Methods for Evaluating the Effects of a Simulated Mine Effluent with Elevated Ionic Concentration to Field Collected Benthic Macroinvertebrates

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ABSTRACT

Increases in dissolved solids and specific conductance result from disturbance in a watershed, which may lead to changes in the biological community of aquatic systems. While previous studies have used specific conductance as a marker for disturbance, only recently has ionic concentration been considered as the causative factor in changes in the biological community. The ionic concentration that limits aquatic communities will be specific to the ionic constituents and the community. Recent literature has attributed mayfly population declines in mining regions to elevated total dissolved solids. However, the causal link between mayfly losses and the water chemistry changes has not been established. The methods described herein were developed to bridge this gap between the suggested impairment threshold from field surveys and the lack of toxicity demonstrated in laboratory testing. These tests will specifically evaluate the benthic macroinvertebrate community response to elevated dissolved solids in a controlled setting. These methods utilize field collected benthic macroinvertebrate communities, which are exposed to simulated mine effluent under laboratory conditions. Specific objectives of the testing described herein were to develop simulated receiving stream water with a composition representative of Appalachian streams receiving mining discharges and to develop laboratory testing methods to evaluate benthic macroinvertebrate community and individual taxa responses to dissolved solids. Measured ionic concentrations of mining influenced streams were used to develop a simulated mine effluent with elevated ionic components similar to that found in West Virginia streams. Benthic macroinvertebrate substrate baskets were colonized in Ash Fork, a relatively undisturbed tributary of Twentymile Creek, in Nicholas County, WV, for 16 days. Additionally, upon retrieval of the substrate baskets, leaf material was collected and placed in mesh bags. The substrate baskets and leaf packs were placed in artificial streams and acclimated

to the laboratory for 24 hours prior to testing. The ionic concentration of the streams was then adjusted to target test concentrations (100 µm/cm to 2400 µm/cm) over a 24–48 hour period. Test endpoints were statistical comparisons of standard benthic community evaluation metrics for the substrate baskets and leaf litter packs. The simulated stream exposures of the field collected organisms were successful, with no significant differences between biological communities at test initiation and those exposed to test conditions in dilute or full strength reconstituted moderately hard water. In water with elevated conductance values ranging from 600 µm/cm to 2400 µm/cm, only one of the endpoints was shown to be statistically significant. However, trends were evident that showed benthic macroinvertebrate taxa responsive to elevated to dissolved solids, represented by conductance, in the range of values tested. Additional evaluations are ongoing utilizing the methods described herein.

INTRODUCTION

Recent literature attributing mayfly declines in mining regions to elevated total dissolved solids (TDS) have failed to establish the causal link between mayfly losses and the water chemistry changes. In fact, laboratory testing of mining effluents with elevated dissolved solids have not generated toxicity to the sensitive United States Environmental Protection Agency (USEPA) test organism, *Ceriodaphnia dubia* (Bitzer 2012; Kennedy et al. 2005) at TDS concentrations well above those reportedly limiting biological communities (USEPA 2011). The conductivity threshold developed by the EPA for the protection of aquatic life, 300 µS/cm, is within the range of standard laboratory test waters (USEPA 2002). Ionic constituent concentrations in a solution with this conductivity would be well below reported literature values for toxicity of common ions such as chloride and sulfate (Soucek 2007; Soucek et al. 2011; Elphick et al. 2010a,b). The use of surrogate test organisms is widely accepted in predicting receiving stream effects of industrial discharges and is the cornerstone of the National Pollutant Discharge Elimination System (NPDES) whole effluent toxicity (WET) testing program. Studies have demonstrated the relationship between laboratory testing endpoints and the response of aquatic communities (Eagleson 1990; USEPA 1991) leading to widespread and continued reliance on the WET tests for aquatic resource protection from specific point sources via the NPDES permitting requirements and, in general, via water quality criteria developed using WET tests. However, there are limitations in the ability to predict real-world effects from laboratory testing due to environmental and organism-related variability (Chapman 2000). One comparison of field and laboratory data used to develop chloride benchmarks protective of aquatic life found good agreement between the alternatives (Elphick 2010a). This close agreement demonstrated for a common ion such as chloride makes the discrepancy between the USEPA's conductivity benchmark and the available ceriodaphnid data more puzzling. To bridge this gap between the suggested impairment threshold from field surveys and the lack of toxicity demonstrated in laboratory testing, methods are being developed which more specifically evaluate the benthic macroinvertebrate community response to elevated TDS. These methods utilize field collected benthic macroinvertebrate communities that are exposed to simulated mine effluent under laboratory conditions. Specific objectives are: to develop a simulated receiving stream water with a composition representative of Appalachian streams receiving mining discharges; and to develop laboratory testing methods to evaluate benthic macroinvertebrate community and individual taxa responses to elevated TDS.

