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Chronic Toxicity Testing In Mining Influenced Streams of West Virginia

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CHRONIC TOXICITY TESTING IN MINING INFLUENCED STREAMS OF WEST VIRGINIA

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Abstract

CHRONIC TOXICITY TESTING IN MINING INFLUENCED STREAMS OF WEST VIRGINIA

By Leah J. Bitzer

Whole effluent toxicity (WET) tests have become a common tool in the evaluation of effluent for discharge acceptability. In this study, four years of toxicity data from 119 sampling locations were analyzed to determine relationships with ions and conductivity as indicators of toxicity. West Virginia Stream Condition Index (WVSCI) scores were also examined to evaluate correlations between stream scores, conductivity, and IC25 endpoints from toxicity results. Conductivity was not an indicator of toxicity in the range of conductivities tested. Streams dominated by mining effluent sometimes exhibited toxicity to *Ceriodaphnia dubia*; however, toxicity was not found to be related to ionic concentration in the range tested. Although mortality and reproductive impairment were often demonstrated in the mining effluent dominated streams, there were no relationships established between survival and reproductive endpoints and the ionic concentrations. Benthic macroinvertebrate communities in the streams sampled indicated some level of impairment. Only a weak relationship was demonstrated between habitat assessment scores and WVSCI scores. No apparent relationship between conductivity and WVSCI was observed.

Chapter 1

Introduction

Coal is a combustible material formed from the remains of living plants that flourished millions of years ago in swamp-like areas. Layers of fallen plant material accumulated and partially decayed in wet environments to form a substance called peat. Over time, peat was compressed under sand and mud and heated by the earth to be transformed into coal (Plummer *et al.*, 1999). Coal is an organic compound primarily composed of carbon, hydrogen, and oxygen with lesser amounts of sulfur and nitrogen (Ragland and Bryden, 2011).

Coal is used to generate heat, produce electricity and make steel and industrial products. Simple burning of coal produces heat for homes and industries. About 88% of the present use of coal in the United States is for generating electricity (Plummer *et al.*, 1999). After oil and natural gas, coal is our third major energy source (Plummer *et al.*, 1999).

Coke is a hard material produced when coal is heated without air at approximately 1,000°C. Coke is used to smelt iron ore for the production of steel. Coal tar, a sticky black liquid derived from coke, is used for paving roads and tarring roofs (EFMR 2009). The extraction and distillation of coal tar into separate compounds produces a variety of products for making drugs, plastics, paints and synthetic fibers (EFMR 2009).

The two main types of coal mining are underground and surface (strip). Underground mining involves the removal of coal deposits, often hundreds of feet below the earth's surface. Shafts or tunnels are dug into the coal layers and widened to allow room for miners and equipment. Surface mining removes the soil and rock over a coal seam to expose the coal. The excess overburden is often stacked in piles to be used to construct original contours after mining or disposed of in constructed fills in valleys or hollows (McElfish and Bier, 1990).

Disposing of large quantities of materials can be a challenge for the mining industry. Erosion from waste rock piles or runoff after heavy rainfall may increase the sediment load of nearby water bodies (Pepper *et al.*, 2006). In addition, mining may modify stream morphology by disrupting a channel, diverting stream flows, and/or changing the slope or bank stability of a stream channel.

Mine drainage has a combination of elements that can interact to cause a variety of effects on aquatic life. In the northern Appalachians and Allegheny Plateau, certain coal strata have higher sulfur content and tend to cause acidic mine drainage (AMD) (Pond *et al.* 2008). Advances in mining technology allow for acid-base accounting in overburden so that alkaline amendments can be made to prevent or minimize the formation of AMD from surface operations (Lottermoser 2010). The overall effect of mine drainage is also dependent on the flow, pH, and alkalinity or buffering capacity of the receiving stream. The higher the concentration of bicarbonate and carbonate ions in the receiving stream, the higher the buffering capacity and the greater the protection of aquatic life from adverse effects of acid mine drainage (Kimmel 1983). Alkaline mine drainage with low concentrations of metals may have little discernible effect on receiving streams, whereas acid mine drainage with elevated metal concentrations discharging into headwater streams or lightly buffered streams can have significant effects on the aquatic life. A study was conducted in western Pennsylvania on the effects of constant and intermittent acid mine drainage on insect fauna. The results showed that, under conditions of constant acid mine drainage, the Odonata, Ephemeroptera and Plecoptera were completely eliminated. The Trichoptera, Megaloptera and Diptera were reduced in number of species. *Ptilostomis* (Trichoptera), *Sialis* (Megaloptera) and *Chironomus attenuatus* (Diptera) were tolerant of the conditions produced by acid mine drainage. The non-benthic Hemiptera and Coleoptera were little affected and developed large populations in the stations damaged by acid mine drainage. Under intermittent acid mine drainage, a diverse but slightly depressed insect fauna was able to develop (Roback and Richardson, 1969).

All natural water contains dissolved minerals. Common ions in freshwater include bicarbonate, sulfate, calcium, sodium, and silica (Weber-Scannell and Duffey, 2007). Adverse effects can occur in aquatic organisms when common ions exceed a certain concentration, when the normal composition (ratio) of ions is not correct, or in some cases when ion concentrations are too low (SETAC 2004a). Several common ions can be toxic to aquatic organisms when present at concentrations above or below biologically-tolerable concentrations (SETAC 2004a). Mount *et al.* (1997) conducted laboratory testing and established a database of the acute toxicity of seven major ions to three freshwater organisms and developed statistical toxicity models.

Conductivity is a measure of the ability of water to pass an electrical current. Specific minerals influence the conductivity value differently. Specific conductance (µS/cm) increases with increasing concentrations of total dissolved solids (Lind 1979). Conductivity above 2,000 µS/cm or TDS above 1,340 mg/L represent conditions that may adversely affect freshwater organisms (Goodfellow *et al.*, 2000). Conductivity in water is affected by inorganic dissolved solids such as chloride, nitrate, sulfate and phosphate anions or sodium, magnesium, calcium, iron, and aluminum cations. Organic compounds like oil, phenol, alcohol, and sugar do not conduct electrical current very well and therefore have a low conductivity when in water (Spellman 2009).

Total dissolved solid (TDS) is a direct measure of all constituents dissolved in water while conductivity is an indirect approximation. Changes in TDS levels and individual cations and anions can occur from a variety of anthropogenic sources including industry and resource extraction such as mining and gas well development (Fillo *et al.*, 1992). Toxicity of TDS to aquatic life depends upon the combinations and concentrations of the ions in solution which may have additive or synergistic properties and is not predictable from TDS concentrations alone (Chapman *et al.*, 2000). There is no sulfate or TDS federal water quality criterion for the protection of freshwater aquatic life. Elevated TDS can be toxic to freshwater animals by causing osmotic stress and affecting the osmoregulatory capability of the organism (McCulloch *et al.*, 1993).

Sulfate is widely distributed in nature and may be present in natural waters at concentrations ranging from a few to several hundred milligrams (Iowa DNR 2007). In coalfield streams, TDS is most often dominated on a mass basis by the dissolved anions sulfate and bicarbonate, with elevated concentrations (relative to reference streams) of calcium, magnesium, sodium, potassium, and chloride also common (Pond *et al.*, 2008; Mount *et al.*, 1997). Kennedy *et al.* (2003) exposed *Ceriodaphnia dubia* to sulfate-dominated mine effluent and observed significant effects on survival and reproduction at specific conductivities of approximately 6,000 and 3,700 μS/cm (approx. 4,200 and 2,590 mg/L TDS), respectively.

The physical alteration of water bodies in West Virginia, including wetlands and streams, are regulated by federal and state statutes under Section 401 (Certification) and Section 404 (Permits) of the Federal Clean Water Act (1972). The Clean Water Act allows industries to discharge effluent to streams as long as the standards that are set in place are met and the aquatic resource is not significantly impacted. Under Section 404 of the Clean Water Act, the United States Army Corps of Engineers regulates the discharge of dredged and/or fill material in waters of the U.S. and under Section 10 of the Rivers and Harbors Act of 1899, the United States Army Corps of Engineers regulates work in navigable waters of the United States. Section 401 of the Clean Water Act requires that any applicant for a Section 404 permit also obtain a Water Quality Certification from the State. The purpose of the certification is to confirm that the discharge of fill materials will be in compliance with the State's applicable Water Quality Standards. The National Pollutant Discharge Elimination System (NPDES) permit program, authorized by the Clean Water Act, controls water pollution by regulating point source discharges into water of the United States (SETAC 2004b).

For coal mining, permits must be submitted to the state and federal agencies to characterize any and all impacts to aquatic resources. The agencies review the applications to ensure that environmental laws are obeyed. If the permit application is granted, the resources are monitored prior to, during, and after any impact to the resource. The results are provided to the federal and state agencies to review the data to ensure the impacts are not exceeding any water quality standards. In the event that the biological community is significantly impacted, the violator is required to mitigate the impact or return the aquatic resource to its pre-impact condition.

Permits often include whole effluent toxicity (WET) tests as a monitoring requirement and sometimes for compliance determination (SETAC 2004b). “WET” is a term used to describe the adverse effects or toxicity to a population of aquatic organisms caused by exposure to an effluent. Toxicity can be experimentally determined in a laboratory by exposing sensitive organisms to effluents using WET tests. WET testing is used to assess and regulate the combined effects of all constituents of a complex effluent rather than the conventional methods of controlling the toxicity of single chemicals or constituents.

WET testing determines the specific toxicity, either acute or chronic, of the effluent being discharged into the streams so that discharges can be regulated to prevent in-stream effects. Acute tests are conducted for 24 - 96 hours and usually focus on how well an organism survives. Chronic tests are conducted for 7 days and evaluate survival, growth and/or reproduction (USEPA 2002). The effluent is collected from a discharge point and sent to a WET testing laboratory. At the lab, a serial dilution is prepared which tests the effluent at full concentration and several dilutions to determine which concentration may not meet the federal or state standard. WET testing exposes laboratory populations of aquatic organisms such as fish, invertebrates, and algae to diluted and undiluted effluent samples under controlled conditions in order to estimate the environmental toxicity of that sample.

The objective of aquatic toxicity tests with effluents is to estimate the "safe" or "no effect" concentration (NOEC) of these discharges, which is generally defined as the concentration with no significant difference in mortality, growth, or reproduction in the test organisms (USEPA 2002). The NOEC is a concentration believed to be protective of aquatic life in the receiving waters. The LC50 is the lethal concentration to 50% of the population. The NOEC is the no observable effects concentration where no statistical differences from the control are observed. The LOEC is the lowest observable effect concentration and the IC25 is the 25% inhibition concentration. The NOEC and the LOEC are determined by significance testing while the LC50 and the IC25 are determined by regression analysis.

Information gained from WET tests is used to evaluate the impact of the effluent sample on survival, growth, reproductive capacity, and normal development of the test population. The data provide an estimate of the concentration above which detrimental impact from the effluent would be predicted to occur in the receiving stream.

In addition to laboratory testing, effects of discharges on aquatic communities are evaluated by monitoring in-stream communities. Benthic macroinvertebrates are the most common stream organism used in biomonitoring due to their importance in the stream community. Benthic macroinvertebrates are fairly ubiquitous and extremely easy to collect (Cummins 1975) and are ideal due to their sedentary nature (Resh and Jackson, 1993). Benthic macroinvertebrate communities are a good bioindicator because they integrate effects of stressors over the life cycle of each taxon (Barbour *et al.*, 1999). According to Southerland and Stribling (1995), benthic macroinvertebrates are used in 90% of the state water quality assessment programs in the United States.

A 0.5 meter kick-net is commonly used to collect a four-sample composite benthic macroinvertebrate sample in riffle/run sections of stream channels. Samples are composited, field sieved, and preserved for identification. Samples are picked and identified to the genus level using appropriate taxonomic keys with a target of 180 organisms (WVDNR 2008). The genus level benthic macroinvertebrate community data are evaluated using a series of metrics which includes taxa richness, Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa richness, percent EPT, percent two dominant taxa, percent Chironomidae, and Hilsenhoff Biotic Index (HBI) (Barbour *et. al.*, 1999). Each metric responds to disturbance in a specific manner. Taxa richness and EPT taxa richness are measures that provide information on overall and EPT-group specific taxonomic variety or diversity of the aquatic community (Barbour *et. al.*, 1999).

EPT are the sensitive Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) taxa. Percent EPT and percent Chironomidae are taxonomic compositions that provide information on the make-up of the community and the relative contribution of a group to the total population (Barbour *et al.*, 1999). Percent two dominant taxa is a composition measure, but it is usually classified with tolerance measures. Tolerance measures are intended to be representative of the relative sensitivity to perturbation and may include numbers of pollution tolerant and intolerant taxa (Barbour *et al.*, 1999). The Hilsenhoff Biotic Index (HBI) is also a tolerance measure which was originally designed to evaluate organic pollution. HBI rates the tolerance of the community on a 0 to 10 scale with 0 being pollution intolerant (Barbour *et. al.*, 1999). The WVSCI is a multi-metric index that combines family level data describing the aforementioned six measures into a single value which is representative of the overall community health. The WVSCI provides a total score for each site with a range from 0 to 100. Each score is also assigned a narrative term (*Unimpaired* and *Impaired*).

WVSCI scores are applicable to kick-net samples which are identified to the family level. As an indicator of overall community health, the WVSCI score is used to evaluate whether the narrative criteria are being met in streams.

Objectives

In recent years, I have been involved with the coal industry monitoring streams and have conducted sampling for WET testing and benthic macroinvertebrate evaluations. I would like to use the available data to answer questions pertaining to the impacts of mining on downstream aquatic resources.

The specific objectives of the stream toxicity study are as follows:

1) Because conductivity is an indicator parameter, the relationship with individual ions, such as sulfate, will be developed and examined as an indicator of toxicity
2) To determine if mining effluent is toxic to sensitive laboratory test organisms
3) If so, to determine whether toxicity is related to the ionic concentration of mining effluent
4) And to demonstrate what level of stream conductivity is associated with toxicity
5) To determine if stream impairment, as indicated by WVSCI scores, is related to laboratory toxicity
6) To determine if stream impairment, as indicated by WVSCI scores, is related to conductivity.

Objectives 1 - 4 will be presented in Chapter 2, and Objectives 5 and 6 will be presented in Chapter 3. Chapter 4 will be a summary and conclusions of the research.

Chapter 2

Toxicity Testing

Introduction

Natural fresh waters contain several ionic constituents at greater than trace levels. Ions such as sodium, calcium, magnesium, potassium, chloride, sulfate, and bicarbonate are required to support aquatic life. Total dissolved solids (TDS) are a measure of all constituents dissolved in water. Elevated levels of TDS have been suggested as stressors to aquatic life in streams influenced by coal mining (Timpano 2010). Sulfate ions are known to be elevated in mining activity area receiving streams (Mount *et al.*, 1997).

Some effluents are toxic because of imbalances in the ion environment to which the test organisms are exposed (Goodfellow *et al.*, 2000). Toxicity can occur if ion concentrations are too low or too high for aquatic organisms to osmoregulate properly.

Aquatic organisms have developed physiological mechanisms to balance water and ion concentrations in their body fluids. A great deal of metabolic energy is spent trying to regulate water and ions (SETAC 2004a). Changes in the concentration or composition of ions over long periods of time can cause an organism to expend too much energy trying to regulate water and ions. This may result in chronic stress affecting important functions and can result in death (SETAC 2004a). The toxicity level and relative toxicity of common ions are well-documented (Mount *et al.*, 1997). There is also much evidence that the presence of two or more ions can ameliorate the expected toxicity and result in lower toxicity levels than expected by individual ionic testing (Soucek and Kennedy, 2005). The relationship between toxicity and specific ions is linear and predictable and can be used in determining safe exposure concentrations (Soucek and

Kennedy, 2005). Currently, no federal water quality criteria exist for the protection of aquatic life for several individual ions like sulfate or for TDS. WET testing has been used as part of the NPDES permitting process to evaluate complex effluents, specifically those with potential toxicity not protected by specific numeric criteria. WET testing has recently been employed to evaluate the toxicity of high conductivity discharges in mining influenced streams.

The water quality downstream of mining activity can have elevated levels of naturally occurring ions including SO_4, Ca, Mg, Fe, Mn, Se, alkalinity, K, acidity, and NO_3/NO_2 (Bryant *et al.*, 2002). In addition to dissolved solids, total suspended solids may also be elevated below mining activities. Sediment runoff is controlled through a series of sediment-control structures and ponds, but excess fine sediment might be increased in streams downstream of valley fills (Wiley and Brogan, 2003). Physical effects, such as increased turbidity from soil erosion, accumulation of coal fines, and smothering of the stream substrate from precipitated metal compounds, may also occur (Parsons 1968; Warner 1971).

The correlation between increasing TDS or conductivity and toxicity may vary with ionic composition and therefore may not be the best predictor of toxicity (Goodfellow *et al.*, 2000). If the conductivity of a freshwater effluent is above 2,000 μs/cm, the concentration of dissolved solids can be high enough to adversely affect freshwater test species (American Petroleum Institute 1998). The objective of the current study is to examine the potential toxicity of mining effluent dominated streams to establish whether mining discharges are toxic to aquatic organisms and to relate toxicity found to specific discharge constituents, specifically those contributing to overall conductivity or TDS measurements.

Materials and Methods

Sampling sites included in this study are selected from watersheds in West Virginia influenced by mining activity (**Figure 1**). Two coal companies were required to conduct semi-annual WET in streams receiving discharges where conductivity values greater than 1,500 µs/cm had been recorded in monthly discharge monitoring. WET testing was conducted December 2008 to September 2011. One coal company (Company 1) conducted testing at 71 sites, with 29 sites being sampled semi-annually (**Table 1**). Company 2 consisted of 48 sampling locations, with 19 sites sampled semi-annually (**Table 2**). Combined, there are 119 WET test results. Some sampling locations were tested multiple times.

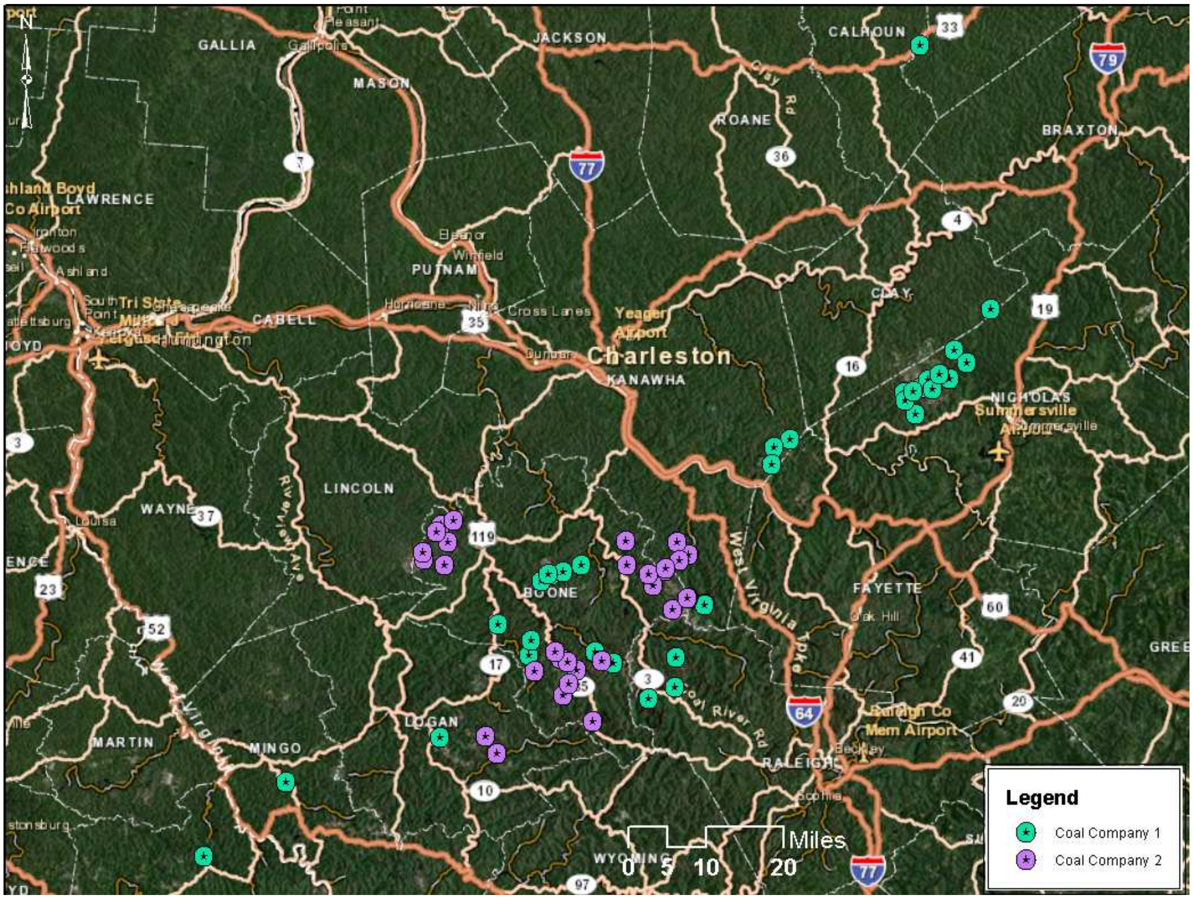

Figure 1 – Sampling Locations for WET Testing

Table 1 - Stream Sampling Locations - Company 1

Mine	Sampling Location
Company 1	Bias Branch
Company 1	Big Creek
Company 1	Downstream Fifteen Mile Fork
Company 1	Hardway Branch
Company 1	Hardway Pond @ Location PM 236
Company 1	Horse Creek
Company 1	Hughes Fork off Bells Creek
Company 1	Laurel Creek
Company 1	Lilly Fork @ PM 89
Company 1	Line Creek
Company 1	Mammoth Site 1
Company 1	Mammoth Site 2
Company 1	Mouth of Robinson Creek
Company 1	Mouth of Robinson Creek @ PM 24 location
Company 1	Mudlick Fork x3
Company 1	Hardway Pond
Company 1	No Name
Company 1	PM 260 Inlet
Company 1	PM 316 Pond
Company 1	Robinson Creek
Company 1	Robinson North @ PM 181
Company 1	Sixmile off Hughes Creek
Company 1	Slip Ridge
Company 1	Spruce Laurel Fork
Company 1	Stollings Fork
Company 1	Taylor Fork
Company 1	Twenty Mile Creek
Company 1	UBB Area of Jarrells Branch
Company 1	Upstream Fifteen Mile Fork
Company 1	West Fork
Company 1	Bandmill Below 016
Company 1	Below 033 on 20 Mile Fork
Company 1	Delbarton below 400
Company 1	ICC Below 031
Company 1	James Creek below 015
Company 1	Mammoth below 004
Company 1	Marfork Below 018
Company 1	029 on Radner Fork of 20 mile
Company 1	013 Robinson North on 20 mile
Company 1	001 on Sugarcamp

Table 2 – Stream Sampling Locations - Company 2

Mine	Sampling Location
Company 2	Ballard Branch
Company 2	Calvin Branch
Company 2	Sycamore Fork
Company 2	Tenmile Fork
Company 2	Cow Creek
Company 2	Joes Creek
Company 2	Left Fork
Company 2	Stanley Fork
Company 2	Jarrell Branch
Company 2	White Oak
Company 2	Mud Lick Branch
Company 2	Jack Smith Branch
Company 2	West Fork
Company 2	Cabin Creek
Company 2	Coal Fork
Company 2	Seng Creek
Company 2	Tom's Fork
Company 2	Little White Oak
Company 2	UNT Left Fork
Company 2	UNT Tenmile Fork
Company 2	UNT Boone Block Hollow
Company 2	Big Horse Creek
Company 2	Pond Fork
Company 2	Moccasin Hollow

WET testing was conducted under low summer/fall conditions and higher flow winter/spring conditions. Field water quality measurements were taken at the time of sample collection by both companies. Field parameters measured included conductivity, pH, temperature, and dissolved oxygen. Additional water quality parameters requiring laboratory analysis were measured at sampling stations for Company 1 from the initiation of WET testing in the spring and fall of 2011. The additional parameters included: total alkalinity, total acidity, turbidity, specific conductance, total sulfates, chlorides, total and dissolved iron, total and dissolved manganese, total and dissolved aluminum, total and dissolved sodium, total and dissolved magnesium, total and dissolved calcium, total and dissolved hardness, total suspended solids, and total dissolved solids. Additional water quality parameters requiring laboratory analysis were measured by Company 2 in the winter of 2011 and fall of 2011. The additional parameters included: bicarbonate, lab tested specific conductance, sulfate, and total dissolve solids. In 2011, additional water quality analyses were collected along with WET testing as described by the West Virginia Department of Environmental Protection (WVDEP) Permitting Guidance for Surface Coal Mining Operations (2010a).

Water for toxicity testing must be collected three times during the course of the seven-day test. Water was collected, to the extent practical, from mid-channel, mid-depth locations. It was collected in dedicated 1 gallon cubitainers and stored in coolers on ice during transport to the laboratory.

The freshwater microcrustaceans *Ceriodaphnia dubia* were used in the seven-day chronic toxicity tests consistent with United States Environmental Protection Agency (USEPA) Method 133. Organism mortality and reproduction were endpoints.

Ceriodaphnia dubia can be used in short-term standardized tests to estimate the acute or chronic toxicity of chemicals, effluents, and freshwater receiving systems (Naddy *et al.*, 1995; Stewart *et al.*, 1990; Nimmo *et al.*, 1990). The use of this animal as a representative aquatic organism in such tests is justified in part because it has a widespread geographic distribution and holds an intermediate position in planktonic food webs; it consumes algae and detritus and, in turn, is consumed by various predators (Stewart and Konetsky, 1998). *Ceriodaphnia dubia* is also convenient to use because it is sensitive to various toxic chemicals, easily reared under laboratory conditions, and has a moderately short life cycle (Mount and Norberg, 1984).

Toxicity tests were conducted at laboratories that were National Environmental Laboratory Accreditation Conference (NELAC) certified. The water samples were diluted at control concentration, 6.25% concentration, 12.5% concentration, 25.0% concentration, 50.0% concentration, and 100.0% concentration.

Results

Field Water chemistry

In 2008, the conductivity ranged from 284 µS/cm at Spruce Laurel Creek to 2,540 µS/cm at Mudlick Fork (**Table 3, Appendix A**). The dissolved oxygen ranged from 9.19 mg/L at Robinson Creek to 11.33 mg/L at Line Creek. The temperature at the time of collection ranged from 3.0°C at Big Creek to 16.1°C at West Fork. The pH ranged from 5.93 S.U. at Taylor Fork to 8.13 S.U. at West Fork.

In 2009, the conductivity ranged from 68 µS/cm at Mammoth Site 2 to 2,990 µS/cm at Joes Creek (**Table 4, Appendix A**). The dissolved oxygen ranged from 9.10 mg/L at Jarrell Branch to 12.50 mg/L at Sixmile off Hughes Creek. The temperature ranged from 1.1°C at upstream fifteen mile to 18.6°C at Jarrell Branch. The pH ranged from 6.72 S.U. at the Downstream Fifteen Mile Fork to 8.57 S.U. at Mammoth Site 1.

In 2010, the conductivity ranged from 222 µS/cm at Little White Oak to 3,074 µS/cm at Twentymile Creek (**Table 5, Appendix A**). The dissolved oxygen ranged from 4.0 mg/L at UBB Area of Jarrells Branch to 14.71 mg/L at White Oak. The temperature ranged from 2.6°C at Horse Creek to 22.5°C at Mudlick Branch. The pH ranged from 5.25 S.U. at No Name to 10.40 S.U. at Mudlick Fork.

In 2011, the conductivity ranged from 105 µS/cm at below 033 on 20 Mile Fork to 2,412 µS/cm at Tom's Fork (**Table 6, Appendix A**). The dissolved oxygen ranged from 6.90 mg/L at Delbarton below 400 to 12.00 mg/L at West Fork. The temperature ranged from 4.0°C at Bandmill below 016 and Marfork below 018 to 26.8°C at ICC below 031. The pH ranged from 6.50 S.U. at below 033 on 20 Mile Fork to 8.94 S.U. at Moccasin Hollow.

Additional water data were collected for Company 1 in the spring and fall of 2011. Spring sampling occurred in May and June of 2011. Fall sampling occurred in October and November of 2011. The pH was in the acceptable range of 6 to 9 S.U. at all sampling sites during the spring and fall sampling events (**Tables 7 and 8, Appendix A**). Total and Dissolved ions measured included iron, manganese, aluminum, sodium, calcium, and hardness concentrations. Total iron did not exceed the water quality standards of 1.5 mg/L and (**Figure 2**). Total manganese exceeded the water quality standard of 1.0 mg/L at the Sugarcamp and Radner sampling locations (**Figure 3**). Total Aluminum did not exceed the water quality standard of 0.75 mg/L at any of the sampling locations (**Figure 4**). Total and dissolved sodium concentrations were the same in the spring and fall of 2011 (**Figure 5**). Total and dissolved ion concentrations for magnesium and calcium were equal, indicating the water is not saturated with respect to those constituents (**Figure 6**).

