Levels of Dissolved Solids Associated with Aquatic Life Effects in Headwater Streams of Virginia's Central Appalachian Coalfield Region

Anthony Joseph Timpano

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
in
Environmental Sciences and Engineering

Stephen H. Schoenholtz
Carl E. Zipper
David J. Soucek
E. Fred Benfield

March 16, 2011
Blacksburg, VA

Keywords: conductivity, salinization of freshwaters, biological integrity, water quality criteria, mountaintop mining

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ABSTRACT

Benthic macroinvertebrate communities in headwater streams influenced by Appalachian coal mining often differ from communities in minimally distrubed streams. Total dissolved solids (TDS) associated with mining have been suggested as stressors to these communities. In studies of such streams conducted to date, both non-TDS stressors and elevated TDS have been present as potential influences on biota. Here the association between dissolved salts and benthic macroinvertebrate community structure was examined using a family-level multimetric index and genus-level taxa sensitivity distributions. Test sites were selected along a gradient of elevated TDS, with non-TDS factors of reference quality. Virginia Stream Condition Index (VASCI) scores were regressed against log-transformed measures of TDS, specific conductance, and sulfate (SO_4^{2-}) using ordinary least squares and quantile regression techniques. Biological effects, as defined by VASCI scores indicating stressed or severely stressed conditions, were observed with increasing probability from 0% at ≤ 190 mg/L TDS to 100% at ≥ 1,108 mg/L TDS, with 50% probability of effects observed at 422 mg/L TDS. Associations between water quality measures and biological condition were variable, with approximately 48% of the variance explained by TDS. Genus-level analysis using a field sensitivity distribution approach indicated 95% of reference genera were observed at sites with TDS ≤ 281 mg/L, and 80% of genera were observed at sites with TDS ≤ 411 mg/L. This is evidence that TDS, specific conductance, or SO_4^{2-} can be used to establish dissolved solids levels for streams influenced by Appalachian coal mining above which aquatic life effects are increasingly probable.

ACKNOWLEDGEMENTS

I thank first my academic advisor/committee co-chair/co-principal investigator Dr. Carl Zipper. His guidance at every turn has been embraced and appreciated, from research details to advice on family time. I appreciate greatly the leadership and counsel of my other committee co-chair/co-principal investigator, Dr. Stephen Schoenholtz. His insights have been invaluable in helping me improve as a professional scientist and writer. Dr. David Soucek, a committee member and a co-investigator for this research, has been immensely valuable as a conceptual sounding board and analytical troubleshooter, particularly with regard to metals toxicity. My final committee member, Dr. E. Fred Benfield, through his feedback during numerous committee meetings, has kept me striving to do the best research I can.

Conduct of this research involved many weeks spent searching for suitable study sites. I could not have accessed such high-quality sites without the generous support of numerous mine permittees and their consultants, as well as private landowners. For their cooperation I and my co-investigators are immensely grateful. They are: Eddie Clapp and Roger Jones, Red River Coal Company; Mark Sproles, Dickenson-Russell Coal Company; Tad Nunley, United Coal Company; Phil Mullins, Skelly & Loy, Inc.; Keith Mohn, Arch Coal, Inc.; Chris Stanley and Dean Childress, Clintwood-Elkhorn Mining Company; Craig Kaderavek, The Forestland Group, LLC; Marty Large, Marty Corp.

I thank also the sponsors of this research: Virginia Department of Environmental Quality, Virginia Department of Mines, Minerals, and Energy, and Powell River Project. Their commitment to funding this important research is greatly appreciated.

I also express my gratitude toward Pat Donovan in the CSES GIS Lab – her expertise was indispensible in getting me set up for site selection; Bobbie Niederlehner in the Stream Ecology Lab – her instruction and assistance with the ion chromatograph was most gracious; and David Mitchem in the Forestry Lab – I got up the ICP-OES learning curve very quickly, thanks to him.

I could not have collected, processed, and analyzed my field samples without the help of numerous graduate and undergraduate students. Thanks to Robert Northington and Trip Krenz for taking time from their own graduate research to help make mine happen. I enjoyed learning from them during the copious windshield time we accumulated together. Thanks also to the hardest working, most enthusiastic group of undergraduates I've had the pleasure of knowing, Jackie Carl, Amanda Eakins, Mindy Forsyth, and Caleb Parks, for their invaluable help in finding sites, collecting samples, running chemical analyses, and sorting macroinvertebrates.

Finally, words cannot express how grateful I am for my wife Autumn. Not only has she put up with me in my single-minded pursuit of this research, but she makes all my hard work worthwhile. She makes a great field assistant too, having helped me sample during a snowy Thanksgiving week. For that and many other reasons, I am forever thankful for her.

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AMD	Acid Mine Drainage
ANOVA	Analysis of Variance
CCC	Criteria Continuous Concentration
CDF	Cumulative Distribution Function
CWA	Clean Water Act
DO	Dissolved Oxygen
EDAS	Ecological Data Application System
EPT	Ephemeroptera, Plecoptera, Trichoptera
FSD	Field Sensitivity Distribution
HBI	Hilsenhoff Biotic Index
ICP-OES	Inductively Coupled Plasma - Optical Emission Spectrometer
IEPA	Illinois Environmental Protection Agency
MDL	Method Detection Limit
MFC	Maximum Field Concentration
MFD	Maximum Field Distribution
OEC/OEC_X	Observed Effect Concentration/Observed Effect Concentration to X% of Taxa
OLS	Ordinary Least Squares
RBP	Rapid Bioassessment Protocols
SC	Specific Conductance
SD	Standard Deviation
SSD	Species Sensitivity Distribution
TDS	Total Dissolved Solids
TMDL	Total Maximum Daily Load
USEPA	U.S. Environmental Protection Agency
VAC	Virginia Administrative Code
VASCI	Virginia Stream Condition Index
VDEQ	Virginia Department of Environmental Quality
VDMME	Virginia Department of Mines, Minerals, and Energy
WVSCI	West Virginia Stream Condition Index

CHAPTER 1. INTRODUCTION

Background

Elevated levels of total dissolved solids (TDS) have been suggested as stressors to aquatic life in Central Appalachian streams influenced by coal mining (*e.g.*, Pond et al. 2008, Pond 2004, Green et al. 2000). In coalfield streams, TDS is most often dominated by the dissolved ions SO_4^{2-} and HCO_3^-, with elevated concentrations (relative to reference streams) of Ca^{2+}, Mg^{2+}, Na^+, K^+, and Cl^- also common (Pond et al. 2008, Mount et al. 1997). At present there are no aquatic life water quality criteria for TDS/ions in the primary coal-producing Central Appalachian states (KY, VA, WV). In these states, aquatic life conditions are assessed for Clean Water Act compliance using measures of benthic macroinvertebrate community composition (*e.g.*, VDEQ 2010).

In mine-influenced streams of the Central Appalachians, in-stream TDS concentration can exceed 2,000 mg/L, whereas background levels are generally < 200 mg/L (Pond et al. 2008). Dissolved ions such as SO_4^{2-} have been shown to cause lethal and sublethal effects to a variety of freshwater invertebrates in laboratory toxicity testing (Soucek and Kennedy 2005, Kennedy et al. 2003). Laboratory bioassays illustrate a clear biological response to elevated TDS, though results differ among studies, suggesting that TDS tolerance varies widely among different test organisms.

Field data have shown that the biotic response to elevated TDS also occurs outside the laboratory with indigenous species. Recent studies of Appalachian coalfield streams have found that benthic macroinvertebrate community composition is altered in coal mining-influenced streams relative to communities in streams uninfluenced by mining (*e.g.*, Pond et al. 2008, Pond 2004, Green et al. 2000). In those studies, most mining-influenced streams had elevated specific conductance/TDS and in all cases one of those water quality parameters was significantly and strongly correlated with biotic community composition change.

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.

Problem Statement & Need for Research

During the period 1992-2002, coal mining and related activities affected > 1,900 km of Appalachian headwater streams with "…an increase of minerals in the water as well as less diverse and more pollutant-tolerant macroinvertebrates and fish species."(USEPA 2005). The potential for impact to aquatic life from elevated TDS may prompt regulatory agencies to develop water quality criteria to protect aquatic life in mining-influenced streams. To do so, policy makers must have knowledge of how biota respond to the pollutant to be regulated.

We have a good understanding of the toxic effects of some mining-related dissolved salts from laboratory experiments with select indicator organisms and we have a clear picture of how mining impacts can affect resident biota, but we lack the knowledge of how communities respond specifically to SO_4^{2-} and HCO_3^- dominated salt solutions (with Ca^{2+} as dominant cation) *in situ* in mining-influenced streams. Recent research has focused on showing that mining affects biota (Pond 2004) and how new bioassessment tools can detect mining-related effects on aquatic biota (Pond et al. 2008). A review of the primary literature reveals that to date, no field studies have been designed specifically to identify the biological effects of TDS by isolating the TDS effect through deliberate selection of study sites where non-TDS stressors are minimized.

Research Observations, Questions, and Objectives

Observations
As characterized using a benthic macroinvertebrate multimetric index, biological condition in Central Appalachian streams draining land disturbed by coal mining is often different from reference condition (Pond 2004, Howard et al. 2001, Green et al. 2000). Virginia benthic macroinvertebrate community index scores are often lower than reference values where concentrations of dissolved solids are elevated. In numerous datasets from various Central Appalachian mined lands, elevated dissolved solids (either as measured or as represented using the surrogate of specific conductance) are strongly and consistently correlated with decreased stream biological condition (Merricks 2007, Pond 2004, Chambers and Messinger 2001).

Research Questions
The goal of this research is to determine levels of TDS (or its surrogates/constituent ions) that are associated with aquatic life effects by defining associations between benthic macroinvertebrate community composition and TDS and related measures in headwater streams of Virginia's Central Appalachian coalfield where non-TDS stressors are minimized.

With the study site population constrained to headwater streams of Virginia's Central Appalachian coalfield region, I addressed the following research questions:

1) Can streams be identified where non-TDS stressors are minimized such that the influence of dissolved solids on biological condition can be more accurately measured?

2) Is there an association between benthic macroinvertebrate community composition and TDS/component ion/specific conductance level?

3) What is the ionic composition of TDS in this study region?

4) Does the ionic composition of TDS influence the association between TDS/component ion/specific conductance and benthic macroinvertebrate community composition?

5) What level of TDS/component ion/specific conductance is associated with benthic macroinvertebrate community composition effects as defined by VASCI score < 60?

6) What TDS/component ion/specific conductance levels are associated with absence of benthic macroinvertebrate genera using a taxa sensitivity distribution approach with field data?

Objectives
This research had the following three objectives for accomplishing the overall goal:

1) Effectively isolate the influence of dissolved solids by finding study sites in streams where non-TDS stressors are minimized.

2) Determine which measure of dissolved solids, be it TDS, specific conductance, or ions/ion combinations, is most strongly associated with biological condition.

3) Define levels of selected water quality measures that are associated with effects to aquatic life, using family- and genus-level benthic macroinvertebrate data.

The research objectives have been addressed using two analytical approaches. Chapter 3 describes the approach of using Virginia's Stream Condition Index (VASCI), a family-level multimetric index of benthic macroinvertebrate community integrity, to determine levels of dissolved solids associated with biological effects in a regulatory context. Chapter 4 describes the second approach, which calculated levels of dissolved solids associated with observance of most taxa by using genus-level field data applied to a taxa sensitivity distribution method of developing water quality criteria. Research methods are described in each analytical chapter. These analytical chapters are preceded in Chapter 2 by a Literature Review. A concluding chapter summarizes and integrates results of the two analytical approaches.

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CHAPTER 2. LITERATURE REVIEW

Biocriteria in Clean Water Act Enforcement

The U.S. Environmental Protection Agency (EPA) is tasked with enforcing the many water quality requirements of the Clean Water Act (CWA §101(d), CWA §309(a)(3)). It is the objective of the Act to "…restore and maintain the chemical, physical, and biological integrity of the Nation's waters." (CWA §101(a)). To that end, the U.S. EPA has developed water quality standards, which consist of two main parts (CWA §303(c)(2)(A)). The first part specifies the designated use(s), or the intended beneficial purpose(s) of a water body (fishing, swimming, drinking water, aquatic life, etc.). The Act states that all waters are designated for the "…protection and propagation of fish and aquatic life and wildlife…" (CWA 102(a)). The second part of a water quality standard consists of the recommended water quality criteria necessary to protect that use. As an example of criteria, waters must be free of toxic metals in toxic amounts so that healthy fish populations may exist. Water quality standards also include antidegradation and implementation policies. Although U.S. EPA is ultimately responsible for enforcing the Act, it is the individual States and Tribes that are responsible for developing and implementing their own water quality protection programs to achieve the goals of the Act (CWA §101(b)).

Each State's water quality program must ensure that all waters of that State are maintained at a level satisfactory for attainment of designated uses. Waters of Virginia must meet the General Standard for aquatic life. In Virginia, the General Standard for aquatic life is comprised of a general designated use and general criteria to attain that use (9 VAC 25-260-10).. At a minimum, all State waters are designated for "…the propagation and growth of a balanced, indigenous population of aquatic life…which might reasonably be expected to inhabit them;" (9 VAC 25-260-10). To protect that use, it is necessary that all waters of Virginia "…shall be free from substances attributable to sewage, industrial waste, or other waste in concentrations, amounts, or combinations which contravene established standards or interfere directly or indirectly with designated uses of such water or which are inimical or harmful to…aquatic life." (9 VAC 25-260-20).

