthic macroinvertebrate community, followed by agricultural and abandoned mined land sedimentation, with active mine effluent having the least severe impact of the three.

The benthos in Parker Run was not subjected to extensive sediment deposition or sediment toxicity. The chronic and periodic acute water column toxicity observed in Parker Run is most likely responsible for the depressed macroinvertebrate community richness in that stream.

The integrative ecotoxicological evaluation employed in this study was different from previous studies of the impact of sedimentation in the literature in that it allowed for the elimination of additive effects of toxins sometimes associated with sedimentation as a cause for benthic macroinvertebrate community impairment. None of the three toxicity bioassay methods employed in Little Leading Creek detected toxicity. It may be concluded, therefore that the extensive sediment deposition observed in Little Leading Creek is the most likely cause of depressed benthic macroinvertebrate community indices observed in that stream.

The effects of the consistent, acute toxicity of Thomas Fork are most likely responsible for its impoverished macroinvertebrate community. Although sediment deposition in this stream was of an even greater magnitude than that seen in Little Leading Creek, the effects of sedimentation were probably secondary to those of low pH and high concentrations of Al and may or may not further affect the community.

Of the various techniques used in this study to differentiate affects of each influence on the stream communities, *in situ* bioassays using *C. fluminea* were the most useful. Benthic macroinvertebrate surveys are ranked next in terms of utility, followed by laboratory *C. dubia* acute toxicity bioassays, water quality analysis, sediment depth profiling, measures of periphyton chlorophyll *a*, and placed last in terms of effectiveness were the 10-day chronic sediment bioassays.

Chapter 2.

A Study of Benthic Macroinvertebrate Community Recovery in Parker Run, Meigs County, Ohio, from a Catastrophic Coal Slurry Spill

Introduction

The accidental introduction of toxic substances into lotic ecosystems can have severe, detrimental effects on benthic macroinvertebrate communities. For example, accidental discharge of fly ash from a settling pond was shown to cause significant reductions in both diversity and abundance of macroinvertebrates in a receiving stream (Specht et al, 1984). A large (~95,000 gallon) gasoline spill was reported by Pontasch and Brusven (1988) to reduce benthic macroinvertebrate densities by 75% to 98% relative to an upstream control. Guiney et al (1987) observed complete elimination of stream benthic invertebrates immediately below the site of an aviation kerosene spill. Similar results also have been shown in experimental additions of insecticides to streams (Whiles and Wallace, 1995).

Long term, toxic inputs may have similar, but more persistent effects on the benthos. Skinner and Arnold (1990) sampled a stream impacted by acid mine drainage (AMD) and found no difference in the distribution of benthic invertebrates from samplings conducted at the same site 12 years earlier. Both of these sampling events detected substantially decreased taxa richness and density values below the AMD input relative to a site above the impact. Chadwick, et al (1986) monitored the recovery of a stream community impacted by metal mining and found that the majority of taxa had not recovered in the 10 years following termination of the disturbance. As pointed out by Niemi et al (1990), long term anthropogenic impacts which impart residual toxicity to the system, as toxic sediment metal concentrations for instance, or impacts that alter physical habitat, require the most time and are the most difficult for an ecosystem to recover.

The recovery of stream ecosystems from disturbance, both natural and anthropogenic, is receiving increasing attention. Several researchers (Cairns, 1990; Wallace, 1990; Yount and Niemi, 1990) have analyzed the factors controlling rates of ecosystem recovery. Among factors identified as important in governing recovery rates of disturbed lotic systems were the presence of upstream colonizing populations, suitable physical and chemical habitat, and a cessation of the original disturbance. Both Cairns (1990) and Kelly and Harwell (1990) have suggested that a community's recovery from a toxic disturbance will be retarded if part of the system continues to be exposed to either the initial or an additional toxic stress.

The Leading Creek watershed in Southeastern Ohio has been the subject of intense study following the emergency dewatering of the Meigs #31 deep coal mine in Meigs Co., Ohio. In June of 1993 the mine was flooded with approximately one billion gallons of AMD from previously sealed and unused portions of the mine. This untreated and partially treated effluent was then pumped into Parker Run, a tributary of Leading Creek and into an adjacent watershed before entering the Ohio River. This effluent had a low pH (~2.5 – 3.5), high conductivity (~4,000 – 8,000 µmhos/cm) and elevated concentrations of several metals including copper (Cu, ~25 - 75 µg/L), iron (Fe, ~50 – 70 mg/L) and zinc (Zn, ~110 – 140 µg/L) (Birge, et al, 1995). In conjunction with numerous federal and state agencies the Leading Creek Improvement plan (LCIP) was developed to assess the recovery of the Leading Creek Watershed and to make recommendations for its further enhancement (Cherry et al, 1998a).

Parker Run, a major tributary to Leading Creek, joins the mainstem at stream km 26.27, approximately in the middle of the 97,000-acre watershed. The stream flows through the Meigs #31 coal mine complex, and is subject to numerous non-point source and one major point source discharge associated with the mine. The major discharge from the mine is a treated effluent that increases the flow of Parker Run by 10 fold and constitutes its entire flow under conditions of low upstream dilution (Latimer, 1999, Ch.1). This effluent adds large quantities of dissolved salts to the stream which contribute to high conductivity (2,140 – 7,000 μmhos/cm), elevated total dissolved solids (TDS, 1,246 – 4,602 mg/L), high concentrations of sodium (Na, 225 – 1,360 mg/L), sulfate ($SO_4^{=}$, 478 – 2,470 mg/L) and chloride (Cl^-, 74 - 456 mg/L) measured at the Parker Run sampling site as described in Chapter 1 of this thesis. The water column in Parker Run has also been observed to be intermittently acutely toxic to *Ceriodaphnia dubia* and to be chronically toxic to *Corbicula fluminea* (Latimer, 1999, Ch.1).

Approximately four years after the emergency dewatering, Parker Run received another environmental impact. Waste slurry from a coal processing area at the Meigs #31 coal mine is pumped to a settling pond for treatment. In early April 1997 a pipeline carrying this waste coal slurry ruptured, allowing an unknown amount of untreated slurry to flow into Parker Run for several hours. The slurry, a black, sooty material with a greasy consistency, coated the streambed to a depth of up to 18 inches (46 cm).

The purpose of this study was to perform an integrative ecotoxicological evaluation of the damage to Parker Run's benthic macroinvertebrate community and its subsequent recovery from the coal slurry spill. To this end several sampling tools were utilized, among them were benthic macroinvertebrate sampling, water quality assessment, chronic sediment bioassays, acute water column toxicity bioassays, and *in situ* toxicity testing. A further goal was to determine what, if any, impact the intermittent acute toxicity of the active coal mine effluent which dominates the flow in Parker Run had on the recovery of this system.

Methods

Study Site

Parker Run, Meigs County, Ohio, is a third order stream, located at Leading Creek km 26.27, in the middle of the Leading Creek watershed. It has a total length of 8.33 km and drains an area of 4,715 acres (Cherry et. al., 1998). This tributary flows through the Meigs #31 coal mine complex and is subjected to numerous types of runoff associated with the mine. As described in the introduction, the main impact on the stream is the treated mine effluent, released directly from settling ponds into the stream. This active coal mine effluent dominates the flow of Parker Run and increases the concentrations of several dissolved ions, in some cases by an order of magnitude. Six stations were established from which to monitor the recovery of Parker Run.

The first station was the Parker Run reference area, designated #1, sampled above the mine complex, adjacent to the mine parking lot. The stream at this point was considerably smaller (<1 – 2 m wide) than lower Parker Run, with a streambed composed mainly of gravel. The second station was the site of the initial coal slurry spill, designated #2, sampled in a riffle section approximately 35m above the entrance of the active mine effluent into Parker Run. At this station Parker Run was roughly 5 – 7m wide, with shallow (≤0.3m deep) flow over a cobble and gravel substrate. Parker Run site #3 was within the effluent raceway, sampled 10 – 15 meters before the effluent joined the mainstem of Parker Run. The raceway was ~1.5 meters wide and characterized by fast, turbulent flow over a large cobble substrate. Site #4 was in a riffle section ~ 40 meters downstream of the confluence of the raceway and Parker Run. At site #4 the stream is wide (~7m) and fast, flowing over large gravel substrate. The next sampling site was at the Parker Run Rd. bridge crossing, approximately 800m downstream of the effluent raceway. The stream at this site was not as wide (~3 – 4m), deeper (~0.5 – 1m), and flowed rapidly over a mixed gravel and sand substrate. The final sampling site, Parker Run #6, was located ~75m above the confluence of Parker Run and Leading Creek. The stream at this site was of approximately the same width and depth as at #5; however, extensive erosion of the stream banks in this area contributed to substantial increases in sediment deposition, observed to be ~8 – 18" deep at this site.

Benthic Macroinvertebrate Sampling

Two replicate benthic macroinvertebrate community samples were taken during each of 4 sampling events, (site #3, the effluent raceway, was excluded from the initial sampling) using a D-frame dip net with 800μm mesh. These samples were taken immediately following the spill (April 1997), and again at 4 (July 1997), 9 (January 1998) and 16 months (July 1998) after the initial slurry spill. Wherever the habitat allowed, four different representative areas of the stream were sampled: riffle, pool, margin and run as per Ohio Environmental Protection Agency (OEPA) protocol for rapid bioassessment of benthic macroinvertebrates (OEPA, 1989). Insects were identified according to the keys in Merritt and Cummins (1996), to genus level where possible. Other macroinvertebrates were identified according to keys in Pennak (1989). Data were summarized using four indices: total abundance, EPT abundance, taxon richness and EPT richness, where EPT stands for Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies), the three most sensitive orders of aquatic insects.

Abiotic Parameters

The water quality at each site was monitored using the following parameters: pH, conductivity (μmhos/cm), total dissolved solids (TDS, mg/L), dissolved oxygen (DO, mg/l), water-column metals (Na, Mn, Fe, Cu, and Al), and other dissolved ions (Cl^-, $SO_4^{=}$). Samples were analyzed for pH, DO, and conductivity in the field or cooled to 4ºC and measured in the laboratory with a Fisher Scientific (Accumet® pH meter 15), YSI (Yellow Springs Instrument Co, inc.) model 54 dissolved oxygen meter, and a Hach Co. model 44600 conductivity/TDS meter respectively. TDS analyses were performed according to standard methods (APHA et al 1992). Metals analysis (Na, Mn, Fe, Cu, and Al) was performed at the inductively coupled plasma spectrometry (ICP) Lab on the Campus of VPI&SU. Sulfate and chloride were analyzed by ion chromatography in the Civil Engineering Department at VPI&SU.

Toxicity Testing

Forty-eight hr, static, acute water-column toxicity tests using <24 hr old *Ceriodaphnia dubia* were performed, testing followed United States Environmental Protection Agency protocol (Weber, 1993). Water that was toxic at 100% concentration was diluted using a 0.5 dilution series (100, 50, 25, 12.5, 6.25% and control) with pristine local stream water as the diluent and control (Sinking Creek, Giles Co., VA) and 48-hr acute toxicity tests were conducted according to USEPA methods (USEPA, 1993).

Ten-day chronic impairment sediment toxicity tests using 5-day old *Daphnia magna* and 10-day old *Chironomus tentans* as the test organisms were performed 4 times: immediately following the spill (April 1997), and 6 (September 1997), 9 (January 1998), and 15 months (June 1998) after the spill. Five replicate 1-liter beakers per site were filled with 200g unsieved site sediment and overlain with 800 ml of reference water (Sinking Creek, Giles Co., VA). All test beakers were aerated (~100 bubbles/minute) except during the April 1997 test, which was not aerated to better mimic the smothering, field conditions immediately following the slurry spill. Both species of test organism, 5 *D. magna* and 10 *C. tentans*, were introduced into the same test beaker. These procedures were modifications of Nebeker, et al (1984) and USEPA (1994).

Three *in situ* toxicity tests were conducted in Parker Run (7/97, 7/98 and 8/98) using *C. fluminea*, the Asian clam. The first test (7/97) included only sites #1,3,4 and 5. Although the second (7/98) test was intended to include all 6 sites, the clams were lost at #6. The final test (8/98) did include all 6 sites.

Clams were collected using clam rakes from the New River near Ripplemead, VA. Clams used in testing had shell lengths between 9.0 – 12.0mm, measured with a Fowler & NSK Max-cal digital micrometer (0.0005”). After measurement, the clams were marked with a file and placed in plastic mesh bags, 5 clams per bag. Five bags were staked in the stream channel at each site, making a total of 25 clams per site. After 35 ± 1 days, the clams were recovered, evaluated for mortality and surviving clams were measured. These procedures were modifications of Cherry and Dobbs (1993).

Statistical Analysis

Statistical analysis, calculation of means and standard deviations were conducted using version 3.2.1 of JMP IN© statistical software (Sall and Lehman, 1996). An alpha (α) level of 0.05 was used in all analyses.

Benthic Macroinvertebrates

Each sample taken (n = 2) during each sampling event was considered a replicate. One-way analysis of variance (ANOVA) or for non-normal data Kruskal-Wallis Rank Sum tests were performed, then Tukey's test were employed in determination of significant differences between sites. When only two sites were compared a T-test was used for normally distributed data and a Wilcoxon Rank Sum test for non-normally distributed data sets.

Acute Water-Column Toxicity Testing

Percent survival of test organisms in the 100% sample concentration and 48-hr LC_{50} values were determined for each 48-hr acute water column test performed. Spearman-Karber LC_{50} values were calculated using the Toxstat® statistical package (Gulley, 1993).

Sediment and In Situ Toxicity Testing

Each beaker of a sediment test was used as a replicate (5 beakers per test) and means were calculated using all replicates (5) from each test. Each bag of five clams was considered a replicate (5 bags per site) and means were calculated using all replicates (20) from all *in situ* toxicity tests (3). Significant differences in percent survival of test organisms, *D. magna*, *C. tentans*, and *C. fluminea*, were determined using one-way ANOVA or Kruskal-Wallis Rank Sum test and Tukey's test. Differences in *D. magna* reproduction, *C. tentans* and *C. fluminea* growth were determined in the same manner, except that replicates with 100% mortality of the test organism in question were excluded from analysis. To compare *C. fluminea* growth and survival between two sites, T-tests were used for normally distributed data and a Wilcoxon Rank Sum test for non-normal data.

Linear Regression Analysis

Simple linear regression analysis was conducted using the JMP IN© statistical software package. Linear regression analysis was performed to make correlations between various physical, biotic and toxicological parameters. Both coefficient of correlation "r" values and "P" values were reported for these analyses.

Results

Benthic Macroinvertebrates

All four parameters calculated for samples collected within 48 hours of the spill showed significant ($P < 0.05$) impairment of the benthic macroinvertebrate community in relation to the reference, site #1 (Figure 1). Total abundance values ranged from 8 – 20 at the 4 sites below the spill compared to 342 at site #1. Similar results were seen for EPT abundance (1.5 – 11 below vs. 248 at #1), taxa richness (3.5 – 5 below vs. 23 at #1), and EPT richness (1.5 – 3 below vs. 7.5 at #1).

After four months, partial recovery of the sites affected by the spill was apparent by the fact that no significant differences were detected among total abundance values (values ranged from 25 - 237.5) for any of the 6 study sites (Figure 2). Somewhat similar results were observed for EPT abundance except that a significant difference was detected between site #1, which had the highest EPT abundance (52.5), and site #5 (3.5) that had the lowest. No other sites were significantly different from one another. There were significant differences in taxon richness values among many of the sites. The spill site, #2, had a significantly higher taxon richness value (27) than those observed at any other sites except the reference (25.5). The taxon richness value calculated for the reference was not, however, significantly higher than that recorded at site #4, immediately downstream of the effluent raceway. EPT richness followed a similar trend, as site #2 had a significantly higher value (5.5) than any site but the reference (3.5) and site #5 (2.5). It is apparent from these data that the benthic macroinvertebrate community had recovered to reference levels at site #2, sites below the active mine effluent had not.

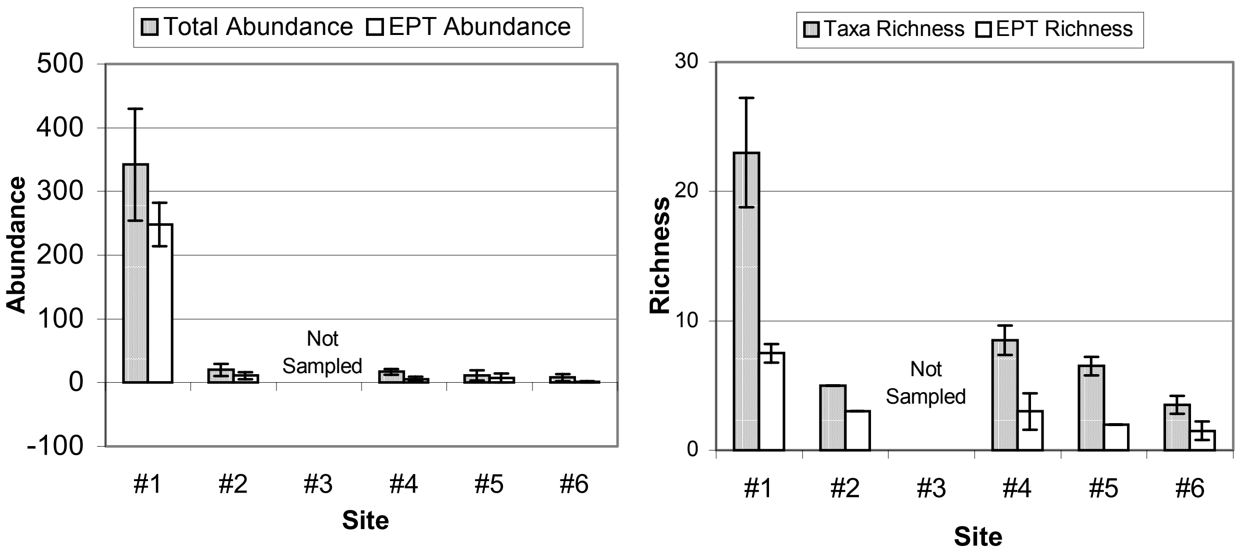


Figure 1. Total and EPT abundance (left) and total taxa and EPT richness (right) of benthic macroinvertebrates sampled immediately following the coal slurry spill, April 1997. Values for all sites are significantly (P<0.05) less than those recorded at the upstream reference (site #1).