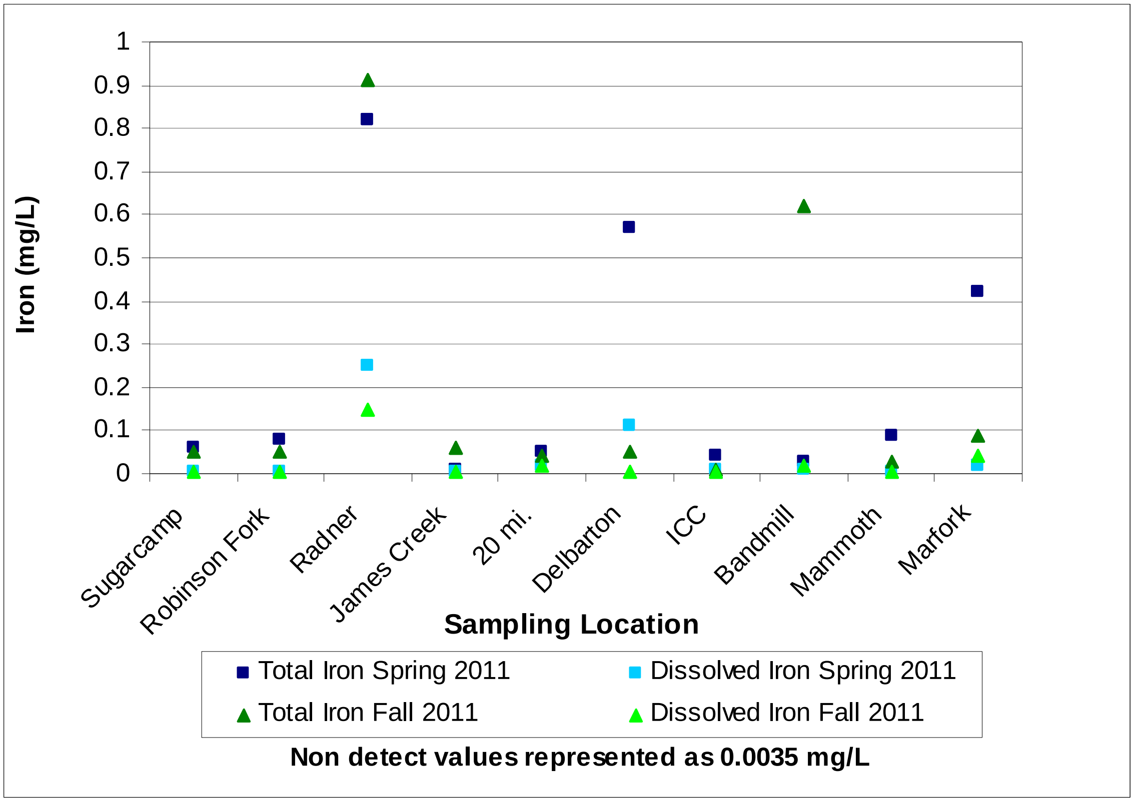

Figure 2 - Total and Dissolved Iron Concentrations from Sites Sampled in the Spring and Fall 2011

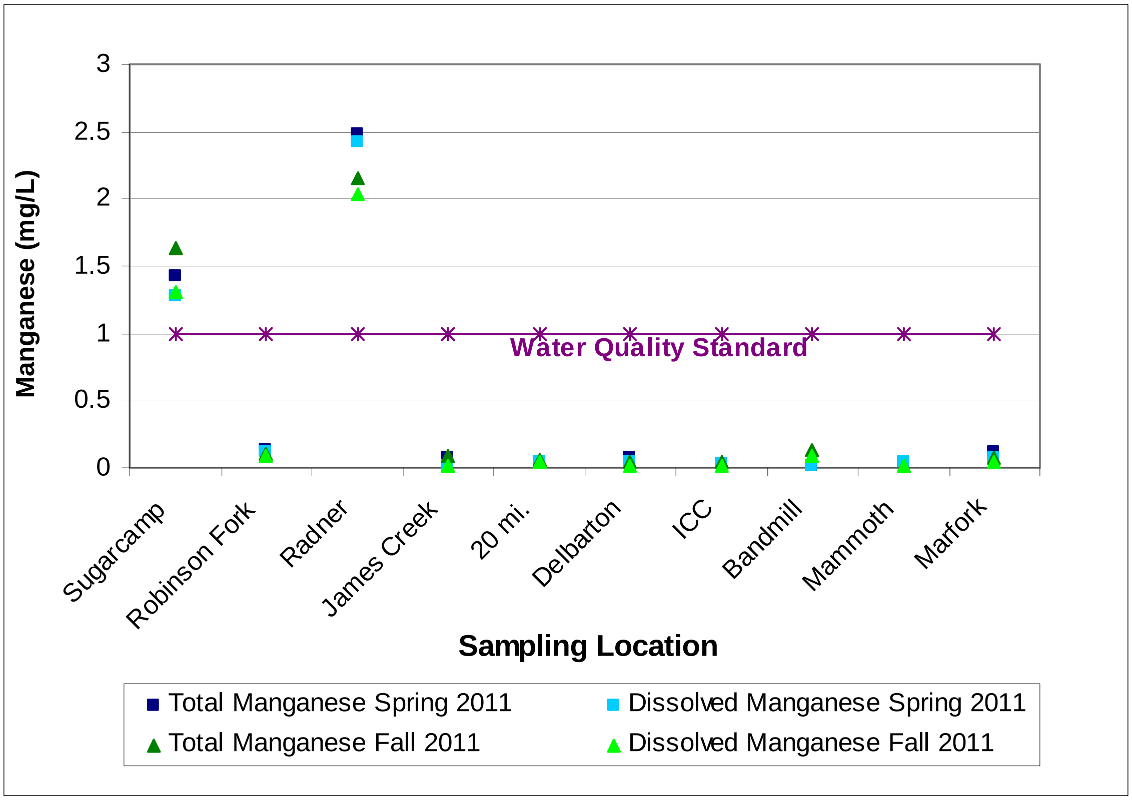

Figure 3 – Total and Dissolved Manganese Concentrations from Sites Sampled in the Spring and Fall 2011

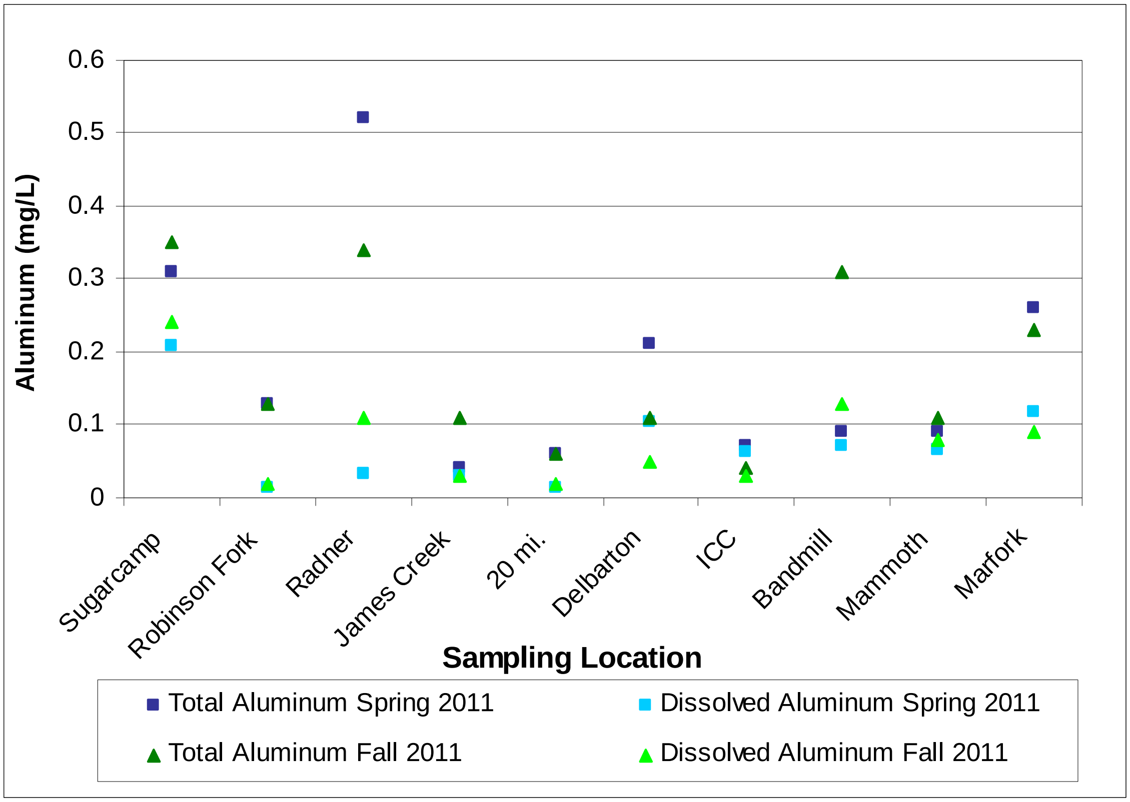

Figure 4 – Total and Dissolved Aluminum Concentrations from Sites Sampled in the Spring and Fall 2011

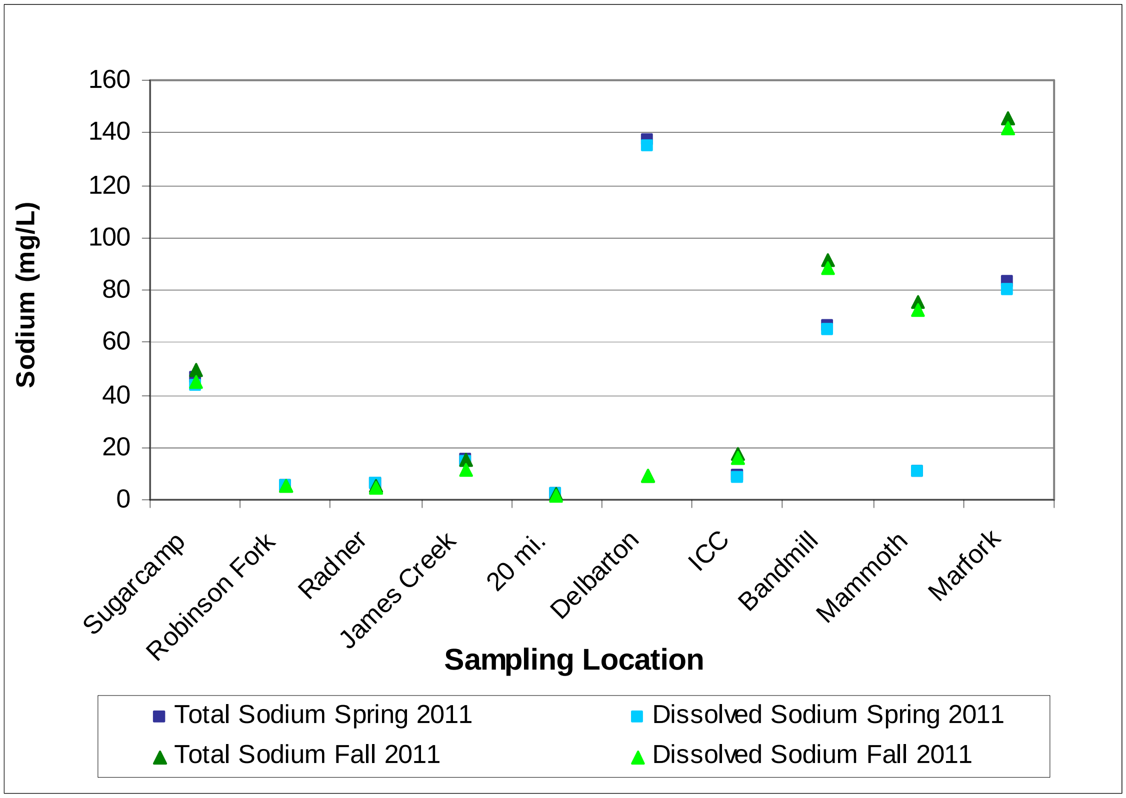

Figure 5 – Total and Dissolved Sodium Concentrations from Sites Sampled in the Spring and Fall 2011

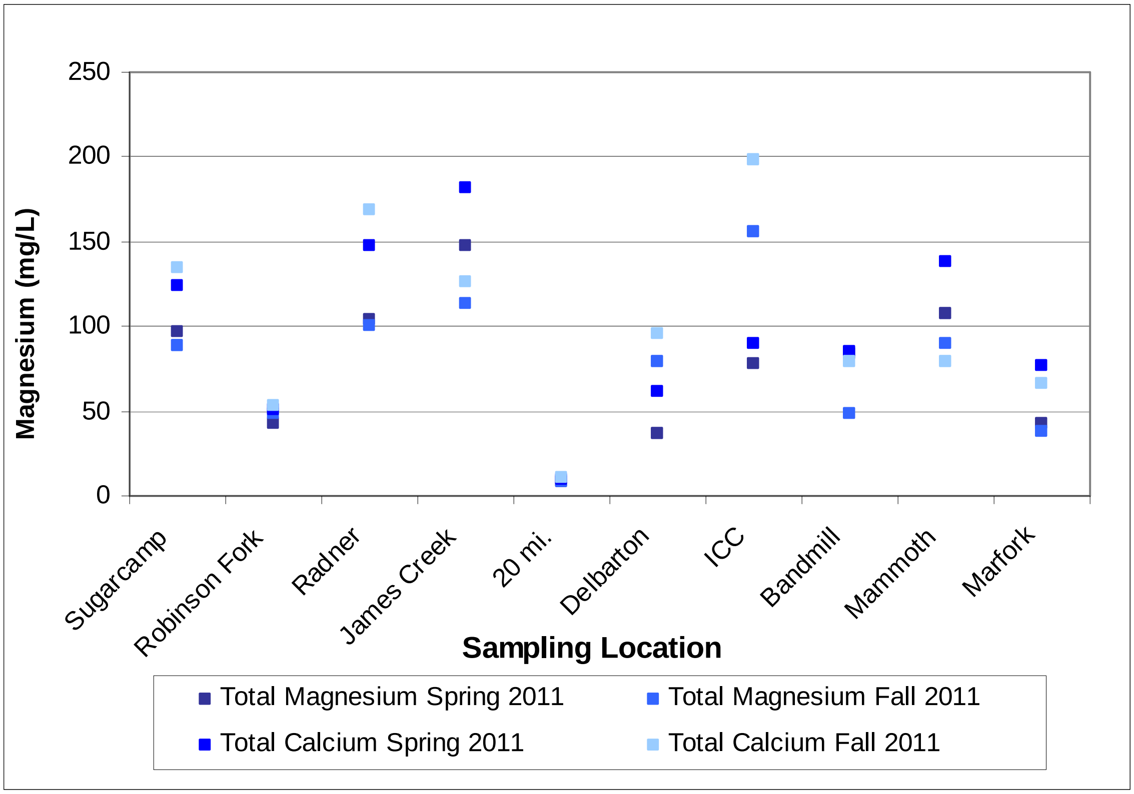

Figure 6 – Total Magnesium and Calcium Concentrations from Sites Sampled in the Spring and Fall 2011

Mount *et al.* (1997) developed a DOS-based model effective in identifying ion toxicity to 3 freshwater organisms (*Ceriodaphnia dubia*, *Daphnia magna*, and *Pimephales promelas*). Using the model developed by Mount, stream ion toxicity for Company 1 was calculated using spring (May/June) and fall (October/November) 2011 water data. Ion imbalance was demonstrated for James Creek, Delbarton, ICC, and Marfork in the spring of 2011 (**Table 9, Appendix A**). Predicted percent survival in 100% stream water ranged from 84.6% to 99.9% in these samples. Ion imbalance was demonstrated for Mammoth, ICC, James Creek, and Delbarton, in the fall of 2011 (**Table 10, Appendix A**). Predicted percent survival in 100% stream water ranged from 84.6% to 99.9% in these samples. When comparing the predicted results from Mount's model to the measured results for 100% stream water survival, the results were consistent except for James Creek and Mammoth in the fall of 2011. Mount's model predicted a survival rate of 84.6% in the 100% concentration; however, the actual result was 100%. James Creek had a predicted survival rate of 99.7%; however, the actual result was 70%.

TSS ranged from non detect to 13 mg/L during the spring of 2011 and non detect to 9 mg/L in the fall of 2011 (**Figure 7**). Radner had the highest TSS in both sampling events.

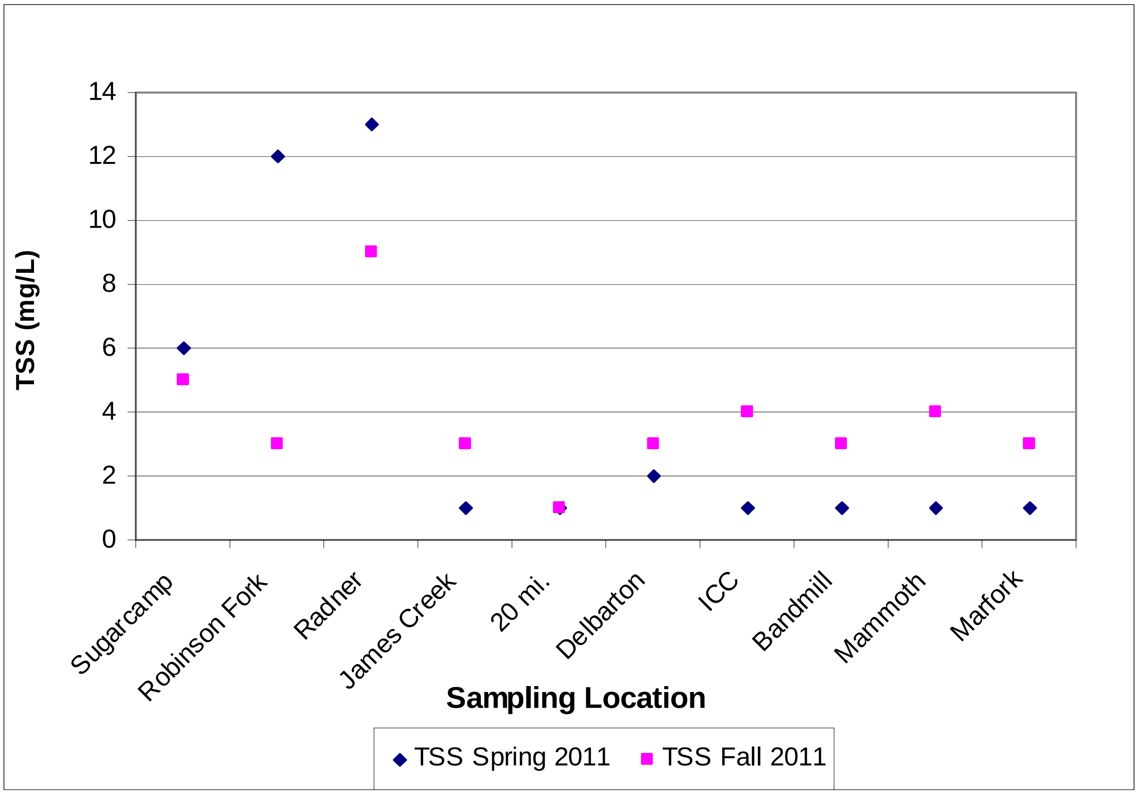

Figure 7 – Total Suspended Solid Concentrations from Sites Sampled in the Spring and Fall 2011

Radner, Sugarcamp, ICC, James Creek and Mammoth were dominated by sulfate in the 2011 sampling (**Figures 8 - 13)**. Total Alkalinity was abundant at Marfork and

Delbarton in the spring of 2011 (**Figures 14 -15**). Potassium levels were estimated based on professional judgment because it was not analyzed in spring and fall 2011.

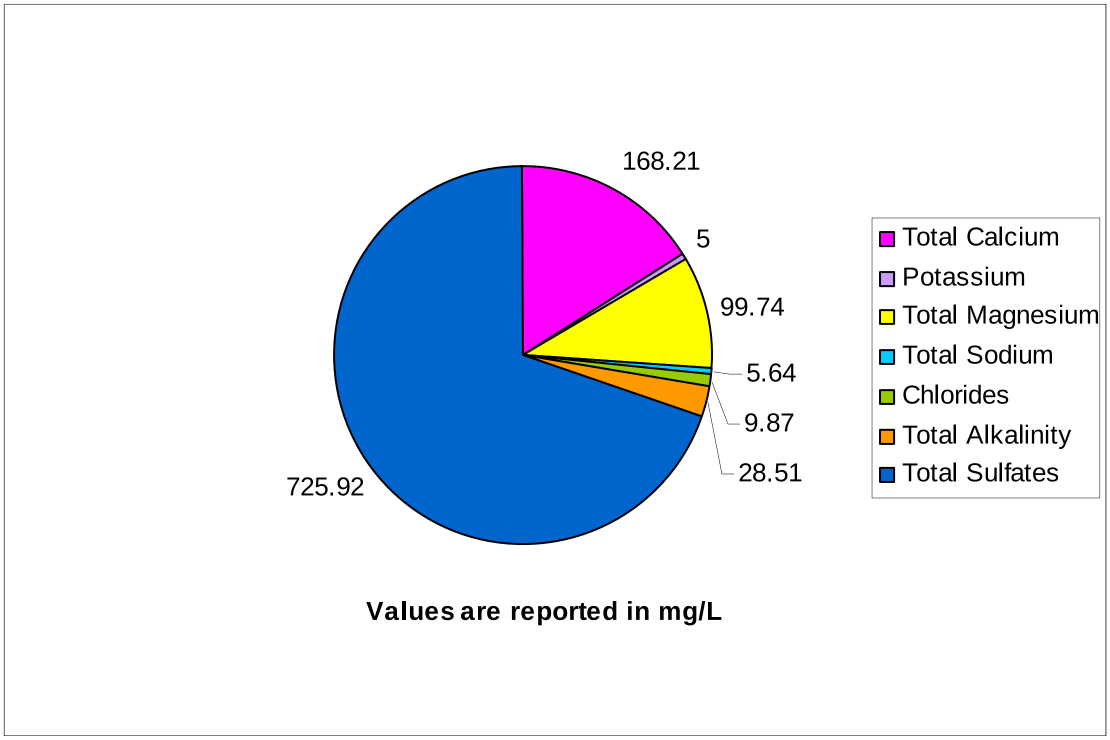

Figure 8 - Major Ionic Constituents in Water Collected from Radner in Fall 2011

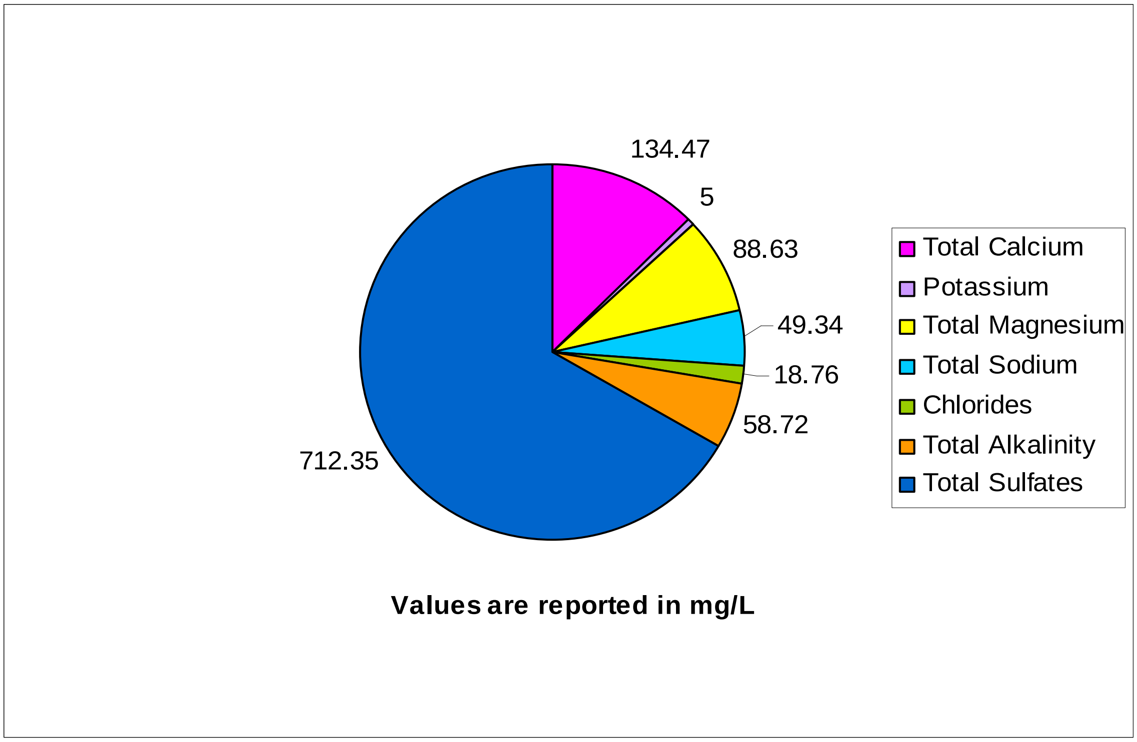

Figure 9 - Major Ionic Constituents in Water Collected from Sugarcamp in Fall 2011

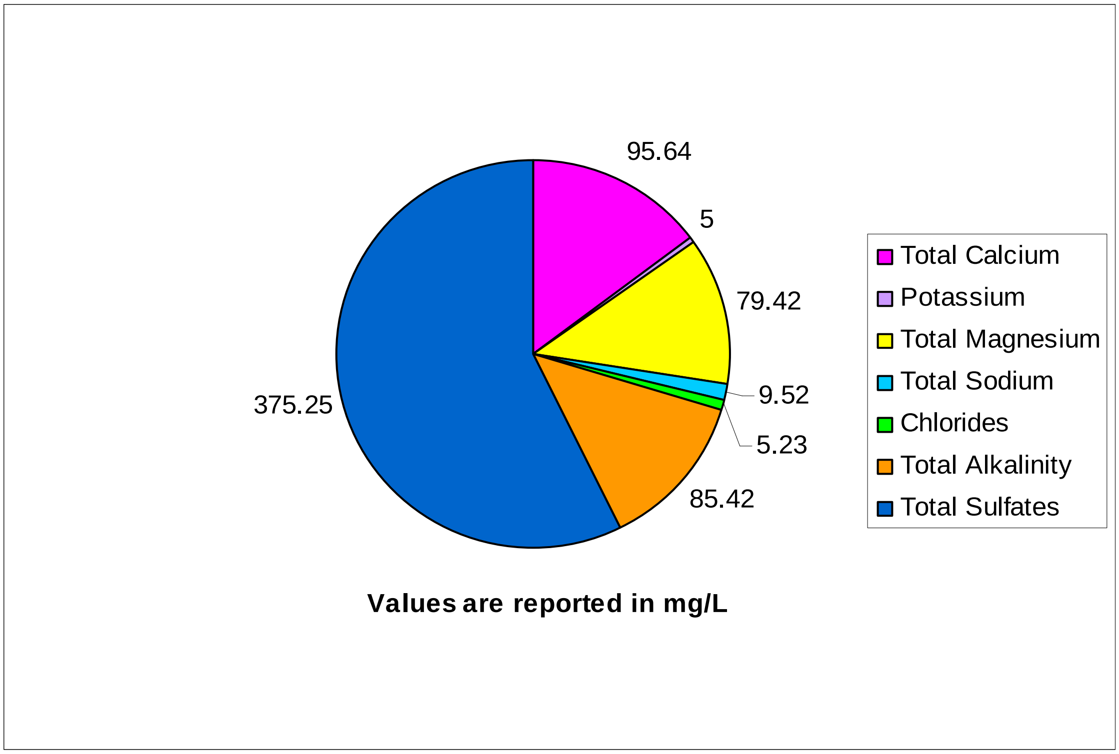

Figure 10 - Major Ionic Constituents in Water Collected from ICC in Fall 2011

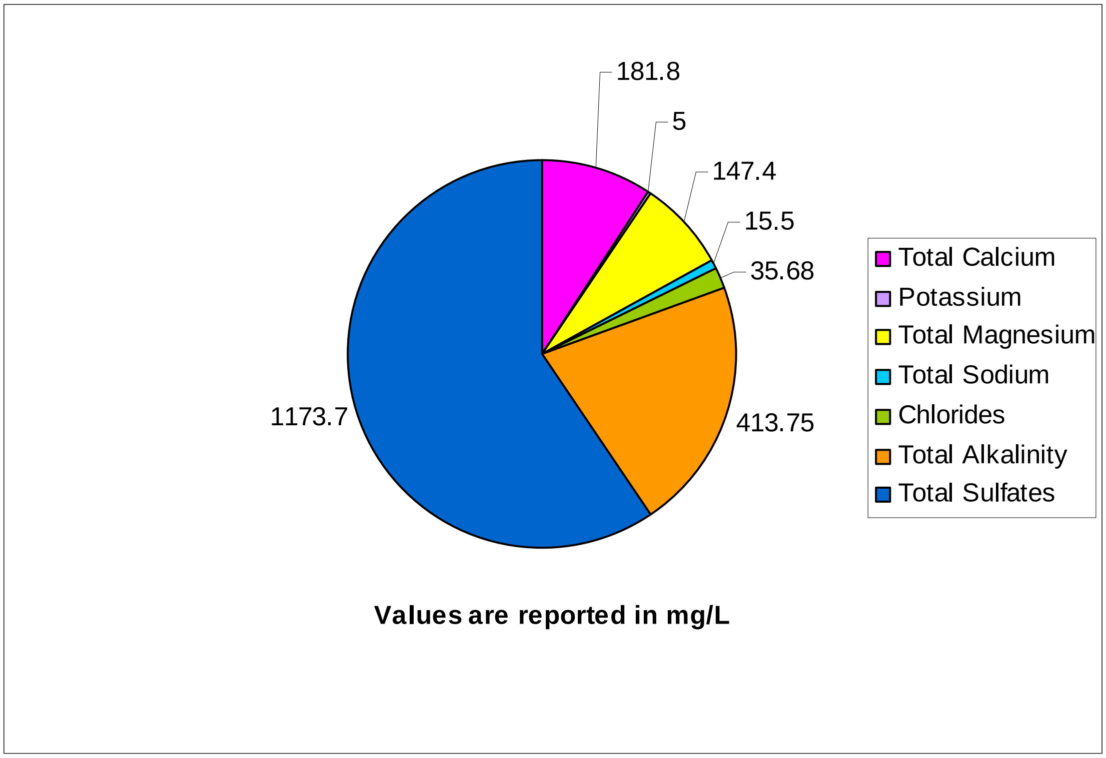

Figure 11 - Major Ionic Constituents in Water Collected from James Creek in Spring 2011