The General Standard serves as a broad water quality goal that is attained by enforcement of water quality criteria. Those criteria may be narrative, as in the general criteria, or they may be numeric, addressing specific pollutants and limiting their concentrations in State waters. Regardless of the criteria type, if they are not met, a water body may be considered impaired and in need of corrective measures to enforce criteria and restore capability to serve designated use. Because of significant regulatory implications for impaired waters, it is important to have scientifically sound methods for determination of use attainment and impairment status.

With numeric water quality criteria, aquatic life use attainment is assumed to occur through indirect measures. Most common pollutants to aquatic life have had numeric water quality criteria developed that are scientifically justified through toxicity testing and other scientific research. It is assumed that if a water body meets those criteria, the aquatic life is sufficiently protected and the water body has attained its designated use (USEPA 1976). Judging attainment of the narrative criteria is more complex and often requires direct measurement of the condition of aquatic life rather than reliance on measurement of a physical or chemical water quality parameter (USEPA 1990).

Detection of a violation of numeric criteria for chemical pollutants, and for physical pollutants such as temperature, is relatively simple – analytical chemical measurements of water samples allow us to detect most pollutants at even trace levels with great reliability. However, no simple analytical procedure exists for determination of whether a water body has met its narrative criteria and attained its general designated use. For General Standard enforcement, aquatic life use attainment is most often measured directly using biocriteria (*e.g.*, VDEQ 2010).

Biocriteria set the target biological condition for a water body. Biological condition can be estimated in many ways, but generally it is characterized by the structural diversity of an aquatic community. Fish communities and benthic macroinvertebrate assemblages are the most common indicator groups used for assessing biological condition in flowing waters. Many States have implemented benthic macroinvertebrate and/or fish monitoring programs, with benthic macroinvertebrates being used in Virginia and surrounding States (*e.g.*, WV, KY) for determination of general standard aquatic life designated use attainment (VDEQ 2010).

The assessment tool of choice is a multimetric index of biological condition that characterizes the quality of the benthic macroinvertebrate community in a stream and compares it to a regional reference quality standard. In Virginia, the index is called *A Stream Condition Index for Virginia Non-Coastal Streams* (Burton & Gerritsen 2003), or VASCI. By assessing the biological condition of an impacted stream relative to that of a desirable regional reference condition, a determination can be made as to whether the impacted stream is attaining its designated aquatic life use and whether it should be considered impaired. A significant benefit to using a multimetric index is that a regional reference condition is built into the index, obviating the need to make comparisons to individual local reference streams each time monitoring is conducted. In the case of the VSCI, the regional reference condition is established as the target condition, or 100%, and streams scoring below 60% do not meet the criteria of the general standard (Burton & Gerritsen 2003).

When a stream fails to meet water quality criteria it will most likely be considered impaired (CWA §303(d)). This situation is largely prevented by enforcement of pollutant limits for industrial dischargers, which are often the most common source of pollutants in streams. However, even if numeric pollutant criteria are met a stream may still be impaired based on failure to meet biocriteria. If biological impairment has been detected, it indicates that something in the water body is still preventing attainment of the general designated use for aquatic life. The cause could be a pollutant or a non-pollutant, but it is usually not readily apparent. In most cases a water quality improvement study must be conducted to determine the maximum amount of a pollutant that the water body can tolerate and still allow aquatic life to propagate. The water quality improvement study specifies the Total Maximum Daily Load (TMDL) of a pollutant allowed in the water body (CWA §303(d)(1)(C)).

Development of a TMDL for a water body is a complex process that involves integrating many types of data to model pollutant loadings and estimate biological condition attainable given certain pollutant reduction scenarios. Foremost in the process is to identify pollutants (collectively called stressors) that are causing the impairment. It is possible that a water body is found to contain pollutants at levels below the numeric criteria. In this case, pollutants without numeric criteria could be stressors to aquatic life. Identification of those stressors and their critical values is of central importance to the rest of the TMDL development process.

Biotic Impacts of Elevated Dissolved Solids

The questions addressed by this research have been developed based on historical and contemporary investigations into use of freshwater invertebrates as bioindicators and effects of dissolved ions on those organisms.

Most freshwater benthic macroinvertebrates are adapted to survive in water of relatively low ionic strength and can be stressed or killed as TDS concentration increases. Elevated TDS can cause toxicity to freshwater organisms through osmotic stress (McCulloch et al. 1993). Dissolved ions have been shown to cause lethal and sublethal effects to a variety of freshwater invertebrates in laboratory toxicity testing. Organisms used in testing have included common indicator species, such as the cladocerans *Ceriodaphnia dubia* and *Daphnia magna*, the amphipod *Hyalella azteca*, and the midge *Chironomus tentans*, as well as species such as the mayfly *Isonychia sp*.

Kennedy et al. (2003) exposed *C. dubia* to SO_4^{2-}-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. Soucek and Kennedy (2005) observed lethal effects of SO_4^{2-} to *H. azteca* (431-607 mg/L), *C. dubia* (2,050 mg/L), and *C. tentans* (14,134 mg/L). Chapman et al. (2000) also found reductions in survival and growth of *C. tentans* from simulated mine effluent of approximately 2,000 mg/L TDS. To investigate the effects of TDS on a mayfly, Kennedy et al. (2004) collected mayflies of the genus *Isonychia* from an unpolluted stream. They then exposed the mayflies to simulated mine effluent for seven days and observed a significant decline in survival at a conductivity of ~1,500 µS/cm (~1,050 mg/L TDS). These laboratory experiments illustrate a biological response to elevated TDS, though the results differ among studies, suggesting that TDS tolerance varies among different test organisms.

Additional research has shown that TDS toxicity is dependent upon the type and combination of ions in solution. Acute toxicity tests conducted by Mount et al. (1997) exposed *C. dubia* and *D. magna* to 2,453 different solutions of various ion combinations. They found the relative toxicity of individual ions to be: $K^+ > HCO_3^- \approx Mg^{2+} > Cl^- > SO_4^{2-}$. They also found that toxicity of K^+, Cl^-, and SO_4^{2-} was reduced in solutions with more than one cation present. Soucek and Kennedy (2005) also found that sodium sulfate toxicity was reduced for *C. dubia* and *H. azteca* when water hardness (Ca^{2+} and Mg^{2+}) was increased. Elevated Cl^- concentration also reduced SO_4^{2-} toxicity to *H. azteca*. These studies suggest that the biological response to instream mineral solutions likely depends on TDS composition and ion interactions.

The Central Appalachian ecoregion spans from western Pennsylvania through western Maryland, West Virginia, southwest Virginia, eastern Kentucky, and terminates in northeast Tennessee (Omernik 1987). Surface and underground mining for coal is conducted throughout the ecoregion, especially in Kentucky, Virginia, and West Virginia. Many studies in those and other coal-producing States have found that stream benthic macroinvertebrate communities are adversely affected by mining activity (Green et al. 2000, Chambers and Messinger 2001, Howard et al. 2001, Pond 2004, Hartman et al. 2005, Freund and Petty 2007, Merricks et al. 2007). Those studies have been invaluable in understanding biotic impacts of mining activity in general and they have succeeded in demonstrating the ability of benthic macroinvertebrate monitoring to detect impaired waters. However, because both non-TDS stressors and elevated conductivity/TDS were concurrent influences on biota in the streams assessed in those studies, much is still unknown about how the benthic macroinvertebrate community responds to specific TDS concentrations and compositions.

Although those studies reported that elevated TDS or surrogates were correlated with poor biological condition, isolation of the TDS variable was beyond the scope of the studies. In West Virginia streams, Green et al. (2000) found a significant negative correlation between the West Virginia Stream Condition Index (WVSCI) and median conductivity (a surrogate for TDS), along with strong positive correlations between the WVSCI and habitat quality. However, they also found a strong negative correlation between median conductivity and habitat quality. Using canonical correspondence analysis to relate invertebrate species distribution to multiple environmental gradients in the Kanawha River Basin of West Virginia, Chambers and Messinger (2001) discovered that 60 percent of the variance in species-environment relation was explained by a combination of chemical and physical measures, including specific conductance, SO_4^{2-}, and substrate particle size. In the Kentucky coalfields, Howard et al. (2001) examined chemical and physical parameters as they related to benthic macroinvertebrate communities in mined, reclaimed, and unmined streams. They found their biological condition indicator to be strongly and negatively correlated to conductivity and sediment deposition. Pond (2004) also noted similar relationships in a study of eastern Kentucky coalfield streams. Pond's data showed that biological condition metrics had the highest correlation to conductivity and habitat quality. The covariation between poor habitat and elevated TDS found in these studies masks the biological response to elevated TDS alone.

In a paired watershed study comparing West Virginia hollow-filled streams with nearly identical and adjacent reference streams, Hartman et al. (2005) could not detect increased sediment deposition in hollow-filled sites. They did, however, detect a significant decline in biological condition concurrent with elevated conductivity. Although that study better isolated the TDS variable, the sample size of four pairs of sites is insufficient to develop a robust relationship between TDS and biotic response. Furthermore, the authors acknowledged that with only one sediment sample per stream, an accurate assessment of sedimentation may not have been accomplished.

Sedimentation is not the only frequent covariate to TDS in mine-influenced catchments. Examining benthic macroinvertebrate communities below hollow fills and sediment control ponds in three West Virginia headwater basins, Merricks et al. (2007) observed reduced richness of sensitive taxa as compared to a regional reference stream. Although specific conductance was elevated below the fills and ponds, there were also several sites where water column Al was higher than chronic water quality criteria for aquatic life. The authors noted that community structure in those streams was affected also by the presence of sedimentation ponds, which affected biodegradable organics in the streams below the ponds and thus had a direct effect on the nature of the stream community. The authors also noted the inability to identify conductivity effect thresholds independent of potential metal toxicity and recommended further study.

In a study with aspects similar to the design used here, Freund and Petty (2007) examined WVSCI response to a gradient of mining-related stressors. They used regression techniques to estimate specific stressor concentrations at which biological impairment was always observed. Their study focused on impacts from acid mine drainage and the data included sites with the stressors of acidic pH and toxic metals. Their findings indicated that specific conductance was less influential to WVSCI response than were pH, Ni, and Al. Although the authors were able to observe a conductivity level associated with biological impairment, effects of specific conductance could not be isolated because of the presence of non-TDS stressors.

Ecological study of the structure and function of the benthic macroinvertebrate community in streams has lead to the adoption of that group of organisms as reliable indicators of stream biological condition because they are ubiquitous, responsive, easy to sample, and integrate cumulative impacts (Barbour et al. 1999). As a result of that adoption, assessment tools have been scientifically developed to allow use of benthic macroinvertebrate community data for assessment of stream biological integrity and designated use attainment determinations.

The three states discussed above have each developed one or more macroinvertebrate multi-metric indexes of biological condition for use in the Central Appalachian coalfield region. These indexes score a stream relative to a pooled regional or statewide reference condition. West Virginia was the first to develop and implement a statewide family-level Stream Condition Index for wadeable streams (Gerritsen et al. 2000). For the headwater streams of the eastern Kentucky coalfields, there are the genus- and family-level Eastern Kentucky Macroinvertebrate Bioassessment Indexes (Pond and McMurray 2002). Virginia also developed its family-level Stream Condition Index for Non-Coastal Streams (Burton and Gerritsen 2003), followed by its validation of the index with additional data (VDEQ 2006). Most recently, U.S. EPA has developed for West Virginia a genus-level index that should allow for better detection of moderate impairments (Pond et al. 2008).

In summary, the available literature provides the following evidence:

- Laboratory experiments with both indicator and indigenous benthic invertebrates describe toxic effects of elevated TDS at the organism scale.
- Field studies demonstrate that mining activities are associated with impacts on stream benthic macroinvertebrates at the community scale, and that these effects are often associated with specific conductance and/or TDS, as well as other stressors.
- Multimetric indexes of benthic macroinvertebrate community structure, such as the Virginia Stream Condition Index and comparable indexes in adjacent States, have been developed and are accepted by CWA implementation agencies as indicators of stream biological integrity.

Given this evidence, it is reasonable to hypothesize that in Virginia coalfield streams, there is a statistically significant association between macroinvertebrate biotic index score and specific conductance/total dissolved solids/component ion concentration. It is also reasonable to expect that such an association would be sufficient to allow determination of a TDS threshold above which biological effects are likely.

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CHAPTER 3. DETERMINING LEVELS OF DISSOLVED SOLIDS ASSOCIATED WITH AQUATIC LIFE EFFECTS IN COALFIELD STREAMS USING A BENTHIC MACROINVERTEBRATE MULTIMETRIC INDEX

Introduction

Background

Environmental disturbances occur globally as a consequence of a variety of anthropogenic influences including agriculture, urbanization, industrial development, and similar activities that are essential to sustaining modern human society. As such, establishing limits for allowable levels of environmental disturbance is an essential societal function. Such limits are necessary to preserve an environmental quality that does not compromise human health and that preserves the capability of natural ecosystems to provide essential functions and services that support human life (MEA 2005, Costanza et al. 1997). Thus, modern societies face questions and decisions about how to limit environmental impacts so as to allow essential economic activity, while also protecting a socially desirable level of environmental quality.