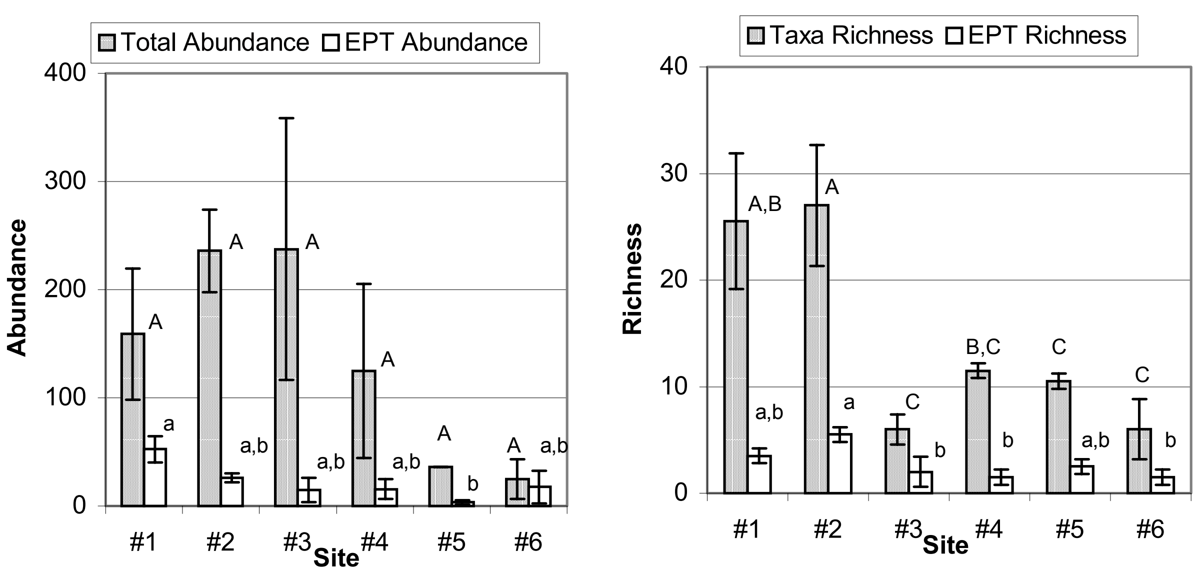
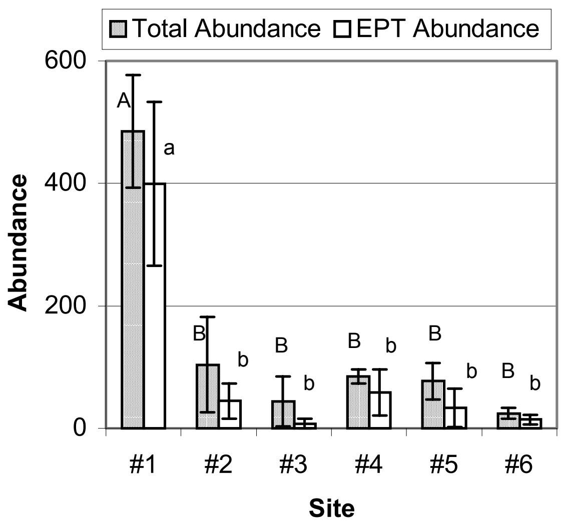


Figure 2. Total and EPT abundance (right) and total taxa and EPT taxa richness (left) of benthic macroinvertebrates sampled in July 1997, 4 months after the coal slurry spill. Values followed by the same letter (lowercase for EPT parameters, uppercase for others) are not significantly different (P<0.05).



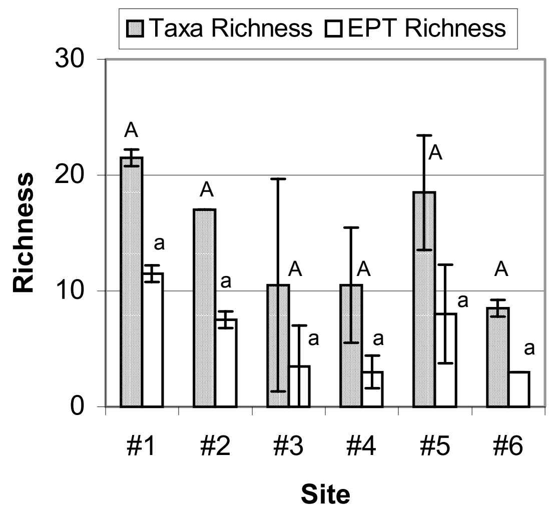
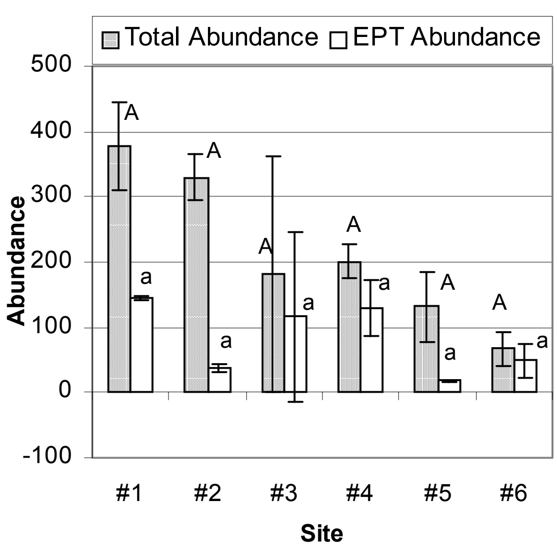


Figure 3. Total and EPT abundance (left) and total taxa and EPT taxa richness (right) of benthic macroinvertebrates sampled in January 1998, 9 months after the coal slurry spill. Values marked with the same letter (lower case for EPT parameters, uppercase for others) are not significantly different (P< 0.05).



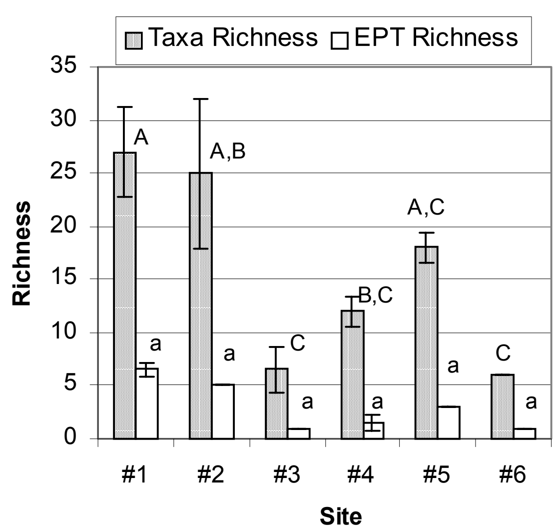


Figure 4. Total and EPT abundance (right) and total taxa and EPT taxa richness (left) of benthic macroinvertebrates sampled in July 1998, 16 months after the coal slurry spill. Values marked with the same letter (lowercase for EPT parameters, uppercase for others) are not significantly different (P< 0.05).

Samples taken in January 1998, 9 months after the initial spill had significant differences in both total abundance and EPT abundance between the reference (485 and 399.5 respectively) and all other sites (ranging from 24.5 – 104.0 for total abundance and from 7.5 –58.5 for EPT abundance) (Figure 3). No significant differences were detected among the remaining sites, which ranged from a high of 104 at site #2 to 24.5 at site #6 for total abundance and 58.5 at site #4 to 7.5 at site #3 for EPT abundance. Although a substantial downward trend from the reference to site #6 was observed for measures of both taxon richness and EPT richness, no significant differences were observed among the sites. Although the one-way ANOVA failed to detect significant differences between the two sites, measures of both taxon and EPT richness were substantially higher at site #2 (17.0 and 7.5 respectively) than at site #4 (10.5 and 3.5 respectively), indicating a lack of benthic macroinvertebrate community recovery below the effluent.

The final sampling effort in July 1998, 16 months after the initial spill, detected no significant differences in measures of either total abundance or EPT abundance among the sites, although a substantial downward trend from site #1 (377.5) to site #6 (67.0) existed for total abundance (Figure 4). Relative to site #1 (27), taxon richness values were significantly depressed at sites #3 (6.5), #4 (12), and #6 (6), no significant differences were observed between sites #2 (25) and #4 (12) or between any of the last 4 sites. Measures of EPT richness were substantially higher at site #1 (6.5) and #2 (5) than at sites #3 (1), #4 (1.5), #5 (3), #6 (1). Again, observed taxon and EPT richness values (25 and 5 respectively) at site #2 were higher than at any site below the effluent (taxon richness ranged from 6 – 18 and EPT richness ranged from 1 - 3), suggesting a continued lack of community recovery below the coal mine effluent.

Relative to the effect of the active coal mine effluent entering above site #4 on the recovery of Parker Run, measures of EPT richness at the initial spill site (#2) which is above the effluent were compared to those at site #4. Of the four sampling events, there was no difference between the sites during only the first event, which was expected since the benthic community at both sites was nearly eliminated by the spill. A T-test was used to determine significant differences between EPT richness values at these two sites for each of the three subsequent sampling events. Significant differences were seen in July 1997 (5.5 vs. 1.5, $P = 0.0299$) and July 1998 (5.0 vs. 1.0, $P = 0.0198$); however, the values (7.5 vs. 3.0) observed in January 1998 must be termed a substantial difference as the P value was 0.0565 for these data. The persistent difference in EPT richness at these two sites indicates that the active effluent has a direct, negative impact on the benthic macroinvertebrate community in Parker Run.

Water Chemistry

Of the 11 water quality parameters monitored in Parker Run (Tables 1 and 2), levels of conductivity, TDS, sulfate ($SO_4^{=}$) and sodium (Na^+) best demonstrate the significant impact of the Meigs #31 mine on Parker Run (Figure 5). Mean conductivity values ranged from 490µmhos/cm (range of 330 – 680µmhos/cm) at site #1 to a high of 5745µmhos/cm (range of 5030 – 6130 µmhos/cm) in the effluent raceway, site #3. The effect of the effluent raceway on the water quality is easily demonstrated by the increase in mean conductivity from site #2 (3232.5 µmhos/cm, range of 1020 – 5310 µmhos/cm) to site #4 (5670 µmhos/cm, range of 4970 – 6100 µmhos/cm). Similar results were observed for TDS, which increased from a mean of 355.5 mg/L (range of 244.4 – 483.3 mg/L) at site #1 to a high of 4202.6 mg/L (range of 3538.2 – 4577.6 mg/L) at site #3. As with conductivity the effluent raceway adds substantial concentrations of TDS to Parker Run, increasing concentrations from a mean of 3008.7 mg/L (range of 1305.7 – 3866.7 mg/L) at site #2 to a mean of 4019mg/L (range of 3196.7 – 4455.3 mg/L) at site #4.

Concentrations of $SO_4^=$ and Na^+ followed the same trends, lowest at site #1 (mean of 111.97 mg/L $SO_4^=$ and 32.93 mg/L Na^+) and highest in or below the effluent (mean of 2,369.4 mg/L $SO_4^=$ and 882.63 mg/L Na^+) (Table 1 and 2). Site #4 had the highest mean $SO_4^=$ concentration (2369.4 mg/L), a substantial increase over the mean concentration at site #2 (1715.43 mg/L). This increase is again the result of high concentrations of $SO_4^=$ (mean of 2281.37 mg/L) present in the effluent discharged between these two sites. Concentrations of Na^+ were highest in the effluent raceway (mean of 882.63 mg/L) which contributes to the increase from a mean of 613.4 mg/L at site #2 to a mean of 837.47 mg/L at site #4.

Mean chloride (Cl^-) levels were also elevated in Parker Run below the mine (432.73 mg/L at site #4 vs. 17.03 at site #1) (Table 1.) Again, the effluent raceway contributes much of this increase as seen by the change in mean Cl^- concentration from site #2 (330.47 mg/L) to site #4 (432.73). As with the parameters discussed in Figure 5, mean Cl^- concentrations decreased with distance downstream of site #3 (354.88 at site #5 and 349.03 at site #6).

Another parameter monitored was pH, the mean levels of which were circum-neutral at all sites, although there was an increase from site #1 (7.24) to sites below the mine, ranging from 7.97 – 8.18 (Table 1). Additionally, concentrations of manganese (Mn), iron (Fe), copper (Cu) and aluminum (Al) were elevated in and below the effluent. Concentrations of Mn ranged from 0.05 mg/L at sites #1 and #2 to 0.18 – 0.45 mg/L below the effluent. Iron followed the same general pattern increasing from 0.042 mg/L at site #1 to a high of 1.11 mg/L at site #6. The mean concentration of Cu increased at site #4 to 0.01 mg/L and ranged from 0.003 – 0.009 mg/L at all other sites. Mean levels of Al ranged from 0.024 mg/L at site #1 to a high of 1.176 mg/L at site #4 (Table 2.). Dissolved oxygen (mg/L) concentrations were at or near saturation during all sampling events and are not reported.

Table 1. Non-metallic water quality parameters measured at 6 Parker Run stations.

Site	Parameter	Mean Value	SD	Range	n
Reference	pH	7.24	0.41	6.70 – 7.71	4
Spill Site		8.18	0.36	7.65 – 8.42	4
Effluent		8.12	0.19	7.80 – 8.37	8
Below Effluent		8.14	0.26	7.77 – 8.39	5
LCIP TS-11		8.06	0.26	7.44 – 8.41	18
Before Confluence		7.97	0.52	7.21 – 8.41	4
Reference	Conductivity (μmhos/cm)	490	171.85	330 – 680	4
Spill Site		3232.5	2092.87	1020 – 5310	4
Effluent		5745	357.13	5030 – 6130	8
Below Effluent		5670	447.72	4970 – 6100	5
LCIP TS-11		5111.67	1326.13	2140 – 7000	18
Before Confluence		4698	1999.88	1722 – 6020	4
Reference	TDS (mg/L)	355.5	120.3	244.4 – 483.3	3
Spill Site		3008.7	1474.9	1305.7 – 3866.7	3
Effluent		4202.6	577	3538.2 – 4577.6	3
Below Effluent		4019	712.6	3196.7 – 4455.3	3
LCIP TS-11		3470.2	1926.8	1245.5 – 4602.2	3
Before Confluence		3304.9	2047.9	950.0 – 4668.5	3
Reference	$SO_4^{=}$ (mg/L)	111.97	90.63	44.6 – 215.0	3
Spill Site		1715.43	1035.52	519.8 – 2325.5	3
Effluent		2281.37	473.01	1795.6 – 2740.5	3
Below Effluent		2369.4	738.2	1523.0 – 2880.0	3
LCIP TS-11		1977.59	571.19	478.0 – 2470.0	18
Before Confluence		1988.43	1200.53	602.9 – 2720.0	3
Reference	Cl^- (mg/L)	17.03	12.17	8.7 – 31.0	3
Spill Site		330.47	174.36	129.6 – 442.8	3
Effluent		438.73	17.11	424.0 – 457.5	3
Below Effluent		432.63	42.44	386.0 – 469.0	3
LCIP TS-11		354.88	97.46	74.0 – 456.0	18
Before Confluence		349.03	177.24	144.6 – 459.5	3

Table 2. Concentrations of various metals measured in 6 Parker Run stations.