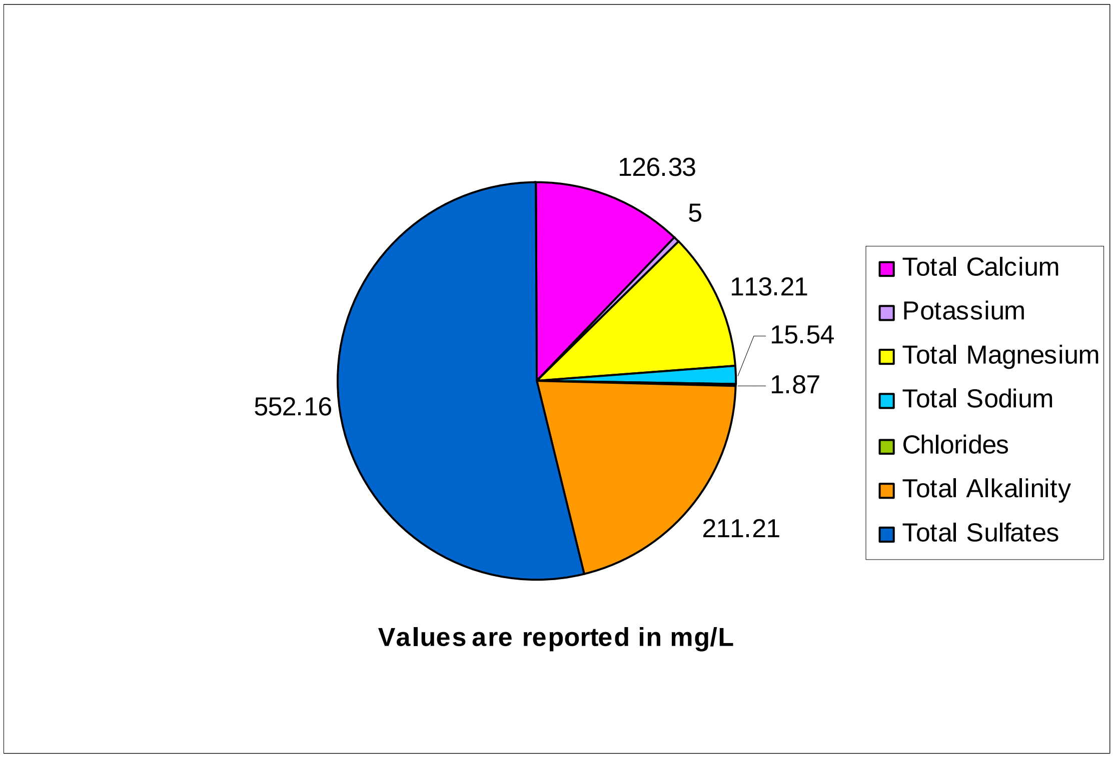

Figure 12 - Major Ionic Constituents in Water Collected from Mammoth in Fall 2011

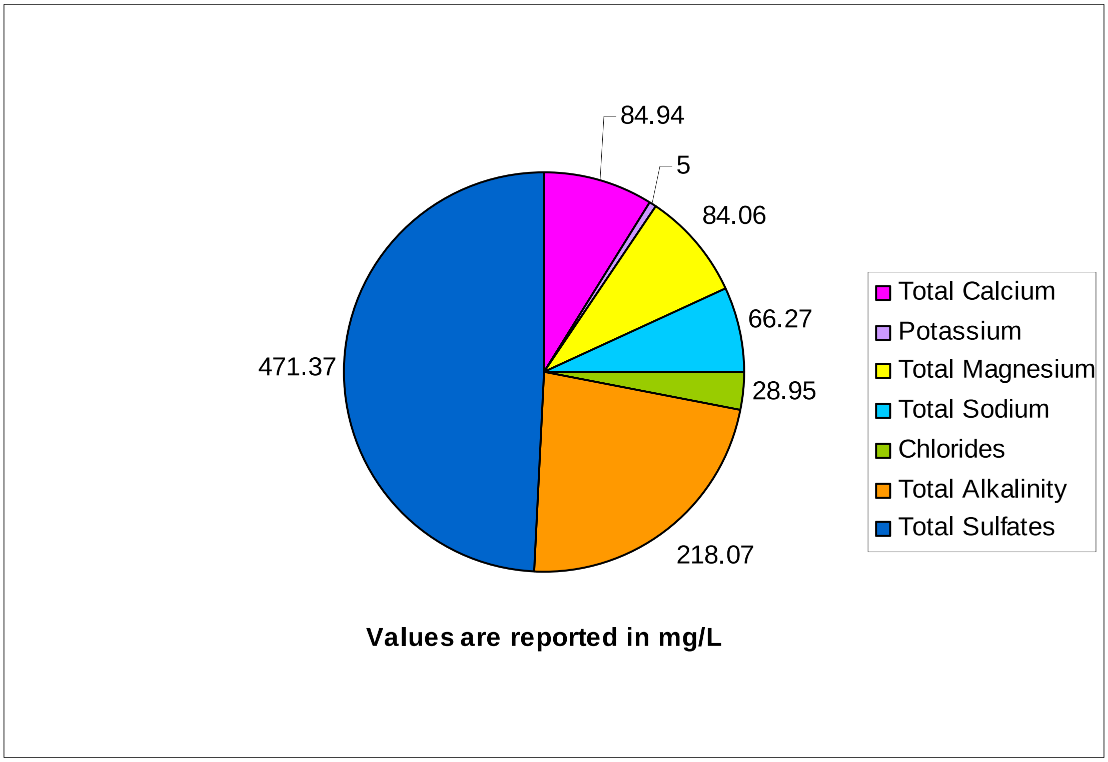

Figure 13 - Major Ionic Constituents in Water Collected from Bandmill in Spring 2011

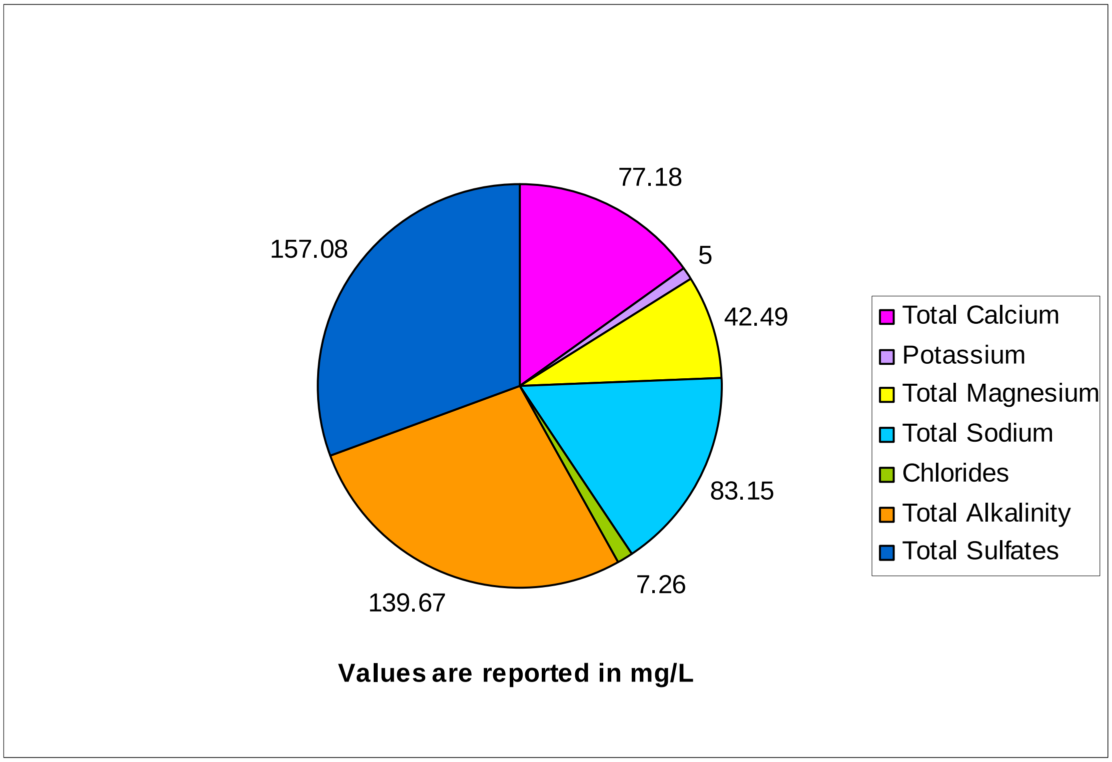

Figure 14 - Major Ionic Constituents in Water Collected from Marfork in Spring 2011

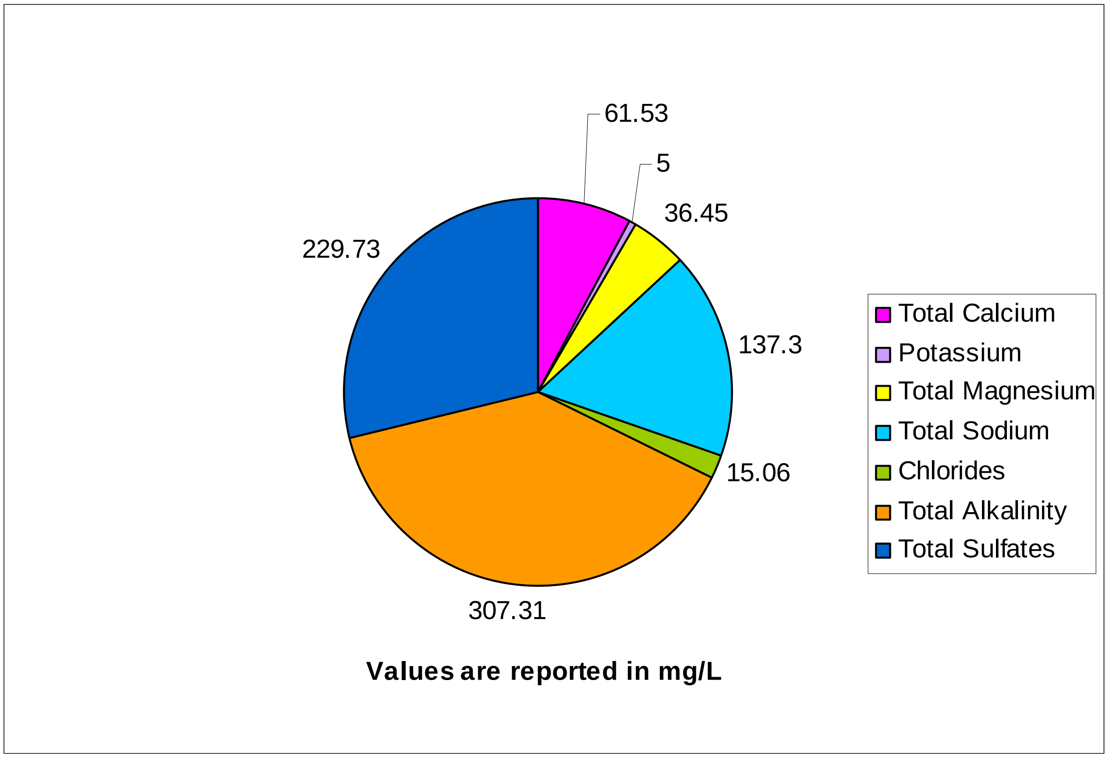

Figure 15 - Major Ionic Constituents in Water Collected from Delbarton in Spring 2011

The strongest relationship was observed between the indicator parameters TDS and conductivity (**Figure 16).** A lower correlation was observed between sulfate and conductivity **(Figure 17)**. There was relationship observed between sulfate and TDS (**Figure 18)**. There was a prominent relationship between magnesium and conductivity **(Figure 19)**. The relationship between conductivity and chloride was weak (**Figure 20**). There was no correlation between bicarbonate and conductivity (**Tables 11-12, Appendix A**) (**Figure 21**).

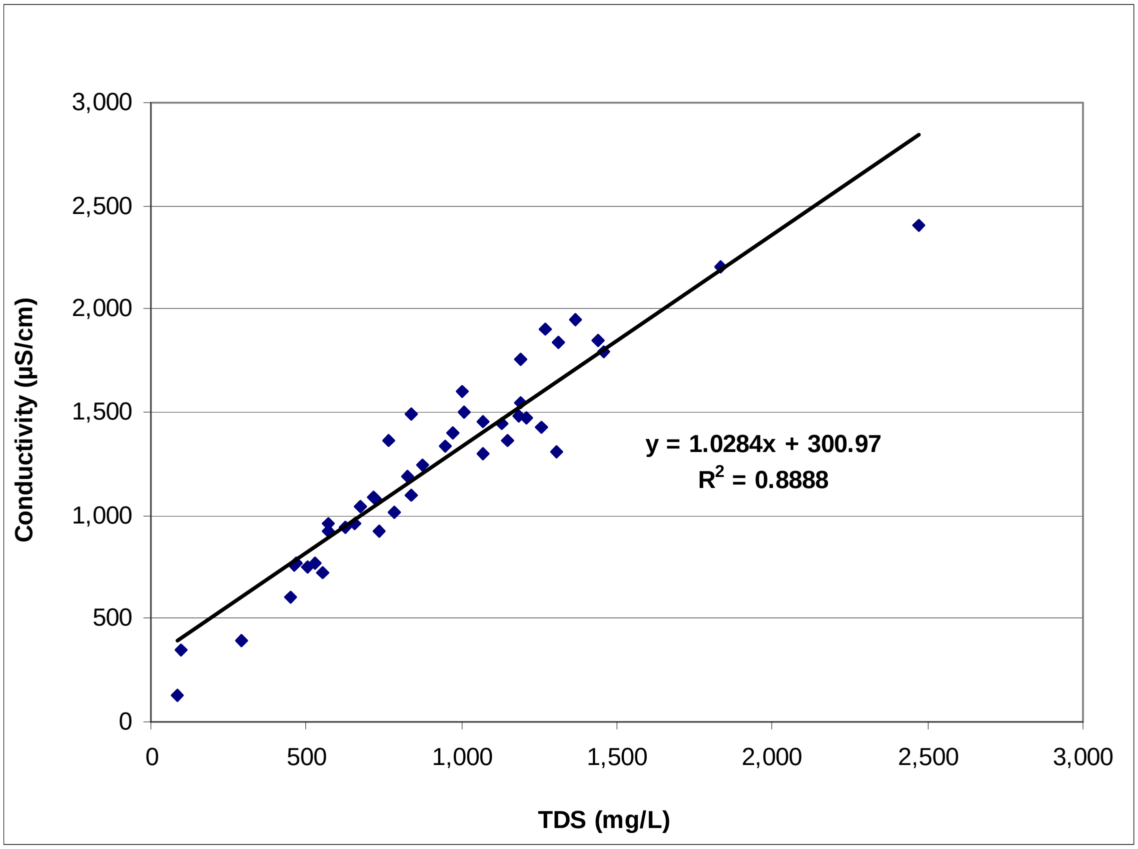

Figure 16 - Relationship between Total Dissolved Solids and Conductivity in Mine Influenced Streams

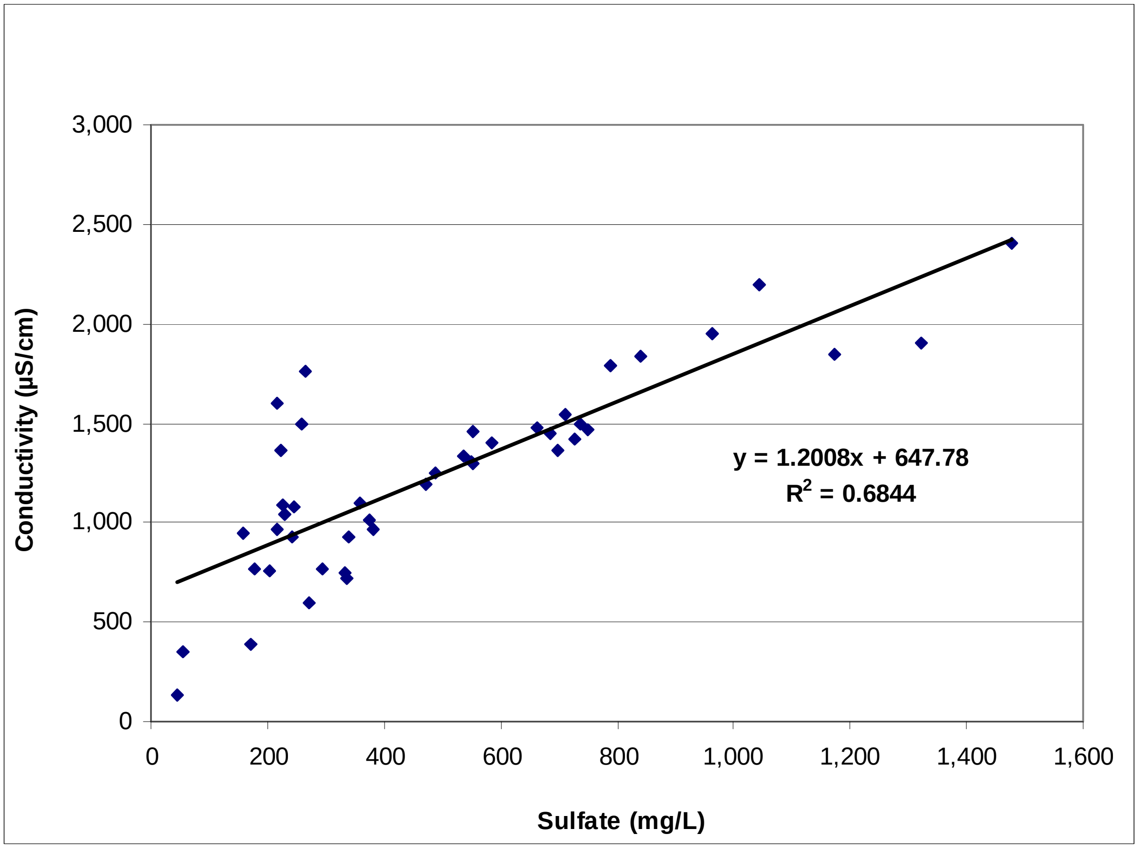

Figure 17 - Relationship between Sulfate and Conductivity in Mine Influenced Streams

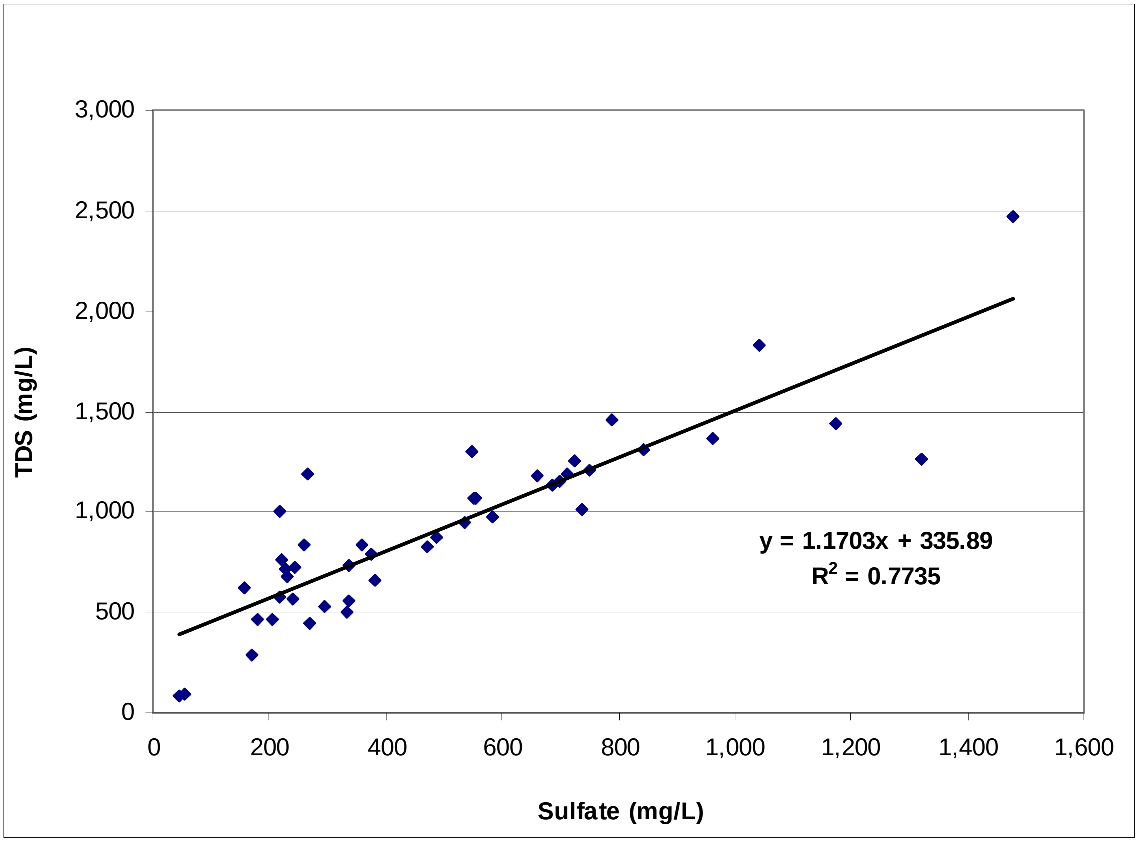

Figure 18 - Relationship between Sulfate and Total Dissolved Solids in Mine Influenced Streams

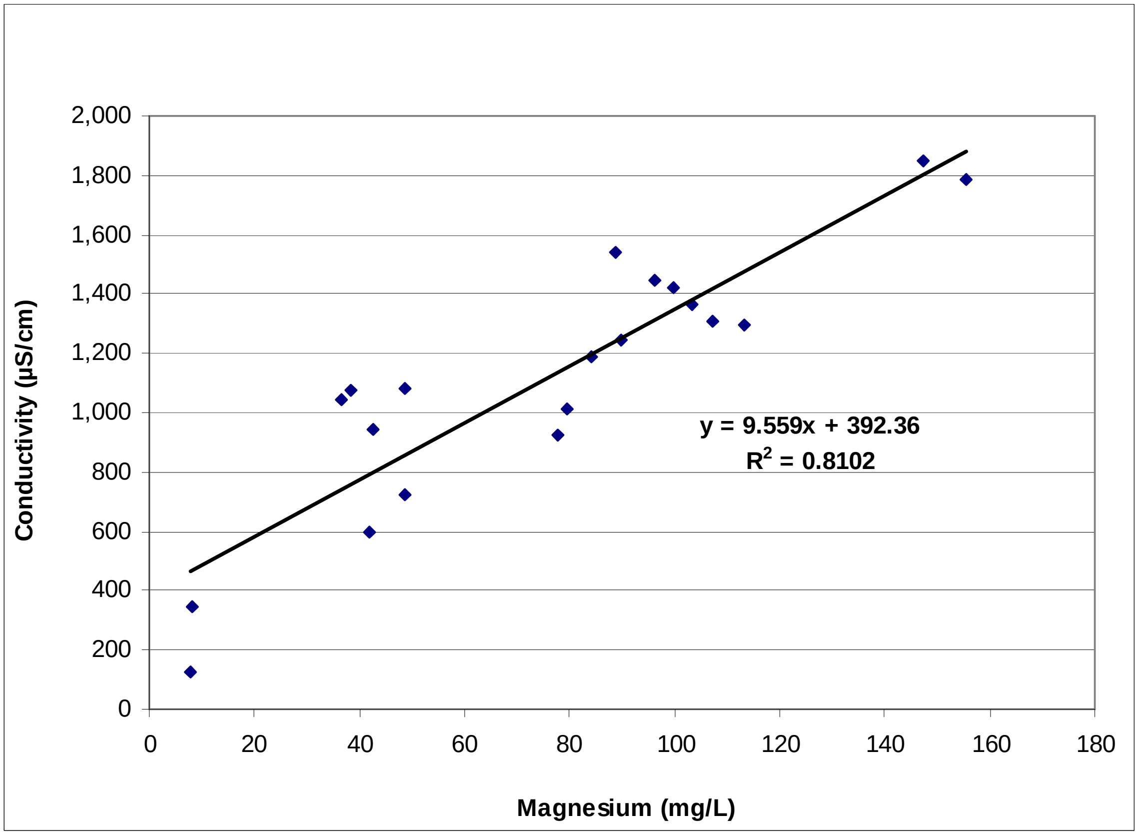

Figure 19 - Relationship between Magnesium and Conductivity in Mine Influenced Streams

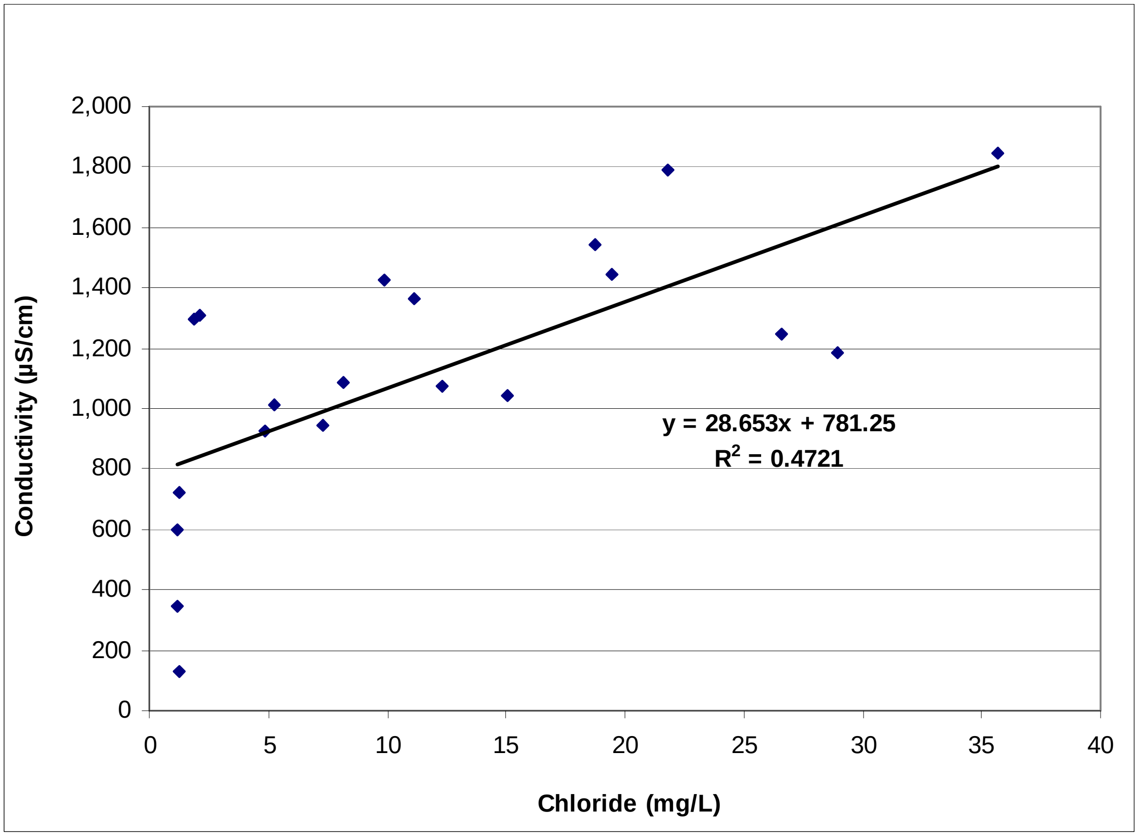

Figure 20 - Relationship between Chloride and Conductivity in Mine Influenced Streams