Currently, such questions are highly relevant and visible to the public in the Appalachian region of the United States, where coal mining is an important industry, but also produces environmental disturbances. Recent studies have found that benthic macroinvertebrate communities in streams below Appalachian surface coal mines, including those that use valley fills for excess spoil disposal, often differ from communities found in non-mined ecosystems (Green et al. 2000, Paybins et al. 2000, Pond 2004, Hartman et al. 2005, Merricks et al. 2007, Pond et al. 2008). Elevated levels of total dissolved solids (TDS) originating from coal mines have been suggested as a primary aquatic life stressor in those streams (*e.g.*, Green et al. 2000, Pond 2004, Pond et al. 2008). The U.S. Clean Water Act, administered by the U.S. Environmental Protection Agency (EPA), is intended to protect the capacity of streams to support aquatic life, and its enforcement in Appalachian mining states relies on benthic macroinvertebrates as indicators of overall biological condition (*e.g.*, VDEQ 2010a). Thus, aquatic ecosystem impacts by TDS from coal mining have become a public issue and a public policy concern (*e.g.*, USEPA 2010).

Salinization of freshwaters, and associated impacts, are not unique to mining-influenced watersheds in Appalachia (Kefford 1998, Goetsch and Palmer 1997, Leland and Fend 1997, Williams 1987). However, in Appalachian streams influenced by coal surface mining, TDS is most often dominated by the dissolved anions SO_4^{2-} and HCO_3^-, with Ca^{2+}, Mg^{2+} as the most prominent cations (Pond et al. 2008, Timpano et al. 2010). Dissolved ions, at concentrations above those that occur in the absence of mining in Appalachian streams, have been shown to cause lethal and sublethal effects to a variety of freshwater invertebrates in laboratory toxicity testing (Soucek and Kennedy 2005, Kennedy et al. 2004, 2003; Chapman et al. 2000). Additional research has shown the toxicity of TDS to test organisms at any given concentration is dependent upon the type and combination of ions in solution (Kennedy et al. 2005, Soucek and Kennedy 2005, Goetsch and Palmer 1997, Mount et al. 1997). The direct applicability of laboratory studies to field conditions in the Appalachians has been questioned, however, because laboratory studies are generally unable to test responses *in situ* of the most sensitive species within Appalachian stream communities (Bodkin et al. 2007). In the most laboratory tests designed to use Appalachian headwater stream invertebrate species, the tests do not evaluate TDS effects to early-life-stage individuals, which are the most sensitive life stages of those organisms (Kefford et al. 2004, 2007).

Although field studies have found altered aquatic communities in streams affected by coal mining, much remains unknown about how benthic macroinvertebrate communities respond to specific TDS concentrations and compositions. In studies conducted by other researchers, both non-TDS stressors and elevated TDS have been present as potential influences on biota in the streams assessed (Pond et al. 2008, Hartman et al. 2005, Howard et al. 2001). Research reported here was conducted to characterize the biotic response to elevated TDS by selecting study streams with a range of TDS concentrations and where non-TDS stressors were minimized. In pursuit of these goals, I evaluated biological condition using the Virginia Stream Condition Index (VASCI), a multimetric index of benthic macroinvertebrate community composition that is used for Clean Water Act enforcement in Virginia's non-coastal streams (Burton and Gerritsen 2003, VDEQ 2010a).

Objectives

The objective of this study was to characterize the association between benthic macroinvertebrate community composition and elevated TDS in selected headwater streams in southwestern Virginia influenced by coal mining activities, where non-TDS stressors are not evident. To that end, I addressed the following questions:

1) Can streams be identified where non-TDS stressors are minimized such that the influence of dissolved solids on biological condition can be more accurately measured?

2) Is there an association between benthic macroinvertebrate community composition and TDS/component ion/specific conductance level?

3) What is the ionic composition of TDS of streams in the study region?

4) Does the ionic composition of TDS influence the association between TDS/component ion/specific conductance and benthic macroinvertebrate community composition?

5) What level of TDS/component ion/specific conductance is associated with benthic macroinvertebrate community composition effects as quantified using the Virginia Stream Condition Index?

Materials and Methods

Site Selection

The goal in choosing test sites was to identify streams with elevated TDS, but where all other observable factors were comparable to reference streams that represent "…the biological condition in places with a minimal amount of human disturbance." (USEPA 2006).

First- and second-order streams (Strahler 1957) within the Virginia portion of Ecoregion 69 (Omernik 1987) were selected for study. I endeavored to located elevated TDS, or "test" sites, meeting non-biological reference criteria (Table 3.1) used for Virginia Clean Water Act implementation studies (Burton and Gerritsen 2003, VDEQ 2006a).

Table 3.1. Abiotic criteria for stream selection.

Parameter or Condition (units or range)	Selection Criterion[1]
Dissolved Oxygen (mg/L)	≥ 6.0
pH	≥ 6.0 & ≤ 9.0
Epifaunal substrate score (0-20)[2]	≥ 11
Channel alteration score (0-20)[2]	≥ 11
Sediment deposition score (0-20)[2]	≥ 11
Bank disruptive pressure score (0-20)[2]	≥ 11
Riparian vegetation zone width score, per bank (0-10)[2]	≥ 6
Total RBP habitat score (0-200)[2]	≥ 140
Residential land use immediately upstream	None

[1]Parameters and numeric selection criteria from Burton and Gerritsen (2003)
[2]RBP habitat, high gradient streams (Barbour et al. 1999)

Streams were chosen by examining a variety of available data using a GIS, augmented by consultation with mine operators, consultants, and regulators with specific knowledge of stream conditions within the study area. Virginia Department of Mines, Minerals, and Energy (VDMME) provided data on water quality, mine permits, and historical surface-mining site locations.

Candidate sites were visited to assess suitability for study. Site reconnaissance allowed verification of current land uses and confirmed minimal catchment disturbance, as per study design. Physicochemical water parameters, including pH and specific conductance, were measured. Physical habitat was evaluated using the qualitative visual estimate approach for high-gradient streams as specified in U.S. EPA's Rapid Bioassessment Protocols (RBP) (Barbour et al. 1999).

In addition to meeting the physicochemical and habitat criteria, all test sites also had to be free from obvious influence from residential land use upstream. This criterion was important to avoid the unpredictable influence of failing septic systems (*e.g.*, dissolved N and P enrichment) or direct stream discharges of household waste (*e.g.*, particulate organic matter, toxics). Other potential sources of non-point source pollution were avoided, including road crossings, bridges, culverts, active logging, non-coal industrial operations or infrastructure (*e.g.*, rail beds), and commercial activity. Finally, accessibility was a practical criterion that had to be met to allow the site to be included in the study. Access permission was obtained for each study site from private landowners and/or mine permittees where necessary.

Field Methods
At each study site, benthic macroinvertebrate and water quality samples were collected up to four times during the study period. Samples were collected during the Spring (March through May) of 2009 and 2010, and Fall (September through November) of 2008 and 2009 biological index periods (VDEQ 2008). Benthic macroinvertebrate collections followed the single-habitat (riffle-run) approach (VDEQ 2008). Approximately 2 m^2 of riffle substrate were sampled using a 0.3 m wide D-frame kicknet with 500 µm mesh. A single composite sample was collected at each site, preserved in 95% ethanol, and returned to the laboratory for sorting and identification.

Water temperature, dissolved oxygen (DO), specific conductance (at 25 °C; henceforth referred to as SC), and pH were measured *in situ* with a calibrated handheld multi-probe meter (Hydrolab Quanta, Hach Hydromet, Loveland, Colorado). Single grab samples of water were collected using vacuum hand pumps and reusable polyethylene filter assemblies (VDEQ 2006b). All samples were stored in acid-rinsed polypropylene Nalgene bottles. Samples for dissolved metals, TDS, alkalinity, and major ions were filtered in the field immediately following collection using acid-rinsed cellulose ester filters with a nominal pore size of 0.45µm. Samples for metals analysis were preserved to pH < 2 with 1+1 concentrated nitric acid. All samples were transported on ice and stored at 4 °C. At each site, all biological and water samples were collected concurrently at base flow. All water quality sampling was conducted upstream of and/or immediately prior to biological sampling. In-stream and riparian habitat quality were assessed during each sample collection using RBP methods (Barbour et al. 1999).

Laboratory Methods
Biological sample processing followed modified VDEQ Biomonitoring Standard Operating Procedures (VDEQ 2008). Each sample was sub-sampled to obtain a 110 (±10%) organism count following RBP methods (Barbour et al. 1999). Benthic macroinvertebrates were identified to the family/lowest practicable taxonomic level.

An inductively coupled plasma - optical emission spectrometer (Varian Vista MPX ICP-OES w/ICP Expert software, Varian Instruments, Walnut Creek, California) was used to measure dissolved Ca^{2+}, Mg^{2+}, K^+, Na^+, and Al, Cu, Fe, Mn, Se, and Zn (APHA 2005). An ion chromatograph (Dionex DX500, Dionex Corp., Sunnyvale, California) was used to measure Cl^- and SO_4^{2-} (APHA 2005); TDS was measured via filtration of known volumes followed by drying at 180°C (APHA 2005), with modifications (0.45 micron cellulose ester filter, field filtration); total alkalinity was measured in an aliquot of filtered sample by titration with standard acid (APHA 2005); and CO_3^{2-}/HCO_3^- were calculated from alkalinity and pH measurements (APHA 2005). Samples were stored at 4 °C and analyzed within holding times of 7 days (TDS), 14 days (alkalinity), 28 days (anions), or 6 months (metals) (APHA 2005). Water chemistry data were examined to determine if trace metals levels exceeding criteria continuous concentrations (CCC) (USEPA 2011, IEPA 2001) were correlated with biological response.

Data Analysis

Virginia Stream Condition Index

The Virginia Stream Condition Index (VASCI) is a multimetric index of benthic macroinvertebrate community composition used to evaluate biological condition of non-coastal streams in Virginia (Burton and Gerritsen 2003, VDEQ 2006a). Using family-level taxonomic data, streams are scored from 0 to 100 relative to a reference condition with 100 being most comparable to reference. For purposes of aquatic life designated use attainment assessment in Virginia, streams with VASCI scores < 60 are considered impaired and streams scoring ≥ 60 are considered unimpaired (VDEQ 2010a).

The VASCI is comprised of eight family-level metrics of benthic macroinvertebrate community composition. The metrics quantify an aspect of community composition, and each has an expected response to increasing catchment disturbance (Table 3.2). A "score", on a 0-100 scale, is assigned for each metric based on each metric's measured value relative to the reference value for that metric (Burton and Gerritsen 2003), and the VASCI is calculated by averaging scores of the eight metrics.

Taxonomic data were entered into VDEQ's Ecological Data Application System (EDAS) relational database (VDEQ 2010b). Biological metrics and VASCI scores were calculated using EDAS regional reference values (Burton and Gerritsen 2003).

Table 3.2. Virginia Stream Condition Index metric descriptions.

Metric	Abbr.	Description	Expected Response to Increasing Disturbance
Total Taxa Richness	Total Taxa	Total number of distinct taxa	Decrease
EPT Taxa Richness	EPT Taxa	Total number of distinct families in the orders Ephemeroptera, Plecoptera, and Trichoptera (mayflies, stoneflies, and caddisflies)	Decrease
Percent Ephemeroptera	% E	Relative abundance of individuals in the order Ephemeroptera	Decrease
Percent Plecoptera and Trichoptera less Hydropsychidae	% PT-H	Relative abundance of individuals in the orders Plecoptera and Trichoptera, with individuals from the generally tolerant Trichoptera family Hydropsychidae excluded	Decrease
Percent Scrapers	% Scrap	Relative abundance of individuals in the functional feeding group Scrapers, which obtain their food by scraping biofilms from solid surfaces	Decrease
Percent Chironomidae	% Chiron	Relative abundance of individuals in the family Chironomidae	Increase
Percent Two Dominant Taxa	% 2 Dom	Summed relative abundance of the two taxa with the greatest number of individuals	Increase
Hilsenhoff Biotic Index	HBI	Organic pollution tolerance index; lower values indicate lower pollution tolerance	Increase

Statistical Analyses

Study sites were divided into two groups: reference sites and test sites. Reference sites were selected for study because their watersheds are minimally disturbed, whereas test sites are characterized by watershed disturbances that have produced elevated TDS, as documented during site selection, but are otherwise comparable to reference. Both site types were included in basic data comparisons. Only test sites were used for regression analyses.

Water quality, habitat, and biotic measures were compared between site categories using Welch's t-test and Mann-Whitney U test for normal and non-normal data, respectively. Correlations among water quality parameters, biotic metrics, and VASCI scores were analyzed using the non-parametric Spearman rank correlation procedure.

Analyses were conducted to test for associations between water quality and biological effects at test sites using ordinary least-squares (OLS) regression applied as a mixed model with VASCI score as dependent variable and sample season as a random effect. Model fit was evaluated by examining coefficients of determination (r^2) and residual diagnostic plots for normality and homoscedasticity. For each water quality parameter evaluated, a model was created for each sampling season, and an All-Year model was created by using all data. General Fall and Spring models were created by using the means of regression coefficients for each pair of sampling season models.

Quantile linear regression was conducted for test sites, again with VASCI as the dependent variable and water quality measure as the independent variable. Results were used to produce simple linear regression models for selected predictors at each of five quantiles of VASCI response. The quantile regression method was applied to these data because of the method's robustness to extremes in the dataset, its allowance for heterogeneous variance, and its ability to quantify how predictor influence on response may vary across the range of response values (Cade and Noon 2003). It is also a useful method for better understanding relationships at multiple points along the data distribution that might go unnoticed when using the mean-based OLS regression (Cade and Noon 2003).