METHODS

Developing Simulated High TDS Stream Water

Available water quality information from the mining influenced region in West Virginia was used to develop simulated stream water with elevated TDS representative of streams receiving mining discharges. The use of simulated stream water in laboratory testing is preferable to collecting actual high conductivity stream water to minimize variability in the tests and to allow for comparisons between tests. Also, by developing the water as a reconstituted test solution, interference by other potential stressors in the water was minimized. In order to develop a simulated receiving stream composition representative of Appalachian streams receiving mining discharges, actual in-stream data were utilized. Ten sampling sites receiving mining discharges in excess of 1,500 µS/cm were sampled semi-annually by a coal company discharging into the streams. The 20 samples were analyzed for common ions, which comprise the dissolved solids component of the stream (Table 1). Potassium was not included in this analysis so the concentration of potassium contained in each sample was estimated. This estimate was established for each sample based on the relationship of potassium concentration to specific conductivity in the Coal River Watershed (r^2 = 0.937) using the regression equation, y = 0.0071x – 0.4845 (Figure 1). Data from the Coal River was obtained from the West Virginia Department of Environmental Protection Agency's Ambient Water Quality Monitoring database available at the agency website. The Coal River was selected from available watersheds because it had the highest correlation between conductivity and potassium and because the watershed had conductivity values similar to the target range for the testing.

The percentage of the common ions comprising the dissolved solids concentration of the 20 stream samples was calculated by determining the ratio of each ion to the total ionic composition of each solution, summing these values, and taking an average for each ion. The measured contributions of the common ionic constituents to the total dissolved solids are shown in Table 2. The simulated high conductivity stream water was prepared using salts which are routinely used in preparing USEPA's synthetic waters (USEPA 2002), which included reagent grade sodium bicarbonate, potassium chloride, calcium sulfate, magnesium sulfate and calcium carbonate. Sodium chloride was also used.

For preparation of the stream water, a target conductivity of 2,400 µS/cm was selected as the highest test concentration. The TDS concentration providing this conductivity was calculated using an online calculator that estimates electrical conductivity from ionic concentrations. A spreadsheet was constructed that allows for entering the weight of the salt to find the weight of each ion, the ratio in the mixture, and the proximity to the target ion concentration. The mixture best representing field conditions was selected. The percentage contribution of each ion to the weight of the total dissolved solids, which was achieved in the final simulated stream water mixture, is shown with the target concentration in Table 2.

Simulated stream water was prepared in a similar manner to the synthetic water used in toxicity testing. Calcium sulfate was difficult to dissolve so, through trial and error, it was established that when preparing the solution, calcium sulfate will be added to 45% of the volume while the remaining salts are added to the second 45% of the volume. Calcium sulfate was mixed with stirring and aeration or continuous mixing with a submersible pump for at least twenty-four hours prior to combining the solutions. The combined mixture was then aerated vigorously or mixed with the submersible pump for 24 hours prior to use in testing. Because the batches are mixed in 20 gallon carboys, measuring from the carboy wall was not accurate. For this reason, after 24 hours the two volumes described above were combined and brought to a conductivity of 2,400 µS/cm with laboratory water. The laboratory water was prepared by deionization and reverse osmosis and was generally maintained with a TDS concentration less than 2 mg/L. Conductivity was used to confirm accuracy of mixing for the 100% test solutions as well as the dilutions used in testing.

Simulated Stream Environments

Streams are constructed from livestock feeding troughs that are five feet in length and 27 inches in width. A center piece constructed from polyvinyl chloride pipe (PVC) measuring three feet in length are placed longitudinally in the streams to provide opportunity for circular flow in the streams. Where needed, aquarium-grade silicone was used in stream construction. Laminar flow is

Table 1. Concentrations of ionic constituents in mining discharge dominated streams sampled for chronic toxicity testing under high flow (May and June) and low flow (October and November) conditions in 2011. Potassium concentrations were not measured but are estimated based on the relationship of potassium concentration to specific conductivity in the Coal River Watershed (r^3 = 0.937) using the regression equation y= 0.0071x–0.4845.

	Unit	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Collection date		5/25/11	5/25/11	5/25/11	5/25/11	5/25/11	6/3/11	6/3/11	6/3/11	6/3/11	6/3/11
Total calcium	mg/L	124.40	50.48	147.20	181.80	9.69	61.53	90.21	84.94	137.50	77.18
Potassium	mg/l	9.16	3.22	8.63	11.80	0.42	6.51	5.96	7.50	8.19	5.94
Magnesium	mg/L	96.17	42.06	103.30	147.40	7.77	36.45	77.58	84.06	107.30	42.49
Total sodium	mg/L	46.70	5.56	6.02	15.50	2.64	137.30	9.40	66.27	11.02	83.15
Chlorides	mg/L	19.42	1.18	11.12	35.68	1.25	15.06	4.85	28.95	2.12	7.26
Total alkalinity	mg/L	54.86	20.17	24.59	413.75	11.89	307.31	93.71	218.07	192.86	139.67
Total sulfates	mg/L	685.04	270.06	697.64	1173.70	44.25	229.73	337.93	471.37	548.34	157.08
Specific conductance	µS/cm	1358	552	1284	1730	127	985	907	1124	1222	905
	Unit	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Collection date		10/26/11	10/26/11	10/26/11	10/31/11	10/26/11	11/2/11	10/31/11	11/2/11	10/26/11	10/31/11
Total calcium	mg/L	134.47	52.69	168.21	198.65	10.25	66.13	95.64	126.33	79.54	78.52
Potassium	mg/l	11.02	3.80	9.60	9.31	0.67	4.79	4.86	7.60	8.49	2.22
Magnesium	mg/L	88.63	48.67	99.74	155.42	8.15	38.29	79.42	113.21	89.81	48.71
Total sodium	mg/L	49.34	5.41	5.64	17.84	2.15	145.21	9.52	15.54	75.21	91.52
Chlorides	mg/L	18.76	1.25	9.87	21.81	1.14	12.35	5.23	1.87	26.55	8.13
Total alkalinity	mg/L	58.72	22.51	28.51	415.62	12.34	298.45	85.42	211.21	225.63	155.22
Total sulfates	mg/L	712.35	335.27	725.92	789.39	55.21	245.10	375.25	552.16	488.93	226.39
Specific conductance	µS/cm	1621	604	1421	1495	163	743	753	1138	1264	381