Site	Parameter	Mean Value	SD	Range	n
Reference	Na (mg/L)	32.93	27.8	16.83 – 65.09	3
Spill Site		613.4	286.4	284.5 – 807.5	3
Effluent		882.63	44.1	834.1 – 920.3	3
Below Effluent		837.47	48.9	787.4 – 885.1	3
LCIP TS-11		869.4	293.23	225.0 – 1360.0	18
Before Confluence		631.53	316.4	275.9 – 881.7	3
Reference	Mn (mg/L)	0.05	NA	NA	1
Spill Site		0.05	NA	NA	1
Effluent		0.33	NA	NA	1
Below Effluent		0.28	NA	NA	1
LCIP TS-11		0.45	0.227	0.1 – 0.78	16
Before Confluence		0.18	NA	NA	1
Reference	Fe (mg/L)	0.042	NA	NA	1
Spill Site		0.199	NA	NA	1
Effluent		0.417	NA	NA	1
Below Effluent		0.368	NA	NA	1
LCIP TS-11		0.44	0.358	0.10 – 1.54	16
Before Confluence		1.11	NA	NA	1
Reference	Cu (mg/L)	0.009	0.008	BDL – 0.014	3
Spill Site		0.009	0.001	BDL – 0.010	3
Effluent		0.007	0.004	BDL – 0.010	3
Below Effluent		0.01	NA	BDL – 0.010	3
LCIP TS-11		0.003	0.003	BDL – 0.010	18
Before Confluence		0.007	0.004	BDL – 0.010	3
Reference	Al (mg/L)	0.024	NA	BDL – 0.024	3
Spill Site		0.083	0.019	BDL – 0.692	3
Effluent		0.908	1.412	0.043 – 2.538	3
Below Effluent		1.176	1.492	BDL – 2.231	3
LCIP TS-11		0.613	0.765	BDL – 1.154	3
Before Confluence		1.291	1.656	BDL – 2.462	3

Chronic Sediment Bioassays

Results of 10-day chronic sediment bioassays conducted on sediments collected immediately following the coal slurry spill suggest sediment dwelling, benthic organisms may have been the most severely affected by the spill, as *C. tentans* survival was significantly depressed at all sites below the reference (Table 3). No significant

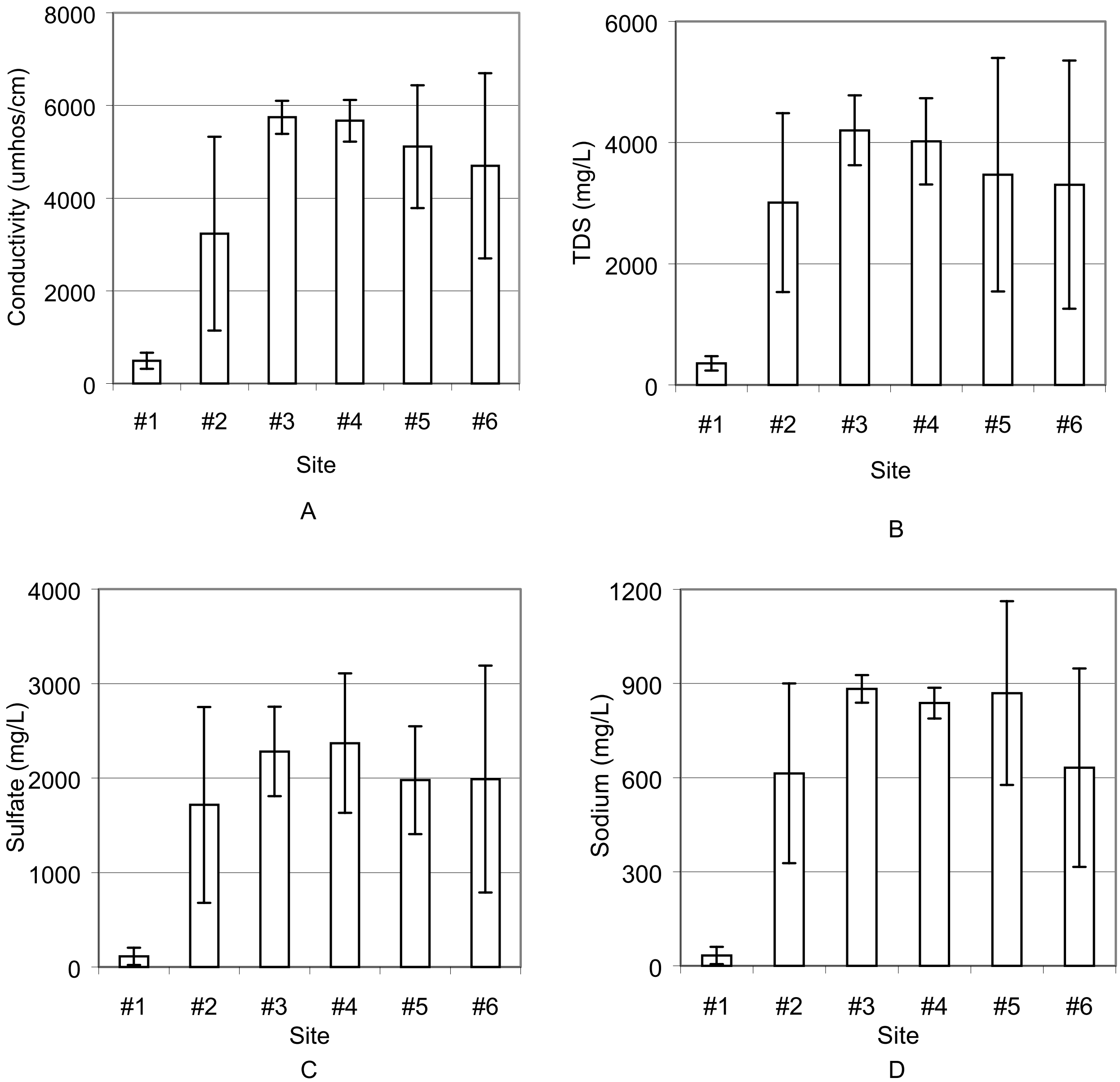


Figure 5 A-D. Mean levels of conductivity (μmhos/cm), TDS (mg/L), sulfate (mg/L) and sodium (mg/L) measured in Parker Run.

differences were detected among any of the sites for mean weight (mg) of *C. tentans*. The lowest *D. magna* survival (48%) was observed at site #5 and the only significant differences were detected between this site, the reference and effluent raceway (96% survival for both sites). All other sites had insignificant differences in survival. Although there appeared to be a substantial downward trend in the data set (84.6 neonates produced at site #1 vs. 33.2 at site #4), due to high variability among the data, no significant differences were detected between any sites for mean *D. magna* reproduction.

Table 3. Mean survival, reproduction and growth data from *Daphnia magna* and *Chironomus tentans* used in 10-day laboratory chronic sediment bioassays on sediment collected immediately following April 1997 coal slurry spill. Values followed by the same letter are not significantly different ($\alpha = 0.05$)

	Daphnia magna						*Chironomus tentans*					
Site	% Survival	SD	Desig-nation	Mean Reproduction	SD	Desig-nation	% Survival	SD	Desig-nation	Mean Weight (mg)	SD	Desig-nation
#1	96%	8.9%	A	84.6	12.21	A	88%	16.4%	A	2.234	1.138	A
#2	80%	14.1%	A,B	43.0	25.42	A	22%	11.0%	B	1.356	0.405	A
#3	96%	8.9%	A	42.4	44.42	A	32%	8.4%	B	0.694	0.686	A
#4	68%	36.3%	A,B	33.2	16.98	A	24%	15.2%	B	0.821	0.528	A
#5	48%	22.8%	B	49.0	28.70	A	28%	24.9%	B	1.163	1.641	A
#6	76%	26.1%	A,B	50.4	32.00	A	50%	15.8%	B	2.462	1.612	A

A chronic sediment bioassay using August 1997 samples resulted in significantly lower mean survival of *D. magna* at site #3 (40% survival) relative to site #6 (100%). No other significant differences in survival of *D. magna* were detected among the sites; however, mean reproduction was significantly lower (0.3 – 6.2) at all sites relative to site #6 (44.0). Mean *C. tentans* survival ranged from a low of 48% at site #5 to a high of 82% at site #6; although, all differences among sites were insignificant. Mean weight of *C. tentans* was significantly higher at sites #1 (4.715 mg), #2 (4.739 mg) and #4 (4.491 mg) relative to site #5 (1.386 mg), no other significant differences were detected among the sites (Table 4).

Table 4. Mean survival, reproduction and growth data from *Daphnia magna* and *Chironomus tentans* used in 10-day laboratory chronic sediment bioassays on sediment collected August 1997. Values followed by the same letter are not significantly different ($\alpha = 0.05$).

	Daphnia magna						*Chironomus tentans*					
Site	% Survival	SD	Desig-nation	Mean Reproduction	SD	Desig-nation	% Survival	SD	Desig-nation	Mean Weight (mg)	SD	Desig-nation
#1	56%	16.7%	A,B	2.2	1.79	B	70%	10.0%	A	4.715	0.812	A
#2	60%	28.3%	A,B	0.8	1.10	B	76%	11.4%	A	4.739	0.288	A
#3	40%	42.4%	B	0.3	0.58	B	70%	14.1%	A	3.462	1.987	A,B
#4	72%	30.3%	A,B	6.2	8.56	B	92%	8.4%	A	4.491	0.745	A
#5	48%	36.3%	A,B	5.5	11.00	B	48%	40.9%	A	1.386	1.601	B
#6	100%	0.0%	A	44.0	13.77	A	82%	19.2%	A	3.658	2.102	A,B

Mean survival of *D. magna* in a chronic bioassay conducted on sediments collected in January 1998 was substantially lower at sites #3 (44%) and #5 (40%) relative to sites #1 (96%), #2 (84%) and #4 (92%). Mean *D. magna* reproduction was significantly depressed at sites #2 (20.8), #3 (24.8) and #5 (45.0) relative to site #4 (77.4); all other sites were similar. Mean survival of C. tentans was significantly depressed at site #5 (22%) relative to site #3 (34%), while survival at both sites was significantly impaired relative to site #1 (80%). Additionally, survival at sites #2 (64%) and #6 (70%) was significantly higher than site #5 (22%). No significant differences were detected among mean *C. tentans* weights, which ranged from 4.909 mg at site #2 to 3.078 mg at site #3 (Table 5).

Chronic sediment bioassays performed on samples collected in June 1998 detected no significant differences among any sites for either test organism. Mean *D. magna* survival ranged from 92% at site #2 to 56% at Site #5, while reproduction ranged from 126.3 at site #5 to 90.8 at site #6. Mean survival of C. tentans ranged from 80% at site #1 to a low of 58% at site #3, while mean weight ranged from a high of 4.813 mg at site #6 to 3.169 mg at site #1 (Table 6).

Table 5. Mean survival, reproduction and growth data from *Daphnia magna* and *Chironomus tentans* used in 10-day laboratory chronic sediment bioassays on sediment collected January 1998. Values followed by the same letter are not significantly different ($\alpha = 0.05$).

	Daphnia magna						*Chironomus tentans*					
Site	% Survival	SD	Designation	Mean Reproduction	SD	Designation	% Survival	SD	Designation	Mean Weight (mg)	SD	Designation
#1	96%	8.9%	A	44.8	10.55	A,B	82%	11.0%	A	3.998	0.865	A
#2	84%	21.9%	A	20.8	12.79	B	64%	15.2%	A,B	4.909	0.714	A
#3	44%	45.6%	A	24.3	17.93	B	34%	19.5%	B	3.078	1.612	A
#4	92%	17.9%	A	77.4	39.07	A	44%	11.4%	A,B,C	3.675	1.248	A
#5	40%	54.8%	A	45.0	2.83	B	22%	22.8%	C	3.429	1.167	A
#6	68%	41.5%	A	34.5	20.89	A,B	70%	30.8%	A,B	3.929	1.736	A

Table 6. Mean survival, reproduction and growth data from *Daphnia magna* and *Chironomus tentans* used in 10-day laboratory chronic sediment bioassays on sediment collected June 1998. Values followed by the same letter are not significantly different ($\alpha = 0.05$).

	Daphnia magna						*Chironomus tentans*					
Site	% Survival	SD	Desig-nation	Mean Reproduction	SD	Desig-nation	% Survival	SD	Desig-nation	Mean Weight (mg)	SD	Desig-nation
#1	84%	26.1%	A	96.6	19.72	A	80%	10.0%	A	3.169	0.524	A
#2	92%	17.9%	A	92.0	27.57	A	66%	15.2%	A	4.029	0.539	A
#3	76%	32.9%	A	104.8	39.33	A	58%	34.2%	A	4.093	1.484	A
#4	68%	36.3%	A	96.6	43.83	A	62%	19.2%	A	4.061	0.717	A
#5	56%	51.8%	A	126.3	35.35	A	68%	16.4%	A	3.403	0.497	A
#6	80%	24.5%	A	90.8	26.53	A	72%	16.4%	A	4.813	1.082	A

The second chronic sediment bioassay conducted in June 1998 used the same sediment, but site-specific overlaying water instead of reference water (Table 7). Similar to the first test, no significant differences in mean survival of *D. magna* (ranging from 96% at sites #1 and #5 to 52% at site #3) were detected among the sites. Significantly lower mean reproduction of *D. magna* was detected at sites #2 (120.6), #4 (120.0), and #6 (123.2) relative to sites #1 (197.4) and #3 (240.7). No significance was detected between any sites for mean survival (ranging from 86% at site #6 to 60% at site #2) or mean weight (ranging from 3.961 mg at site #6 to 2.918 mg at site #1) of *C. tentans* (Table 7).

Table 7. Mean survival, reproduction and growth data from *Daphnia magna* and *Chironomus tentans* used in 10-day laboratory chronic sediment bioassays with overlying site water on sediment collected June 1998. Values followed by the same letter are not significantly different ($\alpha = 0.05$).

	Daphnia magna						*Chironomus tentans*					
Site	% Survival	SD	Desig-nation	Mean Reproduction	SD	Desig-nation	% Survival	SD	Desig-nation	Mean Weight (mg)	SD	Desig-nation
#1	96%	0.1%	A	197.4	43.66	A	74%	28.8%	A	2.918	0.501	A
#2	84%	21.9%	A	120.6	34.33	B	60%	15.8%	A	3.764	0.515	A
#3	52%	48.2%	A	240.7	36.96	A	72%	21.7%	A	2.990	0.831	A
#4	72%	30.3%	A	120.0	23.67	B	74%	13.4%	A	3.337	0.764	A
#5	96%	8.9%	A	178.6	40.75	A,B	84%	8.9%	A	3.337	0.581	A
#6	76%	32.9%	A	123.2	30.85	B	86%	11.4%	A	3.961	0.446	A

Water Column Acute Toxicity

Intermittent 48-hr acute water column toxicity to *C. dubia* was observed in Parker Run. Samples from site #1, the reference, were not acutely toxic and based on its water quality it was not extensively monitored for toxicity. Nor were samples from site #2 toxic, the initial spill site, as survival ranged from 90% – 100%. Test organism survival in 100% concentration of water from sites #3 and #5 (the sites sampled most extensively for acute toxicity, 4 and 10 tests respectively) ranged from 65% to 40% at site #3 and from 100% to 0% at site #5. Forty-eight hr LC_{50} values ranged from 89.09% to >100% at site #3 and from 66.10% to >100% at site #5. Acute toxicity was also observed at sites #4 (survival ranged from 100% to 50%) and #6 (55% survival) (Table 8).

Table 8. Summary of 48-hr acute water column toxicity to *Ceriodaphnia dubia*.

Site	Mean Survival in Undiluted Sample	SD	Range	Mean 48-hr LC50 (when generated)	Range	SD	N
Reference (#1)	100%	0.0%	NA	>100%	NA	0.00%	1
Spill Site (#2)	95%	7.1%	100 - 90%	>100%	NA	0.00%	2
Effluent Raceway (#3)	56%	11.1%	65 – 40%	89.09%	89.09 - >100%	0.00%	4
Below Effluent (#4)	75%	35.4%	100 - 50%	100%	100 - >100%	0.00%	2
LCIP TS-11 (#5)	61%	31.2%	100 - 0%	80.67	66.10 - >100%	13.95%	10
Confluence with L.C. (#6)	55%	0.0%	NA	100%	NA	0.00%	1

In Situ Toxicity

The first of three *in situ* toxicity tests conducted with *C. fluminea* demonstrated significantly impaired growth at sites #3 (0.16mm), #4 (0.10mm), and #5 (0.35mm) relative to site #1 (1.82mm). Although there were no significant differences among mean survival at these sites, survival was decreased to 48% at site #5 and 72% at site #4 relative to 88% at site #1 and 96% at site #3 (Figure 6A).

The second *in situ* test, conducted in July 1998, failed to detect any significant differences among the sites for either mean survival or mean growth of the clams. However, a decrease in mean survival was observed in and below the effluent, 72% at site #3, 44% at site #4 and 60% at site #5 relative to 88% survival at sites #1 and #2. An increase in mean clam growth was observed at sites #5 (1.25mm) and #4 (0.69mm) relative to both sites #1 (0.24mm) and #3 (0.38mm). Additionally, clams placed at site #2 (0.78mm) grew three times as much as clams placed in the reference (#1, 0.24mm) and twice as much as those in the effluent (#3, 0.38mm) (Figure 6B).