Ordinary least squares and quantile regression analyses were conducted using transformed (natural log) values for the water quality measurements, as needed to satisfy analytical requirements.

Biotic effects were defined as VASCI scores < 60. Statistically significant associations among water quality measures and VASCI scores were applied to identify observed effect concentrations (OECs) as water quality levels associated with VASCI = 60. The term "OEC" is used despite uncertainty concerning which water quality variable(s) is/are the primary causative stressor(s); the term is intended to communicate a water-quality level that is associated with a biotic effect observance threshold from a causative stressor.

All statistical analyses were conducted using JMP 8 and SAS 9.2 (SAS Institute, Cary, North Carolina), with test level of $\alpha = 0.05$.

Defining Probabilities of Biotic-Effect Occurrence

Observed effect concentrations modeled using OLS and quantile regression were combined with empirical biotic-effect thresholds to produce probability profiles of biotic-effect occurrence at specific water-quality levels, with OECs derived from OLS regression assumed to correspond with 50% probability of biotic-effect occurrence.

Results

Site Selection

The site selection process yielded 229 candidate sites. Of these, 185 were visited within Wise, Dickenson, Lee, Buchanan, and Russell Counties to verify land uses, habitat quality, and water quality. Twenty-eight sites in first- and second-order headwater streams were selected for study (Tables 3.3 and 3.4). Poor habitat quality was the primary reason for not selecting streams for study. Because of the scarcity of sites satisfying selection criteria, site selection was continued and new sites were added throughout the study period (Table 3.4).

Table 3.3. Site selection summary.

	Reference	Test	Total
Candidate Sites	48	181	229
Sites Visited	36	149	185
Study Sites Selected	6	22	28

The site selection process yielded six reference sites (Figure 3.1). Three, in the Jefferson National Forest (Wise County), approached “natural” or “undisturbed” condition free from significant human disturbance, but with dominant geology different from that of the test sites (Table 3.4). Therefore, three additional reference sites were selected near the Dickenson/Buchanan County border, where dominant geology was similar to test sites. The three additional sites were distributed to nearly encompass the latitudinal extents of the coalfields (Figure 3.1). Twenty-two test sites were located that met selection criteria.

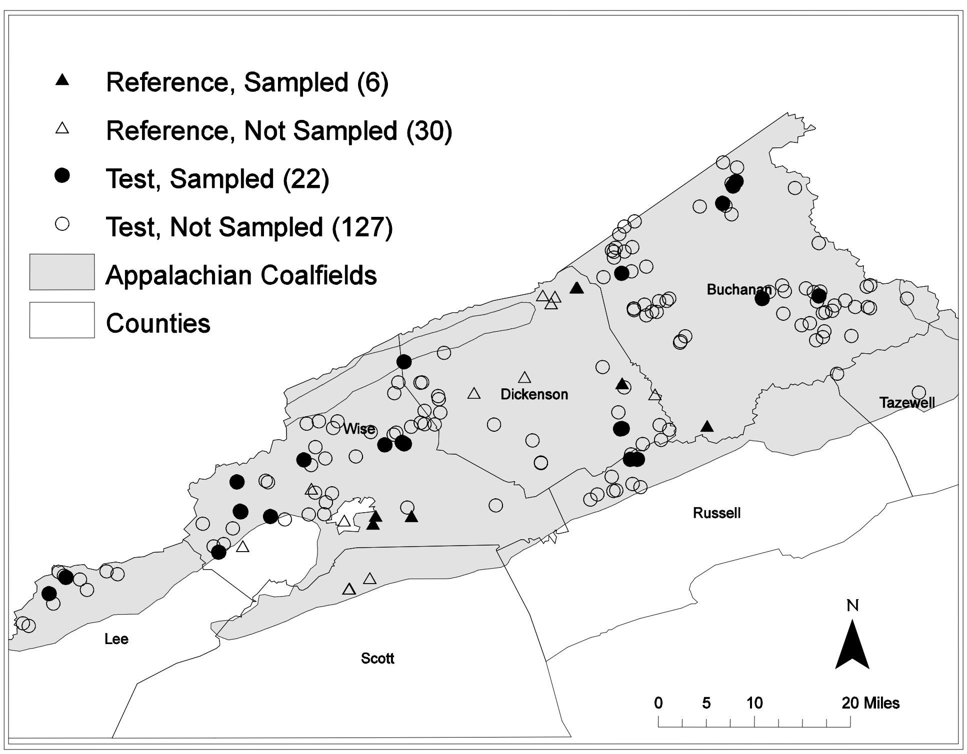


Figure 3.1. Map of visited and selected reference and test site locations in southwestern Virginia.

Table 3.4. Study site information.

Stream	Station ID	Type	Order	Dominant Geologic Formation	County[1]	Lat	Long	Sampled Fall 2008	Spring 2009	Fall 2009	Spring 2010
Burns Creek	BUR	Ref	2	Lee	Wise	36.929	-82.535	x	x	x	x
Clear Creek	CLE	Ref	2	Undivided Mississippian	Wise	36.929	-82.589	x	x	x	x
Copperhead Branch	COP	Ref	1	Norton	Dickenson	37.064	-82.090				x
Crooked Branch	CRO	Ref	2	Norton	Dickenson	37.130	-82.218				x
Eastland Creek	EAS	Ref	1	Undivided Mississippian	Wise	36.917	-82.593	x	x	x	x
Middle Camp Branch	MCB	Ref	1	Norton	Dickenson	37.274	-82.286				x
Birchfield Creek	BIR	Test	2	Wise	Wise	37.036	-82.575	x	x	x	x
Callahan Creek West Fork	CAW	Test	1	Wise	Wise	36.980	-82.797		x	x	x
Cane Branch	CAN	Test	1	Wise	Dickenson	37.160	-82.547			x	x
Fawn Branch	FAW	Test	1	Wise	Lee	36.811	-83.080		x	x	x
Fryingpan Creek	FRY	Test	2	Norton	Dickenson	37.060	-82.218		x	x	x
Fryingpan Creek Right Fork	RFF	Test	2	Norton	Dickenson	37.060	-82.220		x	x	x
Gin Creek	GIN	Test	2	Wise	Lee	36.836	-83.055		x	x	x
Grape Branch	GRA	Test	2	Norton	Buchanan	37.257	-82.007	x	x	x	x
Hurricane Fork	HUR	Test	2	Norton	Buchanan	37.400	-82.067		x	x	x
Jess Fork	JES	Test	2	Wise	Buchanan	37.295	-82.219		x	x	x
Kelly Branch	KEL	Test	2	Wise	Wise	36.935	-82.792			x	x
Kelly Branch UT[2]	KUT	Test	1	Wise	Wise	36.936	-82.792			x	x
Laurel Branch	LAB	Test	2	Norton	Russell	37.014	-82.205		x	x	x
Laurel Fork	LAU	Test	2	Wise	Wise	36.874	-82.825		x	x	x
Mill Branch Left Fork	MIL	Test	2	Wise	Wise	36.927	-82.747	x	x	x	x
Powell River	POW	Test	1	Wise	Wise	37.013	-82.697	x	x	x	x
Race Fork UT[2]	RAC	Test	1	Norton	Buchanan	37.427	-82.050		x	x	x
Richey Branch	RIC	Test	2	Wise	Wise	37.036	-82.546			x	x
Richey Branch UT[2]	RUT	Test	1	Wise	Wise	37.037	-82.544			x	x
Roll Pone Branch	ROL	Test	1	Norton	Russell	37.014	-82.195		x	x	x
Spring Branch	SPR	Test	1	Norton	Buchanan	37.434	-82.046		x	x	x
Spruce Pine Creek	SPC	Test	2	Norton	Buchanan	37.261	-81.922	x	x	x	x

[1]All sites located in southwestern Virginia; [2]Unnamed Tributary

Habitat

Mean total habitat scores, of 177 and 169 for reference and test sites, respectively (Figure 3.2, Table 3.5), were not significantly different ($p = 0.07$). All habitat parameter means were nominally lower for test sites than for reference sites (Table 3.5), with the largest nominal differences recorded for embeddedness, sediment deposition, and bank stability in that order. However, only mean riparian vegetated width scores were significantly different between reference and test sites ($p = 0.0002$), with reference sites scoring higher. Habitat parameters and total score were not significantly correlated to VASCI score. Water quality measures were not correlated to habitat parameters or total score, except bank stability, which was moderately correlated with TDS ($\rho = -0.36$), SC ($\rho = -0.37$), and SO_4^{2-} ($\rho = -0.46$). Test site means were > 85% of reference mean for all habitat parameters and for total score, indicating that test site habitat was comparable to reference (Barbour et al. 1999).

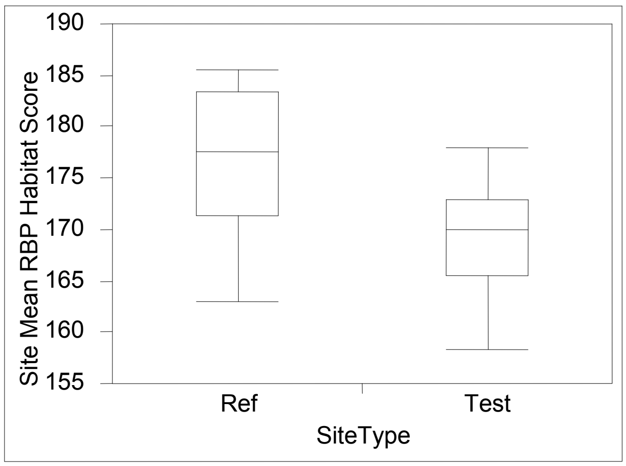


Figure 3.2. Box plot of mean total habitat scores for reference sites (n=6) and test sites (n=21). Mean total habitat scores were not significantly different between site types. Box plots represent 5th, 25th, 50th, 75th, and 95th percentiles.

Table 3.5. Site mean habitat summary data for study sites.

		Substrate/Cover	Embeddedness	Velocity/Depth	Sediment Dep.	Flow	Channel Alt.	Riffle Freq.	Bank Stability L+R	Bank Veg. Protection L+R	Rip. Veg. Width L+R	Total
Reference Sites[1]												
	Mean	18.3	15.4	16.0	13.8	18.7	20.0	18.3	17.3	19.2	20.0	176.7
	SD	1.1	2.0	3.1	1.6	1.2	0.0	1.1	2.3	0.8	0.1	8.2
	Min	17	13	10	12	17	20	17	13	18	20	163
	Max	20	17	19	16	20	20	20	20	20	20	186
Test Sites[2]												
	Mean	17.4	14.2	15.7	12.6	18.2	19.9	18.1	15.5	18.4	18.9*	169.0
	SD	0.8	0.9	1.2	0.7	0.9	0.2	0.8	1.8	1.0	1.0	5.4
	Min	16	12	13	11	17	19	16	11	17	17	158
	Max	19	16	19	14	20	20	19	18	20	20	178

[1]Six sites; [2]21 sites; *Mean is significantly different from reference ($\alpha = 0.05$).

Streamwater Chemistry

Physicochemical Properties

Streamwater temperature, pH, DO, and SC values ranged from 1.7 to 17.5 °C, 6.11 to 8.49, 7.7 to 12.3 mg/L, and 16 to 1,670 µS/cm, respectively across all samples (Table 3.6). Mean test site pH (7.71) and SC (593 µS/cm) were significantly different from reference sites (pH of 7.02, and SC of 31 µS/cm), but streamwater temperature and DO were not significantly different between reference and test sites (Table 3.6).

Table 3.6. Physicochemical summary statistics for study sites.

		Temp °C	pH SU	DO[1] mg/L	SC[2] µS/cm
Reference Sites[3]					
	Mean	11.0	7.02	9.6	31
	SD	4.0	0.45	1.1	27
	Min	1.7	6.11	7.7	16
	Max	14.4	7.80	11.8	116
Test Sites[4]					
	Mean	12.1	7.71*	9.4	593*
	SD	3.3	0.39	0.9	349
	Min	2.5	6.57	7.8	20
	Max	17.5	8.49	12.3	1,670

[1]Dissolved oxygen; [2]Specific conductance; [3]Six sites, 15 samples; [4]21 sites, 63 samples; *Mean is significantly different from reference ($\alpha = 0.05$).

Major Ions and Trace Metals

Mean TDS and major ion concentrations at test sites were significantly higher than at reference sites (Table 3.7). Test site mean TDS was 406 mg/L, whereas reference site mean TDS was 21 mg/L (Table 3.7).

Table 3.7. Total dissolved solids (TDS) and major ion summary statistics for study sites.