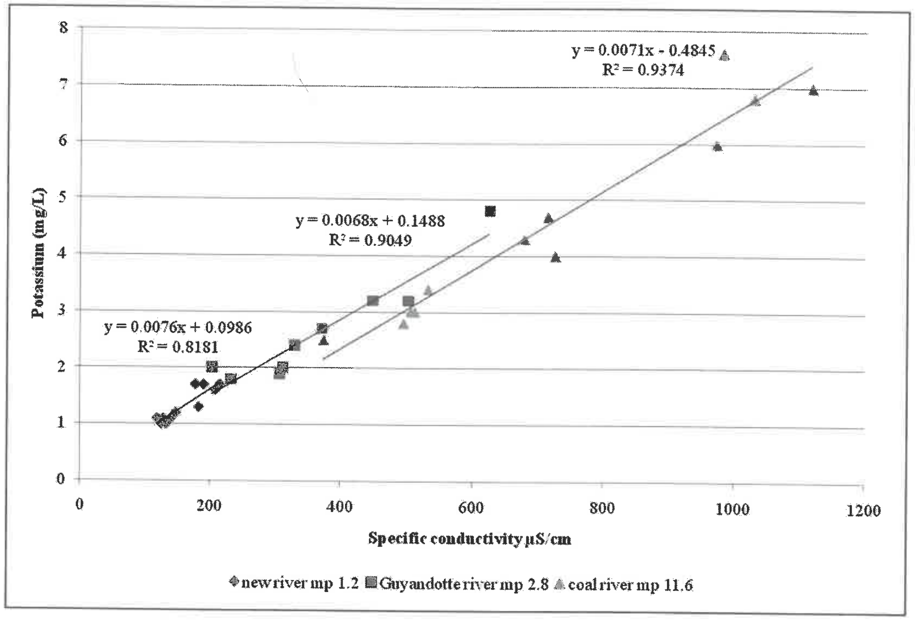

Figure 1. Relationship between potassium and conductance from three WVDEP monitoring stations in mining influenced watersheds. Coal River watershed was selected to estimate potassium due to having the largest dataset and highest correlation coefficient and having conductivity in the range of concentrations being simulated.

Table 2. Estimation of percentage of ionic constituents and the total percentage achieved in the simulated stream water

	% Ionic Constituent Measured (Target)	% Achieved in the Simulated Stream Water
Total Calcium	12.12	11.64
Potassium	0.78	0.61
Magnesium	9.29	7.98
Sodium	5.39	5.55
Chloride	1.32	1.26
Bicarbonate	17.28	13.51
Total Sulfate	54.60	59.45

achieved by using a constructed paddle system, which consists of stainless steel rods attached to a rotisserie motor for consistent flow of approximately 0.01 feet per second. There are paddles that were constructed using five inch diameter plastic lawnmower wheels and four speckling spatulas attached to the rod. The depth of the streams, 7.5 inches, was achieved by placing a draining standpipe in the stream. A gardening shade cloth was suspended over the streams to reduce the light intensity of the ambient laboratory lighting to approximately 45 lux. USEPA's moderately hard water was the dilution water used in testing.

Benthic Macroinvertebrate Community Collection

Benthic macroinvertebrate communities were sampled in streams known to have West Virginia Stream Condition Index (WVSCI) scores in the unimpaired range. In the testing described herein, the community tested was collected in Ash Fork of Twentymile Creek in Nicholas County, West Virginia. Upon stream selection, benthic collection baskets which were 7" × 9" × 3" were deployed in the stream thalwag. The collection baskets were made from bird feeders. The metal baskets were covered in rubber to prevent metal casings exposure to the organisms. The baskets had mesh size of approximately one inch.

At the time of deployment, rocks from the reference site were placed into the baskets and benthic macroinvertebrates were allowed to colonize for 15 days. After the 15 days the baskets were retrieved by placing each individual basket in a two gallon Ziploc bag underwater to ensure there was no drifting of macroinvertebrates out of the baskets. Rock baskets and stream water were transferred to coolers and transferred to the laboratory. Leaf packs were obtained from the stream by randomly selecting available leaf material and placing it into pecan bags. Each bag was then placed in a Ziploc bag and into a cooler. Water samples were collected in laboratory provided bottles with appropriate preservative and taken to BioChem Testing for analysis. After collection, benthic macroinvertebrate baskets were transferred to the laboratory where they were randomly placed gently into the streams. USEPA's moderately hard water was placed in the streams to a depth to cover the baskets. Four benthic macroinvertebrate baskets were transferred into each stream with the stream water and allowed to acclimate for 24 hours prior to initiation of testing. Leaf packs were also transferred into the streams and tied to the stream so they did not move around during testing or become entangled in the paddles. Four replicates of rock baskets and leaf packs were placed into each stream and streams were randomly assigned a conductivity treatment.