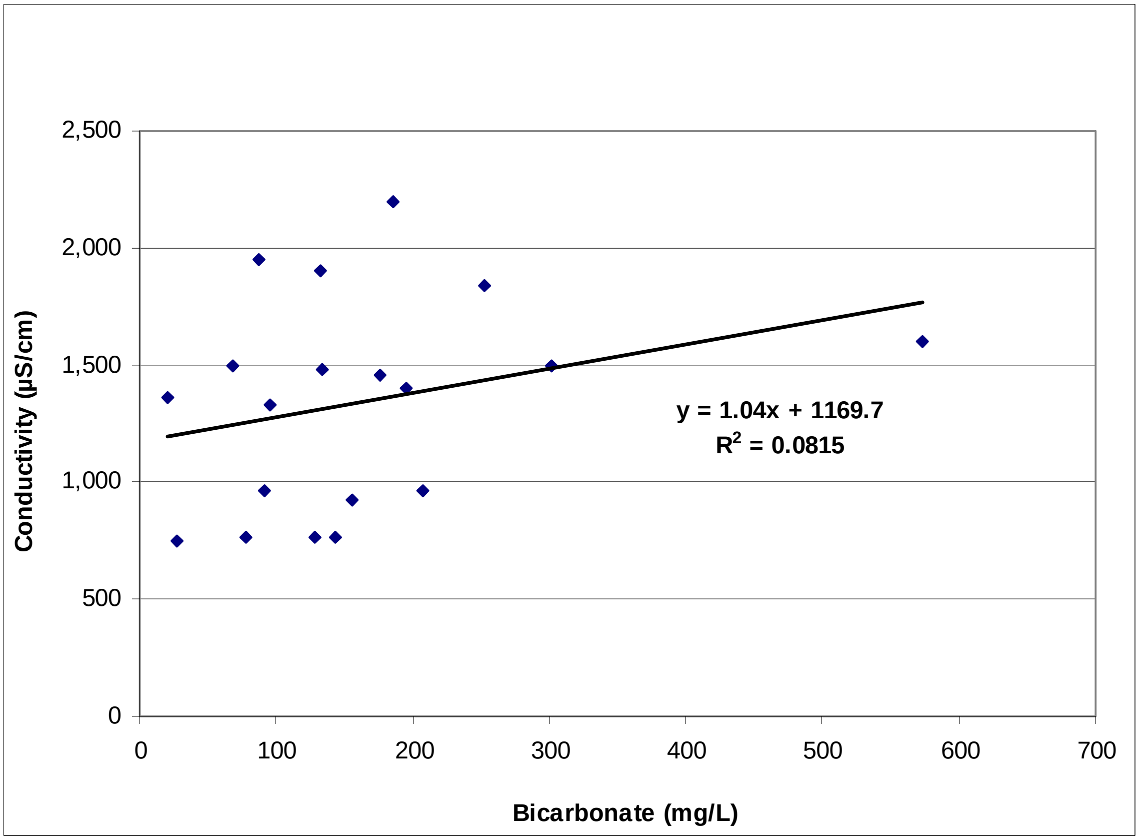

Figure 21 - Relationship between Bicarbonate and Conductivity in Mine Influenced Streams

Control survival of *Ceriodaphnia dubia* was acceptable in each of the tests conducted with 75 of the control treatments having 100% survival. There were 31 tests with 90% survival and 13 tests with 80% control survival (**Tables 13-14, Appendix A**). The percent survival in 100% stream water was 57 streams at 100%, 37 streams at 90%, 16 streams at 80%, 4 streams at 70%, 1 stream at 60%, 2 streams at 50%, and 2 streams at 0% (**Figure 22**). The Lethal Concentration to 50% of the organisms (LC50) was calculated for each test at 48 hours. For the majority of the streams tested, 115 out of 119 streams, the LC50 was >100% stream water or no LC50 was generated. The LC50 at 1 of the streams was 100%; 1 stream had an LC50 of 57% and 1 stream had an LC50 of

50% stream water. Jack Smith Branch had no toxicity demonstrated for the LC50 in fall 2010. The NOEC for survival was 100 for 112 out of the 119 streams. There were 6 streams with an NOEC of 50 and 1 stream with a NOEC of 25.

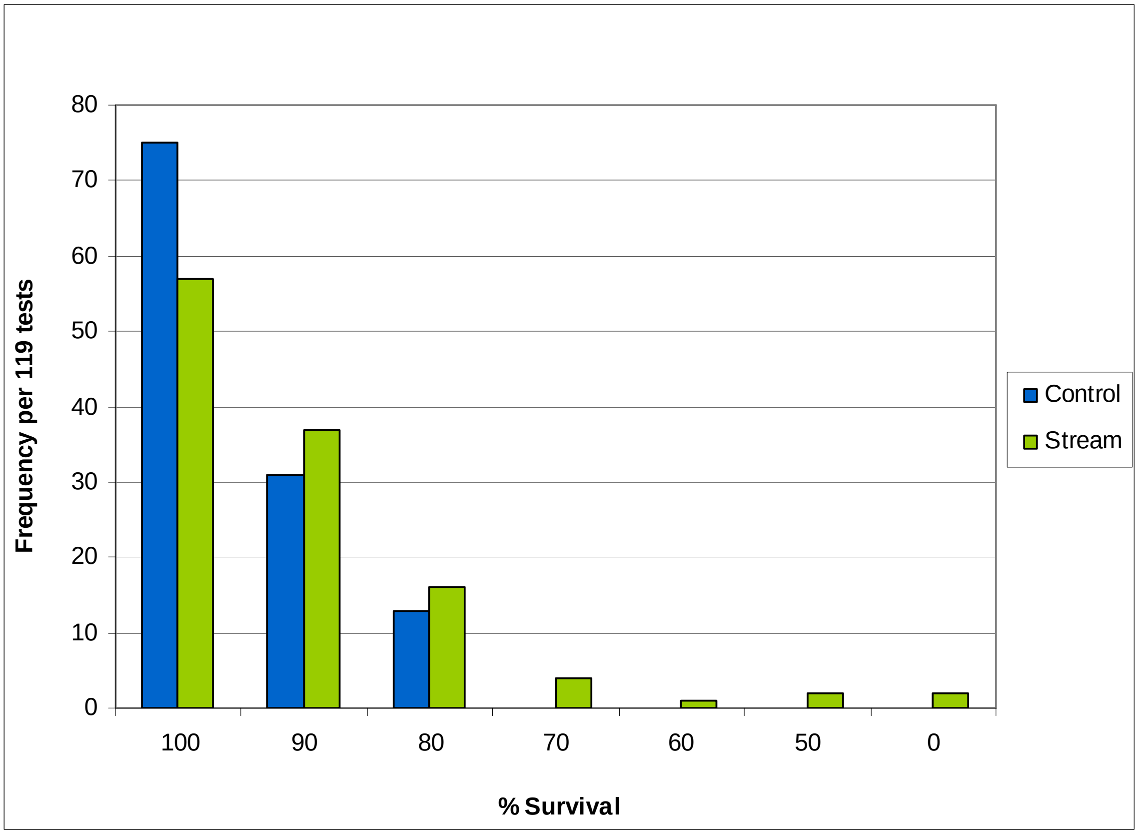

Figure 22 - Percent Ceriodaphnia dubia Survival in Control and 100% Stream Water in Mining Influenced Streams

Stream conductivity, TDS, and sulfate showed no relationship when compared to percent stream survival in 100% stream water (**Figures 23-25**).

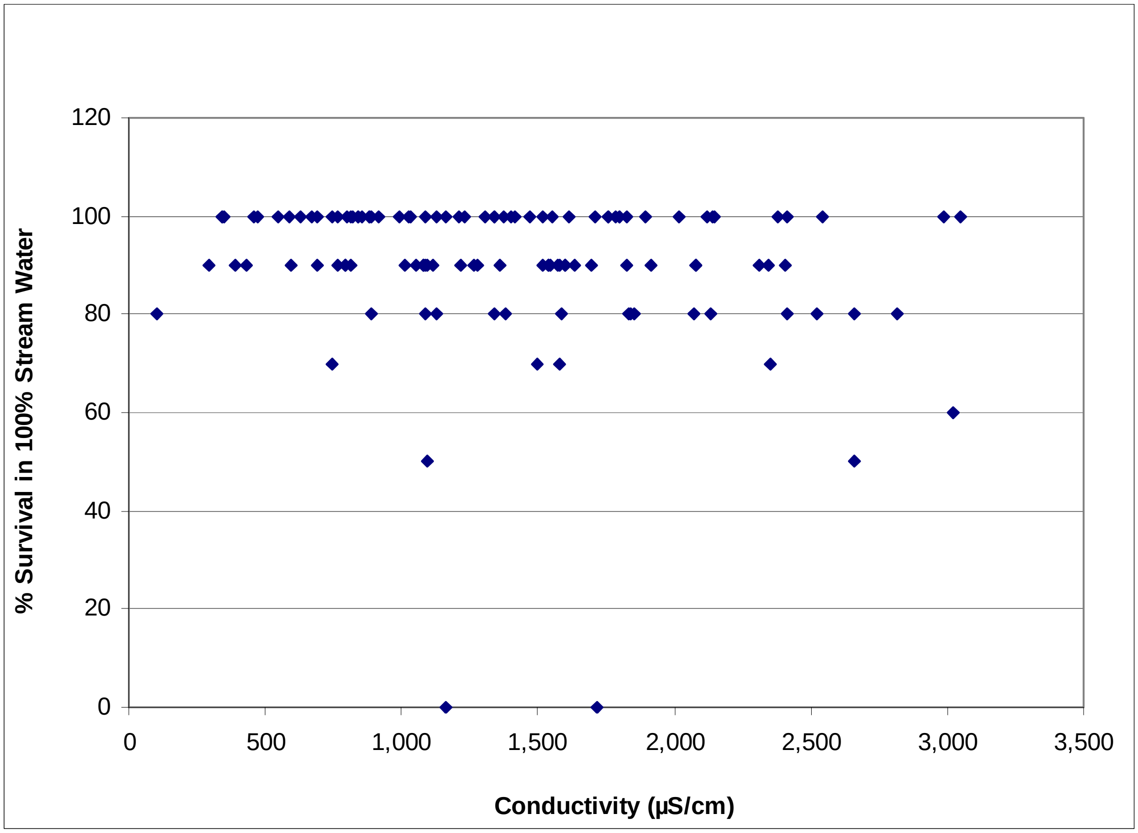

Figure 23 - Relationship between Conductivity and Percent Survival of Ceriodaphnia dubia in 100% Mine Influenced Stream Water

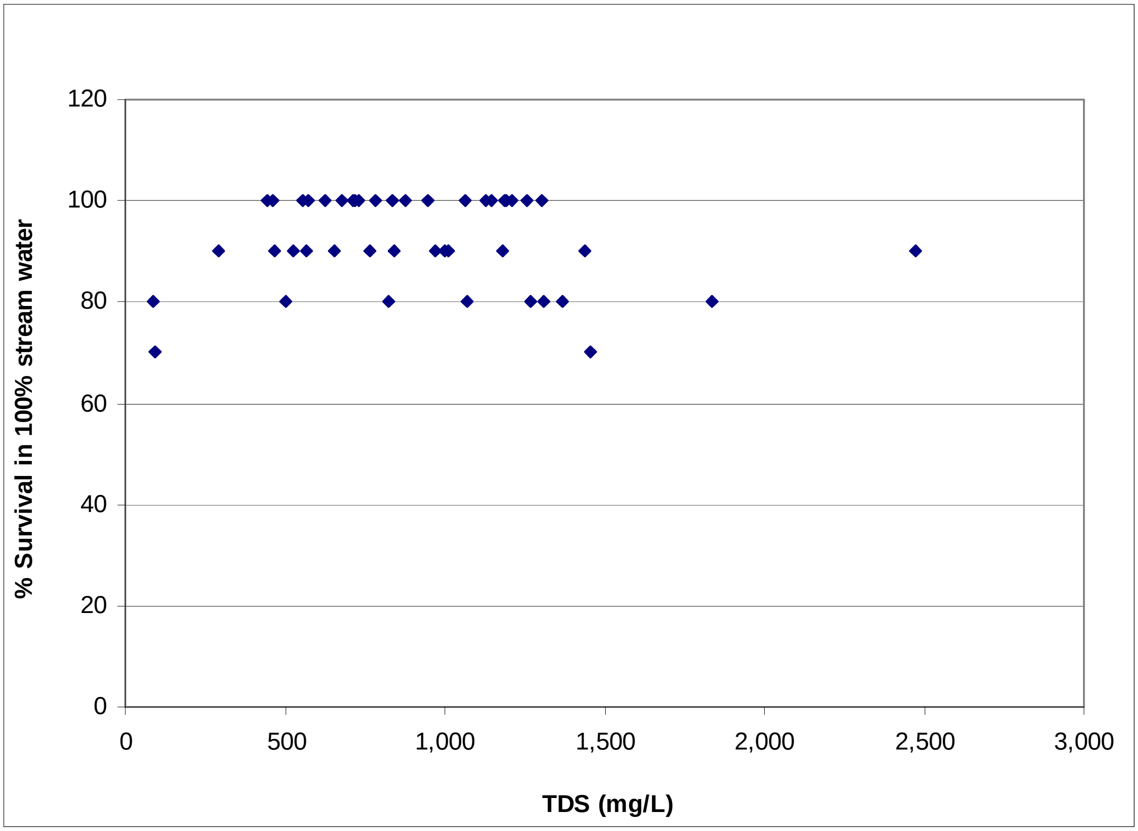

Figure 24 - Relationship between Total Dissolved Solids and Percent Survival of Ceriodaphnia dubia in 100% Mine Influenced Stream Water

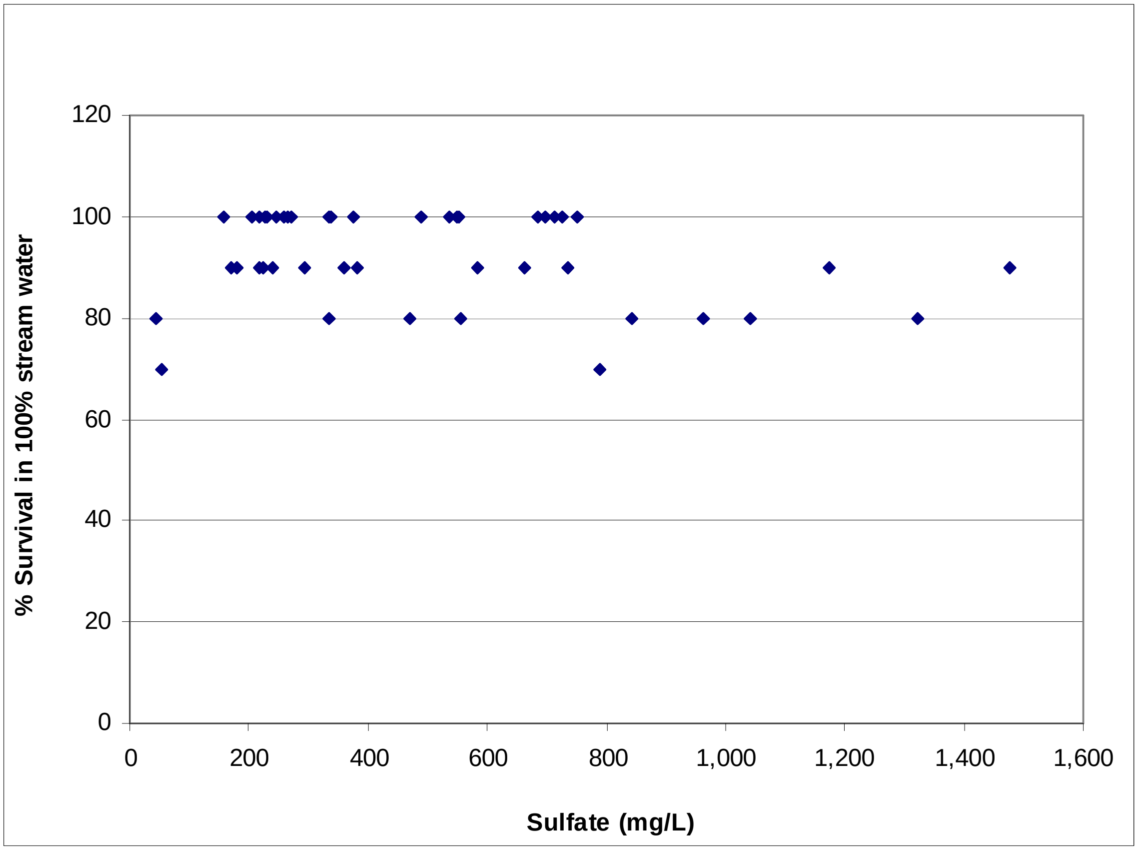

Figure 25 - Relationship between Sulfate and Percent Survival of Ceriodaphnia dubia in 100% Mine Influenced Stream Water

With respect to the sub-lethal reproductive endpoint, 77 of the 119 sites had NOEC concentrations of 100% (**Tables 15-18, Appendix A**). NOEC concentrations at 29 of the stream sampling locations were 50%, 8 sampling locations had NOEC concentrations of 25% and 5 stream locations had an NOEC concentration of 12.5. The Lowest Observable Effect Concentration (LOEC) concentrations were greater than 100% at 76 of the 119 stream sampling locations. LOEC concentrations were 100% at 30 of the sampling locations, 50% at 8 locations and 25% at 5 sampling locations. The IC25, or concentration of stream water which is predicted to result in a 25% reduction in reproduction, was greater than 100% for 61 of the 119 streams. IC25 concentrations ranged from 15-25 for 6 of the streams, 26-50 for 6 of the streams, 51-75 for 24 streams and 76-100 for 22 of the streams.

There was no relationship between the sensitive IC25 endpoint with conductivity, total dissolved solids, and sulfate (**Figures 26-28)**. There was no relationship between conductivity and toxicity in the streams sampled.

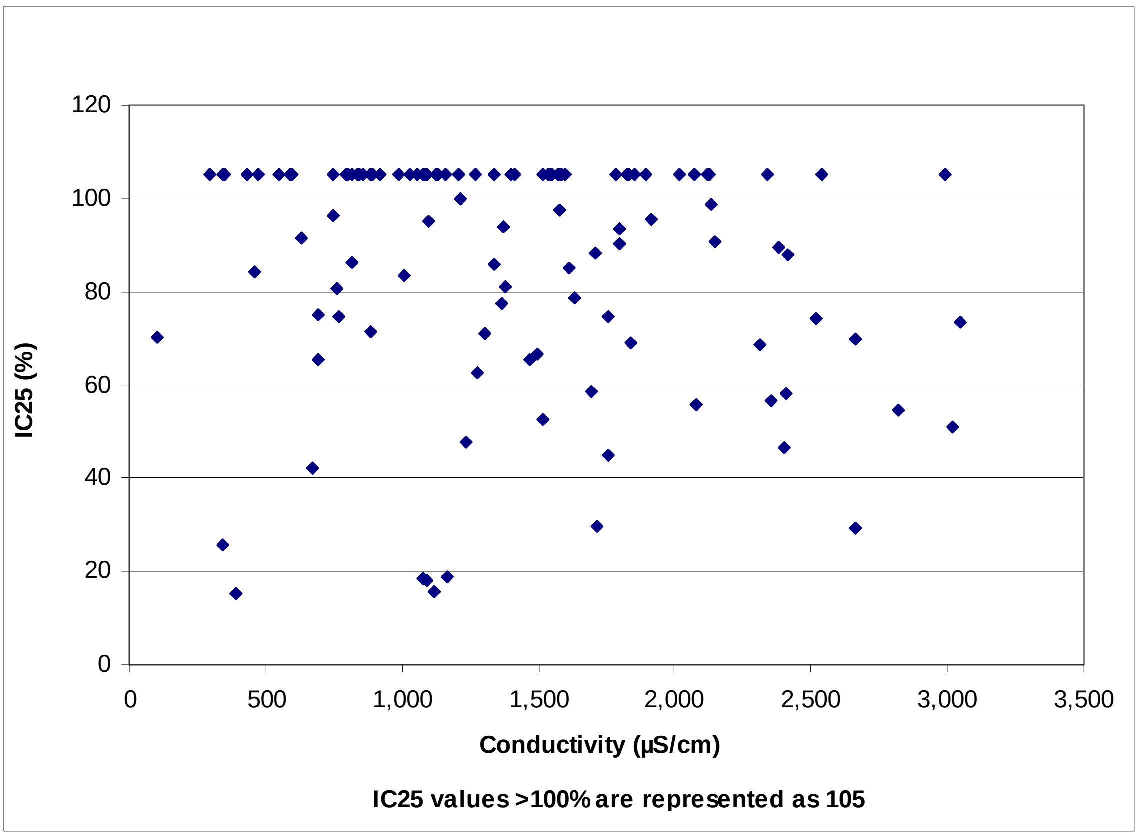

Figure 26 - Relationship between Conductivity and the Reproductive IC25 Endpoint in WET Tests Conducted in Mine Influenced Streams

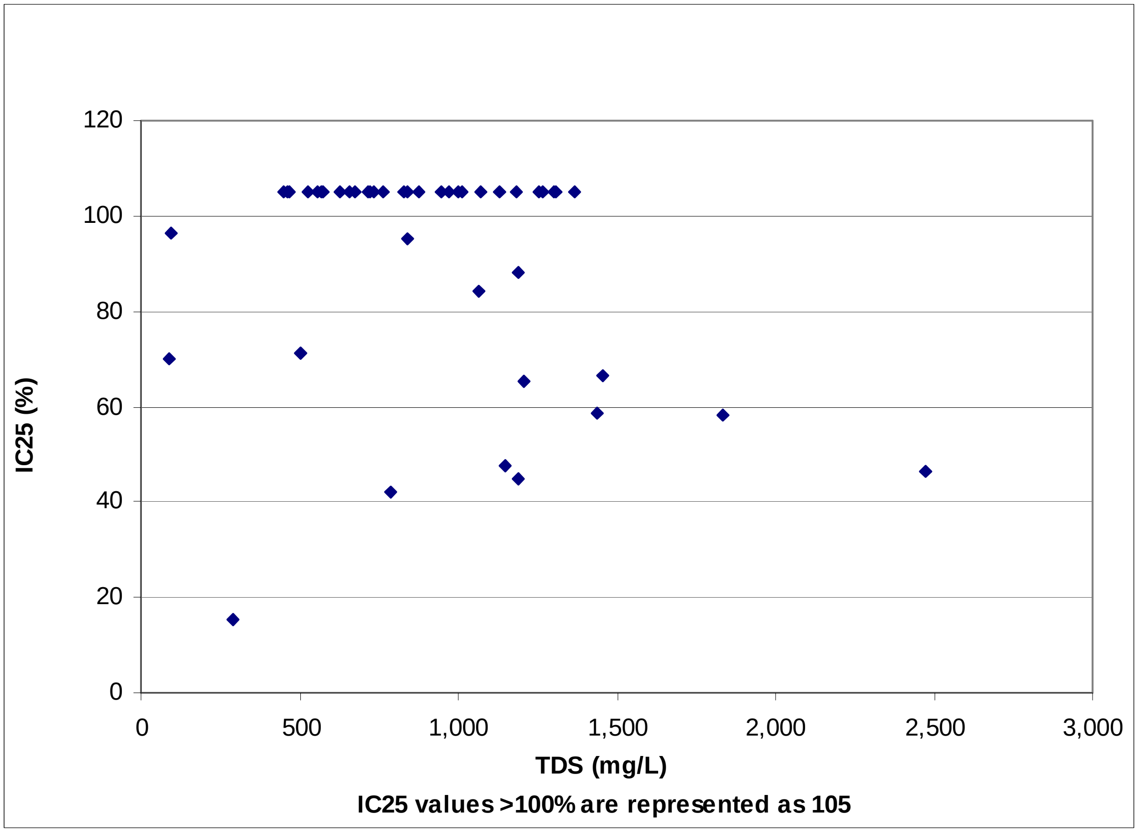

Figure 27 - Relationship between TDS and the Reproductive IC25 Endpoint in WET Tests Conducted in Mine Influenced Streams

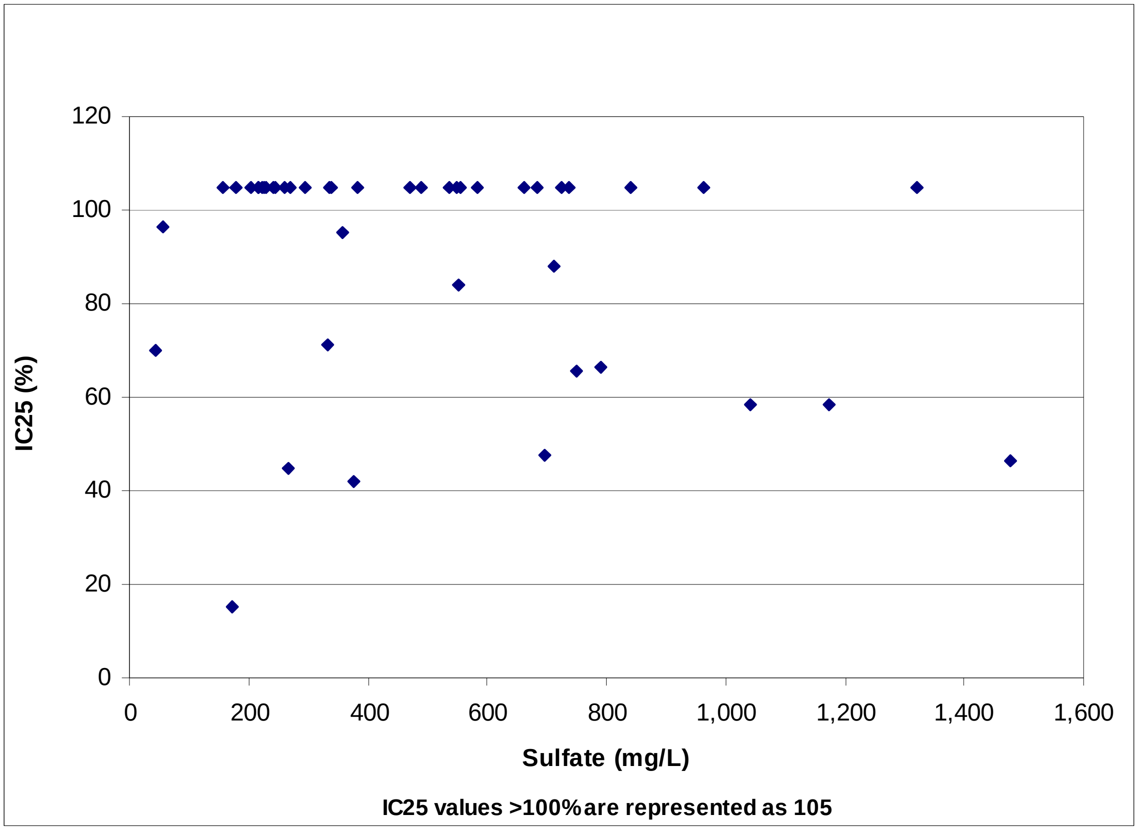

Figure 28 - Relationship between Sulfate and the Reproductive IC25 Endpoint in WET Tests Conducted in Mine Influenced Streams

Discussion

Sulfate dominated the mining effluent; however, Mount *et al.* (1997) observed sulfate as the least toxic ion. There was no correlation between sulfate and percent survival or reproduction of *Ceriodaphnia dubia* (**Figures 25 & 28**). This is important to the mining industry because sulfate is commonly found in mine effluent. Soucek and Kennedy (2005) observed lethal effects of sulfate to *Hyalella azteca* (512 mg/L), *Ceriodaphnia dubia* (2,050 mg/L), and *Chironomus tentans* (14,134 mg/L). The stream water sampled by companies 1 and 2 showed sulfate levels >1,000 mg/L for four of the streams tested. Three out of the four streams had IC25 scores ranging from 46.6% to 58.31% and one stream showed no toxicity in the sensitive reproductive endpoint. It is believed that chloride and hardness influence the toxicity of sulfate to aquatic invertebrates due to alterations in osmoregulation. Dr. Soucek's work revealed that the level of sulfate toxicity is driven by the concentrations of chloride and hardness. The high hardness and chloride concentrations in mining influenced streams would explain why the organisms are so tolerant of the elevated conductivities.