		TDS	SO_4^{2-}	HCO_3^-	Ca^{2+}	Mg^{2+}	Na^+	K^+	Cl^-
					mg/L				
Reference Sites[1]									
	Mean	21	5.9	10.8	2.6	0.8	1.2	1.5	0.6
	SD	19	5.1	10.4	2.7	0.6	0.9	1.4	0.4
	Min	5	2.8	0.7	0.4	0.5	0.4	0.4	0.3
	Max	76	22.1	44.1	12.0	2.6	3.7	5.5	1.4
Test Sites[2]									
	Mean	406*	231.4*	117.6*	61.1*	36.3*	24.8*	3.5*	3.2*
	SD	284	187.2	69.7	41.4	29.7	28.0	3.4	1.5
	Min	16	4.2	5.1	1.5	1.1	0.7	0.3	0.6
	Max	1,378	849.0	301.7	183.9	160.6	135.9	15.1	7.6

[1]Six sites and 15 samples; [2]21 sites and 63 samples; *Mean is significantly different from reference ($\alpha = 0.05$);

Median trace metal concentrations (Table 3.8) were nominally higher in test sites than in reference sites for most metals (Table 3.8). Streamwater dissolved metal concentrations were below method detection limits in 257 of 396 (65%) test site samples (Table 3.8). No measurements exceeded CCC for Al, Cu, Fe, or Mn. Two of 63 samples (3.2%) exceeded hardness-adjusted CCC for Zn. Nine of 63 samples (14.3 %) exceeded CCC for Se (Table 3.8). Correlation analysis revealed no significant associations between metal concentrations > CCC and VASCI score. There was also no significant correlation between metal concentrations > CCC and the error term from the VASCI – SC OLS regression All-Year model.

Table 3.8. Trace metals summary data for study sites.

		Al	Cu	Fe	Mn	Se	Zn
				µg/L			
Reference Sites[1]							
	Median	< 12.6	< 17.7	< 32.3	< 6.7	< 17.1	10.9
	Max	41.9	< 22.8	< 64.9	< 15.7	< 24.1	< 37.3
	# > MDL[2]	7	0	1	6	6	3
	# > CCC[3]					3	3
Test Sites[4]							
	Median	11.9	< 17.7	60.3	15.0	< 17.1	11.4
	Max	50.5	< 22.8	410.9	787.9	28.3	116.0
	# > MDL	37	1	23	44	9	25
	# > CCC					9	2
MDL							
	Mean	8.5	15.6	39.7	7.2	15.6	16.2
	Min	2.8	8.9	22.2	1.7	4.9	4.0
	Max	12.6	22.8	64.9	15.7	24.1	37.3

[1]Six sites, 15 samples; [2]Method Detection Limit, mean of four sample season batches, values below batch MDL reported as "< [MDL value]"; [3]Criteria Continuous Concentration, hardness-adjusted for Cu, Mn, Zn; [4]21 sites, 63 samples;

Ionic Composition

Mean relative ionic composition of streamwater at reference sites was dominated on a mass basis by HCO_3^- (43%) and SO_4^{2-} (26%), followed by Ca^{2+}, Cl^-, Na^+, Mg^{2+}, and K^+ (Figure 3.3a). Mean dissolved ion composition of streamwater at test sites was dominated on a mass basis by SO_4^{2-} (46%) and HCO_3^- (27%), followed by Ca^{2+}, Mg^{2+}, and Na^+ (Figure 3.3b). At test sites, Cl^- and K^+ each comprised approximately 1% of total ion concentration.

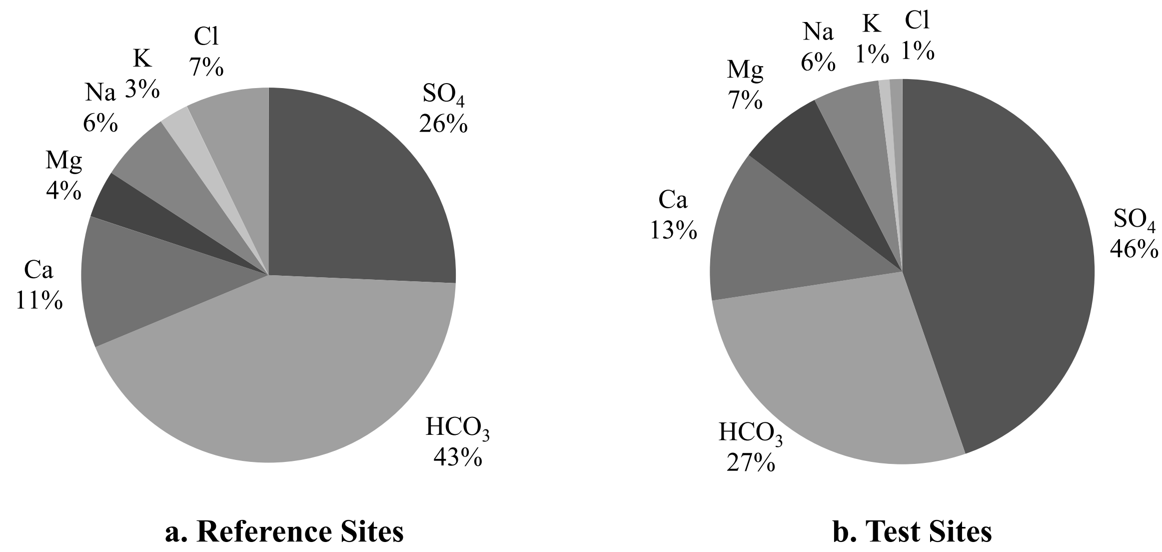


Figure 3.3. Mean relative ionic composition by mass of total dissolved solids for a) reference sites and b) test sites.

Reference sites had significantly higher relative proportions of HCO_3^-, Cl^-, and K^+ than test sites, whereas test sites had significantly higher relative proportions of SO_4^{2-} and Mg^{2+} than reference sites (Table 3.9). There was no significant difference in relative proportion of Ca^{2+} and Na^+ between reference and test sites.

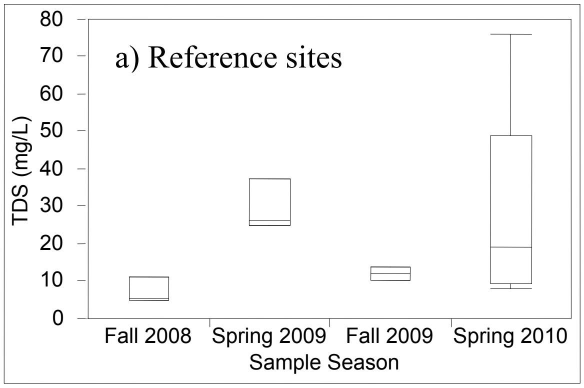
Table 3.9. Relative ionic composition comparison between reference and test sites.

Ion	p[1]	Site Type With Greater Ion Proportion
SO_4^{2-}	< 0.0001	Test
Mg^{2+}	< 0.0001	Test
Ca^{2+}	0.0523	No difference
Na^+	0.3682	No difference
HCO_3^-	<0.0001	Reference
Cl^-	<0.0001	Reference
K^+	< 0.0001	Reference

[1]from one-sided Welch's t-test

Temporal Variability of TDS

There was little seasonal variability of TDS among reference sites (Figure 3.4a). Three additional reference sites with higher TDS were added in Spring 2010, which explains the greater range of values observed in that season than in previous seasons. There was no significant difference in TDS at test sites across four sample seasons (ANOVA, $p = 0.28$), although TDS was nominally higher in Fall (season mean: 500 mg/L) than in Spring (season mean: 371 mg/L) (Figure 3.4b).



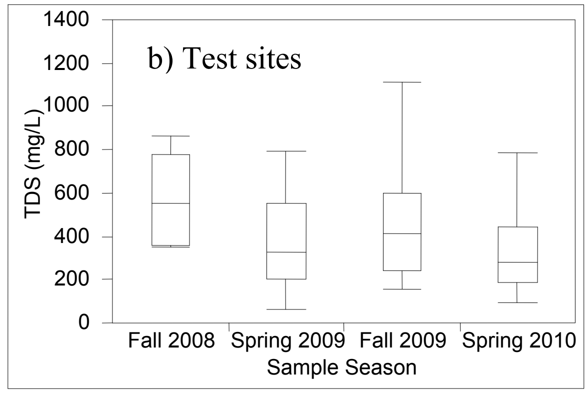


Figure 3.4. Box plots of total dissolved solids (TDS) at a) reference sites and b) test sites by sample season.

Inter-site Variability of TDS Level and Ionic Composition

Laurel Fork (LAU) is a forested second-order stream in southwest Wise County with excellent habitat quality and no evidence of mining in the catchment. During site selection, it was categorized as a test site rather than a reference site because of a recent history of elevated and variable TDS (VDMME unpub. data). Monitoring data obtained from VDMME indicated multiple high-TDS (>200 mg/L with some >500 mg/L) samples in the Spring of 2007 and 2008. However, it exhibited very low TDS for three consecutive seasons of sampling during this study, with composition similar to reference sites (Figure 3.5). Therefore, Laurel Fork was excluded from analyses.

Among test sites, ionic composition of TDS was similar (Figure 3.3b), with two exceptions: Jess Fork and Gin Creek. Jess Fork (JES) is a second-order stream draining surface-mine activity in northwest Buchanan County. Its mean HCO_3^- proportion was 6.6%, whereas mean HCO_3^- proportion of all test sites was 28% (Figure 3.3b). In addition, the mean pH of the site was 7.01 (with a low of 6.57), which was lower than the mean pH of 7.71 for all test sites (Table 3.7).

Gin Creek (GIN) is another site with ionic composition that differs from the bulk of test sites. It is a second-order stream in northern Lee County that receives a discharge from an abandoned deep mine. The ionic composition of water at the sampling station was consistently dominated by HCO_3^- and Na^+ with midrange TDS (Figure 3.6).

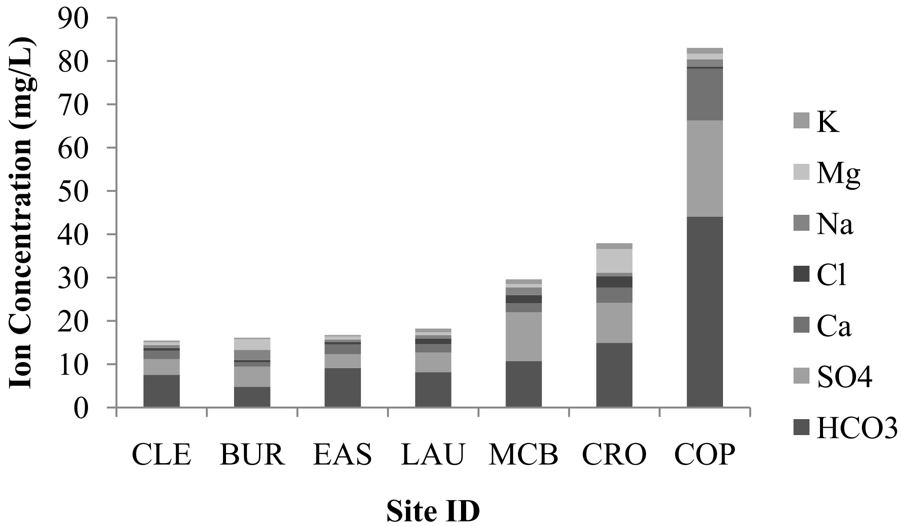


Figure 3.5. Stacked ion concentrations (means) for reference sites and Laurel Fork (LAU).

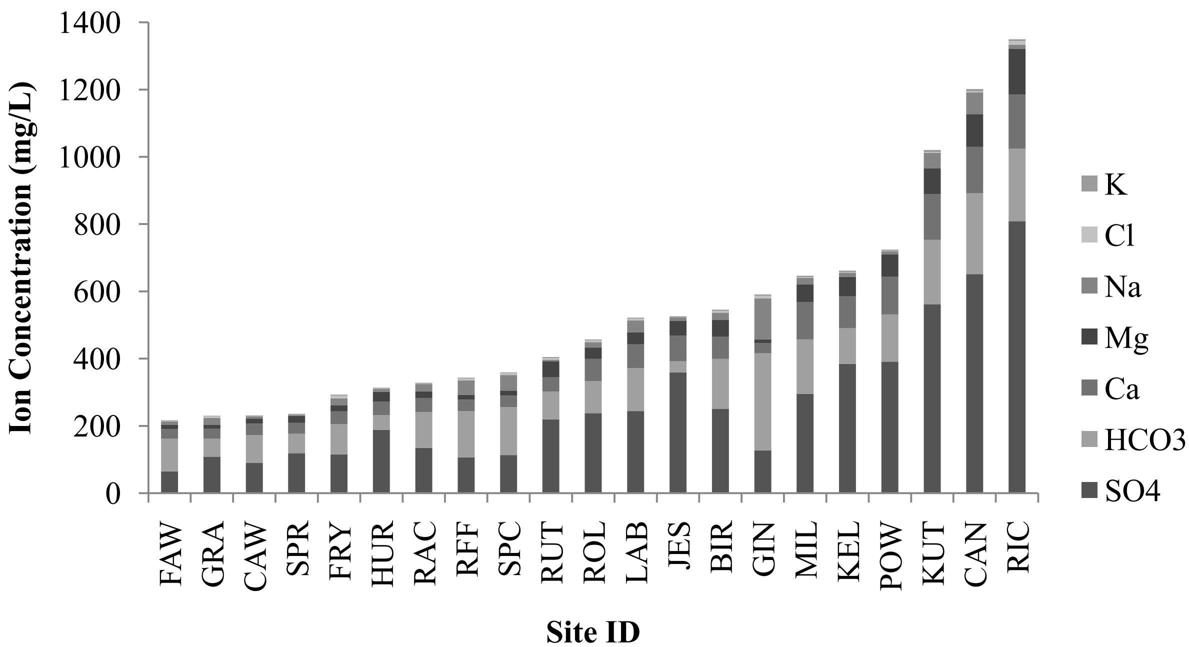


Figure 3.6. Stacked ion concentrations (means) for test sites.