After the 24 hour acclimation period, test solution was introduced into the streams using gravity flow and overflow stream water was allowed to flow out the standpipe. Achieving desired conductivity in each stream occurred over a two day period with the target concentration taking longer to achieve at the higher concentrations. The exposure period was 10 days after the target concentration was obtained. The test was a static renewal test with 10 liters of reconstituted stream water changed over twice daily for a total volume of 20 liters per day changed over. In addition to the leaf packs contributing particulate organic material to the steams during testing, each stream received 5 mL of yeast, cereal leaves and trout chow mix (YCT) and 5 mL of *Selenastrum capricornutum* daily after the morning change over. Water chemistry was conducted daily on test solution prior to change over to ensure water was correctly mixed and aerated prior to use. Water draining from streams was also evaluated to ensure water quality parameters were being maintained in an appropriate range. Daily water chemistry included dissolved oxygen, pH, temperature and conductivity.

After the 10-day exposure period, the paddles were stopped and the baskets were gently placed in a bucket for removal from the simulated stream. In the bucket, the basket was opened and the rocks were rinsed. Contents of the bucket were poured through a sieve, which was rinsed into a tray for macroinvertebrate removal. Leaf packs were similarly removed from the streams and rinsed into a sieve. Benthic macroinvertebrates removed were preserved in 70% ethanol and identified to the lowest practical taxa, usually genus. After the baskets and bags were removed, the stream contents were emptied through a sieve to collect any macroinvertebrates that had abandoned their respective baskets during testing. These benthic macroinvertebrates were sorted to evaluate the number of organisms lost from the baskets during testing.

Common descriptive metrics were calculated for rock basket and leaf pack communities. These included total abundance, taxa richness, abundance of mayflies and

Table 3. Water chemistry demonstrating test conditions for each treatment during the 10-day exposure period of the field collected benthic macroinvertebrate community to simulated stream water with elevated conductivity

	100 µS/cm	300 µS/cm	600 µS/cm	1215 µS/cm	1800 µS/cm	2430 µS/cm
Dissolved oxygen (average) mg/L	9.06	8.95	8.78	8.81	8.75	8.96
Dissolved oxygen (range) mg/L	8.39–9.65	8.59–9.56	1.36–9.48	6.70–9.51	7.07–9.31	8.42–9.49
pH (range) S.U.	7.35–7.70	7.38–8.01	7.40–7.89	7.21–8.29	7.41–8.28	7.44–8.36
Temperature (average) °C	17.71	17.73	17.94	17.74	17.87	17.79
Temperature (range) °C	15.43–20.90	15.39–20.90	15.90–20.90	15.50–20.90	15.88–20.90	15.66–20.90

caddisflies (in the leafpacks), richness of the sensitive Ephemeroptera, Plecoptera and Trichoptera organisms, and percentage of sensitive Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa. Data were evaluated for significance using a one way analysis of variance procedure where the assumption of homogeneity and normality were met. A Kruskal-Wallis one-way analysis of variance procedure was use where the assumptions were not met. Statistical comparisons were made for the evaluation of Day 0 community (rock baskets initially collected from the streams) to the end of test communities from the dilute moderately hard USEPA reconstituted water and the standard reconstituted waters with conductivities of 100 µS/cm and 300 µS/cm, respectively. Statistical comparisons were also made for the community metrics from the four exposure concentrations compared to the two reference water communities. Multiple rock baskets and leaf bags were exposed in each treatment stream such that the experimental design, as with many mesocosm experiments, may be considered pseudoreplicated. Differences between the treatments may result from factors other than the TDS exposure concentrations and should be viewed with this limitation.

RESULTS

Water chemistry evaluations showed that dissolved oxygen, pH and temperature were suitable to support the benthic macroinvertebrate communities exposed during testing (Table 3). Target conductance concentrations were achieved within 24 hours of test initiation in the 100 µS/cm, 300 µS/cm, 600 µS/cm and 1200 µS/cm concentrations. Higher conductance values were harder to achieve and were not obtained until days 4 and 6 for the 1,800 µS/cm and 2,400 µS/cm concentrations, respectively (Figure 2).

Overall, abundance indicated a good representation of in-stream conditions with the average Day 0 abundance of 22 organisms (Table 4) corresponding to an in-stream community with approximately 541 individuals per square meter. This is a reasonable approximation of riffle abundance in a stream representing undisturbed conditions. Upon retrieval from Ash Fork, abundance in the baskets ranged from 14 to 30 organisms representing seven to eight taxa. The community had four to five mayfly taxa represented which represented 93 to 100% of the population (11 to 24 organisms). At the end of the exposure period, abundance in the 100 µS/cm and 300 µS/cm conductance treatments averaged 12.5 and 21.25 in the two waters, respectively (Figure 3a). Taxa richness was lowest in the 100 µS/cm concentration (three to seven taxa), but was not significantly different from the Day 0 collection (Table 4; Figure 3b). A similar trend was seen in mayfly abundance and richness, but there were no significant differences (Figure 4a and b).