Conductivity was not an indicator of toxicity in the stream sampled in this study (**Figures 23 & 26**). Although conductivity above 2,000 µS/cm may adversely affect freshwater organisms (Goodfellow 2000, SETAC 2004a), the conductivities in this study were often recorded at levels greater than 2,000 µS/cm and showed no adverse effect to *Ceriodaphnia dubia* survival. Although conductivity is an important factor in streams influenced by mining, this study shows that conductivity is not correlated with stream toxicity of *Ceriodaphnia dubia*. The thresholds of toxicity to the ceriodaphnid were not established.

Toxicity was observed in streams receiving mine effluent; however, the cause of toxicity is undetermined. Further research is warranted to investigate other factors that may contribute to toxicity, such as TSS, which was only included in a few samples in this study.

CHAPTER 3

Benthic Macroinvertebrates and Toxicity

Introduction

Recent studies have found that benthic macroinvertebrate communities in streams below Appalachian surface coal mines often differ from communities found in non-mined ecosystems. Elevated levels of TDS have been suggested as stressors to aquatic life in Central Appalachian streams influenced by coal mining. Although field studies have succeeded in demonstrating the ability of benthic macroinvertebrate monitoring to identify aquatic community responses to coal mining activity, much remains unknown about how benthic macroinvertebrate communities respond to specific TDS concentrations and compositions in the absence of non-TDS stressors that are often concurrent with elevated TDS levels in mining-influenced streams (Timpano *et al.*, 2010).

Benthic macroinvertebrates do not move around much so they are less able to escape the effects of pollutants that diminish water quality. Therefore, macroinvertebrates can provide reliable information about stream water quality. Their long life cycles allow studies conducted by aquatic ecologists to determine any decline in environmental quality (Spellman 2009). Macroinvertebrates represent an extremely diverse group of aquatic animals and the large numbers of species possess a wide range of responses to stressors such as organic pollutants, sediments, and toxicants.

Concurrent with the toxicity testing described in Chapter 2, one of the coal mining companies (Company 2) was required to conduct annual benthic macroinvertebrate monitoring in streams where samples were collected for WET testing. Three years of monitoring have resulted in 28 paired data points which include toxicity testing and benthic macroinvertebrate community data. The objectives are to determine if stream impairment, as indicated by WVSCI scores, is related to laboratory toxicity; and to determine if stream impairment, as indicated by WVSCI scores, is related to conductivity.

Materials and Methods

Benthic macroinvertebrates were collected using the USEPA's Rapid Bioassessment Protocol (RBP) methods (Barbour *et al.*, 1999). A 0.5 meter kick-net was utilized to collect a four-sample composite in riffle/run sections of the stream channel. Samples were composited, field sieved, and preserved. Samples were then sorted and subsampled with a target of 180 organisms (WVDNR 2008). Samples are identified to the genus level by a biologist familiar with regional taxa using appropriate taxonomic keys (Merritt and Cummins 1996; Stewart and Stark, 2002; Smith 2001).

The genus level benthic macroinvertebrate community data were evaluated using a series of metrics which include Taxa Richness, Ephemeroptera, Plecoptera, and Trichoptera (EPT) Taxa Richness, Percent EPT, Percent Two Dominant Taxa, Percent Chironomidae, and Hilsenhoff Biotic Index (HBI) (**Table 19, Appendix A**). In addition to the genus level consideration of individual metrics described above, family level stream community data were also evaluated using the WVSCI (**Table 20, Appendix A**).

Habitat assessments were completed using the USEPA RBP (Barbour *et al.*, 1999) and WVDEP Watershed Assessment Branch (WAB) Wadeable Benthic Stream Assessment Forms (WVDEP 2010b). At these sampling locations, 10 parameters were evaluated that represent the overall quality of available habitat at each site. Those 10 parameters include epifaunal substrate/available cover, embeddedness, velocity/depth regime, sediment deposition, channel flow status, channel alteration, frequency of riffles, bank stability, vegetative protection and riparian vegetative zone. The results of the visual-based habitat assessment were used to determine the quality of habitat at each sampling location to discern effects of mining discharges as well as support the biological assessment.

Results

None of the 30 streams sampled over the 3 year period scored in the optimal habitat range. Sub-optimal habitat was demonstrated in 20 sampling locations while 8 sampling locations scored in the marginal range (**Tables 21-23, Appendix A**). In 2009, Ballard Branch and Stanley Fork demonstrated marginal habitat due to low frequency of riffles, low channel flow status, and low riparian vegetative zone. In 2010, Mud Lick Branch, Jack Smith Branch, Cabin Creek, and Seng Creek had low scores due to embeddedness, width of undisturbed vegetative zone, low velocity/depth regime, and low channel flow status. In 2011, Jarrell Branch scored in the marginal range due to width of undisturbed vegetative zone, bank vegetative protection, channel alteration, channel flow status, and low velocity/depth regime.

In general, most sites had substrates dominated by cobble and coarse gravel which would provide suitable substrate for benthic macroinvertebrates (**Tables 24-26, Appendix A**). Mud Lick Branch was the only site dominated by bedrock.

Field Water Chemistry

In the fall of 2009, conductivity ranged from 960 µS/cm at Cow Creek to 2,990 µS/cm at Joes Creek (**Table 27, Appendix A**). The dissolved oxygen ranged from 9.10 mg/L at Jarrell Branch to 10.36 mg/L at Tenmile Fork. The temperature ranged from 13.9°C at Calvin Branch and Tenmile Fork to 18.2°C at Jarrell Branch. The pH ranged from 7.66 S.U. at Joes Creek to 8.53 S.U. at Cow Creek. The turbidity ranged from 0.0 NTU at Cow Creek to 10.0 NTU at Stanley Fork. Turbidity could not be recorded at some sites due to a meter malfunction. Stream velocity ranged from 0.45 cfs at Ballard Branch to 6.20 cfs at Jarrell Branch.

In the fall of 2010, conductivity ranged from 222.1 µs/cm at Little White Oak to 2,747 µs/cm at Coal Fork (**Table 28, Appendix A**). Dissolved oxygen ranged from 7.22 mg/L at Big Horse Creek to 9.13 mg/L at Coal Fork. The temperature ranged from 14.2°C at Cabin Creek to 22.5°C at Mud Lick Branch. The pH ranged from 7.77 S.U. at Mud Lick Branch to 8.52 S.U. at Pond Fork. Turbidity ranged from 1.7 NTU at Big Horse Creek to 34.0 NTU at Mud Lick Branch. Stream velocity ranged from 0.04 cfs at Little White Oak to 27.59 cfs at Pond Fork.

In the fall of 2011, conductivity ranged from 622 µs/cm at Pond Fork to 1,965 µs/cm at the UNT Left Fork (**Table 29, Appendix A**). The temperature ranged from 16.0°C at Pond Fork to 18.5°C at the other Pond Fork location. The pH ranged from 7.35 S.U. at the UNT Left Fork to 8.70 S.U. at Cow Creek. Turbidity ranged from 3.2 NTU at Big Horse Creek to 19.0 NTU at Moccasin Hollow. Stream velocity ranged from 0.45 cfs at the UNT Tenmile Fork to 48.14 cfs at Pond Fork. Dissolved oxygen readings were not recorded due to a meter malfunction.

Toxicity tests were conducted at nine sampling locations during the fall of 2009. Eight streams failed to generate an LC50 (lethal concentration to 50% of the organisms) and had NOEC of 100% stream water for the survival endpoint (**Table 30, Appendix A**). One sampling site, on Left Fork, had an LC50 of 100% and an NOEC of 25% stream water for the survival endpoint.

With respect to the reproductive endpoint, 6 of the 9 sampling sites had NOEC concentrations of 100% stream water with LOEC estimated to be greater than 100 % stream water (**Table 31, Appendix A**). Two of the streams, Stanley Fork and Cow Creek had NOEC of 50% and LOEC of 100% while one site, Left Fork, had an NOEC of 25% and an LOEC of 50%. The IC25, or concentration of stream water which is predicted to result in a 25% reduction in reproduction, was greater than 100% for 4 of the 9 streams and ranged from 29.28% to 83.41% for the remaining streams.

Toxicity tests were conducted at 10 sampling locations during the fall of 2010. Nine streams failed to generate an LC50 (lethal concentration to 50% of the organisms) (**Table 32, Appendix A**). Jack Smith Branch had no toxicity demonstrated for the LC50 analysis. All streams sampled also had NOEC of 100 % stream water for the survival endpoint.

With respect to the reproductive endpoint, 7 of the 10 sampling sites had NOEC of 100 % stream water with LOEC estimated to be greater than 100 % stream water (**Table 33, Appendix A**). Three of the streams, Seng Creek, Tom's Fork, and Jack Smith Branch had NOEC of 50% and LOECs of 100%. The IC25 was greater than 100% for 4 of the 10 streams and ranged from 56.61% to 87.74% for the remaining streams.

In fall of 2011, the 9 streams tested did not generate toxicity with no LC50s predictable for mortality (**Table 34, Appendix A**). The streams sampled also had NOEC of 100 % stream water for the survival endpoint.

With respect to the sub-lethal reproductive endpoint, 9 of the 9 sampling sites had NOEC of 100% stream water with LOEC estimated to be 100% stream water or greater (**Table 35, Appendix A**). The IC25 was greater than 100% for each of the 9 streams.

In the fall of 2009, genus level taxa richness ranged from 11 to 23 taxa per site in the benthic macroinvertebrate communities from the 9 streams sampled (**Table 36, Appendix A**). Richness of the sensitive Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa ranged from 3 to 7 genera with the percentage of EPT taxa as high as 86.15 %. Most streams had fairly high values for the metric "percent two dominant taxa" (50 % to 86.54 %) although the "percent Chironomidae" was variable ranging from 7.07 % to 58.39 %. The HBI, a composite tolerance value metric, was lowest in the Left Fork, indicating a sensitive community.

WVSCI scores are calculated for each sampling location. WVSCI is a multi-metric index that presents an overall estimation of community health. Values ranged from 40.88 to 63.65 at the 9 sampling locations (**Table 37, Appendix A**) with each sampling site scoring in the impaired zone except Cow Creek which scored in the "grey zone." The composite benthic sample at Ballard Branch had less than 180 bugs; therefore, a WVSCI score could not be calculated.

In the fall of 2010, genus level taxa richness ranged from 7 to 18 in the benthic macroinvertebrate communities from the streams sampled. Richness of the EPT taxa ranged from 3 to 8 genera with the percentage of EPT taxa as high as 61.14 % (**Table 38,**

Appendix A). The values for the metric "Percent Two Dominant Taxa" ranged from 33.16 % to 84.13 % and the "Percent Chironomidae" varies from 11.92 % to 67.14 %. The HBI was lowest in the Pond Fork indicating a sensitive community. This site was dominated by Ephemeroptera and Trichoptera species.

The WVSCI scores ranged from 29.83 to 69.72 at the sampling locations (**Table 39, Appendix A**) with each sampling site scoring in the impaired zone except West Fork which scored in the "Unimpaired."

In the fall of 2011, genus level taxa richness ranged from 13 to 18 in the benthic macroinvertebrate communities from the streams sampled (**Table 40, Appendix A**). Richness of the EPT taxa ranged from 2 to 8 genera with the percentage of EPT taxa as high as 60.77%. The values for the metric "Percent Two Dominant Taxa" ranged from 46.38 at the UNT Left Fork Creek to 74.29 at Jarrell Branch and the "Percent Chironomidae" varied from 6.22 at Cow Creek to 50.00 at Pond Fork. The HBI was lowest in Cow Creek (4.4) indicating the more sensitive community. This site was dominated by Ephemeroptera, Plecoptera and Trichoptera species.

The WVSCI values ranged from 38.24 at Big Horse Creek to 65.62 at Moccasin Hollow at the sampling locations with each sampling site scoring in the impaired zone except the UNT of Left Fork Creek and Moccasin Hollow (**Table 41, Appendix A**). These sites scored in the "grey zone" which may indicate slight impairment.

Benthic macroinvertebrate communities in the streams sampled indicated some level of impairment. A weak relationship was demonstrated between habitat assessment scores and WVSCI scores (**Figure 29**). No apparent relationships were observed between conductivity and WVSCI or conductivity and the number of taxa present in the sampling locations (**Figures 30-31**).

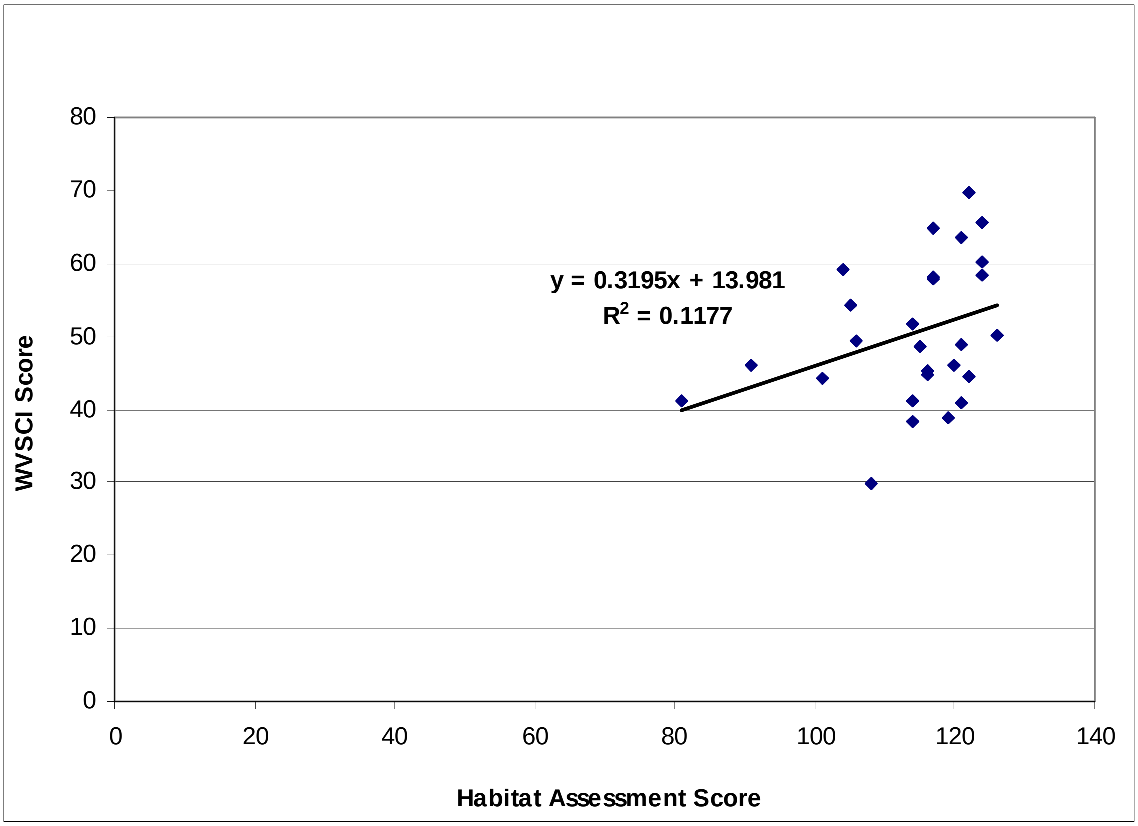

Figure 29 - Relationship between Habitat Assessment Scores and WVSCI Scores in Mine Influenced Streams

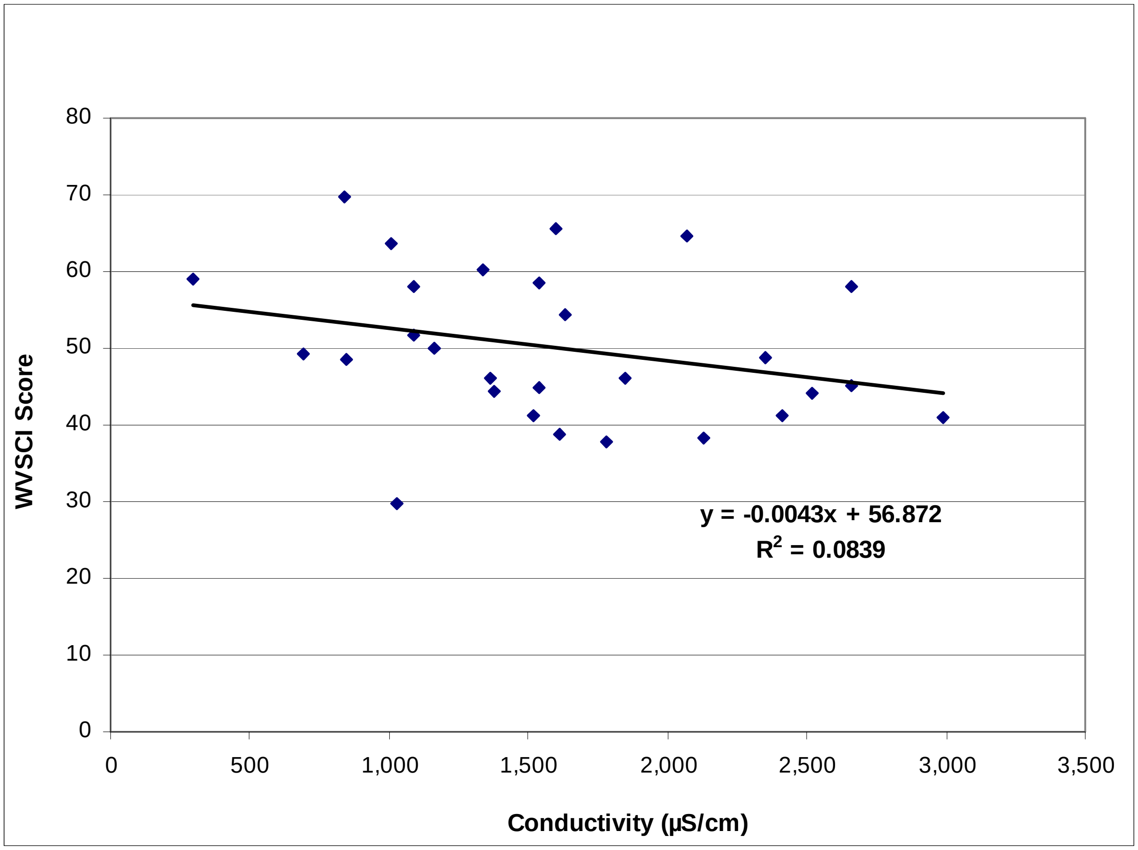

Figure 30 - Relationship between Conductivity and WVSCI Scores in Mine Influenced Streams

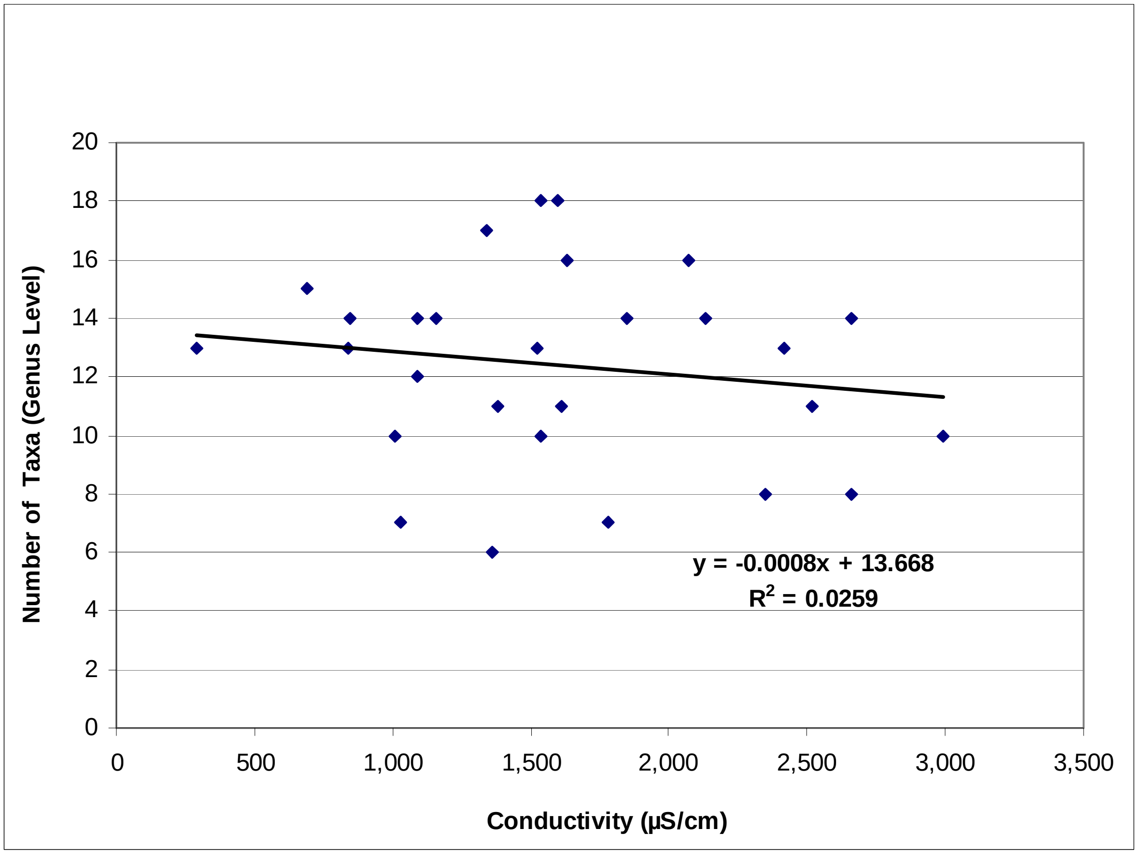

Figure 31 - Relationship between Conductivity and Number of Taxa Present in Mine Influenced Streams

Discussion

Stream impairment, as indicated by WVSCI scores, was unrelated to laboratory toxicity testing outcomes. Streams with the lowest WVSCI scores exhibited no toxicity. Benthic macroinvertebrate communities in the streams sampled indicated some level of impairment, however, no toxicity was observed in the 100% mine effluent stream survival of *Ceriodaphnia dubia*. The toxicity tests conducted on *Ceriodaphnia dubia* occur in a controlled laboratory environment whereas benthic macroinvertebrates live in the environment which is uncontrolled. Macroinvertebrates in the receiving environment are exposed to a wide variety of abiotic and biotic modifying factors that can affect an organism's response to a toxicant (Chapman 1999).

There was no relationship between conductivity and WVSCI scores. A stream can have a low level of specific conductance and a WVSCI score firmly within the range for impairment; conversely, a stream can have a high level of specific conductance and a WVSCI score that indicates the stream is above the threshold for impairment (WVDEP 2010b). WVSCI scores are affected by many factors: habitat, other uses of the stream and the surrounding land, and pollutants unrelated to conductivity (e.g., fecal coliform). Certain stream reaches simply cannot attain a "good" WVSCI score because of those factors (WVDEP 2010b). The Pond-Passmore Study found a shift in the benthic macroinvertebrate community downstream from mining activity, but did not otherwise correlate this finding with any significant or adverse impairment of the ecosystem (Pond *et al.*, 2008).

CHAPTER 4

Conclusions

The toxicity research that I conducted demonstrates that conductivity does not correlate to toxicity. Conductivity measurements in the streams samples exceeded the 300 µs/cm level recently deemed harmful to aquatic life in the Central Appalachians by the EPA (Cormier *et al.*, 2011). Some of the streams tested did exhibit toxicity; however, it was not due to conductivity. This is relevant to the coal industry for many reasons. The most recent news in coal mining involves a mining permit being revoked and one of the issues for the revocation is conductivity (Ward Jr. 2011). This study reveals that conductivity may not be the most important factor affecting aquatic ecosystems. Further investigation is warranted to determine what exactly causes benthic macroinvertebrate impairment and stream water toxicity to laboratory organisms. I think that it would be beneficial to conduct toxicity testing above and below mine effluent discharges to determine if toxicity changes between upstream and downstream sampling locations. Conducting upstream toxicity testing would also give an opportunity to investigate other variables which may result in stream toxicity. Stream sampling locations should also be selected for sampling without focusing on high conductivity. Some of the streams that I sampled during my research exhibited toxicity although the streams had low conductivity.

When evaluating the data I also noticed that some stream sampling locations had low IC25 values but their survival was not impaired. What could be causing these inconsistent values? *Ceriodaphnia dubia* are extremely sensitive organisms (USEPA 2002). Changes in temperature and/or dissolved oxygen during testing could affect the reproductive output of *Ceriodaphnia dubia* (USEPA 2002). It is also possible for the sensitive organisms to become stressed during the daily water changes from poor handling techniques (USEPA 2002). Another factor that may affect both the reproduction and survival of *Ceriodaphnia dubia* is a rain event during the water collection for WET testing. Rain can cause the total suspended solids and turbidity of a stream increase. Filter-feeding invertebrates exposed to high levels of suspended solids can clog feeding structures, reducing feeding efficiency and therefore reducing growth rates, stressing and even killing the organisms (Hynes 1970). Suspended solids were not measured in the study presented here but might be something to include in future research. Also, a possibility for no survival impairment but low IC25 might be higher conductivity or poor water quality in later samples since the 48 hour endpoint is based on the first of 3 samples.

Stream impairment, as indicated by WVSCI scores, were unrelated to laboratory toxicity testing outcomes. Streams with the lowest WVSCI scores exhibited no toxicity. I think that this occurred due to the *Ceriodaphnia dubia* toxicity testing being conducted in a controlled laboratory environment whereas benthic macroinvertebrates live in the environment which is highly variable.

There was no relationship between conductivity and WVSCI scores. I think that stream impairment occurs regardless of conductivity. Many factors can lead to stream impairment. I have sampled all of these streams for benthics and the majority of the streams are affected by human disturbance. Some other factors that come to mind are stream velocity, embeddedness, stream canopy, and substrate. These are all important issues when considering stream impairment.