Correlations between Chemical Parameters

Test site dissolved ion concentrations were positively correlated with one another, and negatively correlated with VASCI (Table 3.10). The measures most strongly correlated with VASCI were TDS (-0.64), SC (-0.63), Ca^{2+} (-0.61), and SO_4^{2-} (-0.58). Pairwise correlations among these four water quality parameters were strong, with correlation coefficients > 0.90 for each pair. Weaker correlations were observed between VASCI and HCO_3^- (-0.42) and Na^+ (-0.35) (Table 3.10).

Table 3.10. Matrix of significant[1] Spearman correlations for major ions, total dissolved solids, specific conductance, and Virginia Stream Condition Index scores of test sites.

	VASCI[2] Score	TDS[3] mg/L	SC[4] μS/cm	Ca^{2+}	SO_4^{2-}	Mg^{2+} mg/L	K^+	HCO_3^-	Na^+
TDS	-0.64								
SC	-0.63	0.99							
Ca^{2+}	-0.61	0.91	0.90						
SO_4^{2-}	-0.58	0.92	0.91	0.94					
Mg^{2+}	-0.55	0.87	0.85	0.95	0.94				
K^+	-0.54	0.90	0.89	0.82	0.83	0.81			
HCO_3^-	-0.42	0.64	0.67	0.45	0.38	0.35	0.63		
Na^+	-0.35	0.34	0.37				0.31	0.67	
Cl^-								0.30	0.58

[1]$\alpha = 0.05$; [2]Virginia Stream Condition Index; [3]total dissolved solids; [4]specific conductance.

Biology

VASCI Metrics

Mean values for all raw VASCI metrics and scores were significantly different between reference and test sites (Table 3.11). Values for Total Taxa Richness, EPT Taxa Richness, % Ephemeroptera, % Scrapers, % Chironomidae, HBI, and VASCI score were all significantly higher in reference sites, whereas % PT-Hydropsychidae and % 2 Dominant Taxa were significantly higher in test sites (Table 3.11). Differences concerning % Chironomidae, HBI, and % PT-Hydropsychidae were in directions counter to those expected in response to disturbance (Tables 3.2 and 3.11).

Table 3.11. Virginia Stream Condition Index raw metrics and score summary for study sites.

		Total Taxa[1]	EPT Taxa[2]	% E[3]	% PT-H[4]	% Scrap[5]	% Chiron[6]	% 2 Dom[7]	HBI[8]	VASCI[9] Score
Reference Sites[10]										
	Mean	17.3	12.1	20.8	38.6	15.2	17.3	46.4	3.4	72.56
	SD	2.3	2.4	13.5	14.3	7.9	11.2	7.6	0.6	7.69
	Min	13	8	2.0	18.0	1.0	0.0	32.7	2.3	60.91
	Max	20	16	41.3	71.3	25.7	40.7	58.3	4.3	84.98
Test Sites[11]										
	Mean	13.0*	8.3*	11.7*	56.6*	4.8*	8.1*	63.7*	2.6*	62.36*
	SD	3.4	3.1	11.4	16.7	5.1	6.5	14.9	0.9	9.87
	Min	5	2	0.0	16.5	0.0	0.0	37.7	1.0	42.76
	Max	22	16	43.0	88.2	18.7	27.0	96.1	5.0	78.24

[1]Total Taxa Richness; [2]EPT Taxa Richness; [3]Percent Ephemeroptera; [4]Percent Plecoptera and Trichoptera less Hydropsychidae; [5]Percent Scrapers; [6]Percent Chironomidae; [7]Percent Two Dominant Taxa; [8]Hilsenhoff Biotic Index; [9]Virginia Stream Condition Index; [10]Six sites, 15 samples; ; [11]21 sites, 63 samples; *Mean is significantly different from reference ($\alpha = 0.05$);

Correlations between VASCI Metrics and TDS, SC, and SO_4^{2-}

Five of the eight individual VASCI metrics were significantly correlated with SC, TDS, and SO_4^{2-} at test sites (Table 3.12). EPT Taxa Richness had the strongest correlation with these three water quality measures (-0.65 to -0.70), followed by Total Taxa Richness (-0.58 to -0.65), % Scrapers (-0.51 to -0.55), % Ephemeroptera (-0.45 to -0.51), and % 2 Dominant Taxa (0.30 to

0.40) (Table 3.12). The VASCI metrics % PT-Hydropsychidae, % Chironomidae, and HBI were not significantly correlated with these selected water quality measures.

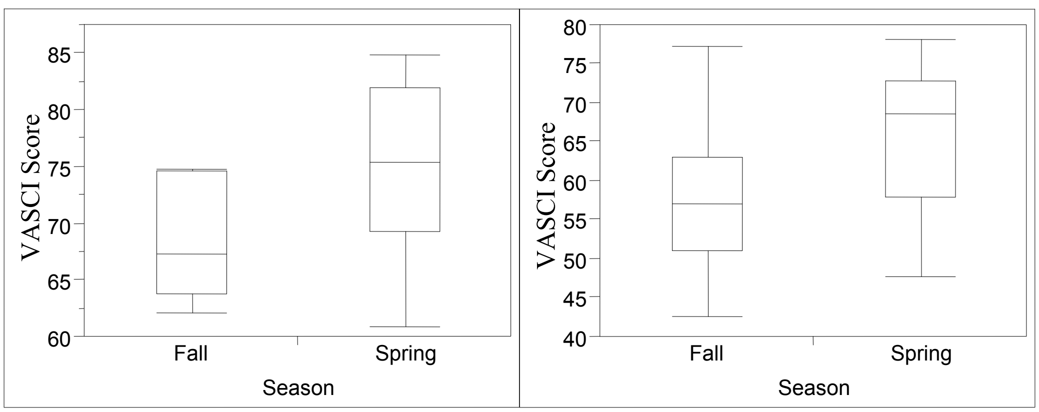
Table 3.12. Matrix of significant[1] Spearman correlation coefficients between Virginia Stream Condition Index metrics and selected water quality measures.

VASCI[2] Metric	SC[3] µS/cm	TDS[4] mg/L	SO_4^{2-} mg/L
EPT Taxa Richness	-0.70	-0.70	-0.65
Total Taxa Richness	-0.64	-0.65	-0.58
% Scrapers	-0.51	-0.52	-0.55
% Ephemeroptera	-0.49	-0.51	-0.45
% 2 Dominant Taxa	0.40	0.42	0.30

[1]α = 0.05; [2]Virginia Stream Condition Index; [3]specific conductance; [4]total dissolved solids.

Seasonality of VASCI Scores

Spring VASCI scores were significantly higher than Fall VASCI scores at both reference and test sites (Figure 3.7). Paired t-tests for sites that were sampled in consecutive Fall-Spring seasons also show a higher VASCI score in Spring than in Fall (Figure 3.8). Mean differences between Spring and Fall VASCI scores for reference and test sites were 7.3 and 6.1, respectively (Figure 3.8).



a. Reference Sites b. Test Sites

Figure 3.7. Box plot of a) reference site and b) test site Virginia Stream Condition Index (VASCI) scores by season (Reference: Fall n = 6, Spring n = 9, p = 0.03; Test: Fall n = 26, Spring n = 37, p = 0.0008).

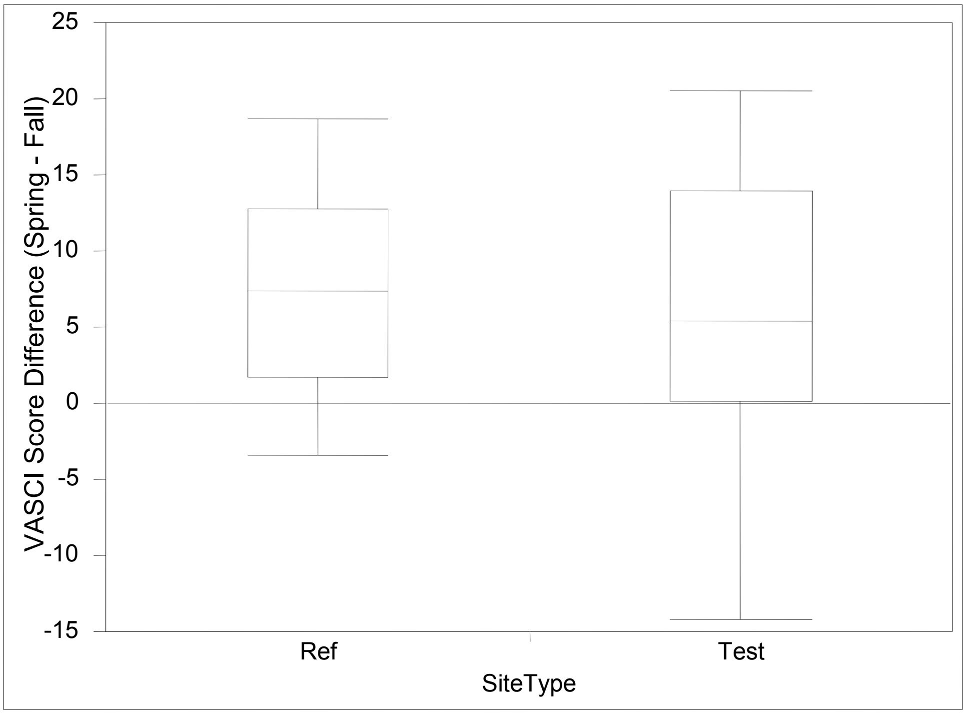


Figure 3.8. Box plot of site-paired seasonal Virginia stream condition index (VASCI) score differences (Spring minus prior Fall) for consecutive fall-spring sample pairs, by site type (Ref n = 6 site pairs, Test n = 26 site pairs). The dashed line indicates zero difference between seasons.

Associations between VASCI Score and Water Quality Parameters

Associations between water quality measures and biological effects (*i.e.*, VASCI < 60) were variable. Reference sites exhibited an absence of biological effects at TDS levels ranging from 8 to 76 mg/L. At test sites, biological effects were noted at TDS levels ranging from > 190 mg/L to 1,378 mg/L, the maximum recorded. All samples with TDS ≥ 1,108 mg/L exhibited biological effects. The lowest biological-effect levels observed for SC and SO_4^{2-} were 332 µS/cm and 70 mg/L, respectively; whereas all samples with SC ≥ 1,366 µS/cm and SO_4^{2-} ≥ 849 mg/L demonstrated biological effects. Within the water quality range above the minimum observed biotic-effect level (*i.e.*, >190 mg/L TDS), benthic macroinvertebrate communities exhibited biological effects inconsistently, but with increasing frequency as TDS increased up to 1,108 mg/L.

Ordinary Least Squares Linear Regression with Mixed Effects

Ordinary Least Squares (OLS) linear regression of test-site VASCI score versus log-transformed TDS with sample season as a random effect was significant for TDS (Figure 3.9). Sample seasons had no significant effect on the model. Regressions of VASCI with other water quality parameters were also significant, with the exception of HCO_3^- (Table 3.13). Multiple regression was not used because of multicollinearity among water quality parameters (Table 3.10). Specific conductance, TDS, and SO_4^{2-} were retained for OEC determinations because they had the strongest relationships (excepting Ca^{2+}) with VASCI score based on r^2 values (Table 3.13). Calcium was not included because it was highly correlated with SO_4^{2-} (Table 3.10), and SO_4^{2-} has been shown to be a good indicator of mining disturbance (*e.g.*, Pond et al. 2008).

The TDS model for Spring yielded a higher OEC (528 mg/L) than the Fall (337 mg/L) model, with the All-Year model (422 mg/L) in between the seasonal models (Table 3.14). Models for SC and SO_4^{2-} followed the same pattern, with values of 768, 634, and 523 µS/cm SC for Spring, Fall, and All-Year models, respectively, and 336, 143, and 219 mg/L SO_4^{2-} for Spring, Fall, and All-Year models, respectively (Table 3.14).