The final *in situ* test, August 1998, detected significantly decreased survival at site #5 (36%) relative to all other sites (values ranged from 100% at sites #1, #2 and #6 to 88% at site #4). Significantly depressed mean growth was also observed at site #3

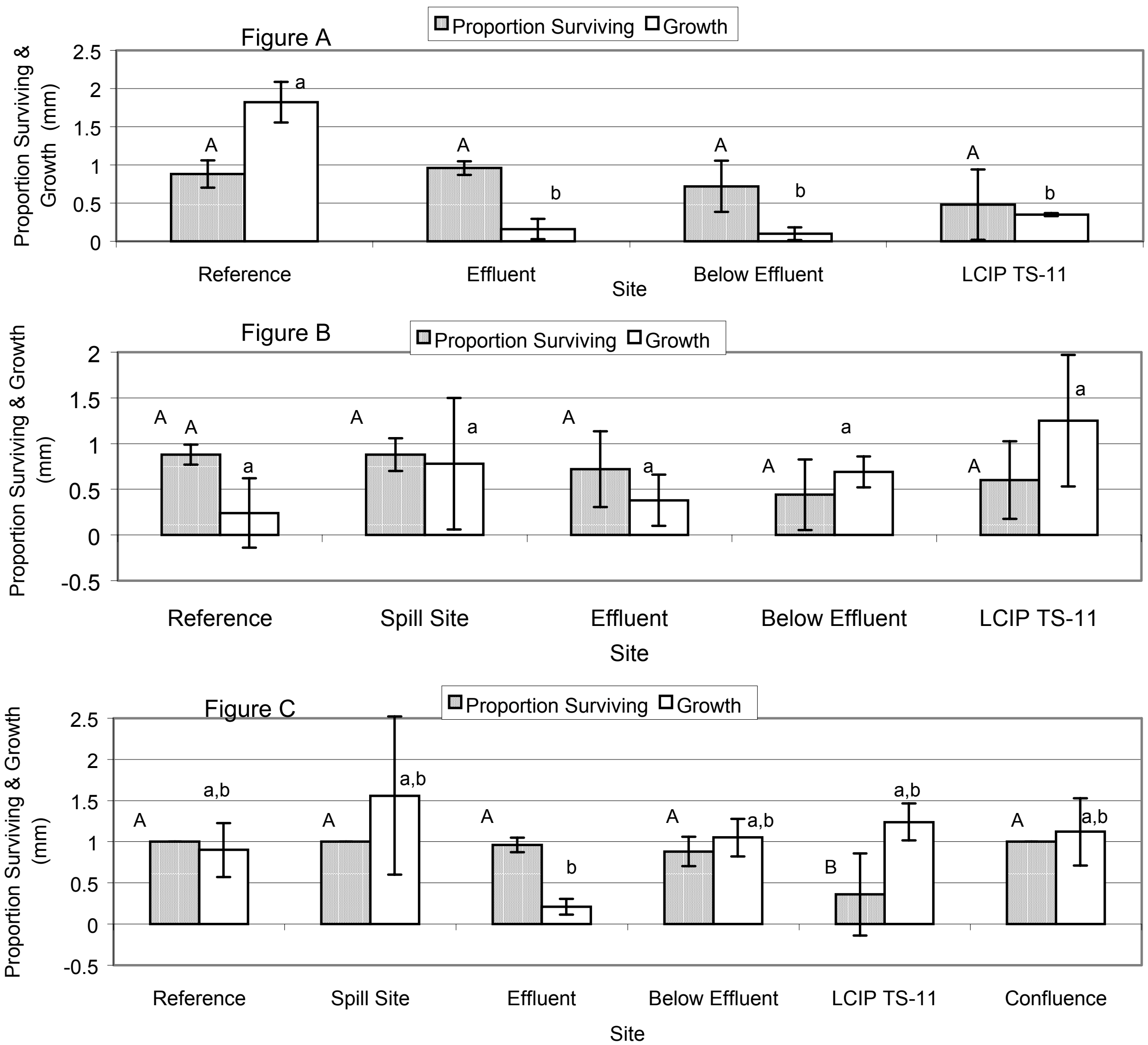


Figure 6 A-C. Summary of mean *Corbicula fluminea* survival and growth (mm) in three *In Situ* toxicity tests conducted July 1997 (Figure 6A), July 1998 (Figure 6B) and August 1998 (6C) in Parker Run. Those values followed by the same letter (uppercase for survival, lowercase for growth) are not significantly different ($\alpha = 0.05$).

(0.21mm) relative to site #2 (1.56mm). Other values ranged from 0.90mm at site #1 to 1.24mm at site #5, although no significant differences were determined among any other sites (Figure 6C).

When all *in situ* toxicity data were summarized from the four sites from which data were collected during each sampling event (#1, #3, #4, #5) substantial downward trends become evident. Mean *C. fluminea* survival decreased steadily from site #1

(92%), to #3 (88%), to #4 (68%) with site #5 (48%) having significantly lower survival than either site #1 or #3. Mean growth was significantly depressed in the effluent raceway (#3, 0.24mm) relative to site #1 (0.99mm) and #5 (0.95mm). Growth was decreased at site #4 (0.60mm), below the effluent; however, the difference was not significant between this and any other site in this analysis (Figure 7).

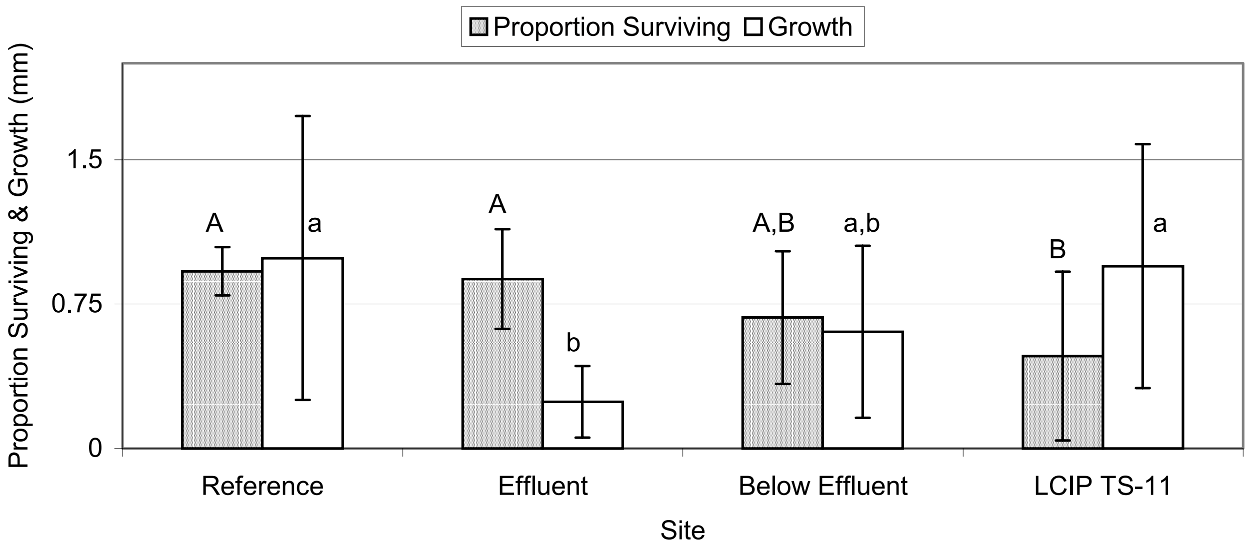


Figure 7. Summary of *Corbicula fluminea in situ* toxicity tests conducted in Parker Run, July 1997, July 1998, and August 1998. Data presented are mean values for those sites from which data were collected during each (n=3) sampling event. Those values denoted by the same letter (uppercase for survival data, lowercase for growth) are not significantly different ($\alpha = 0.05$).

In order to determine the direct effects of the mine effluent on the stream biota, data from both 1998 (as site #2 were not included in the 1997 test) *in situ* toxicity tests were used to compare mean survival and growth at the spill site (#2) and below the effluent (#4) (Figure 8). Mean survival was significantly depressed at site #4 (66%) relative to site #2 (94%). Mean growth, although lower at site #4 (0.89mm) than at site #2 (1.17mm), was not significantly different between these two sites.

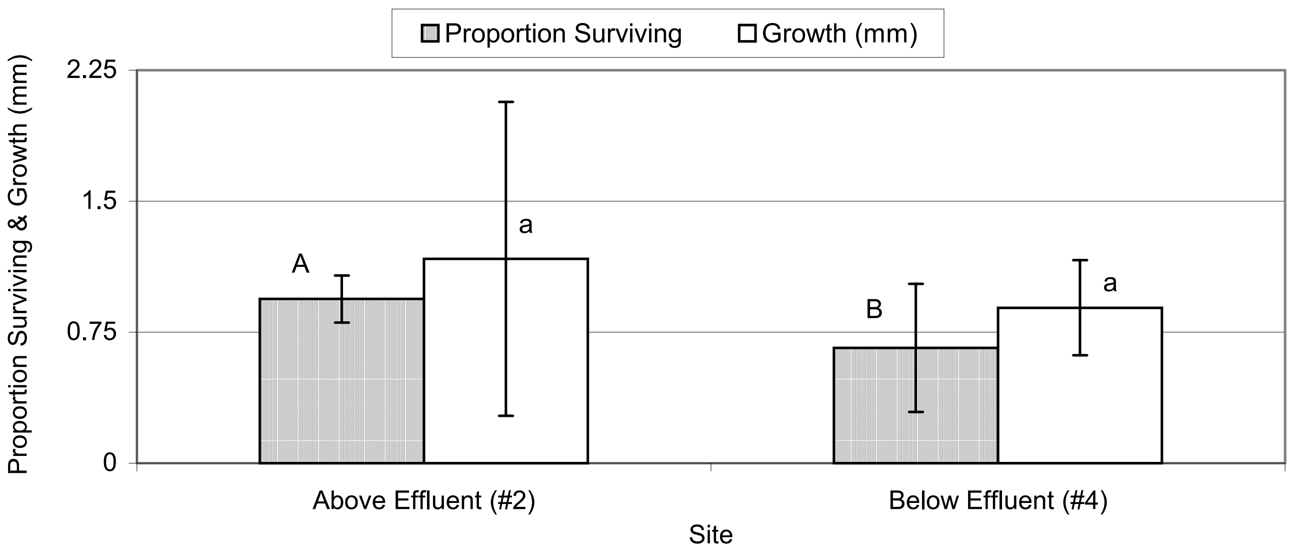


Figure 8. Summary of mean survival and growth of *Corbicula fluminea* placed above and below the Meigs #31 coal mine effluent in Parker Run. Data from 1998 sampling events only as site #2 was not sampled in 1997. Those values denoted by the same letter (uppercase for survival and lowercase for growth) are not significantly different (n=2, α=0.05).

<u>Correlation Analysis</u>

Simple linear regression analysis was used to correlate water chemistry data with both benthic macroinvertebrate EPT richness data and acute *C. dubia* water column toxicity data collected in July and August 1998 (Table 9). When all six sampling sites were included in the analysis TDS (mg/L), conductivity (μmhos/cm), sulfate (mg/L), sodium (mg/L) and chloride (mg/L) were significantly (P = 0.0014 – 0.0049) and negatively (r = -0.750 - -0.816) correlated with EPT richness in Parker Run. Additionally each of these water chemistry parameters were highly significantly (P <0.0001) correlated (r = 0.975 – 0.992) with TDS. Because all of the water chemistry parameters increased by an order of magnitude between the reference and downstream sites, elimination of reference data allowed for a greater resolution among impacted stations. When site #1, the reference, was excluded from the analysis only TDS and conductivity were significantly correlated with EPT richness (P = 0.010, r = $^{-}$0.765 and P = 0.027, r = $^{-}$0.692 respectively). Sulfate, sodium and chloride were partially correlated (r = $^{-}$0.208 - $^{-}$.540) with EPT richness at the lower 5 sites; however, these correlations are insignificant (P = 0.107 – 0.564).

Test organism survival in the 100% concentrations of acute water column toxicity tests were significantly correlated with TDS (P = 0.0424, r = $^{-}$0.649), conductivity (P = 0.0323, r = $^{-}$0.675), and sulfate (P = 0.0371, r = $^{-}$0.662) (Table 8). As with EPT richness data, sodium (P = 0.308, r = -0.359) and chloride (P = 0.120, r = -0.524) were somewhat correlated with test organism survival, but the relation was insignificant. These data, combined with EPT richness correlations suggested that TDS, conductivity and sulfate were important components, influencing both observed water column toxicity and depressed benthic community values in Parker Run. It is likely, however that TDS alone may best explain the observed affect of the mine effluent. Sulfate was a large contributor to the dissolved ion content in Parker Run and was highly correlated (r = 0.989) with TDS. Additionally, conductivity is a very similar measure to, and was highly correlated (r = 0.992) with, TDS.

Table 9. Summary of simple linear regression analysis performed on water chemistry, acute 48-hr *Ceriodaphnia dubia* toxicity, and benthic macroinvertebrate EPT richness data, collected June – August 1998.

Sites included in Analysis	Data Points Correlated			r	P
All 6	EPT Richness	vs.	TDS	-0.812	0.0014
All 6	EPT Richness	vs.	conductivity	-0.816	0.0012
All 6	EPT Richness	vs.	sulfate	-0.798	0.0019
All 6	EPT Richness	vs.	sodium	-0.768	0.0035
All 6	EPT Richness	vs.	chloride	-0.750	0.0049
All 6	TDS	vs.	conductivity	0.992	<0.0001
All 6	TDS	vs.	sulfate	0.989	<0.0001
All 6	TDS	vs.	sodium	0.975	<0.0001
All 6	TDS	vs.	chloride	0.986	<0.0001
Sites 2,3,4,5,6	EPT Richness	vs.	TDS	-0.765	0.010
Sites 2,3,4,5,6	EPT Richness	vs.	conductivity	-0.692	0.027
Sites 2,3,4,5,6	EPT Richness	vs.	sulfate	-0.540	0.107
Sites 2,3,4,5,6	EPT Richness	vs.	sodium	-0.329	0.352
Sites 2,3,4,5,6	EPT Richness	vs.	chloride	-0.208	0.564
All 6	% survival in 100% conc.	vs.	TDS	-0.649	0.0424
All 6	% survival in 100% conc.	vs.	conductivity	-0.675	0.0323
All 6	% survival in 100% conc.	vs.	sulfate	-0.662	0.0371
All 6	% survival in 100% conc.	vs.	sodium	-0.359	0.308
All 6	% survival in 100% conc.	vs.	chloride	-0.524	0.120

Discussion

Benthic Macroinvertebrates

The coal slurry spill had a significant, negative impact on the benthic macroinvertebrate community in Parker Run, reducing mean total abundance and EPT abundance values to 5.8 - 2.3% and 4.4 – 0.6% of those in the upstream reference area, respectively. Community richness values followed this trend as both taxa richness and EPT richness values were significantly depressed to 22 – 15% and 40 – 20% of reference values, respectfully. These initial effects of the coal slurry spill on the benthic community in Parker Run were similar to results reported in other studies of large-scale disturbances of stream ecosystems, both anthropogenic and natural. Crunkilton and Duchrow (1990) reported a large (1.5 million liter) crude oil spill in Asher Creek, Missouri, U.S., to have reduced total abundance of benthic macroinvertebrates to 0.1% of expected values (compared with a reduction to 5.8 – 2.3% in Parker Run). Guiney et al (1987) found total elimination of the benthic community following an aviation kerosene spill in a Pennsylvania stream. A naturally occurring debris flow in a Cascades Mountain stream, Oregon, U.S., reduced benthic macroinvertebrate abundance to less than two-thirds that of an upstream reference area (Lamberti et al 1991).

Sampling conducted four months after the coal slurry spill showed partial recovery at all sites, as no significant differences in total abundance values were detected among the sites. Values of both taxon richness and EPT richness at the spill site (#2) were actually higher than those sampled at the reference site, although the differences were not significant. Furthermore, the initial spill site had significantly higher EPT richness than site #4 below the effluent, an index shown by Wallace et al (1996) to accurately monitor stream ecosystem recovery from disturbance. These data indicated that the benthic community at the initial spill site had recovered within approximately 4 months. However, taxon and EPT richness values at most sites below the mine effluent remained significantly depressed relative to the initial spill site and/or the reference.

The sites below the spill continued to show effects of the spill in January 1998, 9 months following the initial disturbance. Reduced values of total and EPT abundance at all sites below the reference may suggest some continued impact of the spill on the stream community; however there were no significant differences in taxon or EPT richness among any sites. The large difference in abundance between the reference and other sites was accounted for by the presence of a winter stonefly (*Allocapnia* sp.), which constituted 75.7% of the total and 91.9% of the EPT abundance measures at the reference site. A substantial difference in EPT richness (7.5 vs. 3.0, $P = 0.0565$) suggested site #4, below the effluent, had not recovered relative to site #2, above the effluent, where the spill actually occurred.

A depressed benthic macroinvertebrate community was still evident in Parker Run below the effluent in July 1998, 16 months after the spill. Substantially, with some statistically significant differences, depressed taxon richness and EPT richness values were detected at all sites below the effluent. The site of the initial spill once again had significantly higher (5 vs. 1.5, $P = 0.0198$) EPT richness values than site #4, below the effluent.

The recovery of the spill site (#2) was consistent with reported recovery times for total abundance and taxon richness values of less than a year in over 90% of the case studies in the literature reviewed by Niemi et al (1990). The coal slurry spill at this site also conforms to the definition of a pulse disturbance, one that has an immediate influence from which the system recovers to a pre-disturbance state (Bender et al 1984), a definition analogous to that of acute toxicity, a short-term, non-persistent disturbance (Rand 1995). Conversely, sites below the effluent have not recovered to levels exhibited by the community at the site of the spill above the effluent. The response of communities at these sites, specifically site #4, is similar to what one might expect for a press disturbance. A press disturbance, as defined by Bender et al (1984), has a more persistent effect on the stream community, analogous to that of chronic toxicity, a continuous, long-term toxic exposure (Rand 1995).

In short, the benthic community at site #2, the only site exposed to the coal slurry spill, but not the effluent, recovered more rapidly and completely than did the communities at any site below the effluent. These data suggest that the active coal mine effluent entering Parker Run above site #4, but below the initial spill site, has hindered recovery of the stream system from the coal slurry spill.

Water Quality

The Meigs #31 mine contributes high concentrations of several metals and dissolved ions to Parker Run and of the three most prevalent (Na^+, $SO_4^=$ and Cl^-) there is only a USEPA Ambient Water Quality Criterion (AWQC) for the Protection of Aquatic Life for Cl^- (USEPA 1988). These dissolved ions combine to make significant increases in both conductivity and TDS, neither of which have USEPA AWQC regulatory limitations.

As they are relatively innocuous in most freshwater environments, little data are available concerning the toxicity of Na^+, $SO_4^=$, conductivity or TDS. Extensive regression analyses of toxicity test data performed by Mount et al (1997) ranked the relative toxicity of several ions ($Mg^{++} > Cl^- > SO_4^=$) and suggest that toxicity associated with Na^+ or Ca^{++} is due to a corresponding anion. Further, the authors report that toxicity of $SO_4^=$ and Cl^- is reduced in solutions with multiple cations, a situation similar to that in Parker Run (Latimer, Ch. 1).