While no significant differences were found between the community metrics for organisms exposed to the high conductance test concentrations (Table 5), some trends were evident. Abundance was similar at the four test concentration and two reference waters (Figure 5a) as was taxa richness (Figure 5b). A rock basket with 10 Chironomidae and 15 Ephemerellidae in the 300 µS/cm stream treatment created substantial variability in that concentration. Comparison of the sensitive mayfly taxa abundance and

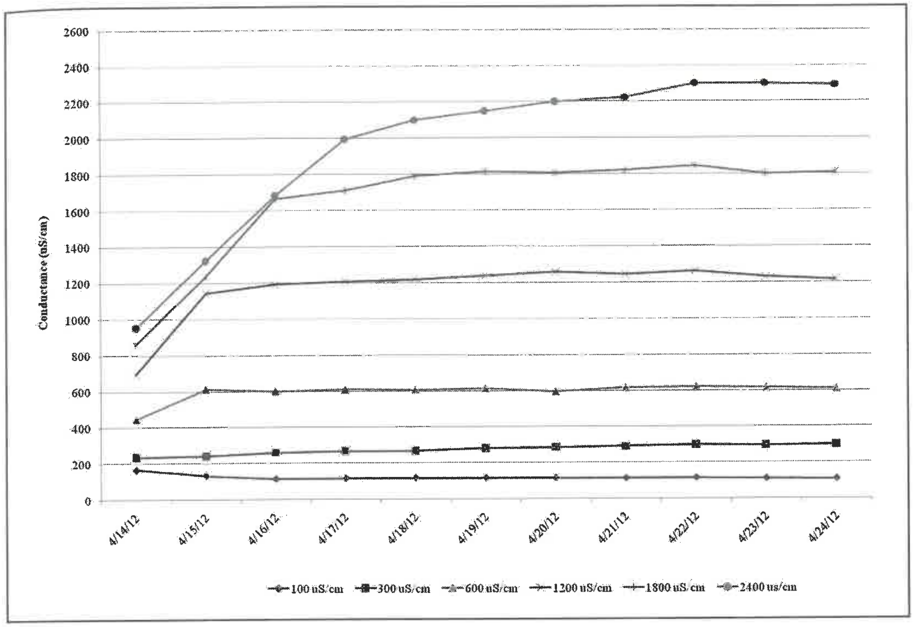

Figure 2. Specific conductance of test solution draining from the simulated stream environments at test initiation (Day 0) and over the 10-day exposure period. Legend value indicates target value. Higher concentrations took longer to achieve due to volumes of change over water necessary to reach the target concentration.

Table 4. Average and range of benthic macroinvertebrate community metrics for the four replicate baskets representing the day of collection compared with reference water communities after the exposure period

	Day 0	100 µS/cm	300 µS/cm	F-Ratio	Prob Level	Chi^2	Prob Level
Abundance	22.5	12.5	21.25	1.61	0.2520		
	14–30	11–17	11–39				
Total # taxa	7.25	5	7.25	2.83	0.1116		
	7–8	3–7	6–7				
# Mayfly taxa	4.25	2.5	3.25	2.64	0.1250		
	4–5	1–4	2–5				
# Stonefly taxa	1.5	1	1.25	0.60	0.5694		
	1–2.	1	0–2				
# Caddisfly taxa	0.75	1.25	1.5	1.17	0.3544		
	0–1	0–2	1–2				
Abundance of mayflies	18.25	8	13	3.32	0.0831		
	11–24	4–14	8–21				
% EPT individuals	93–100	82–100	72–100	1.34	0.3106	2.28	0.3200

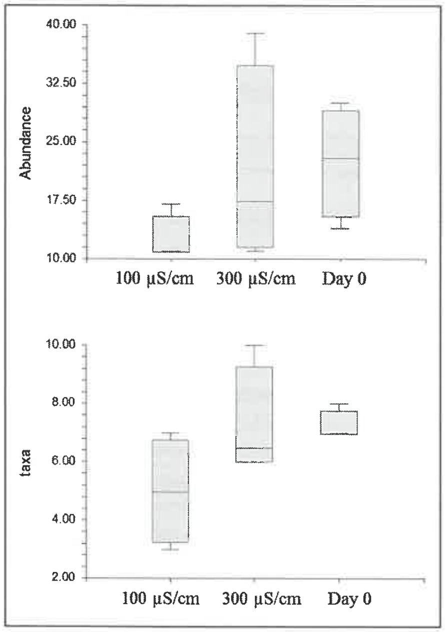

Figure 3. Abundance and taxa richness from the reference streams where field collected benthic macroinvertebrates were exposed to full strength and diluted USEPA reconsituted moderately hard water

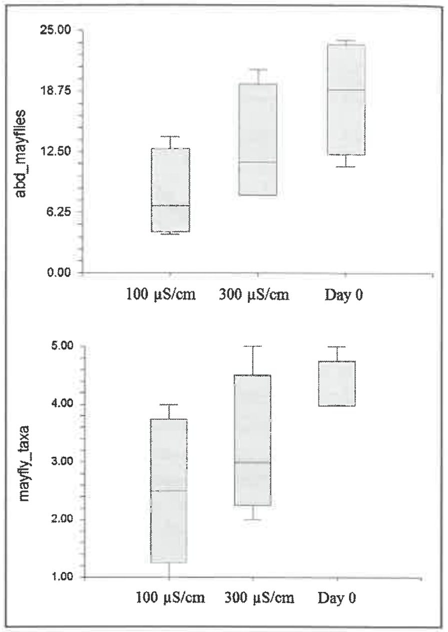

Figure 4. Mayfly abundance and taxa richness from the reference streams where field collected benthic macroinvertebrates were exposed to full strength and diluted USEPA reconstituted moderately hard water