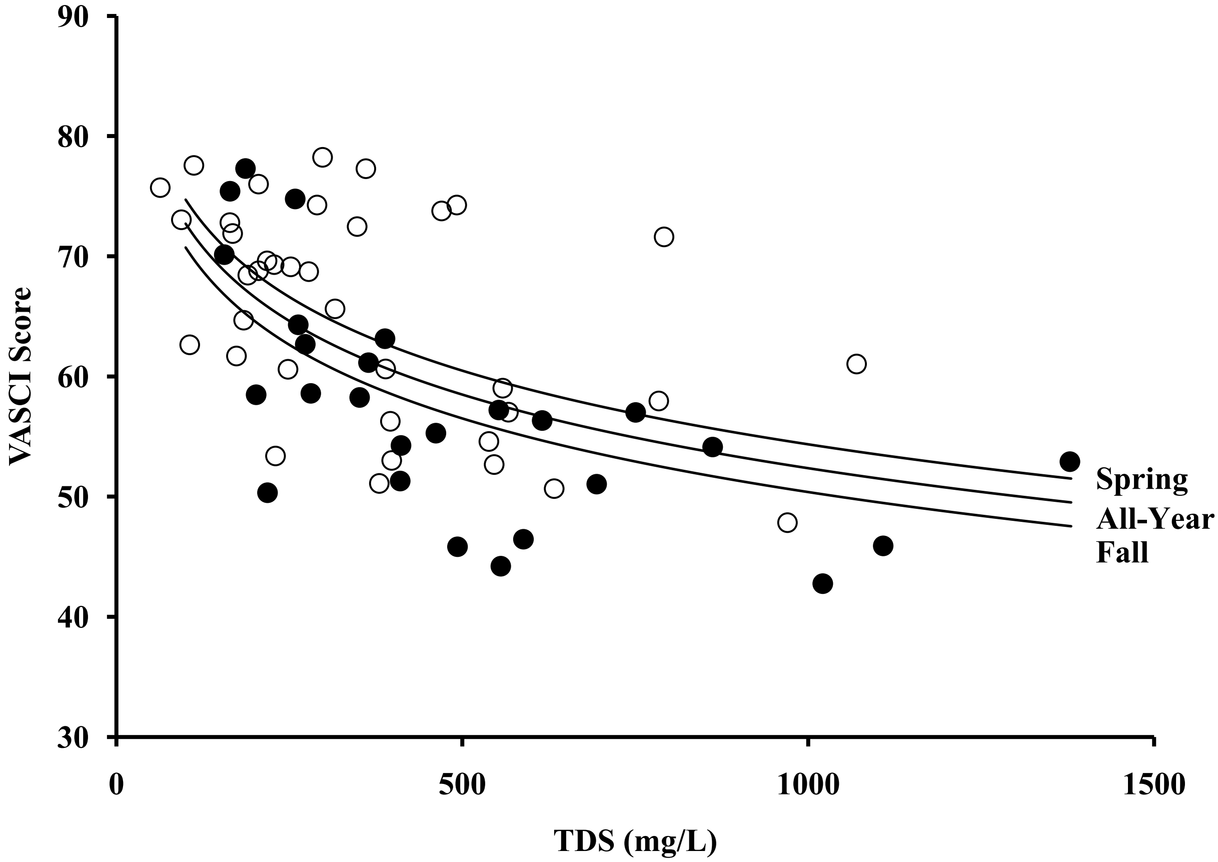


Figure 3.9. Ordinary least squares regression plot of Virginia stream condition index (VASCI) score versus log-transformed total dissolved solids (TDS) with sample season as random effect. The fitted lines for each season and All-Year are shown, along with observed values for Fall samples (solid circles) and Spring samples (open circles). $r^2 = 0.475$, $p < 0.0001$ for the All-Year model.

Table 3.13. Ordinary least squares regression coefficients and r^2 values for All-Year linear model Virginia stream condition index = $\beta_0 + \beta_1[\ln(x)] + \varepsilon$, where x = water quality measure.[1]

Predictor (x)	Units	β_0	β_1	r^2
Total dissolved solids	mg/L	113.42	-8.83816	0.475
SO_4^{2-}	mg/L	101.28	-7.66026	0.474
Ca^{2+}	mg/L	99.42	-9.47779	0.470
Specific conductance	µS/cm	130.31	-10.89646	0.469
Normality	meq/L	85.88	-9.62729	0.461
$SO_4^{2-} + HCO_3^- + Ca^{2+} + Mg^{2+}$	mg/L	118.80	-9.58040	0.458
$SO_4^{2-} + HCO_3^- + Ca^{2+} + Mg^{2+} + Na^+ + Cl^- + K^+$	mg/L	120.58	-9.74379	0.452
Hardness	mg/L as CaCO3	106.88	-8.19283	0.451
$SO_4^{2-} + HCO_3^-$	mg/L	115.91	-9.49492	0.450
Mg^{2+}	mg/L	83.88	-6.65858	0.423
HCO_3^-	mg/L	75.88	-3.10991	0.207

[1]Test sites only. All regressions are significant ($p < 0.0001$), except HCO_3^- ($p = 0.09$).

Table 3.14. Observed effect concentrations for specific conductance, total dissolved solids, and SO_4^{2-}, estimated using ordinary least squares regression.

Model	SC[1] µS/cm	TDS[2] mg/L	SO_4^{2-} mg/L
Spring	768	528	336
All-Year	634	422	219
Fall	523	337	143

[1]specific conductance; [2]total dissolved solids;

Quantile Regression

Quantile linear regression of test-site VASCI score versus log-transformed TDS indicated a significant negative association at the 10th, 25th, 50th, and 75th quantiles, but was not significant at the 90th quantile (Figure 3.10). Regressions of VASCI with SC and SO_4^{2-} also exhibited significant negative associations at all but the 90th quantile (Table 3.15).

For TDS the model for the 75th quantile (637 mg/L) yielded the highest OEC, with decreasing effect concentrations at the 50th (421 mg/L), 25th (263 mg/L), and 10th (142 mg/L) quantile models (Table 3.16). Models for SC and SO_4^{2-} followed the same pattern, with values of, 904, 625, 429, and 281 µS/cm for the SC 75th, 50th, 25th, and 10th quantile models, respectively, and 392, 219, 133, and 48 mg/L for the SO_4^{2-} 75th, 50th, 25th, and 10th quantile models, respectively (Table 3.16).

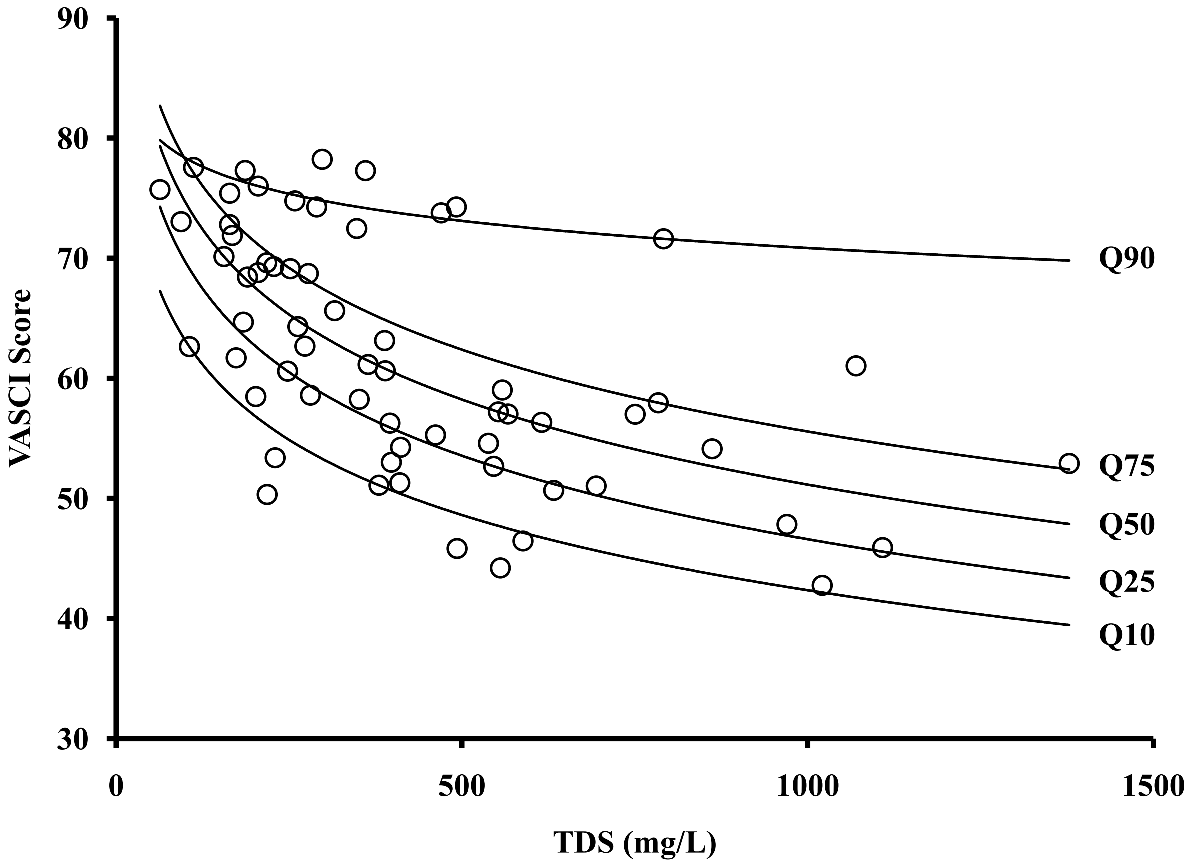


Figure 3.10. Quantile regression fitted lines of Virginia stream condition index (VASCI) versus total dissolved solids (TDS) (log transformed) for 10th, 25th, 50th, 75th, and 90th quantiles, along with observed values (open circles). All models shown except the 90th quantile are significant.

Table 3.15. Quantile regression coefficients for linear equation [Virginia Stream Condition Index score]$_Q$ = $\beta_0 + \beta_1[\ln(x_Q)] + \varepsilon$.

Predictor (x)	Quantile (Q)	β_0	β_1	p-value (β_1)
Specific conductance (µS/cm)				
	75	146.26	-12.6737	0.0003
	50	139.19	-12.3012	<.0001
	25	139.09	-13.0464	<.0001
	10	128.04	-12.0658	0.0013
Total dissolved solids (mg/L)				
	75	123.50	-9.8345	0.0001
	50	121.75	-10.2209	<.0001
	25	115.92	-10.0348	<.0001
	10	104.78	-9.0365	0.0007
SO_4^{2-} (mg/L)				
	75	112.26	-8.7531	0.0013
	50	113.52	-9.9276	<.0001
	25	102.30	-8.6492	0.0019
	10	85.89	-6.6922	0.0436

Table 3.16. Observed effect concentrations for specific conductance, total dissolved solids, and SO_4^{2-} using quantile regression.

Quantile	SC[1] µS/cm	TDS[2] mg/L	SO_4^{2-} mg/L
90	NS[3]	NS	NS
75	903	637	392
50	625	421	219
25	429	263	133
10	281	142	48

[1]specific conductance; [2]total dissolved solids; [3]not significant ($p > 0.05$)

Observed Effect Concentrations

As TDS and associated water quality measures increased beyond the minimum biological effect levels, benthic macroinvertebrate communities exhibited biological effects with increasing frequencies. Observed values for biological effects can be integrated with OLS and quantile regression results (Table 3.17) to describe the probability of biological effect at various water quality levels (Figure 3.11).

The empirical lower OEC bounds were 332 µS/cm, 190 mg/L, and 70 mg/L for SC, TDS, and SO_4^{2-}, respectively (Table 3.17). The empirical upper OEC bounds were 1,366 µS/cm, 1,108 mg/L, and 849 mg/L for SC, TDS, and SO_4^{2-}, respectively. Modeled OECs determined using OLS and quantile regression generally occur between these empirical bounds (Table 3.17 and Figure 3.11).

Table 3.17. Observed effect concentration found to be associated with probabilities of biological effect.

Method	Probability of Biological Effect at OEC[1] (%)	Model/ Quantile (%)	SC[2] (µS/cm)	TDS[3] (mg/L)	SO_4^{2-} (mg/L)
OLS Regression		Fall	523	337	143
	50	All-Year	634	422	219
		Spring	768	528	336
Quantile Regression	10	10	281	142	48
	25	25	429	263	133
	50	50	625	421	219
	75	75	903	637	392
Measured Values	0	All-Year	332	190	70
	100	All-Year	1366	1108	849

[1]Observed Effect Concentration; [2]specific conductance; [3]total dissolved solids.

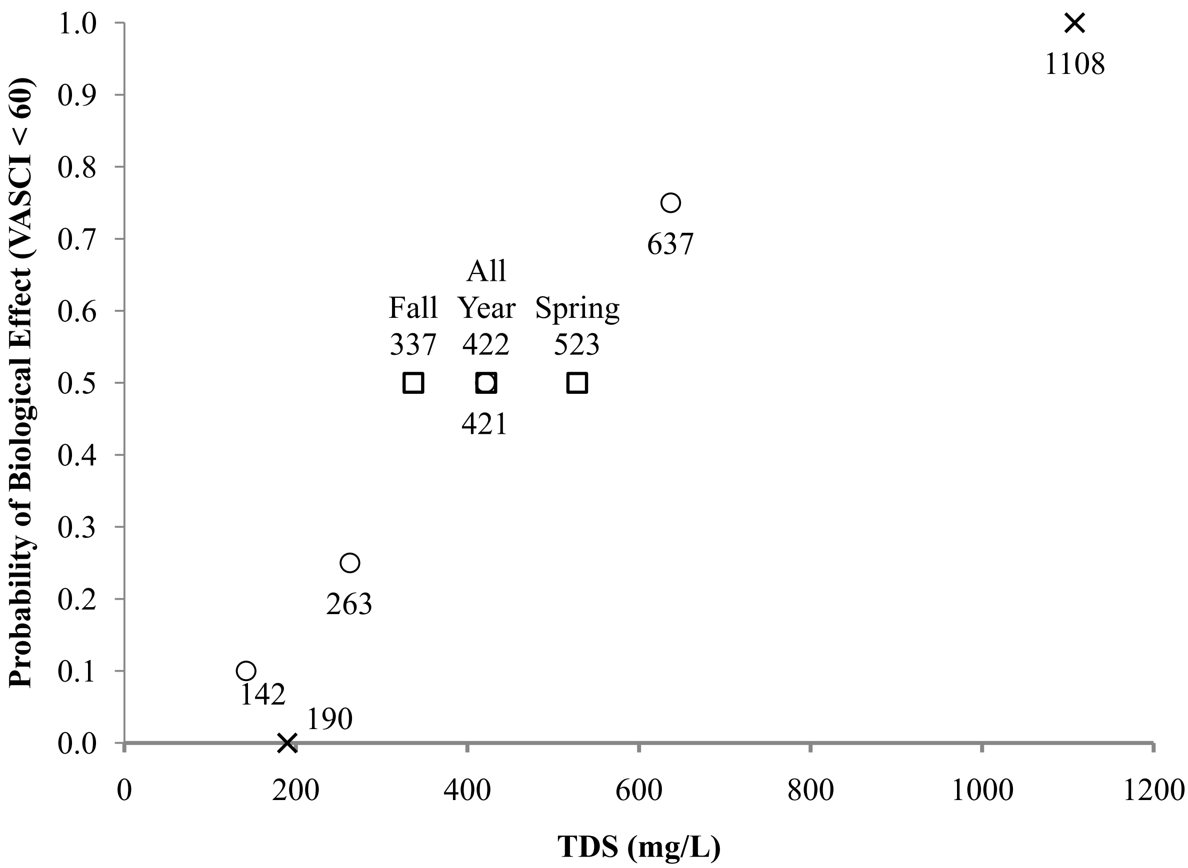


Figure 3.11. Biological effect (VASCI < 60) probability at each observed effect concentration (OEC) for total dissolved solids (TDS). The OECs from three seasonal ordinary least squares (OLS) linear regression models (squares) and four quantiles of the Quantile Regression model (circles) are shown, along with OEC bounds (Xs) from measured TDS. Relative certainty surrounding each point is ordered as follows: observed values > OLS models ~ Q50 > Q25 ~ Q75 > Q10.