If $SO_4^=$ is the toxic element in Na_2SO_4, to the exclusion of Na^+, then based on data presented by Mount et al (1997), the 48-hr $SO_4^=$ LC_{50} to *C. dubia* should be ~2,082 mg/L, a value often exceeded in Parker Run. However, reported $SO_4^=$ levels in Parker Run have never been recorded at similarly calculated 48-hr LC_{50} values of ~3,096 and >5,380 mg/L for the more resistant test organisms, *D. magna* and *Pimephales promelas*, respectively. It should also be noted that these data are considerably lower than the 48-hr LC_{50} of 5,814 mg/L and the 96-hr LC_{50} of 10,275 mg/L for *D. magna* and *P. promelas*, respectively, reported for Na_2SO_4 as $SO_4^=$ by Meyer et al (1985). Additionally, Mount et al (1997) reported that a solution with multiple cations may reduce toxicity of $SO_4^=$ and Cl^- ions in that solution. As Parker Run contains high concentrations of both Na^+ and Ca^{++} ions (Cherry et al 1998), it is realistic to consider such toxicity reductions. Indeed, data reported in the Mount et al (1997) study on the toxicity of a solution with equal parts of two salts (Na_2SO_4 and $CaSO_4$) yielded a mean 48-hr LC_{50} of >3,412 mg/L as $SO_4^=$ to *C. dubia*, a concentration in excess of any reported in Parker Run.

Mean concentrations of Cl^- exceed the USEPA AWQC four-day average concentration of 230 mg/L at every site except the reference. However, measured concentrations did not approach the maximum one-hour average concentration of 860 mg/L at any site (USEPA 1988). Two Cl^- salts (NaCl and $CaCl_2$) tested in solution together were reported by Mount et al (1997) to have a 48-hr LC_{50} to *C. dubia* of 1,935 mg/L as Cl^-. These data are more realistic of the conditions in Parker Run, as the two major cations in solution are Na^+ and Ca^{++} (Cherry et al 1998). It is unlikely, and correlation analysis discussed below support the assertion, that Cl^- had a significant impact on either the benthic macroinvertebrate community or the toxicity of the water column.

Of the other metals present in Parker Run (Mn, Fe, Cu, and Al), only Al was measured at concentrations that may adversely affect aquatic life in Parker Run. At the neutral pH of Parker Run, Fe is not bioavailable and does not contribute to toxicity (Grundl and Delwiche 1993). Manganese toxicity (as LC_{50} values) data reported by Stubblefield et al (1997) for *C. dubia* (12.70 mg/L) and *P. promelas* (7.96 mg/L) are well in excess of the maximum concentration (0.78 mg/L) measured at any site in Parker Run. Finally, neither the 4-day nor the one hour USEPA AWQC for Cu was exceeded at any site in the study (USEPA 1985). The concentrations of Al in Parker Run varied from below detection limits (<1 µg/L) to above 2.0 mg/L at some sites in and below the effluent raceway during a storm event. However, 48-hr *C. dubia* acute toxicity tests conducted on these storm water samples failed to detect any toxicity. It is unlikely that any of these metals had significant, adverse affects on the benthic macroinvertebrate community.

Conductivity (µmhos/cm) and TDS (mg/L) are both measures of ion concentrations in freshwater and as such are closely related to one another. This relationship is typically thought of as a ratio of TDS : conductivity. This ratio may vary anywhere from 0.5 at low solute concentrations to >1.0 as the solute concentration increases (Hem 1982). Because TDS is a more direct measure of the ion concentration of a solution and is highly correlated ($r = 0.992$) with conductivity in this system, its effects will be discussed to the exclusion of conductivity.

Several studies have shown elevated TDS in either field samples or synthetic laboratory effluents to be toxic in laboratory bioassays to several test organisms. Meyer et al (1985) reported that raw oil shale leachates became toxic to *D. magna* and *P. promelas* when the conductivity of the solution reached 7,000 – 8,000 µmhos/cm and TDS reached 6,000 – 7,000 mg/L. These conditions are similar to those observed in Parker Run as acute toxicity appears to have a threshold of approximately 6,000 μmhos/cm. Elevated concentrations of major ions (Na^+, K^+, Cl^-, HCO_3^-, and $CO_3^=$) in a saline oil-field discharge were found responsible for toxicity to *C. dubia* seen by Boelter et al (1992). Similarly, Dickerson et al (1996) reported that acute toxicity to *C. dubia* and *P. promelas* associated with irrigation drain water was due to elevated levels of major ions (Ca^{++}, Mg^{++}, Na^+, K^+, $CO_3^=$, HCO_3^-, $SO_4^=$, and Cl^-) at TDS levels of 12,000 – 18,000 mg/L. Ingersoll et al (1992) used saltwater organisms in testing extremely saline irrigation drain waters. Salinity levels of approximately 19g/L were found to be toxic to *P. promelas*, *D. magna* and *Hyalella azteca*, while elevated concentrations of inorganic ions (B, Mg, K, Na, and Sr) were toxic to saltwater acclimated *Morone saxatilis* and *H. azteca*. Finally, Goetsch and Palmer (1997) reported that the toxicity of a solution with high concentrations of dissolved ions to a South African mayfly (*Tricorythus* sp.) is linked to the conductivity and TDS of that solution. Based on these studies and the above water quality data, it is likely that the elevated concentrations of ions (Na^+, $SO_4^=$, and Cl^-), high conductivity values (~5,000 - >6,000 μmhos/cm) and elevated TDS concentrations (~4,000 mg/L) negatively affect the benthic macroinvertebrate community in Parker Run.

Chronic Sediment Bioassays

Sediment toxicity was evident immediately following the coal slurry spill, as the sediment dwelling midge larvae, *C. tentans*, had significantly reduced survival at all sites impacted by the slurry spill. The dissolved oxygen levels were depressed (2.6 –5.0 mg/L) in the test containers because of the lack of aeration; however, these conditions probably accurately mimicked the environment on the sediment surface to which the stream benthos was exposed. The water column dwelling *D. magna* was not as severely affected as the midge. These results suggest a physically toxic, smothering effect of the coal slurry on the benthic organisms, while the water column remained relatively unaffected. A greater variability in the test results were observed in August 1997, 5 months after the initial spill as reproduction and survival of *D. magna* were reduced at most sites in the stream, while *C. tentans* survival was similar at all sites. Bioassays conducted on sediments collected in January 1998, 9 months after the spill, detected depressed *D. magna* reproduction and *C. tentans* survival. A final battery of bioassays conducted in June 1998, 16 months after the spill, detected no toxicity at any site to either test organism. When site water, rather than reference water, was used to overlay the same sediments, significantly depressed *D. magna* reproduction was detected at several sites (#2, #4, and #6), suggesting that toxicity in Parker Run was more closely related to the water column than the sediments.

Water Column Toxicity

Periodic 48-hr acute water column toxicity to *C. dub*ia was detected at all sites except the reference. The most toxic sample was collected at site #5; however, this sample was taken before the current study was undertaken and was probably no more toxic than the water column at upstream sites. Of the stations from which more than one toxicity test was conducted, site #3, the effluent raceway had the lowest mean survival (56%), followed by sites #5 and #4. This order, again is probably an artifact of the extensive monitoring conducted at site #5 in conjunction with the Leading Creek Improvement Plan development study. It is likely that waters at site #5 were no more toxic than those at site #4. Of two tests conducted above the effluent at site #2, only one detected slight toxicity (90% survival), the other detected none. Water column toxicity was not detected at the reference site, and due to its good water quality, no further testing was conducted.

Several researchers have reported cases of stream benthic macroinvertebrate communities being impaired by associated water column toxicity. Cherry et al (1998b) observed a significant correlation between reductions in benthic macroinvertebrate richness values and acid mine drainage (AMD) influenced acute water column toxicity to *D. magna*. Further, Fucik et al (1991) reported that acute water column toxicity of an AMD influenced stream to *C. dubia*, *Salvelinus fontinalis* and *P. promelas* was correlated with impairment of the benthic macroinvertebrate communities. Although the toxicity in these studies was more persistent than that of Parker Run, similar affects may be expected in the Parker Run system.

In situ Toxicity

The Asian clam, *C. fluminea*, has been used extensively as an *in situ* biomonitoring tool, particularly successful in investigations of diffuse, periodic, or non-point source toxicity (Cherry 1996, Doherty 1990). The first of three *in situ C. fluminea* tests, July 1997, conducted in Parker Run detected significant decreases in growth at sites #3, #4, and #5 (clams were not placed at sites #2 and #6) relative to the upstream reference. The second two *in situ* tests, July and August 1998, yielded more variable results. A general downward trend in survival and growth below site #2 was detected in July 1998 and a general increase in growth with distance from the effluent raceway was detected in August 1998. When data from sites sampled during every event (#1, #3, #4, and #5) were analyzed, all sites demonstrated decreases in survival and growth relative to the reference site. Clams placed in the effluent grew significantly less than those in the reference did and those placed at site #5 had significantly lower survival than those did in either the effluent or the reference. Finally, survival and growth data from sites above (#2) and below (#4) the effluent were analyzed. The clams placed above the effluent had significantly higher survival and substantially increased growth relative to those placed at site #4. These *in situ C. fluminea* data suggest that the effluent discharged from the Meigs #31 mine raceway had significant, toxic affects on both test organisms and the native biota of Parker Run.

Correlation Analysis

Simple linear regression analysis of water chemistry, water column toxicity and benthic macroinvertebrate community (as EPT richness) data, allowed selection of TDS and conductivity as the two factors most related to depressed benthic macroinvertebrate community values observed in Parker Run. As mentioned above, EPT richness was selected as the best measure of benthic macroinvertebrate community recovery in this system based on a study by Wallace et al (1996) in which it was determined to be an accurate measure of stream ecosystem recovery from disturbance. Because all water chemistry parameters included in the analysis (TDS, conductivity, $SO_4^{=}$, Na^{+} and Cl^{-}) increased by an order of magnitude between the reference station and all other sites, data from the reference site were dropped from the analysis in order to gain greater precision. In the reduced (5 station) system, only TDS and conductivity were significantly ($P = 0.010$ and 0.027 respectively) correlated ($r = -0.765$ and -0.692 respectively) with observed EPT richness values. Water column toxicity yielded similar results as percent survival of test organisms in 100% strength stream water was significantly correlated with TDS ($P = 0.0424$, $r = -0.649$), conductivity ($P = 0.0323$, $r = -0.675$) and $SO_4^{=}$ ($P = 0.0371$, $r = -0.662$). However, TDS was highly significantly correlated with both conductivity ($P < 0.0001$, $r = 0.992$) and $SO_4^{=}$ ($P < 0.0001$, $r = 0.989$) suggesting that all three measures are representing the same affect on the test organisms, one of total ions in solution or TDS. This assertion was further supported by literature studies indicating a reduction in the toxicity of $SO_4^{=}$ (Mount et al 1997) in conditions similar to those in Parker Run, as discussed above.

The benthic macroinvertebrate community in Parker Run quickly recovered from the coal slurry spill at the site of the spill, above the entrance of the active mine effluent into the stream. Below the effluent entrance, the community has not recovered to levels measured at either the reference or initial spill sites. Based on the above data and correlation analysis, it is likely that recovery of the benthic macroinvertebrate community in Parker Run has been hindered by the consistently high concentrations of TDS contributed by activities at the Meigs #31 coal mine.

Conclusions

The initial coal slurry spill had an initial catastrophic effect on the benthic macroinvertebrate community in Parker Run with a significant reduction in total abundance to 5.8 – 2.3% of that observed in the upstream reference area. The benthic macroinvertebrate community at the initial spill site (#2), above the entrance of the active coal mine effluent into the stream, recovered more rapidly (~4 – 9 months) and completely than communities at sites below the effluent (>16 months).

Toxicity testing with both *C. dubia* (acute water column) and *C. fluminea* (35-day *in situ*) detected periodic acute and chronic water column toxicity at sites in and below the entrance of the effluent into Parker Run. Simple linear regression analysis demonstrated that both acute water column toxicity to *C. dubia* and reduced benthic macroinvertebrate community (as EPT richness) values were significantly correlated with the high concentration of TDS contributed by the active mine effluent discharged into Parker Run. Further, EPT richness was the most sensitive index of the benthic macroinvertebrate community recovery used in this study.

The disturbance in Parker Run had two different characteristics. The initial coal slurry spill was an acutely toxic, pulse disturbance from which the stream community above the effluent quickly recovered. The sites below the effluent were subjected to a chronically toxic, press disturbance, which hindered full recovery from the initial coal slurry spill. The periodically detected toxicity of the mine effluent, which was significantly correlated with TDS concentrations observed in-stream, was primarily responsible for the incomplete recovery of the benthic macroinvertebrate community in this system.

Chapter 3.

Laboratory Evaluation of Acutely Toxic Components of a Synthetic Active Coal Mine Effluent

Introduction

The United States Environmental Protection Agency (USEPA) has established regulatory limitations on the concentrations of specific ions and substances in effluents and bodies of water for the protection of aquatic life. Currently, there are no federally regulated limitations placed on concentrations of sodium (Na^+), sulfate ($SO_4^=$), total dissolved solids (TDS), or on the conductivity (µmhos/cm) of effluents discharged into freshwater systems. Treated effluent from the Meigs #31 coal mine in Meigs County, Ohio, contributes high concentrations of Na^+ (~900 mg/L), $SO_4^=$ (~2,000 mg/L), Cl^- (~400 mg/L), TDS (~4,000 mg/L) and elevated conductivity (~5,200 µmhos/cm) to the receiving stream, Parker Run. Data gathered during a field study of the recovery of the benthic community in Parker Run from a coal slurry suggested that elevated concentrations of TDS may be responsible for a lack of recovery and persistence of a diminished benthic macroinvertebrate community in the stream (Latimer, 1999, Ch.2). Further, water column toxicity was observed in 48-hr acute *Ceriodaphnia dubia* bioassays conducted on Parker Run water when the conductivity of that water was at or above 6,000 µmhos/cm (Latimer, 1999, Ch. 1).

Several studies have demonstrated toxicity to laboratory bioassay organisms associated with either elevated concentrations of typically innocuous ions (ex: Na^+, K^+, HCO_3^-, and $CO_3^=$), high conductivity (µmhos/cm) or increased concentrations of TDS. Meyer et al (1985) observed raw oil shale leachates to be acutely toxic to both *Daphnia magna* and *Pimephales promelas* when the conductivity and TDS of the solution fell between 7,000 - 8,000 µmhos/cm and 6,000 – 7,000 mg/L, respectively, with magnesium (Mg^{++}) and sulfate ($SO_4^=$) as the major ions (80 - 90% of solute). Further analysis with a reconstituted leachate detected significant, positive correlations between 48-hr *D. magna* LC_{50} values and $SO_4^=$, and between 96-hr *P. promelas* LC_{50} values and both Mg^{++} and conductivity. High concentrations of the major inorganic ions (Na^+, K^+, Cl^-, HCO_3^- and $CO_3^=$) in a saline oil-field discharge were determined by Boelter et al (1992) to cause acute toxicity as 48-hr *C. dubia* LC_{50} values were similar for both raw and reconstituted effluents. The authors suggested that the high salinity (not analyzed; although conductivity values ranged from 6,000 – 6,400 µmhos/cm during the sampling period), a measure similar to TDS, may have had toxic effects which were not considered. Dickerson et al (1996) reported similar acute toxicity to *C. dubia* and *P. promelas* when TDS levels reached 12,000 – 18,000 mg/L due to elevated concentrations of major ions (Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, $SO_4^=$, HCO_3^-, and $CO_3^=$) in irrigation drain water.

In another study of irrigation drain water, Ingersoll et al (1992) used both freshwater and saltwater organisms in an attempt to differentiate between acute toxicity associated with salinity and that of trace elements in solution. Salinity levels of approximately 19 g/L were toxic to *P. promelas*, *D. magna* and *Hyalella azteca*, while elevated concentrations of inorganic ions (B^{+3}, Mg^{++}, K^+, Na^+, and Sr^{++}) were toxic to saltwater acclimated *Morone saxatilis* and *H. azteca*. Finally, Goetsch and Palmer (1997) found that the toxicity of a solution high in dissolved ions to a South African mayfly (*Tricorythus* sp.) is linked to the conductivity and TDS of that solution. Further, the authors determined that the toxicity of the solution was partially dependent upon the type of salt used in elevating the dissolved ions in solution.

The goal of this investigation was to use *C. dubia* acute toxicity bioassays to determine what parameters (Na^+, $SO_4^=$, TDS and conductivity) act, alone or in concert, to cause toxicity. A further goal was to determine whether potential toxicity/regulatory limits could be determined for any of the above 4 parameters.

Methods

Acute Toxicity Bioassays

Forty eight hour, static acute toxicity bioassays using *Ceriodaphnia dubia* (<24 hr) were conducted on four solutions of different salt combinations (Table 1) according to United States Environmental Protection Agency protocols (USEPA, 1993). A 0.75 dilution series was used exclusively in testing solutions 1 (conc. ranged from 1,500 – 475 as mg/L cations), 2 (2,000 – 475), and 4 (2,000 – 633), while a 0.5 dilution series was used to dilute only from the highest (4,000) to the second highest concentration (2,000) in testing solution #3, below that (from 2,000 – 633) a 0.75 dilution was used. Each solution was tested twice and the 48-hr LC_{50} value (with upper and lower 95% confidence limits) for a given parameter was calculated using mean survival and water chemistry data. Spearman Karber 48-hr LC_{50} values were calculated using the Toxstat® statistical package (Gulley, 1993). Moderately hard synthetic water (MHSW) was used as both culture water and as diluent in testing (APHA et al 1992). Organisms were cultured and tests were conducted at 25 ± 1°C, with a photoperiod of 16 hours light, 8 hours dark. Prior to testing, organisms were fed the green algae, *Selenastrum capricornutum* and a mix of yeast, cereal leaves and trout chow (YCT) at a rate of 0.18 ml/30 ml of each per day.