Appendix A

Table 3 - Field Water Chemistry Analysis 2008

Sampling Location	Date	Conductivity (µS/cm)	Temperature (°C)	pH (S.U.)	DO (mg/L)
Big Creek	12/15/2008	918	3.0	8.05	9.70
Big Creek	12/17/2008	454	4.0	7.71	9.60
Big Creek	12/19/2008	719	5.0	7.87	9.30
Hardway Branch	12/11/2008	1,373	9.4	7.07	10.58
Hardway Branch	12/13/2008	1,278	6.6	6.81	11.19
Hardway Branch	1215/2008	1,500	8.1	6.88	10.49
Laurel Creek	12/15/2008	1,550	7.0	7.79	9.50
Laurel Creek	12/17/2008	735	10.2	7.45	10.43
Laurel Creek	12/19/2008	1,130	8.3	6.08	10.45
Line Creek	12/11/2008	883	6.2	7.29	10.85
Line Creek	12/13/2008	574	5.5	6.99	11.33
Line Creek	1215/2008	675	7.0	7.18	10.82
Mudlick Fork	12/15/2008	2,540	8.8	6.24	9.95
Mudlick Fork	12/17/2008	1,622	8.6	6.65	10.62
Mudlick Fork	12/19/2008	2,410	10.4	6.45	10.20
Robinson Creek	12/15/2008	802	7.9	6.32	9.19
Robinson Creek	12/17/2008	336	8.6	7.63	10.64
Robinson Creek	12/19/2008	653	8.8	6.48	10.27
Spruce Laurel Creek	12/15/2008	550	9.5	7.69	9.64
Spruce Laurel Creek	12/17/2008	284	10.0	7.60	10.34
Spruce Laurel Creek	12/19/2008	401	10.4	7.13	10.05
Stollings Fork	12/15/2008	1,826	9.2	6.76	9.20
Stollings Fork	12/17/2008	1,180	9.0	7.48	10.33
Stollings Fork	12/19/2008	1,815	10.4	6.39	9.86
Taylor Fork	12/11/2008	350	7.3	6.23	9.62
Taylor Fork	12/13/2008	403	5.1	5.93	10.56
Taylor Fork	12/15/2008	401	10.9	6.10	9.22
West Fork	12/15/2008	2,120	16.1	6.14	9.86
West Fork	12/17/2008	1,341	13.1	8.13	9.64
West Fork	12/19/2008	1,865	15.4	7.16	9.08

Table 4 - Field Water Chemistry Analysis 2009

Sampling Station	Date Sampled	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)
DS Fifteen Mile Fork	6/22/2009	1,092	9.80	4.1	7.56
DS Fifteen Mile Fork	6/24/2009	1,096	10.10	3.3	7.06
DS Fifteen Mile Fork	6/26/2009	1,073	9.60	1.3	6.72
Laurel Creek	6/29/2009	2,342	10.80	4.5	8.10
Laurel Creek	7/1/2009	1,858	9.80		8.12
Laurel Creek	7/2/2009	1,869	11.40	4.0	8.31
Mammoth Site 1	6/22/2009	815	10.20	4.6	8.57
Mammoth Site 1	6/24/2009	716	10.80	3.6	7.99
Mammoth Site 1	6/26/2009	718	10.20	1.4	7.84
Mammoth Site 2	6/22/2009	631	10.30	3.9	8.50
Mammoth Site 2	6/24/2009	68	10.50	3.3	7.76
Mammoth Site 2	6/26/2009	70	9.80	1.2	7.23
Mouth of Robinson Creek	6/29/2009	2,137	10.90	4.6	7.97
Mouth of Robinson Creek	7/1/2009	2,076	9.80		8.00
Mouth of Robinson Creek	7/2/2009	2,123	11.20	4.1	8.21
Mudlick Fork	6/29/2009	2,818	10.40	3.9	8.18
Mudlick Fork	7/1/2009	2,849	9.60		8.13
Mudlick Fork	7/2/2009	2,884	11.30	4.2	8.36
PM 260 Inlet	6/29/2009	1,578	10.90	4.4	7.71
PM 260 Inlet	7/1/2009	1,539	10.00		7.56
PM 260 Inlet	7/2/2009	1,575	12.00	4.6	7.84
PM 316 Pond	6/29/2009	1,598	11.70	4.0	7.67
PM 316 Pond	7/1/2009	1,589	10.20		7.76
PM 316 Pond	7/2/2009	1,625	11.70	4.1	7.99
Stollings Fork	6/29/2009	1,839	10.60	4.4	8.13
Stollings Fork	7/1/2009	2,351	9.80		8.11
Stollings Fork	7/2/2009	2,373	11.50	4.3	8.33
Upstream Fifteen Mile	6/22/2009	1,078	10.50	3.8	8.39
Upstream Fifteen Mile	6/24/2009	1,071	10.40	3.4	7.82
Upstream Fifteen Mile	6/26/2009	1,062	10.00	1.1	7.63

Sampling Station	Date Sampled	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)
Ballard Branch	9/14/2009	1,783	9.63	16.2	7.83
Ballard Branch	9/16/2009	1,687	9.83	16.6	8.26
Ballard Branch	9/18/2009	1,701	9.96	17.3	8.27
Calvin Branch	9/14/2009	1,090	10.08	14.2	7.75
Calvin Branch	9/16/2009	984	10.33	13.9	7.78
Calvin Branch	9/18/2009	986	10.02	14.7	8.14
Cow Creek	9/14/2009	1,010	9.68	13.0	8.49
Cow Creek	9/16/2009	960	10.17	15.4	8.53
Cow Creek	9/18/2009	975	10.13	15.8	8.54
Hardway Pond	9/28/2009	1,214	11.20	4.6	7.82
Hardway Pond	9/30/2009	1,353	10.20	3.3	7.68
Hardway Pond	10/2/2009	1,396	10.40	4.7	7.81
Jarrell Branch	9/14/2009	2,660	10.13	15.6	8.54
Jarrell Branch	9/16/2009	2,410	9.10	18.2	8.38
Jarrell Branch	9/18/2009	1,994	9.05	18.6	8.38
Joes Creek	9/21/2009	2,730	10.09	15.7	7.92
Joes Creek	9/23/2009	2,880	9.67	17.1	7.69
Joes Creek	9/25/2009	2,990	9.52	16.7	7.66
Laurel Creek	9/28/2009	1,307	10.80	4.2	7.93
Laurel Creek	9/30/2009	1,568	10.40	3.3	8.10
Laurel Creek	10/2/2009	1,651	10.60	5.9	8.17
Left Fork	9/21/2009	2,640	9.72	16.5	8.28
Left Fork	9/23/2009	1,853	9.45	16.4	7.97
Left Fork	9/25/2009	1,842	9.51	16.9	8.01
Mouth of Robinson Creek @ PM 24 location	9/28/2009	1,912	11.10	4.6	8.03
Mouth of Robinson Creek @ PM 24 location	9/30/2009	2,023	10.30	3.3	8.10
Mouth of Robinson Creek @ PM 24 location	10/2/2009	2,037	10.80	6.2	8.15
Mudlick Fork	9/28/2009	2,081	10.90	4.2	8.34
Mudlick Fork	9/30/2009	2,095	10.70	3.3	8.28
Mudlick Fork	10/2/2009	2,264	10.50	6.5	8.33
PM 316 Pond	9/28/2009	1,892	10.50	4.5	7.83
PM 316 Pond	9/30/2009	1,878	9.60	3.3	7.92

Sampling Station	Date Sampled	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)
PM 316 Pond	10/2/2009	1,958	10.80	4.6	7.96
Stanley Fork	9/14/2009	2,520	9.52	16.3	7.11
Stanley Fork	9/16/2009	1,951	9.87	15.1	8.28
Stanley Fork	9/18/2009	1,969	9.99	15.8	8.27
Stollings Fork	9/28/2009	1,267	10.60	4.4	7.67
Stollings Fork	9/30/2009	548	9.80	3.3	7.65
Stollings Fork	10/2/2009	568	10.10	6.6	7.85
Tenmile Fork	9/21/2009	1,378	10.65	13.7	8.36
Tenmile Fork	9/23/2009	1,198	11.04	12.9	7.34
Tenmile Fork	9/25/2009	1,112	10.36	13.9	7.95
White Oak	9/21/2009	1,338	9.69	16.6	8.25
White Oak	9/23/2009	1,199	9.58	16.7	7.90
White Oak	9/25/2009	1,197	9.29	17.6	7.98
Downstream Fifteen Mile Fork	10/5/2009	1,164	10.30	3.8	7.17
Downstream Fifteen Mile Fork	10/7/2009	1,089	11.70	4.3	6.76
Downstream Fifteen Mile Fork	10/9/2009	1,140	10.10	1.4	7.18
Hughes Fork off Bells Creek	10/5/2009	765	11.30	3.7	8.19
Hughes Fork off Bells Creek	10/7/2009	767	12.30	3.9	8.11
Hughes Fork off Bells Creek	10/9/2009	765	11.10	1.7	8.20
Sixmile off Hughes Creek	10/5/2009	889	11.20	4.5	8.18
Sixmile off Hughes Creek	10/7/2009	869	12.50	4.0	8.06
Sixmile off Hughes Creek	10/9/2009	896	10.60	4.5	8.16
Upstream Fifteen Mile	10/5/2009	1,117	11.30	4.8	7.86
Upstream Fifteen Mile	10/7/2009	1,061	11.70	4.1	7.61
Upstream Fifteen Mile	10/9/2009	1,120	11.10	3.0	8.06

Table 5 - Field Water Chemistry Analysis 2010

Sampling Station	Date Sampled	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)
Ballard Branch	2/22/2010	795	11.44	7.2	7.92
Ballard Branch	2/24/2010				
Ballard Branch	2/26/2010	794	12.23	3.0	7.98
Calvin Branch	2/22/2010	748	11.60	7.7	8.15
Calvin Branch	2/24/2010				
Calvin Branch	2/26/2010	866	12.45	3.7	7.06
Cow Creek	2/22/2010	767	11.23	8.2	8.21
Cow Creek	2/24/2010				
Cow Creek	2/26/2010	753	11.88	5.3	8.08
Jarrell Branch	2/22/2010	1,833	11.17	9.3	8.34
Jarrell Branch	2/24/2010				
Jarrell Branch	2/26/2010	1,751	11.32	5.8	8.07
Joes Creek	2/15/2010	1,077	8.38	5.5	8.07
Joes Creek	2/17/2010	1,182	11.11	3.9	8.38
Joes Creek	2/19/2010	1,173	10.80	6.4	7.87
Left Fork	2/15/2010	1,800	8.00	5.6	8.90
Left Fork	2/17/2010	1,710	9.65	4.4	9.04
Left Fork	2/19/2010	1,690	12.83	6.7	8.84
Jack Smith Branch	9/20/2010	690	7.86	19.3	8.17
Jack Smith Branch	9/22/2010	1,242	7.53	18.3	8.14
Jack Smith Branch	9/24/2010	1,337	7.17	21.5	8.14
Stanley Fork	2/22/2010	1,798	10.96	9.8	8.24
Stanley Fork	2/24/2010				
Stanley Fork	2/26/2010	1,824	12.20	5.1	8.11
Tenmile Fork	2/15/2010	1,130	6.31	7.3	8.64
Tenmile Fork	2/17/2010	1,160	10.19	5.7	8.72
Tenmile Fork	2/19/2010	1,126	11.28	4.8	8.01
White Oak	2/15/2010	1,028	5.97	4.4	8.69
White Oak	2/17/2010	1,061	7.13	3.2	8.86
White Oak	2/19/2010	1,077	14.71	5.2	8.89
Sycamore Fork	3/1/2010	594	10.78	8.5	7.90
Sycamore Fork	3/3/2010	602	12.35	5.7	7.93
Sycamore Fork	3/5/2010	614	12.51	6.6	7.76
Horse Creek	4/26/2010	429	12.10	4.6	6.94
Horse Creek	4/28/2010	223	11.50	5.0	6.90
Horse Creek	4/30/2010	286	10.50	5.0	7.31

Sampling Station	Date Sampled	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)
Hardway Pond @ Location PM 235	4/19/2010	2,145	9.00	4.3	7.82
Hardway Pond @ Location PM 235	4/21/2010	2,171	12.40	5.0	8.33
Hardway Pond @ Location PM 235	4/23/2010	2,188	10.70	5.0	8.25
Lilly Fork @ PM 89	4/19/2010	1,340	7.80	4.0	8.13
Lilly Fork @ PM 89	4/21/2010	1,310	11.60	5.0	8.28
Lilly Fork @ PM 89	4/23/2010	1,350	10.50	5.0	7.88
Robinson North @ PM 181	4/19/2010	2,380	8.40	4.2	7.70
Robinson North @ PM 181	4/21/2010	2,325	11.20	5.0	7.95
Robinson North @ PM 181	4/23/2010	2,372	10.80	5.0	7.94
Bias Branch	6/1/2010	392	7.60	4.1	6.47
Bias Branch	6/3/2010	489	9.60	4.3	7.12
Bias Branch	6/4/2010	329	7.90	5.0	6.83
No Name	6/1/2010	1,470	8.20	4.4	7.01
No Name	6/3/2010	1,460	10.20	4.3	7.25
No Name	6/4/2010	1,301	8.40	5.0	6.83
Slip Ridge	6/1/2010	1,096	8.50	4.0	7.49
Slip Ridge	6/3/2010	909	9.90	4.8	7.93
Slip Ridge	6/4/2010	936	8.50	5.0	7.87
Mouth of Robinson Creek	6/1/2010	1,275	8.10	4.6	7.59
Mouth of Robinson Creek	6/3/2010	1,266	10.20	4.6	7.61
Mouth of Robinson Creek	6/4/2010	1,377	8.50	5.0	7.79

Sampling Station	Date Sampled	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)
Mudlick Fork	6/1/2010	2,403	4.90	8.1	8.60
Mudlick Fork	6/3/2010	2,364	4.10	8.2	10.40
Mudlick Fork	6/4/2010	2,253	5.00	8.3	8.20
UBB Area of Jarrells Branch	6/1/2010	1,760	4.00	8.3	8.60
UBB Area of Jarrells Branch	6/3/2010	1,702	4.10	8.3	10.10
UBB Area of Jarrells Branch	6/4/2010	1,723	5.00	8.3	8.60
Bias Branch	9/27/2010	342	7.40	3.2	7.26
Bias Branch	9/29/2010	347	8.30	5.0	7.20
Bias Branch	10/1/2010	357	9.90	5.0	7.82
Big Horse Creek	9/20/2010	1,538	6.82	20.5	7.39
Big Horse Creek	9/22/2010	1,761	7.22	18.8	7.91
Big Horse Creek	9/24/2010	1,793	7.17	22.3	7.94
Cabin Creek	9/27/2010	1,030	8.30	16.3	8.14
Cabin Creek	9/29/2010	1,039	8.89	14.2	8.11
Cabin Creek	10/1/2010	1,050	8.68	14.9	8.09
Coal Fork	9/27/2010	2,414	8.39	16.3	7.67
Coal Fork	9/29/2010	2,747	9.13	14.5	7.92
Coal Fork	10/1/2010	2,863	9.17	14.1	7.89
Horse Creek	9/27/2010	693	7.60	2.6	7.68
Horse Creek	9/29/2010	626	9.30	5.0	7.63
Horse Creek	10/1/2010	579	10.30	5.4	7.86
Little White Oak	9/27/2010	294	6.76	17.1	8.09
Little White Oak	9/29/2010	222	7.75	17.1	7.87
Little White Oak	10/1/2010	343	7.91	15.6	8.38
Mud Lick Branch	9/20/2010	1,363	8.56	18.3	7.73
Mud Lick Branch	9/22/2010	1,319	7.47	22.5	7.77
Mud Lick Branch	9/24/2010	1,406	8.26	20.7	7.83
Mudlick Fork	9/27/2010	3,019	7.70	3.3	7.95
Mudlick Fork	9/29/2010	3,025	8.50	5.0	8.04
Mudlick Fork	10/1/2010	2,977	10.40	5.3	8.22

Sampling Station	Date Sampled	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)
No Name	9/27/2010	1,718	7.60	2.9	7.36
No Name	9/29/2010	2,258	8.40	5.0	7.25
No Name	10/1/2010	2,364	10.30	5.7	5.25
Pond Fork	9/20/2010	840	8.90	22.4	8.67
Pond Fork	9/22/2010	835	8.95	21.4	8.52
Pond Fork	9/24/2010	844	8.22	20.0	8.42
Seng Creek	9/27/2010	1,631	8.44	17.3	8.27
Seng Creek	9/29/2010	1,657	8.65	16.2	8.31
Seng Creek	10/1/2010	1,657	8.45	15.8	8.27
Tom's Fork	9/27/2010	2,351	8.11	18.5	8.16
Tom's Fork	9/29/2010	2,410	7.63	18.0	8.15
Tom's Fork	10/1/2010	2,423	8.29	16.9	8.16
UBB Area of Jarrells Branch	9/27/2010	1,759	7.60	3.0	7.67
UBB Area of Jarrells Branch	9/29/2010	1,746	8.60	5.0	7.76
UBB Area of Jarrells Branch	10/1/2010	1,679	9.60	5.6	7.91
Slip Ridge	9/27/2010	817	6.90	2.6	7.67
Slip Ridge	9/29/2010	917	8.10	5.0	7.89
Slip Ridge	10/1/2010	899	10.70	5.2	8.01
West Fork	9/20/2010	1,610	7.84	19.5	8.16
West Fork	9/22/2010	1,686	8.53	19.3	8.21
West Fork	9/24/2010	1,678	8.95	18.4	8.26
Lilly Fork @ PM 89	10/11/2010	1,579	9.90	4.5	7.58
Lilly Fork @ PM 89	10/13/2010	1,594	9.30	5.0	7.58
Lilly Fork @ PM 89	10/15/2010	1,590	10.40	9.8	7.75
Hardway Pond @ Location PM 236	10/11/2010	1,516	9.30	4.1	7.44
Hardway Pond @ Location PM 236	10/13/2010	2,255	10.80	5.0	8.11
Hardway Pond @ Location PM 236	10/15/2010	2,256	11.50	9.0	8.27
Mouth of Robinson Creek	10/11/2010	2,311	10.50	4.3	7.94

Sampling Station	Date Sampled	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)
Mouth of Robinson Creek	10/13/2010	2,232	10.30	5.0	7.98
Mouth of Robinson Creek	10/15/2010	2,485	10.40	8.9	8.27
Twenty Mile Creek	10/11/2010	3,047	9.50	4.5	8.00
Twenty Mile Creek	10/13/2010	3,014	10.20	5.0	8.03
Twenty Mile Creek	10/15/2010	3,026	9.70	9.0	8.10

Table 6 - Field Water Chemistry Analysis 2011

Sampling Station	Date Sampled	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)
Big Horse Creek	2/14/2011	1,546	10.72	7.0	8.58
Big Horse Creek	2/16/2011	1,582	10.73	7.8	8.60
Big Horse Creek	2/18/2011	1,589	9.70	10.4	8.51
Cabin Creek	2/21/2011	1,055	9.32	9.1	8.64
Cabin Creek	2/23/2011	1,038	10.79	5.8	8.52
Cabin Creek	2/25/2011	886	9.59	9.7	8.72
Coal Fork	2/21/2011	1,827	9.80	8.1	8.16
Coal Fork	2/23/2011	1,605	11.16	5.8	8.01
Coal Fork	2/25/2011	758	9.97	8.5	8.22
Jack Smith Branch	2/14/2011	816	10.85	7.3	8.76
Jack Smith Branch	2/16/2011	813	10.97	8.4	8.75
Jack Smith Branch	2/18/2011	825	10.41	10.3	8.73
Little White Oak	2/21/2011	887	9.00	10.3	8.49
Little White Oak	2/23/2011	872	10.19	8.2	8.32

Sampling Station	Date Sampled	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)
Little White Oak	2/25/2011	543	11.25	6.9	8.46
Mud Lick Branch	2/14/2011	1,403	9.72	7.8	7.76
Mud Lick Branch	2/16/2011	1,409	9.97	8.0	8.64
Mud Lick Branch	2/18/2011	811	8.80	11.4	8.11
Pond Fork	2/14/2011	795	11.31	7.0	8.42
Pond Fork	2/16/2011	803	11.40	7.5	8.69
Pond Fork	2/18/2011	832	9.53	9.8	8.61
Seng Creek	2/21/2011	1,588	9.61	9.0	8.35
Seng Creek	2/23/2011	1,559	10.73	7.3	8.36
Seng Creek	2/25/2011	1,325	10.89	7.2	8.47
Tom’s Fork	2/21/2011	2,412	9.47	9.4	8.42
Tom’s Fork	2/23/2011	2,369	10.15	7.6	8.36
Tom’s Fork	2/25/2011	2,026	10.41	7.1	8.40
West Fork	2/14/2011	1,570	12.00	11.4	8.92
West Fork	2/16/2011	1,805	9.89	14.3	8.63
West Fork	2/18/2011	1,679	8.30	13.3	8.64
Below 033 on 20 Mile Fork	5/23/2011	105	8.90	4.4	6.91
Below 033 on 20 Mile Fork	5/25/2011	127	8.80	18.5	6.94
Below 033 on 20 Mile Fork	5/27/2011	136	9.20	14.0	7.36
James Creek below 015	5/23/2011	1,692	8.60	4.3	7.93
James Creek below 015	5/25/2011	1,730	8.80	18.8	7.80
James Creek below 015	5/27/2011	1,745	9.40	10.6	7.97
Bandmill Below 016	6/1/2011	1,127	7.40	25.7	8.38
Bandmill Below 016	6/3/2011	1,124	9.30	15.1	8.38
Bandmill Below 016	6/6/2011	1,143	9.60	4.0	8.38
029 on Radner Fork of 20 mile	5/23/2011	1,232	9.70	4.9	6.90

Sampling Station	Date Sampled	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)
029 on Radner Fork of 20 mile	5/25/2011	1,284	9.60	5.1	7.72
029 on Radner Fork of 20 mile	5/27/2011	1,312	9.90	9.7	7.06
013 Robinson North on 20 mile	5/23/2011	472	9.00	4.1	7.13
013 Robinson North on 20 mile	5/25/2011	552	9.00	19.0	6.87
013 Robinson North on 20 mile	5/27/2011	589	9.30	12.6	7.22
001 on Sugarcamp	5/23/2011	1,341	9.60	4.9	8.59
001 on Sugarcamp	5/25/2011	1,358	8.50	18.8	8.51
001 on Sugarcamp	5/27/2011	1,433	8.70	13.0	8.40
Delbarton Below 400	6/1/2011	990	6.90	26.1	8.47
Delbarton Below 400	6/3/2011	985	8.40	16.1	8.50
Delbarton Below 400	6/6/2011	974	10.10	4.1	8.47
ICC Below 031	6/1/2011	882	7.60	26.8	8.04
ICC Below 031	6/3/2011	907	9.30	15.5	7.97
ICC Below 031	6/6/2011	879	10.20	4.0	8.33
Mammoth below 004	6/1/2011	1,207	7.70	26.5	8.21
Mammoth below 004	6/3/2011	1,222	9.80	15.2	8.20
Mammoth below 004	6/6/2011	1,185	9.60	4.8	8.23
Marfork Below 018	6/1/2011	857	7.70	24.6	8.17
Marfork Below 018	6/3/2011	905	9.70	15.4	8.15
Marfork Below 018	6/6/2011	884	10.40	4.0	8.19
Big Horse Creek	9/19/2011	2,014	9.30	17.6	8.10

Sampling Station	Date Sampled	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)
Big Horse Creek	9/21/2011	1,768	10.10	17.1	7.69
Big Horse Creek	9/23/2011	1,609	8.90	16.9	8.14
Cow Creek	9/26/2011	1,054	9.50	18.0	8.29
Cow Creek	9/28/2011	744	8.60	16.1	8.70
Cow Creek	9/30/2011	876	8.90	15.6	8.35
Jarrell Branch	9/26/2011	1,473	8.50	18.9	8.35
Jarrell Branch	9/28/2011	1,388	8.20	17.8	8.63
Jarrell Branch	9/30/2011	1,484	8.50	17.5	8.53
Moccasin Hollow	9/26/2011	1,391	9.00	18.7	8.30
Moccasin Hollow	9/28/2011	1,351	8.20	17.4	8.12
Moccasin Hollow	9/30/2011	1,445	8.50	15.3	8.94
UNT Boone Block Hollow	9/19/2011	1,443	8.60	17.3	8.10
UNT Boone Block Hollow	9/21/2011	1,398	8.90	17.0	8.01
UNT Boone Block Hollow	9/23/2011	2,053	8.70	17.4	8.26
UNT Left Fork	9/19/2011	1,968	9.20	15.2	7.41
UNT Left Fork	9/21/2011	1,965	9.40	17.3	7.35
UNT Left Fork	9/23/2011	1,792	8.30	16.9	6.70
UNT Tenmile Fork	9/19/2011	1,745	9.40	14.3	7.74
UNT Tenmile Fork	9/21/2011	1,818	8.90	16.9	7.87
UNT Tenmile Fork	9/23/2011	1,981	8.30	17.3	8.19
Pond Fork	9/26/2011	802	9.20	17.9	8.24
Pond Fork	9/26/2011	1,117	8.90	19.3	8.39
Pond Fork	9/28/2011	622	8.60	16.0	8.42
Pond Fork	9/28/2011	766	8.90	18.5	8.64
Pond Fork	9/30/2011	751	8.60	15.0	8.41
Pond Fork	9/30/2011	891	9.10	16.9	8.39
Bandmill Below 016	10/31/2011	1,084	10.20	4.1	8.47
Bandmill Below 016	11/2/2011	1,138	11.00	4.6	8.25

Sampling Station	Date Sampled	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)
Bandmill Below 016	11/4/2011	1,202	10.70	5.0	8.20
Below 033 on 20 Mile Fork	10/24/2011	747	8.00	5.0	7.10
Below 033 on 20 Mile Fork	10/26/2011	163	7.30	5.0	7.05
Below 033 on 20 Mile Fork	10/28/2011	147	11.40	5.0	6.50
Delbarton below 400	10/31/2011	591	10.70	4.4	8.30
Delbarton below 401	11/2/2011	743	11.10	4.1	8.14
Delbarton below 402	11/4/2011	777	9.70	5.0	8.13
ICC Below 031	10/31/2011	671	10.80	4.2	8.19
ICC Below 031	11/2/2011	753	10.90	4.9	7.92
ICC Below 031	11/4/2011	767	10.70	5.0	7.80
James Creek below 015	10/31/2011	1,495	10.80	4.6	8.20
James Creek below 015	11/2/2011	1,379	10.80	4.9	7.98
James Creek below 015	11/4/2011	1,367	10.10	5.0	7.79
Mammoth below 004	10/24/2011	460	8.20	4.6	7.52
Mammoth below 004	10/26/2011	1,264	8.60	4.8	8.04
Mammoth below 004	10/28/2011	1,137	11.60	5.0	8.08
Marfork Below 018	10/31/2011	340	10.60	4.7	8.21
Marfork Below 018	11/2/2011	381	10.90	4.6	7.66
Marfork Below 018	11/4/2011	324	10.60	5.0	6.94
029 on Radner Fork of 20 mile	10/24/2011	1,414	8.20	4.9	7.29
029 on Radner Fork of 20 mile	10/26/2011	1,421	8.20	4.6	7.26
029 on Radner Fork of 20 mile	10/28/2011	1,211	11.50	5.0	7.52

Sampling Station	Date Sampled	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)
13 Robinson North on 20 mile	10/24/2011	2,018	8.30	4.8	7.63
13 Robinson North on 20 mile	10/26/2011	604	8.10	4.6	6.94
13 Robinson North on 20 mile	10/28/2011	522	11.80	4.7	6.60
001 on Sugarcamp	10/24/2011	1,710	8.30	4.7	7.52
001 on Sugarcamp	10/26/2011	1,621	8.10	4.6	7.62
001 on Sugarcamp	10/28/2011	1,515	11.20	4.9	7.80

Table 7 - Company 1 Additional Water Data Spring 2011

Parameter	Sugarcamp 5/25/2011	Robinson Fork 5/25/2011	Radner 5/25/2011	James Creek 5/25/2011	20 mi. 5/25/2011	Unit	MDL	Method
Field pH	8.30	6.60	6.56	7.80	6.18	S.U.		Field Test SM204500H B
Total Alkalinity	54.86	20.17	24.59	413.75	11.89	mg/L	0.31	SM202320B
Total Acidity	<0.63	<0.63	<0.63	<0.63	<0.63	mg/L	0.63	SM202310B
Turbidity	10.80	9.40	20.80	3.40	2.80	NTU	0.10	EPA 180.1
Sp. Cond.	1,445	600	1,362	1,847	128	µS/cm	1.9	EPA 120.1
Total Sulfates	685.04	270.06	697.64	1,173.70	44.25	mg/L	0.1	EPA 300.0
Chlorides	19.42	1.18	11.12	35.68	1.25	mg/L	0.07	EPA 300.0
Total Iron	0.06	0.08	0.82	0.01	0.05	mg/L	0.007	EPA 200.7
Dissolved Iron	<0.007	<0.007	0.25	<0.007	0.02	mg/L	0.007	EPA 200.7
Total Manganese	1.42	0.14	2.48	0.08	0.05	mg/L	0.036	EPA 200.7
Dissolved Manganese	1.280	0.120	2.420	<0.036	0.040	mg/L	0.036	EPA 200.7
Total Aluminum	0.31	0.13	0.52	0.04	0.06	mg/L	0.010	EPA 200.7
Dissolved Aluminum	0.209	0.014	0.034	0.030	0.013	mg/L	0.010	EPA 200.7
Total Sodium	46.70	5.56	6.02	15.50	2.64	mg/L	0.007	EPA 200.7
Dissolved Sodium	43.27	5.24	5.83	14.62	2.58	mg/L	0.007	EPA 200.7
Total Magnesium	96.17	42.06	103.30	147.40	7.77	mg/L	0.006	EPA 200.7