Table 5. Average and range of benthic macroinvertebrate community metrics calculated for benthic macroinvertebrate samples retrieved from rock baskets from each treatment concentration following 10-day exposures to elevated dissolved solids in simulated stream waters

	100 µS/cm	300 µS/cm	600 µS/cm	1215 µS/cm	1800 µS/cm	2430 µS/cm	F-Ratio	Prob Level	Chi^2	Prob Level
Abundance	12	21.25	17.75	13	12	13	1.09	0.4017		
	11–17	11–39	8–24	9–17	6–20	8–19				
Total # taxa	5	7.25	7	7.5	6	8.25	1.04	0.4223		
	3–7	6–7	5–10	6–10	4–10	5–12				
# Mayfly taxa	2.5	3.25	3.5	3.5	2.25	2.25	1.74	0.1763		
	1–4	2–5	3–4	3–5	2–3	2–3				
# Stonefly taxa	1	1.25	0.5	1.5	1.5	1.75	0.64	0.6722		
	1	0–2	0–2	0–4	0–3	1–3				
# Caddisfly taxa	1.25	1.5	1.25	0.5	1	1.75	1.30	0.3066		
	0–2	1–2	1–2	0–1	0–2	1–3				
Abundance of mayflies	8	13	11.5	6.5	6.75	3.5	2.53	0.0668		
	4–14	8–21	5–20	4–10	4–9	2–6				
% EPT Individuals	82–100	72–100	67–92	45–89	83–90	44–89			8.07	0.1526

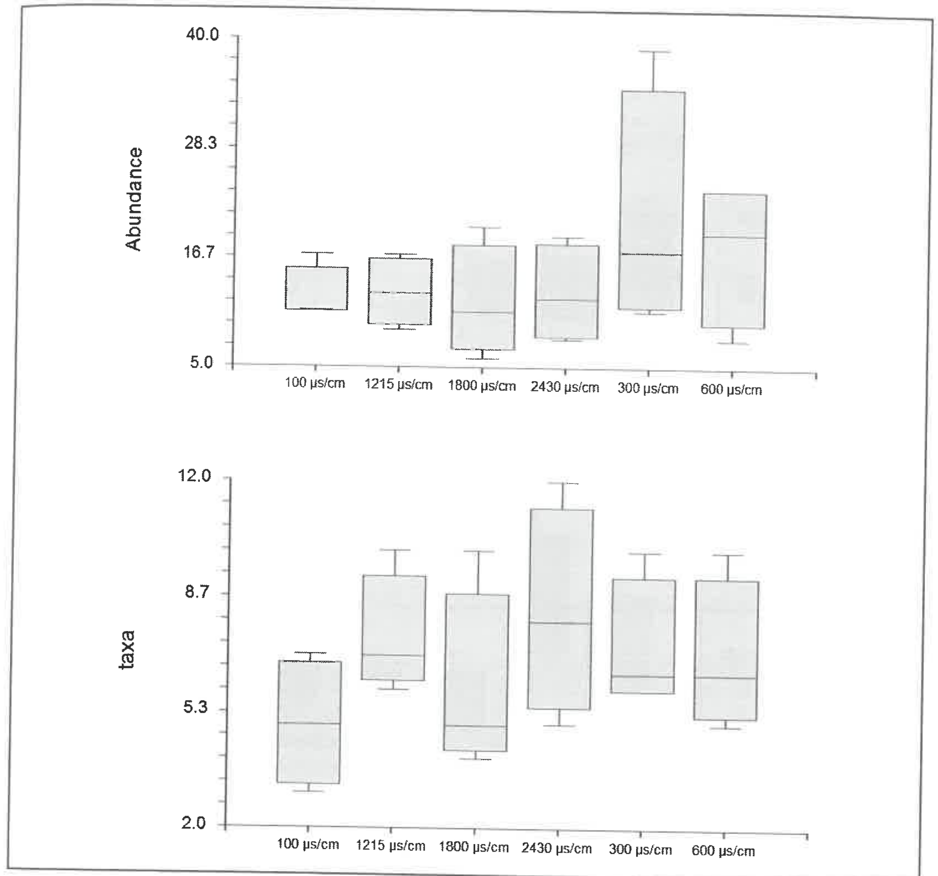

Figure 5. Abundance and taxa richness from the artificial stream exposures of field collected benthic macroinvertebrates to simulated stream waters with elevated conductance

richness values also showed no statistical differences between the treatments concentrations. However, mayfly abundance was lowest at the highest conductance concentration and taxa richness was lowest at the 1,800 µS/cm and 2,400 µS/cm concentrations (Figure 6a and b).