Chemical Analysis

Two samples (beginning and end) from each test concentration were analyzed for each parameter. Water from each concentration was analyzed for pH, conductivity (µmhos/cm), and dissolved oxygen (DO, mg/L) using an Accumet® pH meter (model 15), YSI (Yellow Springs Instrument Co, inc.) model 51B dissolved oxygen meter, and a Hach Co. model 44600 conductivity/TDS meter, respectively. TDS (mg/L) measurements were made according to Standard Methods (APHA, et al 1992) with one modification in that samples were dried overnight at 80°C before being dried at 180 ± 2°C for 1 hour.

Table 1. Summary of amount of each salt added to MHSW to create each test solution (# 1-4). Total nominal concentrations of Na^+ (mg/L) and cations (mg/L) are also listed.

<table>
<tr><th>Solution #</th><th colspan="20">Amount of each salt added to solution</th><th>Nominal conc. of Na^+ (mg/L) in 100% sol.</th><th>Nominal conc. of cations (mg/L) in 100% sol.</th></tr>
<tr><td>1</td><td colspan="10">6.52 ml NaOH (10 N)</td><td colspan="10">~1.95 ml H_2SO_4 (36 N)</td><td>1,500</td><td>1,500</td></tr>
<tr><td>2</td><td colspan="4">1.235 g Na_2SO_4</td><td colspan="4">1.017 g NaCl</td><td colspan="4">1.478 g $NaNO_3$</td><td colspan="4">1.461 g $NaHCO_3$</td><td colspan="4">1.426 g CH_3COONa</td><td>2,000</td><td>2,000</td></tr>
<tr><td>3</td><td colspan="4">3.960 g $MgSO_4$</td><td colspan="4">1.510 g CaCl</td><td colspan="4">2.068 g KNO_3</td><td colspan="4">2.000 g $CaCO_3$</td><td colspan="4">2.852 g CH_3COONa</td><td>800</td><td>4,000</td></tr>
<tr><td>4</td><td colspan="5">2.475 g $MgSO_4$</td><td colspan="5">1.700 g $CaSO_4$</td><td colspan="5">1.544 g Na_2SO_4</td><td colspan="5">1.114 g K_2SO_4</td><td>500</td><td>2,000</td></tr>
</table>

All metals analysis was conducted at the inductively coupled plasma spectrometry (ICP) Laboratory on the campus of VPI&SU. Only Na^+ concentrations were analyzed in water from solutions 1 and 2. Concentrations of Na^+, K^+, Mg^{++}, and Ca^{++} were measured in water from solutions 3 and 4. Concentrations of sulfate ($SO_4^=$) were measured in water from solution #4. Additionally, $SO_4^=$ (mg/L) data used to calculate LC50 values in solutions 1-3 were nominal measures calculated from measured concentrations of the corresponding salt in solution. Sulfate concentrations were analyzed by ion chromatography in the Civil Engineering Department at VPI& SU. All values reported were means from each replicate set of tests.

Results

Acute Toxicity Bioassays

Conductivity, TDS, Na^+ and $SO_4^=$ 48-hr LC_{50} values to *C. dubia* varied markedly with the composition of the solution tested (Table 2, Figure 1). Bioassays conducted on solution 1, with Na^+ and $SO_4^=$ as the two major ions, yielded the highest mean Na^+ 48-hr LC_{50} (1044.3 mg/L), the second highest $SO_4^=$ (2250.76 mg/L), and the second lowest conductivity (4416 µmhos/cm) and TDS (3536.6 mg/L) LC_{50} values. Tests of solution 2, comprised of 5 different Na^+ salts, resulted in the second highest Na^+ (885.1 mg/L), and the lowest $SO_4^=$ (352.5 mg/L), conductivity (3572 µmhos/cm), and TDS (2639.7 mg/L) 48-hr LC_{50} values. The lowest Na^+ (342.2 mg/L), the second lowest $SO_4^=$ (1271.4 mg/L), and the second highest conductivity (4808 µmhos/cm) and TDS (4241.1 mg/L) LC_{50} values were determined for solution 3, made up of 5 salts of 4 separate cations. The final solution tested, #4, composed of 4 different $SO_4^=$ salts, yielded the highest conductivity (6264 µmhos/cm), TDS (6448.0 mg/L), and $SO_4^=$ (4519.4 mg/L) LC_{50} values and the second lowest Na^+ (519.3 mg/L) value.

Table 2. Summary of 48-hr *Ceriodaphnia dubia* LC_{50} values for conductivity, TDS, Na^+ and $SO_4^=$ from each of the four solutions tested. Solution 1 contained sodium and sulfate, solution 2 contained 5 sodium salts, solution 3 contained salts of 4 different cations with 5 anions, and solution 4 contained 4 different sulfate salts with different cations.

	48-hr LC_{50} Value			
Solution Tested	Conductivity (µmhos/cm)	TDS (mg/L)	Na^+ (mg/L)	$SO_4^=$ (mg/L)
1	4,416	3,536.60	1,044.27	2,250.76
2	3,572	2,639.70	885.09	352.5
3	4,808	4,241.10	342.21	1,271.39
4	6,264	6,448	519.28	4,519.43

The highest LC_{50} values for conductivity (4807 and 6264 µmhos/cm) and TDS (4241.1 and 6448.0 mg/L) were calculated for solutions 3 and 4 respectively, which both contained multiple cations (Na^+, K^+, Mg^{++} and Ca^{++}). These solutions also received the least toxicity from Na^+, as calculated LC_{50} values for Na^+ in these solutions were the lowest among the 4 solutions tested.

The toxicity of solutions containing Na^+ as the dominant cation (solutions 1 and 2) appeared to be controlled by the concentration of Na^+ in solution more than the conductivity, TDS or $SO_4^=$ concentration in solution. The LC_{50} values for conductivity and TDS of solutions 3 and 4, both with multiple cations, were much higher than those measured in the first two solutions. The toxicity of $SO_4^=$ varied widely with the solution tested and appeared to have the least effect on the toxicity of the first three solutions. $SO_4^=$ may have had an influence on the toxicity of solution 4 as the LC_{50} (4519.4 mg/L) value was more than twice that of the $SO_4^{=}$ LC_{50} value calculated for any other solution (other values ranged from 352.5 – 2250.8 mg/L).

Another factor that may have affected the toxicity of each solution was the total cation concentration (mg/L). The total cation LC_{50} for solution 3 (1201.2 mg/L) was higher than, but similar to that of solution 1 (1044.3 mg/L as Na^+), and considerably higher than the LC_{50} for solution 2 (885.1 mg/L as Na^+). However, the total cation LC_{50} for solution 4 (1905.0 mg/L) was much higher than for any other solution.

These data suggest that toxicity of solutions with Na^+ as the dominant cation, and one or more commonly innocuous anions in solution, is controlled by the concentration of Na^+ in that solution. Further, the acute toxicity of Na^+ appears to have a threshold of approximately 900 – 1,000 mg/L. However, the toxicity of solutions that have $SO_4^{=}$ as the dominant ion, with several major cations in solution, is controlled by the total amount of dissolved ions or TDS, similarly measured as the conductivity of a solution.

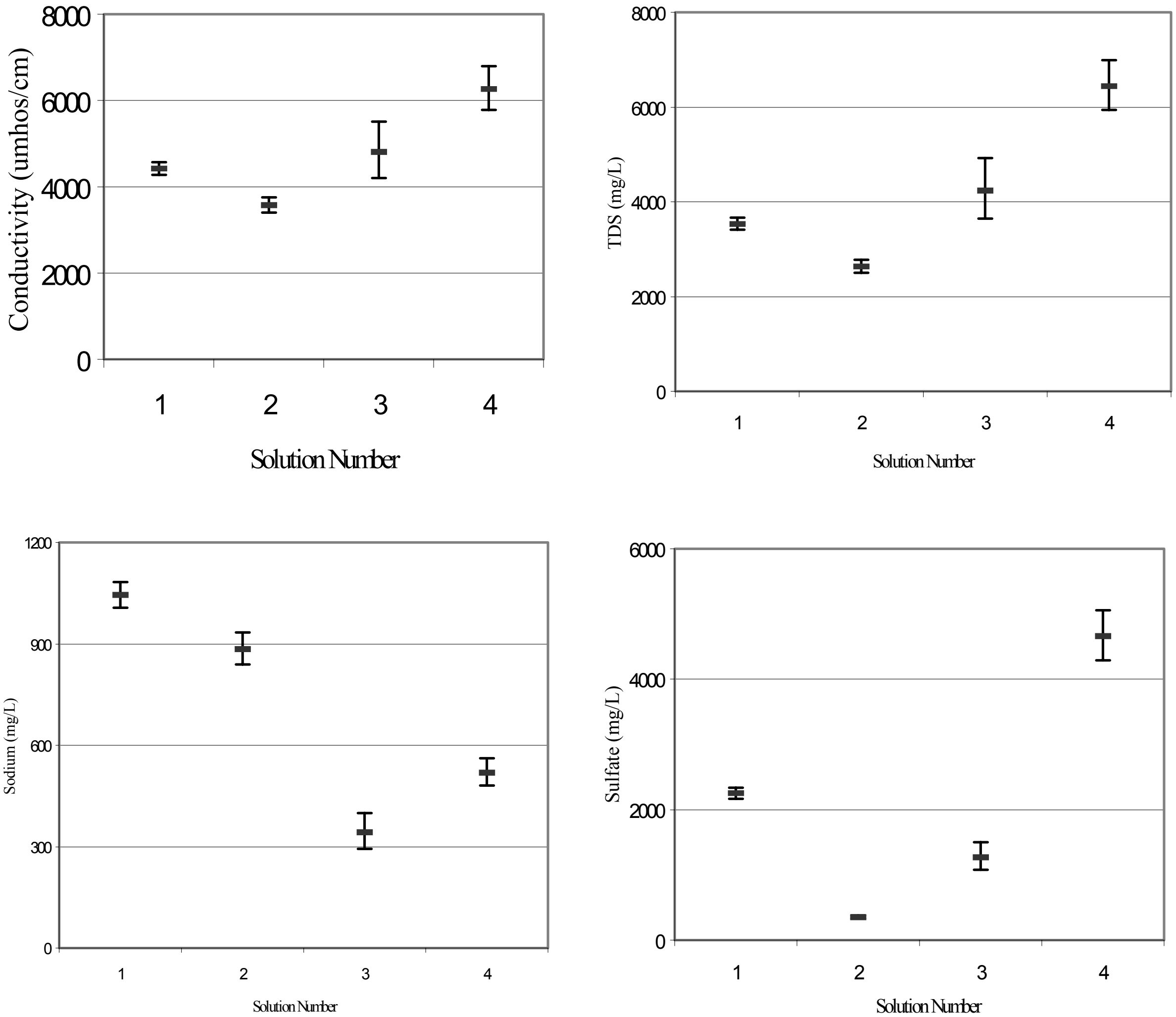


Figure 1 A-D. Summary of 48-hr *Ceriodaphnia dubia* LC_{50} values for conductivity (µmhos/cm), TDS (mg/L), sodium (mg/L) and sulfate (mg/L) from each of the four solutions tested. Solution 1 contained sodium and sulfate, solution 2 contained 5 sodium salts, solution 3 contained salts of 4 different cations with 5 anions, and solution 4 contained 4 different sulfate salts with different cations. Error bars are 95% confidence intervals.

.

Discussion

The results of this study were similar to that reported by other researchers in the literature. The toxicity of solutions with high concentrations of dissolved ions is dependent upon the ions making up the solution. Goetsch and Palmer (1997) in a study of the differential toxicity of high salt solutions made up of NaCl and $NaSO_4$ to a South African mayfly, determined that not only was the toxicity related to the total concentration of TDS in that solution, but that the type of salt(s) in solution strongly controlled the toxicity. Mount et al (1997) conducted a laboratory investigation of the acute toxicity of over 2,900 solutions, in an attempt to rank the toxicity of several common major ions (K^+, HCO_3^-, Mg^{++}, Cl^-, $SO_4^=$, Na^+ and Ca^{++}) that typically contribute to solutions with high concentrations of TDS. The authors stated that while the toxicity of a solution is correlated with increasing TDS or conductivity, these are not reliable tools in determining the toxicity of that solution. However, neither TDS nor conductivity were included in their statistical modeling of the toxic elements. Further, Mount et al (1997) concluded that any toxicity associated with Na^+ is attributable to the corresponding anion (ex: Cl^- or $SO_4^=$), and that Na^+ is not a major contributor to the toxicity of a solution.

The results of this investigation differ from those of Mount et al (1997) in that current data seem to point to a toxicological limit of Na^+ to *C. dubia* when in solution with one or more common anions. The toxic effects of $SO_4^=$ were somewhat less easy to deduce from data generated in testing the four solutions. Mount et al (1997) concluded that $SO_4^=$ was a more toxic constituent than Na^+ and that its toxicity may be reduced when in solution with more than one cation. The 48-hr LC_{50} for $SO_4^=$ generated from solution 4, with multiple cations in solution (4,519.4 mg/L) was much higher than that calculated for a solution with only one cation (solution 1, 2,250.8 mg/L). However, similar LC_{50} values were generated for Na^+ in solutions containing several major anions (solution 2, 885.1 mg/L) and a solution with $SO_4^=$ as the only major anion (solution 1, 1,044.3). These results suggest that Na^+ had a similar effect on the toxicity of a solution with $SO_4^=$ as the only other major ion (solution 1), as it did for one with multiple anions. These data support the conclusion that in solutions with high concentrations of Na^+ as the major cation with one or more common anions, Na^+ does contribute to the toxicity of that solution. Further, the acutely toxic effects of Na^+ to *C. dubia* begin to manifest themselves when Na^+ concentrations reach ~850 – 1,000 mg/L.

The LC_{50}'s generated for conductivity and TDS were higher for a solution containing several cations and $SO_4^=$ as the major anion (solution 4) than for a solution with several major cations and anions (solution 3). The LC_{50} data from solution 4 may represent the diminished toxicity of $SO_4^=$ (LC_{50} of 4,519.4 mg/L) in solution with several cations, as suggested by Mount et al (1997), or it may more accurately reflect the toxic influence of the total concentration of dissolved ions, measured as TDS. It is probable that the LC_{50} values generated for conductivity (6,364 µmhos/cm) and TDS (6,448.0 mg/L) for solution 4 represent an upper tolerance level of *C. dubia* to concentrations of ions in solution, regardless of the individual toxic contributions of each ion. These measures of toxic concentrations of dissolved solids are similar to others reported in the literature to be lethal to cladocerans. The salinity of a solution, a measure similar to that of TDS, was reported by Ingersoll et al (1992) to be toxic to *D. magna* at concentrations of 8,000 – 10,000 mg/L. As *D. magna* are a more robust cladoceran species than *C. dubia*, these data are probably comparable. Boelter et al (1992) suggested that high salinity, in an effluent with conductivity values ranging from 6,000 – 6,400 µmhos/cm, may have contributed to observed *C. dubia* acute toxicity. Hence, it is reasonable to conclude that solutions with conductivity values in excess of ~6,250 µmhos/cm and TDS concentrations above ~6,400 will be acutely toxic to *C. dubia*, no matter the toxicity of individual ion constituents.

Conclusions

The use of several solutions of multiple salt mixtures allowed a high degree of precision to be achieved in separating the toxic influences of the four parameters in question (Na^+, $SO_4^=$, conductivity and TDS). The results allowed differentiation between the toxicity of two ions commonly found in solution together in active coal mine effluent (Na^+ and $SO_4^=$) by testing each ion in solution with different common cations or anions.

The toxicity of a solution with a high concentration of TDS is largely dependent upon the salts used to elevate the TDS of that solution. When present in concentrations of approximately 900 – 1,000 mg/L, Na^+ contributes to the acute toxicity of a solution to *C. dubia*. Acute toxicity of solutions, in which $SO_4^=$ is the major anion accompanied by several common cations (Mg^{++}, Ca^{++}, K^+, and Na^+), was observed when the concentration of TDS approached 6,500 mg/L and conductivity exceeded 6,000 µmhos/cm. These values appear to represent the upper tolerance limits of *C. dubia* to solutions with high concentrations of common ions.

The contribution of $SO_4^=$ to the toxicity of a solution with high concentration of Na^+ (~1,000 mg/L) or TDS (6,000 mg/L) appears to be secondary to the toxic contribution of Na^+ or the total concentration of dissolved ions, measured as TDS.

While the current study probably did not provide a broad enough base upon which to establish regulatory limits, it does demonstrate that toxicological thresholds start at ~1,000 mg/L Na^+, ~6,000 µmhos/cm conductivity, and ~6,400 mg/L TDS.

Introduction

Literature Cited

1. Birge, W.J, D.J. Price, M.D. Kercher, and J.R. Shaw. 1995. Transport, Fate and Effects of Metals in Sediment Systems Associated with the Raccoon and Leading Creek Watersheds, Southeastern Ohio. Technical Report submitted to United States Department of Justice.

2. Consent Decree and Settlement Agreement. 1995. United States vs. Southern Ohio Coal Company. United States District Court for the Southern District of Ohio, Eastern Division.

3. Cherry, D.S., R.J. Currie, H.A. Latimer, J. Cairns, Jr., J.E. Babendreier, R.B. Diz, D. Gallager, D.M. Johnson, and M.M. Yeager. 1998. Leading Creek Improvement Plan. Southern Ohio Coal Company, Meigs Mine No. 31, Leading Creek Improvement Plan. Final Report.