Parameter	Sugarcamp 5/25/2011	Robinson Fork 5/25/2011	Radner 5/25/2011	James Creek 5/25/2011	20 mi. 5/25/2011	Unit	MDL	Method
Dissolved Magnesium	92.88	41.02	102.70	142.60	7.21	mg/L	0.006	EPA 200.7
Total Calcium	124.40	50.48	147.20	181.80	9.69	mg/L	0.007	EPA 200.7
Dissolved Calcium	119.20	48.79	145.70	173.50	8.06	mg/L	0.007	EPA 200.7
Total Hardness	706.65	299.25	792.95	1,060.95	56.19	mg/L		SM202340B
Dissolved Hardness	680.12	290.75	786.73	1,020.46	49.82	mg/L		SM202340B
TSS	6.00	12.00	13.00	<2.00	<2.00	mg/L	2.00	SM202540D
TDS	1,131	447	1,149	1,439	86	mg/L	2.00	SM202540C
Temperature	21.40	15.80	16.60	15.80	15.00	°C		Field Test SM202550 B
Flow	0.668	1.780	0.222	0.858	1.780	cfs		Field Test
Field pH	8.44	7.54	8.25	7.98	7.85	S.U.		Field Test SM204500H B
Total Alkalinity	307.31	93.71	218.07	192.86	139.67	mg/L	0.31	SM202320B
Total Acidity	<0.63	<0.63	<0.63	<0.63	<0.63	mg/L	0.63	SM202310B
Turbidity	7.10	1.80	3.70	4.50	18.60	NTU	0.10	EPA 180.1
Sp. Cond.	1,045	923	1,188	1,308	943	µS/cm	1.9	EPA 120.1
Total Sulfates	229.73	337.93	471.37	548.34	157.08	mg/L	0.1	EPA 300.0
Chlorides	15.06	4.85	28.95	2.12	7.26	mg/L	0.07	EPA 300.0
Total Iron	0.57	0.04	0.03	0.09	0.42	mg/L	0.007	EPA 200.7
Dissolved Iron	0.110	0.010	0.010	<0.007	0.020	mg/L	0.007	EPA 200.7

Parameter	Sugarcamp 5/25/2011	Robinson Fork 5/25/2011	Radner 5/25/2011	James Creek 5/25/2011	20 mi. 5/25/2011	Unit	MDL	Method
Total Manganese	0.080	0.030	<0.036	0.040	0.120	mg/L	0.036	EPA 200.7
Dissolved Manganese	0.050	0.030	<0.036	0.040	0.070	mg/L	0.036	EPA 200.7
Total Aluminum	0.21	0.07	0.09	0.09	0.26	mg/L	0.010	EPA 200.7
Dissolved Aluminum	0.105	0.063	0.071	0.066	0.118	mg/L	0.010	EPA 200.7
Total Sodium	137.30	9.40	66.27	11.02	83.15	mg/L	0.007	EPA 200.7
Dissolved Sodium	135.10	8.46	64.59	10.99	79.65	mg/L	0.007	EPA 200.7
Total Magnesium	36.45	77.58	84.06	107.30	42.49	mg/L	0.006	EPA 200.7
Dissolved Magnesium	33.67	75.92	82.88	104.30	37.48	mg/L	0.006	EPA 200.7
Total Calcium	61.53	90.21	84.94	137.50	77.18	mg/L	0.007	EPA 200.7
Dissolved Calcium	58.50	86.10	81.76	131.50	71.71	mg/L	0.007	EPA 200.7
Total Hardness	303.74	544.73	558.25	785.20	367.69	mg/L		SM202340B
Dissolved Hardness	284.73	527.63	545.45	757.86	333.40	mg/L		SM202340B
TSS	2.00	<2.00	<2.00	<2.00	<2.00	mg/L	2.00	SM202540D
TDS	676	733	827	1,304	625	mg/L	2.00	SM202540C
Temperature	20.00	16.90	18.00	17.60	17.30	°C		Field Test SM202550 B
Flow	3.03	2.61	2.92	0.65	4.85	cfs		Field Test

Table 8 - Company 1 Additional Water Data Fall 2011

Parameter	Sugarcamp 10/26/2011	Robinson Fork 10/26/2011	Radner 10/26/2011	Mammoth 10/26/2011	20 mi. 10/26/2011	Unit	MDL	Method
Field pH	7.98	6.51	6.77	7.74	6.33	S.U.		Field Test SM204500H B
Total Alkalinity	58.72	22.51	28.51	211.21	12.34	mg/L	0.31	SM202320B
Total Acidity	<0.63	<0.63	<0.63	<0.63	<0.63	mg/L	0.63	SM202310B
Turbidity	9.80	8.80	11.23	4.20	3.35	NTU	0.10	EPA 180.1
Sp. Cond.	1,541	721	1,423	1,299	346	µS/cm	1.9	EPA 120.1
Total Sulfates	712.35	335.27	725.92	552.16	55.21	mg/L	0.1	EPA 300.0
Chlorides	18.76	1.25	9.87	1.87	1.14	mg/L	0.07	EPA 300.0
Total Iron	0.05	0.05	0.91	0.06	0.04	mg/L	0.007	EPA 200.7
Dissolved Iron	<0.007	<0.007	0.150	<0.007	0.020	mg/L	0.007	EPA 200.7
Total Manganese	1.64	0.11	2.15	0.09	0.06	mg/L	0.036	EPA 200.7
Dissolved Manganese	1.31	0.09	2.03	0.02	0.04	mg/L	0.036	EPA 200.7
Total Aluminum	0.35	0.13	0.34	0.11	0.06	mg/L	0.010	EPA 200.7
Dissolved Aluminum	0.24	0.02	0.11	0.03	0.02	mg/L	0.010	EPA 200.7
Total Sodium	49.34	5.41	5.64	15.54	2.15	mg/L	0.007	EPA 200.7

Parameter	Sugarcamp 10/26/2011	Robinson Fork 10/26/2011	Radner 10/26/2011	Mammoth 10/26/2011	20 mi. 10/26/2011	Unit	MDL	Method
Dissolved Sodium	45.14	5.16	4.82	11.70	1.87	mg/L	0.007	EPA 200.7
Total Magnesium	88.63	48.67	99.74	113.21	8.15	mg/L	0.006	EPA 200.7
Dissolved Magnesium	83.21	44.82	93.69	106.91	7.98	mg/L	0.006	EPA 200.7
Total Calcium	134.47	52.69	168.21	126.33	10.25	mg/L	0.007	EPA 200.7
Dissolved Calcium	128.50	47.13	157.46	123.71	10.05	mg/L	0.007	EPA 200.7
Total Hardness	745.28	317.51	821.64	786.98	66.72	mg/L		SM202340B
Dissolved Hardness	701.6	303.68	813.55	742.12	58.94	mg/L		SM202340B
TSS	5.00	3.00	9.00	3.00	<2.00	mg/L	2.00	SM202540D
TDS	1,189.42	554.21	1,256.00	1,067.00	95.00	mg/L	2.00	SM202540C
Temperature	18.21	15.40	15.91	16.45	16.21	°C		Field Test SM202550 B
Flow	0.54	1.82	0.22	0.69	1.91	cfs		Field Test
Field pH	7.68	7.81	7.61	8.14	8.35	S.U.		Field Test SM204500H B
Total Alkalinity	85.42	415.62	155.22	225.63	298.45	mg/L	0.31	SM202320B
Total Acidity	<0.63	<0.63	<0.63	<0.63	<0.63	mg/L	0.63	SM202310B
Turbidity	2.10	2.50	11.40	3.60	6.54	NTU	0.10	EPA 180.1
Sp. Cond.	1,013	1,789	1,084	1,246	1,075	µS/cm	1.9	EPA 120.1
Total Sulfates	375.25	789.39	226.39	488.93	245.10	mg/L	0.1	EPA 300.0
Chlorides	5.23	21.81	8.13	26.55	12.35	mg/L	0.07	EPA 300.0

Parameter	Sugarcamp 10/26/2011	Robinson Fork 10/26/2011	Radner 10/26/2011	Mammoth 10/26/2011	20 mi. 10/26/2011	Unit	MDL	Method
Total Iron	0.05	0.01	0.62	0.03	0.09	mg/L	0.007	EPA 200.7
Dissolved Iron	<0.007	<0.007	0.020	<0.007	0.040	mg/L	0.007	EPA 200.7
Total Manganese	0.050	0.050	0.130	<0.036	0.070	mg/L	0.036	EPA 200.7
Dissolved Manganese	0.010	0.010	0.090	<0.036	0.040	mg/L	0.036	EPA 200.7
Total Aluminum	0.11	0.04	0.31	0.11	0.23	mg/L	0.010	EPA 200.7
Dissolved Aluminum	0.05	0.03	0.13	0.08	0.09	mg/L	0.010	EPA 200.7
Total Sodium	9.52	17.84	91.52	75.21	145.21	mg/L	0.007	EPA 200.7
Dissolved Sodium	8.87	15.91	88.68	72.33	141.82	mg/L	0.007	EPA 200.7
Total Magnesium	79.42	155.42	48.71	89.81	38.29	mg/L	0.006	EPA 200.7
Dissolved Magnesium	75.31	151.79	45.35	84.20	35.71	mg/L	0.006	EPA 200.7
Total Calcium	95.64	198.65	78.52	79.54	66.13	mg/L	0.007	EPA 200.7
Dissolved Calcium	90.10	194.31	73.11	76.52	62.81	mg/L	0.007	EPA 200.7
Total Hardness	588.72	1,163.21	395.21	611.27	296.38	mg/L		SM202340B
Dissolved Hardness	554.90	1,127.85	354.65	593.59	275.41	mg/L		SM202340B
TSS	3.00	4.00	3.00	4.00	3.00	mg/L	2.00	SM202540D
TDS	785	1,456	714	875	721	mg/L	2.00	SM202540C

Parameter	Sugarcamp 10/26/2011	Robinson Fork 10/26/2011	Radner 10/26/2011	Mammoth 10/26/2011	20 mi. 10/26/2011	Unit	MDL	Method
Temperature	16.23	15.90	15.30	16.33	17.31	°C		Field Test SM202550 B
Flow	2.89	2.31	3.62	2.75	2.89	cfs		Field Test

Table 9 – Ion Imbalance Calculations from the GRI model from Spring 2011

Sampling Location	% Charge Difference	% Survival 100% Stream Water
Sugarcamp 5/25/2011	2.05	99.3
Robinson Fork 5/25/2011	5.88	99.9
Radner 5/25/2011	6.31	99.7
James Creek 5/25/2011	37.7	84.6
20 Mile 5/25/2011	11.26	100
Delbarton 6/3/2011	17.18	99.2
ICC 6/3/2011	26.96	99.7
Bandmill 6/3/2011	-0.27	98.4
Mammoth 6/3/2011	10.73	99.2
Marfork 6/3/2011	63.21	99.9

Table 10 – Ion Imbalance Calculations from the GRI model from Fall 2011

Sampling Location	% Charge Difference	% Survival 100% Stream Water
Sugarcamp 10/26/2011	2.05	99.3
Robinson Fork 10/26/2011	5.88	99.9
Radner 10/26/2011	6.31	99.7
Mammoth 10/26/2011	37.7	84.6

Sampling Location	% Charge Difference	% Survival 100% Stream Water
20 Mile 10/26/2011	11.26	100
ICC 10/31/2011	17.18	99.2
James Creek 10/31/2011	26.96	99.7
Marfork 10/31/2011	-0.27	98.4
Bandmill 11/2/2011	10.73	99.2
Delbarton 11/2/2011	63.21	99.9

Table 11 - Company 2 Additional Water Data Winter 2011

Date	Sampling Location	Bicarbonate (mg/L)	Conductivity (µS/cm)	Sulfate (mg/L)	TDS (mg/L)
Winter 2011	Big Horse Creek	133.7	1,480	662.0	1,183
Winter 2011	Jack Smith Branch	77.5	766	294.3	527
Winter 2011	Pond Fork	143.3	764	179.0	466
Winter 2011	Mud Lick Branch	95.2	1,333	536.7	947
Winter 2011	West Fork	573.3	1,597	216.7	1,003
Winter 2011	Coal Fork	20.1	1,361	222.7	766
Winter 2011	Seng Creek	176.7	1,453	554.0	1,072
Winter 2011	Cabin Creek	91.7	965	381.7	656
Winter 2011	Tom's Fork	186.0	2,200	1,042.7	1,833
Winter 2011	Little White Oak	26.9	750	333.3	503

Table 12 - Company 2 Additional Water Data Fall 2011

Date	Sampling Location	Bicarbonate (mg/L)	Conductivity (µS/cm)	Sulfate (mg/L)	TDS (mg/L)
Fall 2011	UNT Boone Block Hollow	195.7	1,400	584.0	971
Fall 2011	Big Horse Creek	132.7	1,903	1,321.7	1,267
Fall 2011	Cow Creek	155.0	926	241.3	569
Fall 2011	Jarrell Branch	301.3	1,493	260.0	838
Fall 2011	UNT Left Fork	87.9	1,947	963.3	1,367
Fall 2011	Pond Fork	207.7	961	218.0	574
Fall 2011	Moccasin Hollow	68.9	1,500	736.7	1,009
Fall 2011	UNT Tenmile Fork	253.0	1,840	842.0	1,310
Fall 2011	Pond Fork	128.7	762	203.7	462

Table 13 - Survival of *Ceriodaphnia dubia* Seven-day Chronic Toxicity Tests in 2008

Test Start Date	Sampling Location	% Control Survival	% Survival in 100% Stream Water	LC50	NOEC	Initial Field Conductivity (µS/cm)
12/12/2008	Hardway Branch	90	100	>100	100	1,373
12/12/2008	Line Creek	90	100	>100	100	883
12/12/2008	Taylor Fork	90	100	>100	100	350
12/15/2008	Big Creek	100	100	>100	100	918
12/16/2008	Laurel Creek	100	100	>100	100	1,550
12/16/2008	Mudlick Fork	90	100	>100	100	2,540
12/16/2008	Robinson Creek	100	100	>100	100	802
12/16/2008	Spruce Laurel Fork	90	100	>100	100	550
12/16/2008	Stollings Fork	80	100	>100	100	1,826
12/16/2008	West Fork	100	100	>100	100	2,120

Table 14 - Survival of *Ceriodaphnia dubia* Seven-day Chronic Toxicity Tests 2009

Test Start Date	Sampling Location	% Control Survival	% Survival in 100% Stream Water Survival	LC50	NOEC	Initial Field Conductivity (µS/cm)
6/23/2009	Downstream Fifteen Mile Fork	100	50	>100	50	1,092
6/23/2009	Mammoth Site 2	100	100	>100	100	631
6/23/2009	Upstream Fifteen Mile Fork	100	90	>100	100	1,078
6/23/2009	Mammoth Site 1	100	100	>100	100	815
6/30/2009	PM 260 Inlet	80	70	>100	100	1,578
6/30/2009	Laurel Creek	80	90	>100	100	2,342
6/30/2009	PM 316 Pond	90	90	>100	100	1,598
6/30/2009	Mouth of Robinson Creek	100	100	>100	100	2,137
6/30/2009	Stollings Fork	100	80	>100	100	1,839
6/30/2009	Mudlick Fork	90	80	>100	100	2,818
9/14/2009	Ballard Branch	90	100	>100	100	1,783
9/14/2009	Calvin Branch	90	80	>100	100	1,090
9/14/2009	Stanley Fork	100	80	>100	50	2,520
9/14/2009	Cow Creek	100	90	>100	50	1,010
9/14/2009	Jarrell Branch	80	80	>100	100	2,660
9/21/2009	White Oak	90	80	>100	100	1,338
9/21/2009	Joes Creek	100	100	>100	100	2,990
9/21/2009	Tenmile Fork	90	80	>100	100	1,378
9/21/2009	Left Fork	100	50	100	25	2,660
9/29/2009	Hardway Pond	100	90	>100	100	1,214
9/29/2009	Laurel Creek	90	100	>100	100	1,307
9/29/2009	PM 316 Pond	100	100	>100	100	1,892
9/29/2009	Mouth of Robinson Creek @ PM 24 location	100	90	>100	100	1,912
9/29/2009	Mudlick Fork	100	90	>100	100	2,081
9/29/2009	Stollings Fork	80	90	>100	100	1,267
10/06/2009	Downstream Fifteen Mile Fork	100	0	57	50	1,164
10/6/2009	Sixmile off Hughes Creek	100	100	>100	100	889

Test Start Date	Sampling Location	% Control Survival	% Survival in 100% Stream Water Survival	LC50	NOEC	Initial Field Conductivity (µS/cm)
10/6/2009	Upstream Fifteen Mile Fork	100	90	>100	100	1,117
10/6/2009	Hughes Fork off Bells Creek	100	100	>100	100	765

Table 15 - Survival of *Ceriodaphnia dubia* Seven-day Chronic Toxicity Tests 2010

Test Start Date	Sampling Location	% Control Survival	% Survival in 100% Stream Water	LC50	NOEC	Initial Field Conductivity (µS/cm)
2/15/2010	Tenmile Fork	90	100	>100	100	1,130
2/15/2010	White Oak	80	100	>100	100	1,028
2/15/2010	Left Fork	90	100	>100	100	1,800
2/15/2010	Joes Creek	90	90	>100	100	1,077
2/15/2010	Sycamore Fork	90	90	>100	100	594
2/22/2010	Cow Creek	100	90	>100	100	767
2/22/2010	Jarrell Branch	100	80	>100	100	1,833
2/22/2010	Stanley Fork	100	100	>100	100	1,798
2/22/2010	Ballard Branch	100	90	>100	100	795
2/22/2010	Calvin Branch	90	100	>100	100	748
4/20/2010	Hardway Pond @ Location PM 236	100	100	>100	100	2,145
4/20/2010	Lilly Fork @ PM 89	100	100	>100	100	1,340
4/20/2010	Robinson North @ PM 181	100	100	>100	100	2,380
4/27/2010	Horse Creek	100	90	>100	100	429
6/2/2010	No Name	100	100	>100	100	1,470
6/2/2010	Slip Ridge	100	90	>100	100	1,096
6/2/2010	Bias Branch	100	90	>100	100	392
6/2/2010	UBB Area of Jarrells Branch	100	100	>100	100	1,760
6/2/2010	Mudlick Fork	100	90	>100	100	2,403
6/2/2010	Mouth of Robinson Creek	100	90	>100	100	1,275

Test Start Date	Sampling Location	% Control Survival	% Survival in 100% Stream Water	LC50	NOEC	Initial Field Conductivity (µS/cm)
9/20/2010	Mud Lick Branch	90	90	>100	100	1,363
9/20/2010	Jack Smith Branch	100	100	NTD*	100	690
9/20/2010	Big Horse Creek	80	90	>100	100	1,538
9/20/2010	Pond Fork	100	100	>100	100	840
9/20/2010	West Fork	100	100	>100	100	1,610
9/27/2010	Cabin Creek	90	100	>100	100	1,030
9/27/2010	Coal Fork	100	100	>100	100	2,414
9/27/2010	Seng Creek	100	90	>100	100	1,631
9/27/2010	Tom's Fork	100	70	>100	100	2,351
9/27/2010	Little White Oak	100	90	>100	100	294
9/28/2010	Slip Ridge	100	100	>100	100	817
9/28/2010	Horse Creek	100	90	>100	100	693
9/28/2010	Bias Branch	100	100	>100	100	342
9/28/2010	UBB Area of Jarrells Branch	100	100	>100	100	1,759
9/28/2010	Mudlick Fork	100	60	>100	50	3,019
9/28/2010	No Name	100	0	50	50	1,718
9/29/2010	Mudlick Fork	100	90	>100	100	2,081
10/12/2010	Mouth of Robinson Creek	100	90	>100	100	2,311
10/12/2010	Twenty Mile Creek	90	100	>100	100	3,047
10/12/2010	Lilly Fork @ PM 89	100	90	>100	100	1,579
10/12/2010	Hardway Pond @ Location PM 236	100	90	>100	100	1,516

Table 16 - Survival of *Ceriodaphnia dubia* Seven-day Chronic Toxicity Tests 2011

Test Start Date	Sampling Location	% Control Survival	% Survival in 100% Stream Water	LC50	NOEC	Initial Field Conductivity (µS/cm)
2/14/2011	Mud Lick Branch	90	100	>100	100	1,403
2/14/2011	Jack Smith Branch	100	90	>100	100	816
2/14/2011	Big Horse Creek	100	90	>100	100	1,546
2/14/2011	Pond Fork	90	90	>100	100	795
2/14/2011	West Fork	100	90	>100	100	1,570

Test Start Date	Sampling Location	% Control Survival	% Survival in 100% Stream Water	LC50	NOEC	Initial Field Conductivity (µS/cm)
2/21/2011	Cabin Creek	90	90	>100	100	1,055
2/21/2011	Coal Fork	100	90	>100	100	1,827
2/21/2011	Seng Creek	80	80	>100	100	1,588
2/21/2011	Tom's Fork	100	80	>100	100	2,412
2/21/2011	Little White Oak	80	80	>100	100	887
5/24/2011	Below 033 on 20 Mile Fork	100	80	>100	100	105
5/24/2011	James Creek below 015	100	90	>100	100	1,692
5/24/2011	029 on Radner Fork of 20 mile	100	100	>100	100	1,232
5/24/2011	013 Robinson North on 20 mile	100	100	>100	100	472
5/24/2011	001 on Sugarcamp	100	100	>100	100	1,341
6/2/2011	Bandmill Below 016	100	80	>100	100	1,127
6/2/2011	Delbarton below 400	100	100	>100	100	990
6/2/2011	ICC Below 031	100	100	>100	100	882
6/2/2011	Mammoth below 004	100	100	>100	100	1,207
6/2/2011	Marfork Below 018	100	100	>100	100	857
9/19/2011	UNT Left Fork Creek	90	80	>100	100	2,070
9/19/2011	UNT Tenmile Fork	90	80	>100	100	1,850
9/19/2011	UNT Boone Block Hollow	80	90	>100	100	1,540
9/19/2011	Big Horse Creek	80	80	>100	100	2,130
9/26/2011	Pond Fork	90	100	>100	100	844
9/26/2011	Cow Creek	80	90	>100	100	1,090
9/26/2011	Jarrell Branch	100	100	>100	100	1,520
9/26/2011	Pond Fork	100	100	>100	100	1,160
9/26/2011	Moccasin Hollow	90	90	>100	100	1,600
10/25/2011	Below 033 on 20 Mile Fork	100	70	>100	100	747
10/25/2011	Mammoth below 004	90	100	>100	100	460

Test Start Date	Sampling Location	% Control Survival	% Survival in 100% Stream Water	LC50	NOEC	Initial Field Conductivity (µS/cm)
10/25/2011	029 on Radner Fork of 20 mile	90	100	>100	100	1,414
10/25/2011	013 Robinson North on 20 mile	100	100	>100	100	2,018
10/25/2011	001 on Sugarcamp	90	100	>100	100	1,710
11/1/2011	Bandmill Below 016	100	100	>100	100	1,084
11/1/2011	Delbarton below 400	80	100	>100	100	591
11/1/2011	ICC Below 031	100	100	>100	100	671
11/1/2011	James Creek below 015	90	70	>100	100	1,495
11/1/2011	Marfork Below 018	100	100	>100	100	340

Table 17 - Reproduction of *Ceriodaphnia dubia* Seven-day Chronic Toxicity Tests in 2008

Test Start Date	Sampling Location	NOEC	LOEC	IC25	Initial Field Conductivity (µS/cm)
12/12/2008	Hardway Branch	100	>100	93.95	1,373
12/12/2008	Line Creek	100	>100	>100.00	883
12/12/2008	Taylor Fork	100	>100	>100.00	350
12/15/2008	Big Creek	100	>100	>100.00	918
12/16/2008	Laurel Creek	100	>100	>100.00	1,550
12/16/2008	Mudlick Fork	100	>100	>100.00	2,540
12/16/2008	Robinson Creek	100	>100	>100.00	802
12/16/2008	Spruce Laurel Fork	100	>100	>100.00	550
12/16/2008	Stollings Fork	100	>100	>100.00	1,826
12/16/2008	West Fork	100	>100	>100.00	2,120

Table 18 - Reproduction of *Ceriodaphnia dubia* Seven-day Chronic Toxicity Tests in 2009

Test Start Date	Sampling Location	NOEC	LOEC	IC25	Initial Field Conductivity (µS/cm)
6/23/2009	Downstream Fifteen Mile Fork	12.5	25	17.90	1,092
6/23/2009	Mammoth Site 2	50.0	100	91.50	631
6/23/2009	Upstream Fifteen Mile Fork	12.5	25	18.40	1,078
6/23/2009	Mammoth Site 1	50.0	100	86.20	815
6/30/2009	PM 260 Inlet	100.0	>100	97.40	1,578
6/30/2009	Laurel Creek	100.0	>100	>100.00	2,342
6/30/2009	PM 316 Pond	100.0	>100	>100.00	1,598
6/30/2009	Mouth of Robinson Creek	100.0	>100	98.60	2,137
6/30/2009	Stollings Fork	50.0	100	69.10	1,839
6/30/2009	Mudlick Fork	50.0	100	54.50	2,818
9/14/2009	Ballard Branch	100.0	>100	>100.00	1,783
9/14/2009	Calvin Branch	100.0	>100	>100.00	1,090
9/14/2009	Stanley Fork	50.0	100	74.25	2,520
9/14/2009	Cow Creek	50.0	100	83.41	1,010
9/14/2009	Jarrell Branch	100.0	>100	69.94	2,660
9/21/2009	White Oak	100.0	>100	>100.0	1,338
9/21/2009	Joes Creek	100.0	>100	>100.0	2,990
9/21/2009	Tenmile Fork	100.0	>100	81.25	1,378
9/21/2009	Left Fork	25.0	50	29.28	2,660
9/29/2009	Hardway Pond	100.0	>100	100.00	1,214
9/29/2009	Laurel Creek	50.0	100	71.00	1,307
9/29/2009	PM 316 Pond	50.0	100	>100	1,892
9/29/2009	Mouth of Robinson Creek @ PM 24 location	50.0	100	95.40	1,912
9/29/2009	Mudlick Fork	25.0	50	55.70	2,081
9/29/2009	Stollings Fork	100.0	>100	>100.00	1,267
10/06/2009	Downstream Fifteen Mile Fork	12.5	25	18.70	1,164
10/6/2009	Sixmile off Hughes Creek	100.0	>100	>100.00	889
10/6/2009	Upstream Fifteen Mile Fork	25.0	50	15.70	1,117
10/6/2009	Hughes Fork off Bells Creek	100.0	>100	80.60	765

Table 19 - Benthic Macroinvertebrate Metrics and Their Response to Disturbance

Metric	Description
Taxa Richness	Number of distinct taxa present
EPT Taxa Richness	Number of Ephemeroptera, Plecoptera, and Trichoptera taxa present
Percent EPT	Percentage of sample which is composed of the sensitive EPT individuals
Percent Two Dominant Taxa	Measures the dominance of the two most abundant taxa as a percentage
Percent Chironomidae	Percentage of sample which is composed of the family Chironomidae
Hilsenhoff Biotic Index (HBI)	Abundance-weighted average tolerance of assemblage of organisms (Scale of zero to 10)

Table 20
WVSCI Scoring Criteria

> 68.0 UNIMPAIRED
> 60.6 to 68.0 GREY ZONE
< 60.6 IMPAIRED

Table 21 - Reproduction of *Ceriodaphnia dubia* Seven-day Chronic Toxicity Tests in 2010

Test Start Date	Sampling Location	NOEC	LOEC	IC25	Initial Field Conductivity (µS/cm)
2/15/2010	Tenmile Fork	100.0	>100	>100.00	1,130
2/15/2010	White Oak	100.0	>100	>100.00	1,028
2/15/2010	Left Fork	50.0	100	90.47	1,800
2/15/2010	Joes Creek	100.0	>100	>100.00	1,077
2/15/2010	Sycamore Fork	100.0	>100	>100.00	594

Test Start Date	Sampling Location	NOEC	LOEC	IC25	Initial Field Conductivity (µS/cm)
2/22/2010	Cow Creek	50.0	100	74.72	767
2/22/2010	Jarrell Branch	100.0	>100	>100.00	1,833
2/22/2010	Stanley Fork	50.0	100	93.62	1,798
2/22/2010	Ballard Branch	100.0	>100	>100.00	795
2/22/2010	Calvin Branch	100.0	>100	>100.00	748
4/20/2010	Hardway Pond @ Location PM 236	50.0	100	90.60	2,145
4/20/2010	Lilly Fork @ PM 89	50.0	100	85.70	1,340
4/20/2010	Robinson North @ PM 181	100.0	>100	89.60	2,380
4/27/2010	Horse Creek	100.0	>100	>100.00	429
6/2/2010	No Name	50.0	100	65.50	1,470
6/2/2010	Slip Ridge	100.0	>100	95.10	1,096
6/2/2010	Bias Branch	12.5	25	15.40	392
6/2/2010	UBB Area of Jarrells Branch	25.0	50	45.00	1,760
6/2/2010	Mudlick Fork	25.0	50	46.60	2,403
9/20/2010	Mouth of Robinson Creek	50.0	100	62.70	1,275
9/20/2010	Mud Lick Branch	100.0	>100	77.40	1,363
9/20/2010	Jack Smith Branch	50.0	100	65.35	690
9/20/2010	Big Horse Creek	100.0	>100	>100.00	1,538
9/20/2010	Pond Fork	100.0	>100	>100.00	840
9/27/2010	West Fork	100.0	>100	84.92	1,610
9/27/2010	Cabin Creek	100.0	>100	>100.00	1,030
9/27/2010	Coal Fork	100.0	>100	87.74	2,414
9/27/2010	Seng Creek	50.0	100	78.49	1,631
9/27/2010	Tom’s Fork	50.0	100	56.61	2,351
9/28/2010	Little White Oak	100.0	>100	>100.00	294
9/28/2010	Slip Ridge	100.0	>100	>100.00	817
9/28/2010	Horse Creek	100.0	>100	75.00	693
9/28/2010	Bias Branch	100.0	>100	25.70	342
9/28/2010	UBB Area of Jarrells Branch	50.0	100	74.60	1,759
9/28/2010	Mudlick Fork	25.0	50	51.10	3,019
9/29/2010	No Name	12.5	25	29.60	1,718
10/12/2010	Mudlick Fork	25.0	50	55.70	2,081
10/12/2010	Mouth of Robinson Creek	50.0	100	68.80	2,311
10/12/2010	Twenty Mile Creek	50.0	100	73.50	3,047
10/12/2010	Lilly Fork @ PM 89	100.0	>100	>100.00	1,579