As would be expected, leaf packs were found to have a community substantially different from the rock baskets. The leaf pack community was dominated by Trichoptera, specifically the Limnephiled caddisfly *Pycnopsyche,* and by Oligochaetes. Abundance in the leaf packs ranged from 12 to 100 individuals and was not significantly different between the reference waters (100 µS/cm and 300 µS/cm) and the four treatment concentrations (Table 6). Similarly, there were no differences between the treatments and reference waters with respect to taxa richness. However, mayfly taxa richness was significantly reduced in the leaf packs ($X^2 = 11.04$, $p = 0.05$) although no differences were found in the between treatment comparisons (Figure 7a). Caddisfly abundance was also reduced, although not significantly so, in the 2,400 µS/cm concentration (Figure 7b).

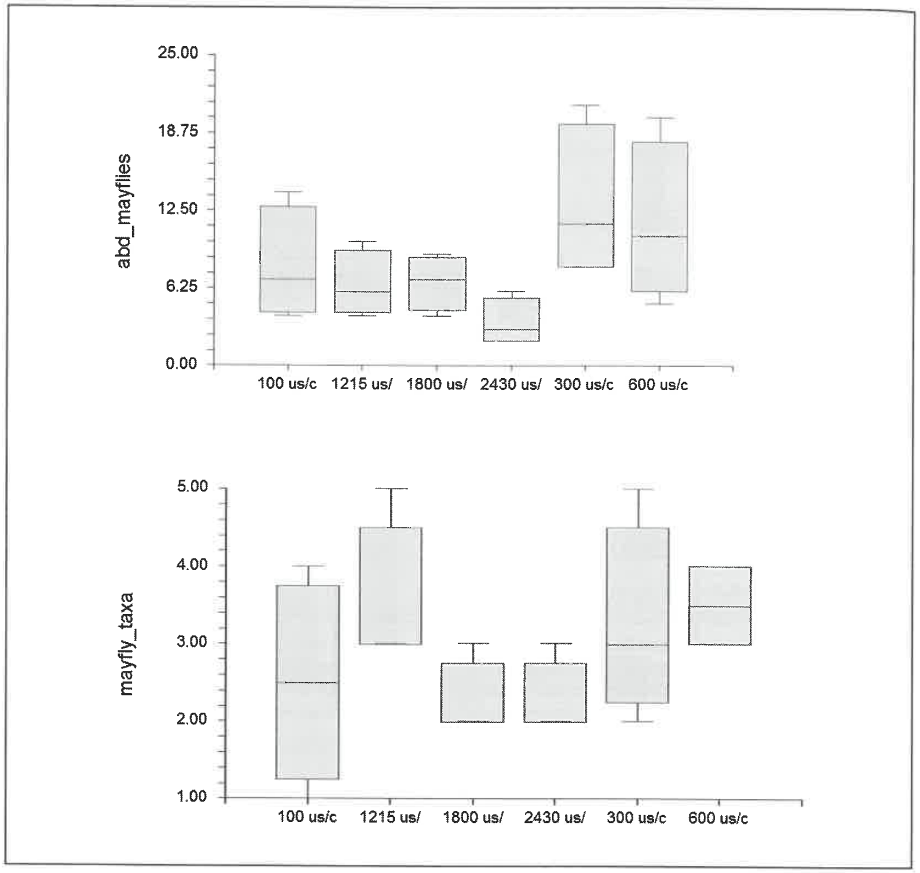

Figure 6. Mayfly abundance and taxa richness from the artificial stream exposures of field collected benthic macroinvertebrates to simulated stream waters with elevated conductance

DISCUSSION

The testing described herein was developed to bridge the gap between the suggested impairment threshold from field surveys summarized in the USEPA benchmark document (USEPA 2011) and the lack of toxicity demonstrated in laboratory testing. These methods are being developed that more specifically evaluate the benthic macroinvertebrate community response to elevated TDS. These methods utilize field collected benthic macroinvertebrate communities that are exposed to simulated mine effluent under laboratory conditions. Specific objectives were to develop a simulated receiving stream composition representative of Appalachian streams receiving mining discharges and to develop laboratory testing methods to evaluate benthic macroinvertebrate community and individual taxa responses to TDS.

Laboratory prepared reconstituted water was successfully developed that simulated natural stream waters receiving mining discharges with elevated conductance. The target concentrations were achieved quickly at lower concentrations but were harder to obtain at higher conductivities.

Table 6. Average and range of benthic macroinvertebrate community metrics calculated for benthic macroinvertebrate samples retrieved from leaf packs from each treatment concentration following 10-day exposures to elevated dissolved solids in simulated stream water

	100 µS/cm	300 µS/cm	600 µS/cm	1215 µS/cm	1800 µS/cm	2430 µS/cm	F-Ratio	Prob Level	Chi^2	Prob Level
Abundance per gram	35.25	79.75	52.25	64.5	50.75	67.25	0.97	0.46		
	12–70	46–99	32–72	49–100	43–58	41–85				
Total # taxa	7	9.5	9	10.25	9	8.75	0.82	0.55		
	4–12	8–11	7–10	9–12	7–13	5–12				
# Mayfly taxa	1.75	2.25	2.25	2	1.25	1	1.52	0.23		
	1–3	2–3	1–3	1–3	0–2	0–2				
# Stonefly taxa	2.5	2.25	2	2	1.75	2	0.16	0.97		
	1–4	2–3	0–4	1–3	1–3	0–4				
# Caddisfly taxa	0.75	1.5	2	1.75	2	2.25	2.22	0.10		
	0–2	1–2	1–3	1–2	1–3	2–3				
Abundance of mayflies	8	14	8.25	4.5	1.75	2	1.26	0.32	11.04	0.05
	1–15	3–25	3–16	2–6	0–4	0–4				
Abundance of caddisflies	18	23	28.5	25.5	38.5	3.25	1.12	0.39		
	2–30	6–37	13–58	2–50	5–75	0–8				
% EPT individuals	54–92	26–92	45–94	42–76	26–74	10–72			2.77	0.74

In future testing, less water will be used in the streams during the 24-hour acclimation period so that less dilution will occur when the streams are being brought to target concentrations.