4. Maloney, M. 1994. American Electric Power, Southern Ohio Coal Company, Meigs No. 31 Mine. Water Quality and Sediment Quality Studies. U.S. Environmental Protection Agency, Region 5. Eastern District Office, Westlake, OH.

Chapter One

Literature Cited

1. American Public Health Association, American Water Works Association and Water Environment Federation. 1992. Standard Methods for the Examination of Water and Wastewater, 18th edition. American Public Health Association, 1015 Fifteenth St., NW, Washington, DC 20005.

2. Birge, W.J, D.J. Price, M.D. Kercher, and J.R. Shaw. 1995. Transport, Fate and Effects of Metals in Sediment Systems Associated with the Raccoon and Leading Creek Watersheds, Southeastern Ohio. Technical Report submitted to United States Department of Justice. 44pp.

3. Bjornn, T.C., M.A. Brusven, M.P. Molnau, J.H. Milligan, R.A. Klamt, R. Chacho, and C. Schaye. 1977. Transport of Granitic Sediment in Streams and its Effects on Insects and Fish. University of Idaho, College of Forestry, Wildlife and Range Sciences, Bulletin Number 17.

4. Boelter, A.M., F.N. Lamming, A.M. Farag, and H.L. Bergman. 1992. Environmental Effects of Saline Oil-field Discharges on Surface Waters. Environmental Toxicology and Chemistry. 18: 1187-1195.

5. Chapman, G.A, et al. Effects of Water Hardness on the Toxicity of Metals to *Daphnia magna*. United States Environmental Protection Agency, Corvallis, OR. (Manuscript)

6. Culp, J.M., F.J. Wrona, and R.W. Davies. 1986. Response of Stream Benthos and Drift to Fine Sediment Deposition versus Transport. Canadian Journal of Zoology. 64:1345-1351.

7. Cherry, D.S. and M.G. Dobbs. 1993. Duquesne Light Company, Beaver Valley Power Station, Ohio River In-Situ Corbicula Growth Study. Technical Report submitted to Duquesne Light Company, Shippingport, PA 15077.

8. Cherry, D.S. 1996. State of the Art of *In Situ* Testing (Transplant Experiments) in Hazard Evaluation. SETAC News. 16(5): 24-25.

9. Cherry, D.S. and R.J. Currie. 1997. Benthic Macroinvertebrate Assemblages, Habitat Assessment, Laboratory Chronic and In Situ Sediment Toxicity Testing in the Ely Creek Watershed Restoration Project Plan. Final Report. Submitted to Virginia Division of Mined Land Reclamation.

10. Cherry, D.S., R.J. Currie, H.A. Latimer, J. Cairns, Jr., D.M. Johnson, and M.M. Yeager. 1998a. Leading creek Improvement Plan. Southern Ohio Coal

Company Meigs Mine No. 31 Leading Creek Improvement Plan. Submitted to United States Fish and Wildlife Service.

11. Cherry, D.S., D.J. Soucek, R.J. Currie and H.A. Latimer. 1998b. Benthic Macroinvertebrate Assemblages, Habitat Assessment, Laboratory Chronic and In-Situ Sediment Toxciity Testing in the Puckett's Creek Watershed Restoration Project: Final Report. Submitted to Virginia Division of Mined Land Reclamation.

12. Dance, K.W. and H.B.N. Hynes. 1980. Some Effects of Agricultural Land Use on Stream Insect Communities. Environmental Pollution (Series A). 22:19-28.

13. Dickerson, K.W., W.A. Hubert, and H.L. Bergman. 1996. Toxicity Assessment of Water from Lakes and Wetlands Receiving Irrigation Drain Water. Environmental Toxicology and Chemistry. 5:1097-1101.

14. Doherty, F.G. 1990. The Asiatic Clam, *Corbicula* spp., as a Biological Monitor in Freshwater Environments. Environmental Monitoring and Assessment. 15:143-181.

15. Driscoll, C.T., J.P. Baker, J.J. Bisogni and C.L. Schofield. 1980. Nature (London). 284:161.

16. Fucik, K.W., J. Herron and D. Fink. 1991. The Role of Biomonitoring in Measuring the Success of Reclamation at a Hazardous Waste Site. Aquatic Toxicology and Risk Assessment: Fourteenth Volume, ASTM STP 1124, Mayes, M.A. and M.G. Barron, Eds., American Society for Testing and Materials, Philadelphia, 1991, pp. 212-220.

17. Graham, A.A. 1990. Siltation of Stone-Surface Periphyton in Rivers by Clay-Sized Particles from low Concentrations in Suspension. Hydrobiologia 199: 107-115.

18. Grundl, T. and J. Delwiche. 1993. Kinetics of Ferric Oxyhydroxide Precipitation. Journal of Contaminant Hydrology. 14:71-87.

19. Gulley, D.D. 1993. Toxstat® 3.3. University of Wyoming, Department of Zoology and Physiology. Laramie, Wyoming 82071.

20. Helliwell, S., G.E. Batley, T.M. Florence, and G.G. Lumsden. 1983. Environmental Technology Letters. 4:141.

21. Herlihy, A.T., P.R. Kaufmann, and M.E. Mitch. 1990. Regional Estimates of Acid Mine Drainage Impact on Streams in the Mid-Atlantic and Southeastern United States. Water, Air, and Soil Pollution. 50:91-107.

22. Ingersoll, C.G., F.J. Dwyer, S.A. Burch, M.K. Nelson, D.R. Buckler and J.B. Hunn. 1992. The Use of Freshwater and Saltwater Animals to Distinguish between the Toxic Effects of Salinity and Contaminants in Irrigation Drain Water. Environmental Toxicology and Chemistry. 11:503-511.

23. Jop, K.M. and A.M. Askew. 1994. Toxicity Identification Evaluation Using a Short-term Chronic Test with Ceriodaphnia dubia. Bulletin of Environmental Contamination and Toxicology. 53:91-97.

24. Kelly, M. 1988. Chapter 8. Effects of Acid Mine Drainage on the Biota. In, Mining and the Freshwater Environment. Elsevier Applied Science, New York. Pp. 108-126.

25. Kratz, K.W., S.D. Cooper and J.M. Melack. 1994. Effects of Single and Repeated Experimental Acid Pulses on Invertebrates in a High Altitude Sierra Nevada Stream. Freshwater Biology. 32:161-183.

26. Lemly, A.D. 1982. Modification of Benthic Insect Communities in Polluted Streams: Combined Effects of Sedimentation and Nutrient Enrichment. Hydrobiologia. 87:229-245.

27. Lenat, D.R. 1984. Agriculture and Stream Water Quality: a Biological Evaluation of Erosion Control Practices. Environmental Management. 8(4): 333-344.

28. McCahon, C.P., A.F. Brown, M.J. Poulton, and D. Pascoe. 1989. Effects of Acid, Aluminum and Lime additions on Fish and Invertebrates in a Chronically Acidic Welsh Stream. Water, Air and Soil Pollution. 45:345-359.

29. McCauley, D.J., L.T. Brooke, D.J. Call, C.A. Lindberg. 1986. Acute and chronic Toxicity of Aluminum to *Ceriodaphnia dubia* at Various pH's. University of Wisconsin, Superior.

30. McClelland, W.T. and M.A. Brusven. 1980. Effects of Sedimentation on the Behavior and Distribution of Riffle Insects in a Laboratory Stream. Aquatic Insects. 2 (3): 161-169.

31. McElfish, J.M., Jr. and A.E. Beier. 1990. Environmental Regulation of Coal Mining, SMCRA's Second Decade. Environmental Law Institute Monograph. Environmental Law Institute. Washington, D.C.

32. Merritt, R.W. and K.W. Cummins. 1996. An Introduction to the Aquatic Insects of North America, 3rd edition. Kendall/Hunt Publishing Company, Dubuque, Iowa 52002.

33. Meyer, J.S., D.A. Sanchez, J.A. Brookman, D.B. McWhorter, and H.L. Bergman. 1985. Chemistry and Aquatic Toxicity of Raw Oil Shale Leachates from Piceance Basin, Colorado. Environmental Toxicology and Chemistry. 4:559-572.

34. Mills, A.L. 1985. In Klein, D. and R.L. Tate, Eds., Soil Reclamation Processes, Marcel Dekker, Inc. New York, pp. 35-81.

35. Nebeker, A.V., M.A. Cairns, J.H. Gakstatter, K.W. Malueg, G.S. Schuytema and D.F. Krawczyk. 1984. Biological Methods for Determining Toxicity of Contaminated Freshwater Sediment to Invertebrates. Environmental Toxicology and Chemistry. 3:617-630.

36. OEPA. 1991. Biological and Water Quality Study of the Southeast Ohio River Tributaries. Ecological Assessment Section, Nonpoint Source Program Management Section, Surface Water Section, Southeast District Office.

37. OEPA. 1989. Biological Criteria for the Protection of Aquatic Life: Volume III: Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Macroinvertebrate Communities. Ecological Assessment Section, Division of water Quality Planning and Assessment.

38. Ormerod, S.J., P. Boole, C.P. McCahon, N.S. Weatherley, D. Pascoe, and R.W. Edwards. 1987. Short-term Experimental Acidification of a Welsh Stream: Comparing the Biological Effects of Hydrogen ions and Aluminium. Freshwater Biology. 17:341-356.

39. Pennak, R.W. 1989. Fresh-Water Invertebrates of the United States, 3rd edition. John Wiley & Sons, Inc. New York.

40. Ramsey, D.L., and D.G. Brannon. 1988. Predicted Acid Mine Drainage Impacts to the Buckhannon River, WV, U.S.A. Water, Air and Soil Pollution. 39:1-14.

41. Roback, S.S., and J.W. Richardson. 1969. The Effects of Acid Mine Drainage on Aquatic Insects. Proceedings of the Academy of Natural Science of Philadelphia. 121:81-107.

42. Sall, J. and A. Lehman. 1996. JMP Start Statistics. Version 3.2.1. SAS Institute Inc.

43. Scullion, J. and R.W. Edwards. 1980. The effects of Coal Industry Pollutants on the Macroinvertebrate Fauna of a Small River the South Wales Coalfield. Freshwater Biology. 10:141-162.

44. Skinner, W.D. and D.E. Arnold. 1990. Short Term Biotic Response before and during the Treatment of an Acid Mine Drainage with Sodium Carbonate. Hydrobiologia. 199:229-235.

45. Snoeyink, V.L. and D. Jenkins. 1980. Water Chemistry. John Wiley & Sons, New York.

46. Soucek, D.J., D.S. Cherry, R.J. Currie, H.A. Latimer, and G.C. Trent. 1999. Laboratory to Field Validation in an Integrative assessment of an Acid Mine Drainage Impacted Watershed. In review.

47. Stubblefield, W.A, S.F. Brinkman, P.H. Davies, T.D. Garrison, J.R. Hockett and M.W. McIntyre. 1997. Effects of water Hardness on the Toxicity of Manganese to developing Brown Trout (*Salmo trutta*). Environmental Toxicology and Chemistry. 16(10): 2082-2089.

48. Stumm, W. and J.J. Morgan. 1970. Aquatic Chemistry. Wiley-Interscience, New York.

49. USEPA. 1981. Ambient Water Quality Criteria for Zinc. Office of Water Regulations and Standards, Washington, D.C. 20460.

50. USEPA. 1985. Ambient Water Quality Criteria for Copper. Office of Water Regulations and Standards, Washington, D.C. 20460. EPA 440/5-84-031.

51. USEPA. 1986. Quality Criteria for Water, 1986. Office of Water Regulations and Standards, Washington, D.C. 20460. May 1, 1986. EPA 440/5-86-001.

52. USEPA. 1990. The Quality of our Nation's Water: A summary of the 1988 National Water Quality Inventory. U.S. Environmental Protection Agency, EPA Report 440/4-90-005, Washington, D.C.

53. USEPA. 1994. Methods for Measuring the Toxicity of Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates. EPA/600/R-94/024

54. Vinyard, G.L. 1996. A Chemical and biological Assessment of water Quality Impacts from Acid Mine Drainage in a First Order mountain Stream, and a Comparison of Two bioassay Techniques. Environmental Technology. 17:273-281.

55. Waters, T.F. 1995. Sediment in Streams: Sources, Biological Effects, and Control. American Fisheries Society Monograph 7. American Fisheries Society. Bethesda, MD.

56. Weatherley, N.S., S.J. Ormerod, S.P. Thomas, R.W. Edwards. 1988. The Response of Macroinvertebrates to Experimental Episodes of low pH with different forms of Aluminium, during a Natural Spate. Hydrobiologia. 169:225-232.

57. Weber, C.I. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. 1993. EPA/600/4-90/027F. U.S. Environmental Protection Agency. 293pp.

58. Welch, E.B., J.M. Jacoby, R.R. Horner, and M.R. Seeley. 1988. Nuisance Biomass Levels of Periphytic Algae in Streams. Hydrobiologia. 157: 161-168.

59. Whiting, E.R. and H.F. Clifford. 1983. Invertebrates and Urban Runoff in a Small Northern Stream, Edmonton, Alberta, Canada. Hydrobiologia. 102:73-80.

Chapter Two

Literature Cited

1. American Public Health Association, American Water Works Association and Water Environment Federation. 1992. Standard Methods for the Examination of Water and Wastewater, 18th edition. American Public Health Association, 1015 Fifteenth St., NW, Washington, DC 20005.

2. Bender, E.A., T.J. Case, and M.E. Gilpin. 1984. Perturbation Experiments in Community Ecology: Theory and Practice. Ecology. 65: 1-13.

3. Birge, W.J., D.J. Price, M.D. Kercher, and J.R. Shaw. 1995. Transport, Fate and Effects of Metals in Sediment Systems Associated with the Raccoon and Leading Creek Watersheds, Southeastern Ohio. Technical report submitted to the United States Department of Justice. 44pp.

4. Boelter, A.M., F.N. Lamming, A.M. Farag, and H.L. Bergman. 1992. Environmental Effects of Saline Oil-Filed Discharges on Surface Waters. Environmental Toxicology and Chemistry. 11: 1187-1195.

5. Cairns, J., Jr. 1990. Lack of Theoretical Basis for Predicting Rate and Pathways of Recovery. Environmental Management. 14(5): 517-526.

6. Chadwick, J.W., S.P. Canton, and R.L. Dent. 1986. Recovery of Benthic Invertebrate Communities in Silver Bow Creek, Montana, Following Improved Metal Mine Wastewater Treatment. Water, Air, and Soil Pollution. 28: 427-438.

7. Cherry, D.S. 1996. State of the Art of In Situ Testing (Transplant Experiments) in Hazard Evaluation. SETAC News. 16(5): 24-25.

8. Cherry, D.S. and M.G. Dobbs. 1993. Duquesne Light Company, Beaver Valley Power Station, Ohio River In-Situ Corbicula Growth Study. Technical Report submitted to Duquesne Light Company, Shippingport, PA 15077.

9. Cherry, D.S., R.J. Currie, H.A. Latimer, J. Cairns, Jr., D.M. Johnson, and M.M. Yeager. 1998a. Leading Creek Improvement Plan. Southern Ohio Coal Company, Meigs Mine No. 31, Final Report. Submitted to the United States Fish and Wildlife Service.

10. Cherry, D.S., D.J. Soucek, R.J. Currie, and H.A. Latimer. 1998b. Benthic Macroinvertebrtae 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.

11. Crunkilton, R.L. and R.M. Duchrow. 1990. Impact of a Massive Crude Oil Spill on the Invertebrate Fauna of a Missouri Ozark Stream. Environmental Pollution. 63: 3-13.

12. Dickerson, K.K., W.A. Hubert, and H.L. Bergman. 1996. Toxicity Assessment of Water from Lakes and Wetlands Receiving Irrigation Drain Water. Environmental Toxicology and Chemistry. 15(7): 1097-1101.

13. Doherty, F.G. 1990. The Asiatic Clam, *Corbicula sp*p., as a Biological Monitor in Freshwater Environments. Environmental Monitoring and Assessment. 15: 143-181.

14. Fucik, K.W., J. Herron and D. Fink. 1991. The Role of Biomonitoring in Measuring the Success of Reclamation at a Hazardous Waste Site. Aquatic Toxicology and Risk Assessment: Fourteenth Volume, ASTM STP 1124, Mayes, M.A. and M.G. Barron, Eds., American Society for Testing and Materials, Philidelphia, 1991, pp. 212-220.

15. Goetsch, P.A. and C.G. Palmer. 1997. Salinity Tolerances of Selected Macroinvertebrates of the Sabie River, Kruger National Park, South Africa. Archives of Environmental Contamination and Toxicology. 32: 32-41.

16. Grundl, T. and J. Delwiche. 1993. Kinetics of Ferric Oxyhydroxide Precipitation. Journal of Contaminant Hydrology. 14: 71-87.

17. Gulley, D.D. 1993. Toxstat® 3.3. University of Wyoming, Department of Zoology and Physiology. Laramie, Wyoming 82071.

18. Guiney, P.D., J.L. Sykora, and G. Keleti. 1987. Environmental Impact of an Aviation Kerosene Spill on Stream Water Quality in Cambria County, Pennsylvania. Environmental Toxicology and Chemistry. 6: 977-988.

19. Hem, J.D. 1982. Chapter 4. Conductance: A Collective Measure of Dissolved Ions. In: Water Analysis, Volume I, Inorganic Species, Part 1. Minear, R.A. and L.H. Keith (eds.). Academic Press, New York.