Test Start Date	Sampling Location	NOEC	LOEC	IC25	Initial Field Conductivity (µS/cm)
10/12/2010	Hardway Pond @ Location PM 236	50.0	100	52.70	1,516

Table 22 - Reproduction of *Ceriodaphnia dubia* Seven-day Chronic Toxicity Tests in 2011

Test Start Date	Sampling Location	NOEC	LOEC	IC25	Initial Field Conductivity (µS/cm)
2/14/2011	Mud Lick Branch	100.0	>100	>100.00	1,403
2/14/2011	Jack Smith Branch	100.0	>100	>100.00	816
2/14/2011	Big Horse Creek	100.0	>100	>100.00	1,546
2/14/2011	Pond Fork	100.0	>100	>100.00	795
2/14/2011	West Fork	100.0	>100	>100.00	1,570
2/21/2011	Cabin Creek	100.0	>100	>100.00	1,055
2/21/2011	Coal Fork	100.0	>100	>100.00	1,827
2/21/2011	Seng Creek	100.0	>100	>100.00	1,588
2/21/2011	Tom's Fork	100.0	>100	58.31	2,412
2/21/2011	Little White Oak	50.0	100	71.27	887
4/20/2011	Lilly Fork @ PM 89	50.0	100	85.70	1,340
5/24/2011	Below 033 on 20 Mile Fork	50.0	100	70.10	105
5/24/2011	James Creek below 015	50.0	100	58.60	1,692
5/24/2011	029 on Radner Fork of 20 mile	25.0	50	47.80	1,232
5/24/2011	013 Robinson North on 20 mile	100.0	>100	>100.00	472
5/24/2011	001 on Sugarcamp	100.0	>100	>100.00	1,341
6/2/2011	Bandmill Below 016	100.0	>100	>100.00	1,127
6/2/2011	Delbarton below 400	100.0	>100	>100.00	990
6/2/2011	ICC Below 031	100.0	>100	>100.00	882
6/2/2011	Mammoth below 004	100.0	>100	>100.00	1,207
6/2/2011	Marfork Below 018	100.0	>100	>100.00	857
9/19/2011	UNT Left Fork Creek	100.0	>100	>100.00	2,070
9/19/2011	UNT Tenmile Fork	100.0	>100	>100.00	1,850
9/19/2011	UNT Boone Block Hollow	100.0	>100	>100.00	1,540
9/19/2011	Big Horse Creek	100.0	>100	>100.00	2,130
9/26/2011	Pond Fork	100.0	>100	>100.00	844
9/26/2011	Cow Creek	100.0	>100	>100.00	1,090
9/26/2011	Jarrell Branch	100.0	100	>100.00	1,520

Test Start Date	Sampling Location	NOEC	LOEC	IC25	Initial Field Conductivity (µS/cm)
9/26/2011	Pond Fork	100.0	>100	>100.00	1,160
9/26/2011	Moccasin Hollow	100.0	>100	>100.00	1,600
10/25/2011	Below 033 on 20 Mile Fork	100.0	>100	96.50	747
10/25/2011	Mammoth below 004	50.0	100	84.20	460
10/25/2011	029on Radner Fork of 20 mile	100.0	>100	>100.00	1,414
10/25/2011	013 Robinson North on 20 mile	100.0	>100	>100.00	2,018
10/25/2011	001 on Sugarcamp	100.0	>100	88.10	1,710
11/1/2011	Bandmill Below 016	100.0	>100	>100.00	1,084
11/1/2011	Delbarton below 400	100.0	>100	>100.00	591
11/1/2011	ICC Below 031	50.0	100	42.20	671
11/1/2011	James Creek below 015	50.0	100	66.50	1,495
11/1/2011	Marfork Below 018	100.0	>100	>100.00	340

Table 23 - Habitat Assessment for Benthic Macroinvertebrate Toxicity Sampling Fall 2009

Habitat Category/Parameter	Highest Possible Score	Station			
		Ballard Branch	Calvin Branch	White Oak Creek	Joes Creek
Epifaunal Substrate/Available Cover	20	11	15	14	11
Embeddedness	20	11	10	7	13
Velocity/Depth Regime	20	9	10	13	13
Sediment Deposition	20	14	16	14	15
Channel Flow Status	20	7	9	15	14
Channel Alteration	20	15	15	15	13
Frequency of Riffles (or bends)	20	6	10	14	12
Bank Stability	20	16	10	14	12
Vegetative Protection	20	16	16	14	14
Riparian Vegetative Zone Width	20	7	3	4	4
Total	**200**	**112**	**114**	**124**	**121**
Assessment category		***Marginal***	***Suboptimal***	***Suboptimal***	***Suboptimal***
Epifaunal Substrate/Available Cover		20	14	15	15
Embeddedness		20	12	6	13
Velocity/Depth Regime		20	15	12	14
Sediment Deposition		20	11	8	12
Channel Flow Status		20	14	7	11
Channel Alteration		20	15	15	15
Frequency of Riffles (or bends)		20	14	12	11
Bank Stability		20	11	8	14
Vegetative Protection		20	12	16	12
Riparian Vegetative Zone Width		20	4	2	4
Total		**200**	**122**	**101**	**121**
Assessment category		***Suboptimal***	***Marginal***	***Suboptimal***	***Suboptimal***

Table 24 - Habitat Assessment for Benthic Macroinvertebrate Toxicity Sampling Fall 2010

Habitat Category/Parameter	Highest Possible Score	Station				
		Mud Lick Branch	Jack Smith Branch	Big Horse Creek	Pond Fork	West Fork
Epifaunal Substrate/Available Cover	20	8	13	14	14	14
Embeddedness	20	2	13	10	10	8
Velocity/Depth Regime	20	7	14	13	14	12
Channel Alteration	20	8	14	14	14	14
Sediment Deposition	20	14	13	14	14	14
Frequency of Riffles (or bends)	20	13	12	13	15	13
Channel Flow Status	20	12	12	13	14	14
Bank Stability	20	12	7	13	13	12
Bank Vegetative Protection	20	13	6	10	12	14
Width of Undisturbed Vegetative Zone	20	2	2	2	2	4
Total	**200**	**91**	**106**	**116**	**122**	**119**
RBP Assessment Category		***Marginal***	***Marginal***	***Sub-Optimal***	***Sub-Optimal***	***Sub-Optimal***

Benthic Macroinvertebrate Substrate	20	8	14	15	15	14
Trash Index	20	12	5	11	8	11
Remoteness Rating	20	4	6	5	4	6
Epifaunal Substrate/Available Cover	20	10	14	11	14	10
Embeddedness	20	10	13	9	14	12
Velocity/Depth Regime	20	12	9	12	14	6
Channel Alteration	20	13	14	13	14	14
Sediment Deposition	20	12	14	13	13	14
Frequency of Riffles (or bends)	20	13	12	14	14	9
Channel Flow Status	20	12	11	11	12	6

Habitat Category/Parameter	Highest Possible Score	Station				
		Mud Lick Branch	Jack Smith Branch	Big Horse Creek	Pond Fork	West Fork
Bank Stability	20	10	11	10	12	9
Bank Vegetative Protection	20	11	12	10	9	12
Width of Undisturbed Vegetative Zone	20	5	4	2	5	12
Total	**200**	**108**	**114**	**105**	**121**	**104**
RBP Assessment Category		***Marginal***	***Sub-Optimal***	***Marginal***	***Sub-Optimal***	***Marginal***
Benthic Macroinvertebrate Substrate	20	9	14	10	14	6
Trash Index	20	5	14	10	13	11
Remoteness Rating	20	4	7	5	6	13

Table 25 - Habitat Assessment for Benthic Macroinvertebrate Toxicity Sampling Fall 2011

Habitat Category/Parameter	Highest Possible Score	Station				
		UNT Left Fork	UNT Tenmile Fork	UNT Boone Block Hollow	Big Horse Creek	Pond Fork
Epifaunal Substrate/Available Cover	20	10	12	13	13	14
Embeddedness	20	6	9	11	13	11
Velocity/Depth Regime	20	13	8	12	7	15
Channel Alteration	20	14	12	15	11	10
Sediment Deposition	20	10	12	6	11	12
Frequency of Riffles (or bends)	20	13	11	11	10	14
Channel Flow Status	20	15	14	14	13	14
Bank Stability	20	14	14	16	16	12
Bank Vegetative Protection	20	16	18	16	16	9

Habitat Category/Parameter	Highest Possible Score	Station				
		UNT Left Fork	UNT Tenmile Fork	UNT Boone Block Hollow	Big Horse Creek	Pond Fork
Width of Undisturbed Vegetative Zone	20	6	10	10	4	4
Total	**200**	**117**	**120**	**124**	**114**	**115**
RBP Assessment Category		***Sub-Optimal***	***Sub-Optimal***	***Sub-Optimal***	***Sub-Optimal***	***Sub-Optimal***

Benthic Macroinvertebrate Substrate	20	10	13	13	13	14
Trash Index	20	7	10	14	16	11
Remoteness Rating	20	7	6	13	4	5

Table 25 (Continued) - Habitat Assessment for Benthic Macroinvertebrate Toxicity Sampling Fall 2011

Habitat Category/Parameter	Highest Possible Score	Station			
		Cow Creek	Jarrell Branch	Pond Fork	Moccasin Hollow
Epifaunal Substrate/Available Cover	20	13	11	14	14
Embeddedness	20	11	10	13	13
Velocity/Depth Regime	20	14	6	14	15
Channel Alteration	20	14	5	14	13
Sediment Deposition	20	13	11	13	14
Frequency of Riffles (or bends)	20	12	6	13	15
Channel Flow Status	20	14	14	14	15
Bank Stability	20	12	12	12	11
Bank Vegetative Protection	20	10	4	13	8
Width of Undisturbed Vegetative Zone	20	4	2	6	6

Habitat Category/Parameter	Highest Possible Score	Station			
		Cow Creek	Jarrell Branch	Pond Fork	Moccasin Hollow
Total	**200**	**117**	**81**	**126**	**124**
RBP Assessment Category		***Sub-Optimal***	***Marginal***	***Sub-Optimal***	***Sub-Optimal***

Benthic Macroinvertebrate Substrate	20	13	6	13	13
Trash Index	20	13	11	11	14
Remoteness Rating	20	4	4	5	6

Table 26 - Inorganic Substrate Components Results for Benthic Macroinvertebrate Toxicity Sampling Fall 2009

Sampling Station	Bedrock	Boulder	Cobble	Gravel	Sand	Silt	Clay
Ballard Branch	0	5	35	30	20	10	0
Calvin Branch	5	10	60	10	10	5	0
White Oak Creek	0	15	30	25	20	10	0
Joes Creek	0	15	40	20	20	5	0
Tenmile Fork	0	5	40	35	10	10	0
Stanley Fork	0	5	60	10	20	5	0
Cow Creek	10	15	40	20	10	5	0
Jarrell Branch	0	5	45	30	10	10	0
Left Fork	0	20	20	40	10	10	0

Table 27 - Inorganic Substrate Components Results for Benthic Macroinvertebrate Toxicity Sampling Fall 2010

Sampling Station	Bedrock	Boulder	Cobble	Coarse Gravel	Fine Gravel	Sand	Silt & Fines	Clay
Mud Lick Branch	60	10	5	10	10	5	0	0
Jack Smith Branch	0	10	40	10	10	10	10	0
Big Horse Creek	0	10	30	20	20	10	20	0
Pond Fork	0	25	50	5	10	10	0	0
West Fork	0	20	30	40	5	0	5	0
Cabin Creek	0	10	60	10	10	10	0	0
Coal Fork	0	5	40	30	10	5	10	0
Seng Creek	0	40	20	20	10	10	0	0
Tom’s Fork	0	20	40	20	10	10	0	0
Little White Oak	0	10	60	20	10	0	0	0

Table 28 - Inorganic Substrate Components Results for Benthic Macroinvertebrate Toxicity Sampling Fall 2011

Sampling Station	Bedrock	Boulder	Cobble	Coarse Gravel	Fine Gravel	Sand	Silt & Fines	Clay
UNT Left Fork	10	5	35	25	15	5	5	0
UNT Tenmile Fork	0	0	45	25	20	5	5	0
UNT Boone Block Hollow	0	0	40	25	10	15	10	0
Big Horse Creek	0	0	40	25	15	10	10	0
Pond Fork	0	5	40	20	20	10	10	0
Cow Creek	10	30	40	10	5	5	0	0
Jarrell Branch	0	0	30	40	20	10	0	0

Sampling Station	Bedrock	Boulder	Cobble	Coarse Gravel	Fine Gravel	Sand	Silt & Fines	Clay
Pond Fork	0	10	40	20	20	10	0	0
Moccasin Hollow	0	10	20	20	40	10	0	0

Table 29 – Field Water Chemistry Results for Benthic Macroinvertebrate Sampling Fall 2009

Sampling Station	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)	Turbidity (NTU)	Flow (cfs)
Ballard Branch	1,687	9.83	16.6	8.26	6.3	0.45
Calvin Branch	984	10.33	13.9	7.78	3.0	0.59
Stanley Fork	1,951	9.87	15.1	8.28	10.0	2.86
Cow Creek	960	10.17	15.4	8.53	0.0	3.23
Jarrell Branch	2,410	9.10	18.2	8.38	5.7	0.80
White Oak	1,197	9.29	17.6	7.98		6.20
Joes Creek	2,990	9.52	16.7	7.66		2.28
Tenmile Fork	1,112	10.36	13.9	7.95		3.48
Left Fork	1,842	9.51	16.9	8.01		2.82

Table 30 – Field Water Chemistry Results for Benthic Macroinvertebrate Sampling Fall 2010

Sampling Station	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)	Turbidity (NTU)	Flow (cfs)
Mud Lick Branch	1,319	7.47	22.5	7.77	34.0	0.76
Jack Smith Branch	1,242	7.53	18.3	8.14	8.5	0.86
Big Horse Creek	1,761	7.22	18.8	7.91	1.7	2.11

Sampling Station	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (°C)	pH (S.U.)	Turbidity (NTU)	Flow (cfs)
Pond Fork	835	8.95	21.4	8.52	3.0	27.59
West Fork	1,686	8.53	19.3	8.21	4.9	13.40
Cabin Creek	1,039	8.89	14.2	8.11	11.0	26.10
Coal Fork	2,747	9.13	14.5	7.92	4.1	6.76
Seng Creek	1,657	8.65	16.2	8.31	2.8	2.45
Tom's Fork	2,410	7.63	18.0	8.15	5.4	18.54
Little White Oak	222	7.75	17.1	7.87	3.6	0.04

Table 31 – Field Water Chemistry Results for Benthic Macroinvertebrate Sampling Fall 2011

Sampling Station	Conductivity (µS/cm)	Temperature (°C)	pH (S.U.)	Turbidity (NTU)	Flow (cfs)
UNT Left Fork	1,965	17.3	7.35	4.1	0.79
UNT Tenmile Fork	1,818	16.9	7.87	3.6	0.45
UNT Boone Block Hollow	1,398	17.0	8.01	4.2	0.46
Big Horse Creek	1,768	17.1	7.69	3.2	7.00
Pond Fork	622	16.0	8.42	4.0	19.67
Cow Creek	744	16.1	8.70	4.5	3.31
Jarrell Branch	1,388	17.8	8.63	6.0	1.43
Pond Fork	766	18.5	8.64	4.9	48.14
Moccasin Hollow	1,351	17.4	8.12	19.0	0.77

Table 32 - Survival of *Ceriodaphnia dubia* in Seven-day Toxicity Tests Fall 2009

Receiving Stream	% Control Survival	% Survival in 100% Stream Water	LC50	NOEC	Initial Field Conductivity (µS/cm)
Ballard Branch	90	100	>100.0	100	1,783
Calvin Branch	90	80	>100.0	100	1,090
White Oak	90	80	>100.0	100	1,338
Joes Creek	100	100	>100.0	100	2,990
Tenmile Fork	90	80	>100.0	100	1,378
Stanley Fork	100	80	>100.0	50	2,520
Cow Creek	100	90	>100.0	50	1,010
Jarrell Branch	80	80	>100.0	100	2,660
Left Fork	100	50	100.0	25	2,640

Table 33 - Results of Reproductive Comparisons for *Ceriodaphnia dubia* Seven-day Toxicity Tests Fall 2009

Receiving Stream	NOEC	LOEC	IC25	Initial Field Conductivity (µS/cm)
Ballard Branch	100	>100.0	>100.00	1,783
Calvin Branch	100	>100.0	>100.00	1,090
White Oak	100	>100.0	>100.00	1,338
Joes Creek	100	>100.0	>100.00	2,990
Tenmile Fork	100	>100.0	81.25	1,378
Stanley Fork	50	100.0	74.25	2,520
Cow Creek	50	100.0	83.41	1,010
Jarrell Branch	100	>100.0	69.94	2,660
Left Fork	25	50.0	29.28	2,640

Table 34 - Survival of *Ceriodaphnia dubia* in Seven-day Toxicity Tests Fall 2010

Receiving Stream	% Control Survival	% Survival in 100% Stream Water	LC50	NOEC	Initial Field Conductivity (µS/cm)
Mud Lick Branch	90	90	>100.0	100	1,363
Jack Smith Branch	100	100	NTD*	100	690
Big Horse Creek	80	90	>100.0	100	1,538
Pond Fork	100	100	>100.0	100	840
West Fork	100	100	>100.0	100	1,610
Cabin Creek	90	100	>100.0	100	1,030
Coal Fork	100	100	>100.0	100	2,414
Seng Creek	100	90	>100.0	100	1,631
Tom’s Fork	100	70	>100.0	100	2,351
Little White Oak	100	90	>100.0	100	294

NTD* - No Toxicity Demonstrated

Table 35 - Results of Reproductive Comparisons for *Ceriodaphnia dubia* Seven-day Toxicity Tests Fall 2010

Receiving Stream	NOEC	LOEC	IC25	Initial Field Conductivity (µS/cm)
Mud Lick Branch	100	>100.0	77.40	1,363
Jack Smith Branch	50	100.0	65.35	690
Big Horse Creek	100	>100.0	>100.00	1,538
Pond Fork	100	>100.0	>100.00	840
West Fork	100	>100.0	84.92	1,610
Cabin Creek	100	>100.0	>100.00	1,030
Coal Fork	100	>100.0	87.74	2,414
Seng Creek	50	100.0	78.49	1,631
Tom’s Fork	50	100.0	56.61	2,351
Little White Oak	100	>100.0	>100.00	294

Table 36 - Survival of *Ceriodaphnia dubia* in Seven-day Toxicity Tests Fall 2011

Receiving Stream	% Control Survival	% Survival in 100% Stream Water	LC50	NOEC	Initial Field Conductivity (µS/cm)
UNT Left Fork Creek	90	80	>100.0	100	2,070
UNT Tenmile Fork	90	80	>100.0	100	1,850
UNT Boone Block Hollow	80	90	>100.0	100	1,540
Big Horse Creek	80	80	>100.0	100	2,130
Pond Fork	90	100	>100.0	100	844
Cow Creek	80	90	>100.0	100	1,090
Jarrell Branch	100	100	>100.0	100	1,520
Pond Fork	100	100	>100.0	100	1,160
Moccasin Hollow	90	90	>100.0	100	1,600

Table 37 - Results of Reproductive Comparisons for *Ceriodaphnia dubia* Seven-day Toxicity Tests Fall 2011

Receiving Stream	NOEC	LOEC	IC25	Initial Field Conductivity (µS/cm)
UNT Left Fork Creek	100	>100.0	>100.0	2,070
UNT Tenmile Fork	100	>100.0	>100.0	1,850
UNT Boone Block Hollow	100	>100.0	>100.0	1,540
Big Horse Creek	100	>100.0	>100.0	2,130
Pond Fork	100	>100.0	>100.0	844
Cow Creek	100	>100.0	>100.0	1,090
Jarrell Branch	100	100.0	>100.0	1,520
Pond Fork	100	>100.0	>100.0	1,160
Moccasin Hollow	100	>100.0	>100.0	1,600

Table 38 - Genus Level Benthic Macroinvertebrate Summary for Toxicity Sampling Fall 2009

Station	Sample Method	Taxa Richness	EPT Taxa Richness	Percent EPT	Percent Two Dominant Taxa	Percent Chironomidae	HBI
Ballard Branch	Kicknet	11	3	21.52	64.56	46.84	4.8
Calvin Branch	Kicknet	16	5	46.45	57.92	26.23	5.9
White Oak Creek	Kicknet	23	6	49.04	53.85	23.56	4.7
Joes Creek	Kicknet	13	7	22.98	69.57	58.39	4.8
Tenmile Fork	Kicknet	14	6	40.22	63.13	45.25	4.5
Stanley Fork	Kicknet	12	4	34.38	77.08	48.96	4.8
Cow Creek	Kicknet	12	6	61.96	50.00	7.07	4.2
Jarrell Branch	Kicknet	18	6	27.78	65.66	56.57	5.1
Left Fork	Kicknet	11	4	86.15	86.54	10.00	1.7

Table 39 - Family Level Benthic Macroinvertebrate Summary for Toxicity Sampling Fall 2009

Station	Sample Method	Taxa Richness	EPT Taxa Richness	Percent EPT	Percent Two Dominant Taxa	Percent Chironomidae	HBI	WVSCI Total Score	WVSCI Scoring Criteria
Ballard Branch	Kicknet	7	1	21.52	68.35	46.84	5.8**	**	**
Calvin Branch	Kicknet	12	2	46.45	64.48	26.23	6.0	51.67	Impaired

Station	Sample Method	Taxa Richness	EPT Taxa Richness	Percent EPT	Percent Two Dominant Taxa	Percent Chironomidae	HBI	WVSCI Total Score	WVSCI Scoring Criteria
White Oak Creek	Kicknet	17	4	49.04	65.38	23.56	5.4	60.27	Impaired
Joes Creek	Kicknet	10	4	22.98	70.81	58.39	6.2	40.88	Impaired
Tenmile Fork	Kicknet	11	4	40.22	82.12	45.25	6.0	44.40	Impaired
Stanley Fork	Kicknet	11	3	34.38	77.08	48.96	5.4	44.25	Impaired
Cow Creek	Kicknet	10	4	61.96	53.26	7.07	5.1	63.65	Grey Zone
Jarrell Branch	Kicknet	14	4	27.78	71.21	56.57	6.1	45.23	Impaired
Left Fork	Kicknet	8	2	86.15	86.54	10.00	3.7	57.96	Impaired

**** Less than 180 organisms**

Table 40 - Genus Level Benthic Macroinvertebrate Summary for Toxicity Sampling Fall 2010

Station	Sample Method	Taxa Richness	EPT Taxa Richness	Percent EPT	Percent Two Dominant Taxa	Percent Chironomidae	HBI
Mud Lick Branch	Kicknet	7	3	40.00	50.00	30.00	6.2
Jack Smith Branch	Kicknet	18	7	33.67	75.88	48.74	6.1
Big Horse Creek	Kicknet	13	5	35.96	71.92	46.31	5.9
Pond Fork	Kicknet	18	8	61.14	33.16	11.92	4.2
West Fork	Kicknet	14	6	21.54	72.82	61.54	6.1
Cabin Creek	Kicknet	8	4	11.76	84.13	66.67	5.8
Coal Fork	Kicknet	14	7	28.17	77.46	67.14	6.0
Seng Creek	Kicknet	18	8	35.64	61.70	39.89	5.5
Tom’s Fork	Kicknet	10	6	60.40	56.44	34.65	5.2
Little White Fork	Kicknet	15	7	55.93	66.10	28.81	5.9

Table 41 - Family Level Benthic Macroinvertebrate Summary for Toxicity Sampling Fall 2010

Station	Sample Method	Taxa Richness	EPT Taxa Richness	Percent EPT	Percent Two Dominant Taxa	Percent Chironomidae	HBI	WVSCI Total Score	WVSCI Scoring Criteria
Mud Lick Branch	Kicknet	6	2	40.00	60.00	30.00	6.0	46.10	Impaired
Jack Smith Branch	Kicknet	15	6	33.67	77.89	48.74	5.8	49.38	Impaired
Big Horse Creek	Kicknet	10	4	35.96	74.88	46.31	5.7	44.82	Impaired
Pond Fork	Kicknet	13	6	61.14	48.19	11.92	4.6	69.79	Unimpaired
West Fork	Kicknet	11	4	21.54	77.95	61.54	6.0	38.86	Impaired
Cabin Creek	Kicknet	7	3	11.76	84.31	66.67	6.2	29.83	Impaired
Coal Fork	Kicknet	13	6	28.17	87.32	67.14	5.8	41.24	Impaired
Seng Creek	Kicknet	16	6	35.64	69.68	39.89	5.7	54.40	Impaired
Tom's Fork	Kicknet	8	4	60.40	80.69	34.65	5.5	48.82	Impaired
Little White Fork	Kicknet	13	6	55.93	67.80	28.81	5.3	59.12	Impaired

Table 42 - Genus Level Benthic Macroinvertebrate Summary for Toxicity Sampling Fall 2011

Station	Sample Method	Taxa Richness	EPT Taxa Richness	Percent EPT	Percent Two Dominant Taxa	Percent Chironomidae	HBI
*UNT Left Fork	Kicknet	16	8	49.28	46.38	17.39	5.3
UNT Tenmile Fork	Kicknet	14	3	12.05	54.22	42.17	6.4
*UNT Boone Block Hollow	Kicknet	18	5	46.63	58.55	20.73	14.0
*Big Horse Creek	Kicknet	14	2	1.24	71.43	43.48	6.1
*Pond Fork	Kicknet	14	7	21.70	68.40	50.00	5.4
Cow Creek	Kicknet	14	5	60.77	57.89	6.22	4.4
*Jarrell Branch	Kicknet	13	4	8.57	74.29	41.90	5.1
Pond Fork	Kicknet	14	7	39.89	64.48	48.09	5.6
Moccasin Hollow	Kicknet	18	8	51.98	56.50	34.46	5.2

*** all organisms identified in kicknet sample**

Table 43 - Family Level Benthic Macroinvertebrate Summary for Toxicity Sampling Fall 2011

Station	Sample Method	Taxa Richness	EPT Taxa Richness	Percent EPT	Percent Two Dominant Taxa	Percent Chironomidae	HBI	WVSCI Total Score	WVSCI Scoring Criteria
UNT Left Fork *	Kicknet	14	6	49.28	53.62	17.39	5.2	64.75	Grey Zone
UNT Tenmile Fork	Kicknet	13	2	12.05	54.22	42.17	5.8	46.01	Impaired
UNT Boone Block Hollow *	Kicknet	13	4	46.63	60.10	20.73	5.2	58.52	Impaired
Big Horse Creek *	Kicknet	11	2	1.24	71.43	43.48	5.6	38.24	Impaired
Pond Fork *	Kicknet	13	6	21.70	68.40	50.00	5.6	48.50	Impaired
Cow Creek	Kicknet	11	4	60.77	77.99	6.22	4.9	58.02	Impaired
Jarrell Branch *	Kicknet	12	3	8.57	74.29	41.90	5.6	41.23	Impaired
Pond Fork	Kicknet	10	5	39.89	64.48	48.09	5.4	50.09	Impaired
Moccasin Hollow	Kicknet	17	7	51.98	56.60	34.46	5.0	65.62	Grey Zone

***all organisms identified in kicknet sample**

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Appendix A

Table 1 – Stream Sampling Locations Company 1

Table 2 – Stream Sampling Locations Company 2

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Table 4 - Field Water Chemistry Analysis 2009

Table 5 - Field Water Chemistry Analysis 2010

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Table 43 - Genus Level Benthic Macroinvertebrate Summary for Toxicity Sampling Fall 2011

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