The simulated streams functioned well with minimal loss of organisms in the reference streams during the course of the 10-day exposure period. Dissolved oxygen was maintained in sufficient range during testing. The feeding regime appears sufficient to maintain the organisms based on maintenance of the communities during the test period. The change over rate of 10 liters twice per day was sufficient to maintain water quality and did not flush the food sources for the organisms as had been noticed in preliminary testing. Benthic macroinvertebrate baskets and leaf packs collected and maintained sufficient abundances; however, some material was lost to the streams and accumulated in the bottom. In future testing, the technique to remove baskets from the stream will be altered to reduce the material lost upon test breakdown. Although not statistically significant, organisms in the 100 µS/cm concentration did not appear to perform as well as the 300 µS/cm concentration in several metrics. This trend will be evaluated in future testing to determine if the invertebrates, particularly the mayflies, have a preference for the water with higher dissolved solids.

In the elevated conductance treatments, with values ranging from 600 µm/cm to 2,400 µm/cm, only one of the endpoints was shown to be statistically significant. However, trends were evident which show benthic macroinvertebrate taxa responsive to elevated to dissolved solids. The mayfly abundance was consistently reduced in the highest conductance treatments, although only significantly so in the leaf packs at the 2,400 µm/cm. Caddisfly abundance was also reduced at this conductance level. These findings indicate the organisms are responsive to the simulated stream water and the elevated TDS in the range of exposures tested. Additional evaluations are ongoing utilizing the methods described herein and expanding on these methods. Specific findings of this preliminary research being more fully evaluated include the poor performance of the organisms in the lowest ionic strenght treatment; the

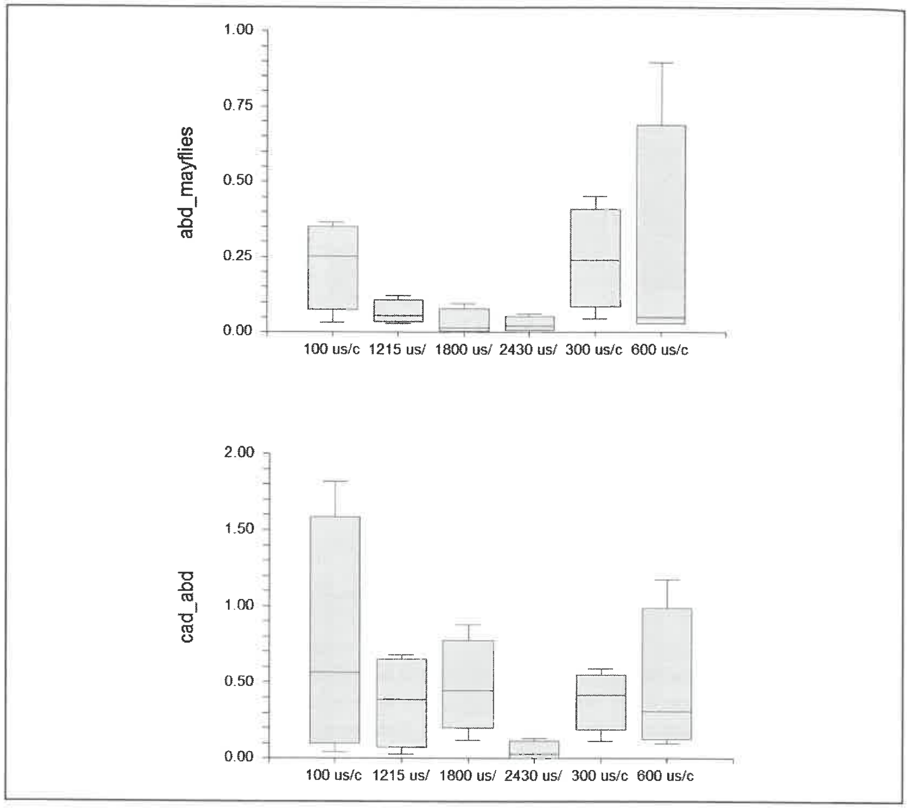

Figure 7. Mayfly abundance and taxa richness from the artificial stream exposures of field collected benthic macroinvertebrates to simulated stream waters with elevated conductance. Abundance values are presented per kilogram of leaf material.

definition of the conductance level where impairment to the biological communities are apparent; consideration of the effects of dissolved solids on individual taxa; and caddisfly sensitivity, which appears in a similar range as mayfly impairment in this limited dataset. Based on this initial assessment, the exposure and testing methods may be modified to increase the number of organisms utilized in the testing and to improve recovery from the simulated stream environments.

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