20. Ingersoll, C.G., F.J. Dwyer, S.A. Burch, M.K. Nelson, D.R. Buckler and J.B. Hunn. 1992. The Use of Freshwater and Saltwater Animals to Distinguish between the Toxic Effects of Salinity and Contaminants in Irrigation Drain Water. Environmental Toxicology and Chemistry. 11:503-511.

21. Kelly, J.R. and M.A. Harwell. Indicators of Ecosystem Recovery. Environmental Management. 14(5): 527-545.

22. Lamberti, G.A., S.V. Gregory, L.R. Ashkenas, R.C. Wildman, and K.M.S. Moore. 1991. Stream Ecosystem Recovery Following a Catastrophic Debris Flow. Canadian Journal of Fisheries and Aquatic Sciences. 48: 196-208.

23. Latimer, H.A. 1999. An Ecotoxicological Evaluation of Active Coal Mine Effluent, Sedimentation, and Acid Mine Drainage in Three Tributaries of Leading Creek, Meigs County, Ohio. Master's Thesis, Virginia Polytechnic Institute and State University, Chapter 1.

24. Merritt, R.W. and K.W. Cummins. 1996. An Introduction to the Aquatic Insects of North America, 3rd edition. Kendall/Hunt Publishing Company, Dubuque, Iowa 52002.

25. Meyer, J.S., D.A. Sanchez, J.A. Brookman, D.B. McWhorter, and H.L. Bergman. 1985. Chemistry and Aquatic Toxicity of Raw Oil shale Leachates from Piceance Basin, Colorado. Environmental Toxicology and Chemistry. 4: 559-572.

26. Mount, D.R., D.D. Gulley, J.R Hockett, T.D. Garrison, and J.M. Evans. 1997. Statistical Models to Predict the Toxicity of Major Ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas* (Fathead Minnows). Environmental Toxicology and Chemistry. 16(10): 2009-2019.

27. Nebeker, A.V., M.A. Cairns, J.H. Gakstatter, K.W. Malueg, G.S. Schuytema, and D.F. Krawczyk. 1984. Biological Methods for Determining Toxicity of Contaminated Freshwater Sediment to Invertebrates. Environmental Toxicology and Chemistry. 3: 617-630.

28. Niemi, G.J., P. DeVore, N. Detenbeck, D. Taylor, A. Lima, and J. Pastor. 1990. Overview of Case Studies on Recovery of Aquatic Systems from Disturbance. Environmental Management. 14(5): 571-587.

29. OEPA. 1989. Biological Criteria for the Protection of Aquatic Life: Volume III: Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Macroinvertebrate Communities. Ecological Assessment Section, Division of water Quality Planning and Assessment.

30. Pennak, R.W. 1989. Fresh-Water Invertebrates of the United States, 3rd edition. John Wiley & Sons, Inc. New York.

31. Pontasch, K.W. and M.A. Brusven. 1988. Macroinvertebrate Response to a Gasoline Spill in Wolf Lodge Creek, Idaho, USA. Archive fur Hydrobiologia. 113(1): 41-60.

32. Rand, G.M. 1995. Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment. 2nd Ed. Taylor & Francis. Washington, D.C. 20005. 1125 pp.

33. Sall, J. and A. Lehman. 1996. JMP Start Statistics. Version 3.2.1. SAS Institute Inc.

34. Skinner, W.D. and D.E. Arnold. 1990. Short Term Biotic Response Before and During the Treatment of an Acid Mine Drainage with Sodium Carbonate. Hydrobiologia. 199: 229-235.

35. Specht, W.L., D.S. Cherry, R.A. Lechleitner, and J. Cairns, Jr. 1984. Structural, Functional, and Recovery Responses of Stream Invertebrates to Fly Ash Effluent. Canadian Journal of Fisheries and Aquatic Sciences. 41(6): 884-896.

36. Stubblefield, W.A., S.F. Brinkman, P.H. Davies, T.D. Garrison, J.R. Hockett and M.W. McIntyre. 1997. Effects of Water Hardness on the Toxicity of Manganese to Developing Brown Trout (*Salmo trutta*). Environmental Toxicology and Chemistry. 16(10): 2082-2089.

37. USEPA. 1988. Ambient Water Quality Criteria for Chloride. Office of Water Regulations and Standards, Washington, D.C. 20460. EPA 440/5-88-001.

38. USEPA. 1993. Methods for Measuring the acute Toxicity of effluents and Receiving Waters to Freshwater and Marine Organisms. EPA/600/4-90/027F.

39. USEPA. 1994. Methods for Measuring the Toxicity of Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates. EPA/600/R-94/024.

40. Wallace, J.B. 1990. Recovery of Lotic Macroinvertebrate Communities from Disturbance. Environmental Management. 14(5): 605-620.

41. Wallace, J.B., J.W. Grubaugh, and M.R. Whiles. 1996. Biotic Indices and Stream Ecosystem Processes: Results from an Experimental Study. Ecological Applications. 6(1): 140-151.

42. Whiles, M.R. and J.B. Wallace. 1995. Macroinvertebrate Production in a Headwater Stream during Recovery from Anthropogenic Disturbance and Hydrologic Extremes. Canadian Journal of Fisheries and Aquatic Sciences. 52(11): 2402-2422.

Chapter Three

Literature Cited

1. American Public Health Association, American Water Works Association and Water Environment Federation. 1992. Standard Methods for the Examination of Water and Wastewater, 18^{th} edition. American Public Health Association, 1015 Fifteenth St., NW, Washington, DC 20005.

2. Boelter, A.M., F.N. Lamming, A.M. Farag, and H.L. Bergman. 1992. Environmental Effects of Saline Oil-Field Discharges on Surface Waters. Environmental Toxicology and Chemistry. 11: 1187-1195.

3. Dickerson, K.A., W.A. Hubert, and H.L. Bergman. 1996. Toxicity Assessment of Water From Lakes and Wetlands Receiving Irrigation Drain Water. Environmental Toxicology and Chemistry. 15(7): 1097-1101.

4. Goetsch, P.A. and C. G. Palmer. 1997. Salinity Tolerances of Selected Macroinvertebrates of the Sabie River, Kruger national Park, South Africa. Archives of Environmental Contamination and Toxicology. 32: 32-41.

5. Gulley, D.D. 1993. Toxstat® 3.3. University of Wyoming, Department of Zoology and Physiology. Laramie, Wyoming 82071.

6. Ingersoll, C.G., F.J. Dwyer, S.A. Burch, M.K. Nelson, D.R. Buckler, and J.B. Hunn. 1992. The Use of Freshwater and Saltwater Animals to Distinguish between the Toxic Effects of Salinity and Contaminants in Irrigation Drain Water. Environmental Toxicology and Chemistry. 11: 503-511.

7. Latimer, H.A. 1999. An Ecotoxicological Evaluation of Active Coal Mine Effluent, Sedimentation, and Acid Mine Drainage in Three Tributaries of Leading Creek, Meigs County, Ohio. Master's Thesis, Virginia Polytechnic Institute and State University. Chapter 2.

8. Meyer, J.S., D.A. Sanchez, J.A. Brookman, D.B. McWhorter, and H.L. Bergman. 1985. Chemistry and Aquatic Toxicity of Raw Oil Shale Leachates from Piceance Basin, Colorado. Environmental Toxicology and Chemistry. 4: 559-572.

9. Mount, D.R., D.D. Gulley, J.R. Hockett, T.D. Garrison, and J.M. Evans. 1997. Statistical Models to Predict the Toxicity of Major Ions to *Ceriodaphnia Dubia*, *Daphnia magna*, and *Pimephales promelas* (Fathead Minnows). Environmental Toxicology and Chemistry. 16(10): 2009-2019.

10. USEPA. 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Office of research and development, Washington, D.C. EPA/600/4-90/027F.

Revised April 1999

Curriculum Vitae

Henry A. Latimer II

Office: Department of Biology
Virginia Polytechnic Institute and State University
2006 Derring Hall
Blacksburg, VA 24061
(540) 231-9071
Email: hlatimer@vt.edu

Present Position: Teaching Assistant
Department of Biology
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

Date and Place of Birth: 30 December 1972
Fairfax, Virginia

Education: January 1997 - Present: Enrolled as a Master of Science candidate
Virginia Polytechnic Institute and State University
Department of Biology
Major: Aquatic Ecotoxicology
Major Professor: Dr. D.S. Cherry
Expected date of graduation: May 1999
GPA: 3.92

Bachelor of Science, Biology
Mary Washington College
Fredericksburg, Virginia 22401
Graduation date: May 1995

Experience: January 1998 – May 1999: Teaching Assistant – Virginia Polytechnic Institute and State University (VPI&SU)
January 1997 – January 1998: Research Assistant – VPI&SU
Summer 1996 - Private contractor - Sampling for rare Odonates, mussels, and amphipods - Virginia Division of Natural Heritage

Training:

Benthic macroinvertebrate collection and identification.
Conduction of acute and chronic toxicity tests using *Ceriodaphnia dubia*, *Daphnia magna*, *Pimephales promelas* (fathead minnow), *Mysidopsis bahia*, and *Menidia beryllina* (inland silverside).

Conduction of *in situ* toxicity tests using *Corbicula flumenia* (Asian clam).
Conduction of chronic sediment toxicity tests using *D. magna* and *Chironomus tentans.*
Maintenance of cultures of *C. dubia, D. magna, D. pulex* and *C. tentans*.

Current Research:

Analysis and comparison of the ecotoxicological effects of active coal mine effluent, acid mine drainage, and agricultural run-off on three tributaries of Leading Creek, OH. Special research emphasis is placed on the toxic aspects of active mine effluent, related to elevated concentrations of sodium and total dissolved solids.

Memberships:

Society for Environmental Toxicology and Chemistry (SETAC)
American Society for Surface Mining and Reclamation (ASSMR)
The Honor Society of Phi Kappa Phi

Research Grants:

1. Cherry, D.S. Prn. Invg., Co-Invgs., R.J. Currie, H.A. Latimer, J.E. Babendreier and D. Gallagher. Leading Creek Improvement Plan renewal, American Electric Power Company, Columbus, Ohio. January 1, 1998 – December 31, 1998. $90,000.

2. Cherry, D.S., C.E. Zipper, R.J. Currie, D.J. Soucek, and H.A. Latimer. Streamlined Feasibility Study for Ecosystem Restoration: Interim Feasibility report for Ely and Puckett's Creeks in the Powell River Watershed, VA. Submitted November 1998 to U.S. Army Corps of Engineers and Virginia Department of Mines, Minerals and Energy. $6,075

Posters Presentations:

1. H.A. Latimer, R.J. Currie, D.S. Cherry, and J.H. Van Hassel. Recovery and Reconnaissance of Three Tributaries in the Leading Creek Watershed, Meigs County, Ohio. SETAC, 18th Annual Meeting, San Francisco, CA, November 19, 1997.

2. H.A. Latimer, D.S. Cherry, R.J. Currie, J.E. Babendreier and J.H. Van Hassel. Recovery Potential of Parker Run from Intermittently Toxic Coal Mine Effluent and a Coal Slurry Spill, Meigs County, OH. SETAC, 19th Annual Meeting, Charlotte, NC, November 19, 1998.

3. R.J. Currie, D.S. Cherry, H.A. Latimer, J.H. Van Hassel, and J.E. Babendreier. Recovery and Enhancement Plan Development for the Leading Creek Watershed, Meigs County, Ohio. ASSMR, 15th National Meeting, St. Louis, MO, May 16-21, 1998.

4. D.J. Soucek, R.J. Currie, D.S. Cherry, H.A. Latimer, and G.C. Trent. 1998. Benthic Macroinvertebrate Assemblages and Sediment Toxicity Testing in the Ely Creek Watershed Restoration Project. ASSMR, 15th National Meeting, St. Louis, MO, May 16-21, 1998.

Platform Presentations:

1. R.J. Currie, D.S. Cherry, J.H. Van Hassel, J.E. Babendreier, D.M. Johnson, and H.A. Latimer. Recovery, Restoration and Enhancement Plan Development for the Leading Creek Watershed, Meigs County, Ohio. SETAC 18th Annual Meeting, San Francisco, CA, November 19, 1997.

2. J.E. Babendreier, D.S. Cherry, D.L. Gallagher, R.J. Currie and H.A. Latimer. Integrating Risk Assessment of Coal Mining Activities Using GIS, Water Chemistry and Toxicity Assays. Abstract submitted to SETAC, Modeling and Measuring the Vulnerability of Ecosystems at Regional Scales for Use in Ecological Risk Assessment and Risk Management. August 1998, Seattle, WA.

3. R.J. Currie, D.S. Cherry, J.E. Babendreier, H.A. Latimer, D.M. Johnson, and J.H. Van Hassel. Watershed Enhancement Plan Development for Leading Creek after Dewatering of the Meigs 31 Coal Mine in Ohio. SETAC, 19th Annual Meeting, Charlotte, NC, November 18, 1998.

4. J.E. Babendreier, D.L. Gallagher, D.S. Cherry, R.J. Currie and H.A. Latimer. Scale Dependence of Corbicula fluminea Growth Rates Determined *In Situ*. SETAC, 19th Annual Meeting, Charlotte, NC, November 18, 1998.

5. J.E. Babendreier, D.L. Gallagher, D.S. Cherry, R.J. Currie and H.A. Latimer. A Data Screening Tool for Watershed Ecological Risk Assessment to Evaluate Complex Multi-Disciplinary Monitoring Programs. SETAC, 19th Annual Meeting, Charlotte, NC, November 18, 1998.

Publications:

1. Babendreier, J.E., D.L. Gallagher, D.S. Cherry, R.J. Currie, and H.A. Latimer. Measures of Hydrology, Habitat, Toxicity and Biodiversity. Proceedings, 1998 American Society of Civil Engineers, March 22-26, 1998.

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

3. Cherry, D.S., R.J. Currie, D.J. Soucek, H.A. Latimer, and G.C. Trent. 1997. An Integrative Assessment of a Watershed Impacted by Abandoned Mined

Land Discharges. Accepted with revisions by Environmental Pollution, February 1997.

4. Soucek, D.J., D.S. Cherry, R.J. Currie, H.A. Latimer and G.C. Trent. 1998. Laboratory to Field Validation in an Integrative Assessment of an Acid Mine Drainage Impacted Watershed. Accepted with revisions by Environmental Toxicology and Chemistry, April 1999.

Papers in Preparation:

1. Latimer, H.A., D.S. Cherry, R.J. Currie. An Ecotoxicological Evaluation of Active Coal Mine Effluent, Sedimentation, and Acid Mine Drainage in Three Tributaries of Leading Creek, Meigs County, Ohio.

2. Latimer, H.A., D.S. Cherry, R.J. Currie. A Study of Benthic Macroinvertebrate Community Recovery in Parker Run, Meigs County, Ohio, from a Catastrophic Coal Slurry Spill.

3. Latimer, H.A. and D.S. Cherry. Laboratory Evaluation of Acutely Toxic Components of a Synthetic, Active Coal Mine Effluent.

Technical Reports:

1. Cherry, D.S., R.J. Currie and H.A. Latimer. 1997. 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. Virginia Polytechnic Institute and State University. Blacksburg, Virginia.

2. Cherry, D.S., R.J. Currie and H.A. Latimer. Chronic Effluent Toxicity Test Report. 1997. Submitted to International Paper Company, Woronoco Mill, Valley View Avenue, Russell, Massachusetts, 01097.

3. Cherry, D.S., R.J. Currie, H.A. Latimer, J.E. Babendreier, R.B. Diz, D. Gallager, D.M. Johnson, and M.M. Yeager. 1997. Leading Creek Improvement Work Plan. Southern Ohio Coal Company, Meigs Mine No. 31, Leading Creek Improvement Plan. Second – Sixth Quarterly Reports.

4. Cherry, D.S., R.J. Currie, H.A. Latimer, J.E. Babendreier, R.B. Diz, D. Gallager, D.M. Johnson, and M.M. Yeager. 1997. Leading Creek Improvement Work Plan. Southern Ohio Coal Company, Meigs Mine No. 31, Leading Creek Improvement Plan. Final Report.

5. Cherry, D.S., D.J. Soucek, R.J. Currie and H.A. Latimer. Benthic Macroinvertebrate Assemblages, Habitat Assessment, Laboratory Chronic and In Situ Sediment Toxicity Testing in the Straight Creek Watershed Restoration Project: Preliminary and Second Reports. Submitted to the Virginia Division of Mined Land Reclamation, Big Stone Gap, VA.

6. Cherry, D.S., D.J. Soucek, R.J. Currie and H.A. Latimer. Benthic Macroinvertebrate Assemblages, Habitat Assessment, Laboratory Chronic and In Situ Sediment Toxicity Testing in the Straight Creek Watershed Restoration Project: Final Report. Submitted to the Virginia Division of Mined Land Reclamation, Big Stone Gap, VA.

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

8. Cherry, D.S., R.J. Currie, H.A. Latimer, and D.J. Soucek. 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. Virginia Polytechnic Institute and State University. Blacksburg, Virginia.

9. Cherry, D.S., R.J. Currie, H.A. Latimer, and D.J. Soucek. 1999. 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. Virginia Polytechnic Institute and State University. Blacksburg, Virginia.

10. Cherry, D.S., R.J. Currie, H.A. Latimer and D.J. Soucek. 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.

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

References:

1. Dr. Donald S. Cherry, Virginia Polytechnic Institute and State University, Department of Biology, Blacksburg, VA 24061, (540) 231-6766.

2. Dr. George Simmons, Virginia Polytechnic Institute and State University, Department of Biology, Blacksburg, VA 24061, (540) 231- 8925.

3. Dr. David M. Johnson, Ferrum College, Life Science Division, PO Box 2357, Ferrum, VA 24088, (540) 365-4364.

Other references available upon request.