Discussion

Site Selection for Minimizing Influence from Non-TDS Stressors
Near-reference-quality streams with elevated TDS are rare in Virginia's Central Appalachian ecoregion, because most of the region's streams are influenced by legacy landuses including agriculture, mining, contemporary mining, infrastructure, and commercial, industrial, and/or residential development. The extensive effort undertaken to locate test sites with abiotic conditions comparable to those of reference sites was successful in minimizing biotic influence from non-TDS stressors.

Ionic Composition of TDS
Test site TDS was dominated by SO_4^{2-} and HCO_3^-, which is typical of alkaline mine drainage in the region (Pond et al. 2008, Timpano et al. 2010). In addition, the dominant cation in streamwater TDS was Ca^{2+}. This is a different matrix from those tested in the laboratory, where Na^+ is most often the dominant cation (Kennedy 2005, Soucek and Kennedy 2005). TDS at test sites exhibited ionic compositions that differed from those at reference sites, because SO_4^{2-} was the dominant ion at test sites, but HCO_3^- was the dominant ion at reference sites. Because it has been documented that ionic composition influences ion toxicity (Soucek and Kennedy 2005, Mount et al. 1997), one of the objectives of this study was to determine if ionic composition influenced the associations between TDS and biotic condition. However, evaluation of the effects of differing ionic compositions on VASCI score was not possible, because the study sites, with few exceptions, had similar relative ionic composition (Figure 3.3b).

Seasonality of VASCI Scores
Spring VASCI scores were generally higher than same-site prior-Fall VASCI scores, at both reference and test sites (Figure 3.8). This observation is different from the results of VASCI development studies, which found no difference in VASCI scores between seasons (Burton and Gerritsen 2003, VDEQ 2006a). Those studies, however, were conducted at sites located throughout Virginia's non-coastal region, with only a few sites located within the current study area. The finding that seasonal VASCI differences occur at reference sites suggests that there may be natural seasonal differences in benthic macroinvertebrate communities that influence VASCI scores for headwater streams in the study region.

Seasonality of VASCI scores in the coalfield region is relevant to the application of water quality policies and/or practices to protect aquatic life, because at present the VASCI makes no distinction between seasons and VDEQ requires streams to score ≥ 60 year-round (VDEQ 2010a). If further research were to conclude that naturally occurring seasonal differences in benthic macroinvertebrate communities cause VASCI scores to be lower in Fall for headwater streams in this part of Virginia, that finding would be relevant to VASCI application for water quality assessments in such streams. Although my reference sites scored lower in Fall, all reference VASCI scores were > 60 during that season (Figure 3.7a).

Further study of reference-quality streams in the Central Appalachian ecoregion of Virginia would confirm whether a natural seasonal difference in VASCI score exists. Such study would also improve understanding of how the VASCI's current calibration is influencing results of its application in the Central Appalachian region of Virginia, a region that was not heavily represented during VASCI development (Burton and Gerritsen 2003, VDEQ 2006a).

Variability of TDS
There was no indication of seasonal TDS differences at my study sites collectively, although TDS was nominally higher in Fall than in Spring (Figure 3.4b). However, for any sample site there are at most four samples spread over 21 months. I cannot be certain that the TDS concentration I observed at any given site represents the concentration that is driving biological response at that site, because it is possible that some sites may have had higher TDS concentrations than were measured.

Frequency, duration, and intensity of exposure can affect the biotic impact of a stressor (USEPA 2000). Benthic macroinvertebrate communities are a good bioindicator because they integrate effects of stressors over the life cycle of each taxon (Barbour et al. 1999). Early life stages (eggs and hatchlings) of benthic insects can be ≤ 50% as tolerant of salinity as their older counterparts (Kefford et al. 2004). Because it is the mature specimens that are the target of rapid bioassessment sampling (mature specimens facilitate reliable identification), it is quite possible that the TDS observed at the time of collection may not represent the TDS to which the more-sensitive eggs and hatchlings were exposed. Therefore, more-frequent TDS monitoring over the course of one or more years would be necessary to more accurately characterize the pattern of TDS exposure throughout life cycles of benthic organisms, such that biota-limiting levels of TDS can be more accurately determined.

For these reasons, it cannot be determined whether the stressor levels measured during biotic sampling are indeed the levels that influenced biotic conditions that were observed. This assessment therefore defined biological effect as simply the observation of VASCI < 60, and then determined stressor levels at the time of sampling that were associated with those biological observations. The term "observed effect concentration" is not used in a causal context for this study; it is the water quality level observed at the time of biological sampling that is associated with observation of an explicitly defined biological effect (VASCI < 60).

Variability of VASCI-TDS Associations
Water quality – VASCI associations were found to be highly variable, with water quality explaining < 50% of VASCI variability (Table 3.14). The source of this variability is unknown, but several candidate causes can be suggested. Natural seasonal differences in VASCI score, as observed in reference sites, may be partly responsible (Figure 3.7a). Precision of the VASCI, as developed, is estimated at +/- 7.9 points (Burton and Gerritsen 2003). Unknown and unobserved non-TDS stressors may be influencing VASCI in addition to TDS. As an example, mean stream temperatures at test sites were nominally higher than at reference sites, but I have no data for summer maximum temperatures; seasonal thermal regime is an important factor affecting aquatic insect communities (Vannote and Sweeney 1980). Though not considered a stressor *per se*, an unknown factor is the temporal pattern of TDS exposure, which could influence biotic response (USEPA 2000). Because TDS was only measured twice per year, it is not known whether the benthic macroinvertebrate community was exposed to a higher, biological effect-inducing level of TDS at some time prior to sampling.

Comparisons with Other Studies
This study derived OECs that were higher than those found in other investigations of benthic macroinvertebrate communities in coal-mining-influenced Appalachian streams (Pond et al. 2008, Green et al. 2000). The differences may be due in part to the fact that the present study examined biotic condition associations with TDS where influence from non-TDS stressors was minimized, as well as to differences in methods for defining biological effects of significance.

In a study of West Virginia streams influenced by mixed land uses, including mining valley fills, Green et al. (2000) estimated, using least squares linear regression with five seasons of data, that an SC of 426 µS/cm corresponded to "good or better" conditions on the West Virginia Stream Condition Index (WVSCI). The present study, using OLS regression of four seasons of data, found that SC of 634 µS/cm was associated with a 50% probability of biological effects, while some observations at higher levels failed to exhibit biological effects. The lower effect concentration observed by Green et al. (2000) could be due to combined effects of TDS and non-TDS stressors, and to differences in what constitutes a "biological effect". Mining-influenced sites in that study included those with residential influences, and habitat quality was correlated with both SC and biotic condition.

Pond et al. (2008) found all sites > 500 µS/cm impaired using a genus-level benthic macroinvertebrate index in West Virginia streams. However, the authors noted that habitat quality explained some of the variance of the index. I did not observe biological effects at all sites until SC was ≥ 1,366 µS/cm, which is closer to the 1,500 µS/cm survival effect concentration observed by Kennedy et al. (2004) for the mayfly genus Isonychia when exposed to simulated mine drainage in the laboratory. One factor contributing to the finding of impaired sites at SC >500 µS/cm by Pond et al. (2008) was likely the nature of the biological-effect definition that those authors employed. That study used a biotic index specifically designed to differentiate stressed sites from reference sites, where SC >500 µS/cm was an *a priori* criterion for site classification.

In a survey of West Virginia streams influenced by acid mine drainage (AMD), Freund and Petty (2007) found benthic macroinvertebrate assemblages to be impaired at all sites where SC was > 501 µS/cm or SO_4^{2-} was > 213.2 mg/L, values which are lower than the corresponding values (1,366 µS/cm SC, and 849 mg/L SO_4^{2-}) identified by my study. Their definition of biotic effect was based on the West Virginia Stream Condition Index (WVSCI), a 6-parameter multimetric index that is not identical to the VASCI, so it is not surprising that their findings of maximum threshold levels differed. They also found that pH and Al concentration both explained more variance in WVSCI than did SC or SO_4^{2-}, a likely consequence of the fact that their streams were selected for study based on AMD influence; thus, it is likely that factors in addition to SC and SO_4^{2-} were having influence on the biotic effects observed in their study.

Determination of TDS Levels Associated with Aquatic Life Effects
Selecting a stressor level to protect aquatic life is a regulatory decision driven by the measure of biotic condition that is considered socially desirable or acceptable. This analysis used the VASCI as a biotic condition measure. The VASCI is applied within Virginia using VASCI = 60 as a biotic impairment threshold. Thus, analysis has been presented on that basis, while recognizing that other measures of what is a "socially acceptable" biotic condition may be employed.

One essential question is whether TDS, SC, or a component ion such as SO_4^{2-} would be best employed as a biotic effect predictor. The results of this study, conducted in mining-influenced streams where SO_4^{2-} is the dominant anion and Ca^{2+} is the dominant cation, did not indicate one measure over another as the best choice. In test streams, TDS concentration, SC, and SO_4^{2-} concentration were all highly correlated, and potential application of each as a biological effect predictor gives parallel results.

These results do not indicate a single TDS/ion/SC level as an obvious choice for aquatic life protection in the regulatory context. Each of the candidate OECs carries with it a degree of risk of erroneously predicting biotic condition at the OEC, given that biotic condition (as measured by the VASCI) can vary widely at any single concentration. Thus, choice of an OEC as predictive of a certain biotic condition, within a regulatory context, would require a decision concerning allocation of prediction error probabilities. These results are presented within that context.

Although the biological effect probability plot (Figure 3.11) can be used to select an OEC to satisfy one of many desired error probabilities, some constraints are evident. First, biological effects (VASCI < 60) were observed in all samples higher than 1,366 µS/cm SC, 1,108 mg/L TDS, and 849 mg/L SO_4^{2-} (Table 3.17), suggesting these higher levels may be associated with very high probability of aquatic life effects in streams of the region. Second, no biological effects were observed in all samples lower than 332 µS/cm SC, 190 mg/L TDS, and 70 mg/L SO_4^{2-} (Table 3.17), suggesting these lower levels may be associated with very low probability of aquatic life effects in regional streams. Finally, Fall and Spring OLS models must have a biological effect probability less than and greater than 50%, respectively, if the water quality level is applied year-round, because the All-Year model criterion applied year-round would have a 50% impairment probability. If either the Fall or Spring model OEC is applied seasonally, then biological effect probability would be 50% for that season (Table 3.17). The biological effect probabilities for other OECs can be used to approximate the biological effect probability associated with any given concentration (Figure 3.11). Which model or combination to choose depends critically on the management goals for the stream(s) in question. Regardless of goals, there are reasonable choices that are supported by these data that should satisfy a range of management objectives.

Limitations of Data Interpretation

It is important to note that interpretability of these data is limited because the study design was not statistically unbiased in the manner by which sites were selected. I employed a strict targeted approach to site selection in order to isolate TDS effects. In that way, this study was less like a spatially-balanced probabilistic survey and more similar to a laboratory toxicity test where a gradient of treatment levels are assigned to experimental units free from influence by confounding factors. However, I did not have control of the specific treatment levels in this study and thus the frequency distribution of observations is not even across the gradient of TDS. I made multiple sampling visits to a changing number of sites across sample seasons; thus, the data set is not seasonally balanced. Regression analysis describes the association between VASCI and TDS in a manner that is independent of the sampling distribution of these data. For that reason, I avoided using distribution-based analyses (*e.g.*, Paul and McDonald 2005, USEPA 2010). Despite these limitations, my results provide strong support for the use of TDS and/or a highly correlated measure such as SC or SO_4^{2-} as a water quality measure that can be interpreted as an aquatic life effect predictor in these streams.

Conclusions

I was able to effectively minimize the influence of non-TDS stressors at the selected study sites. This offers a novel approach because many studies in the region have observed influence from TDS-covariate stressors. The additional time cost to implement this approach yielded a clear benefit to my dataset, because it is free of major influence by non-TDS stressors.

I observed a significant negative associations between measures of biological condition and TDS/SC/SO_4^{2-}. Relationships were strongest with family-level richness of the generally sensitive orders Ephemeroptera, Plecoptera, and Trichoptera. Increasing TDS was also associated with decreased total richness, as well as lower Ephemeroptera and scraper abundance.

I characterized TDS composition and found it to be generally similar across test sites and dominated by SO_4^{2-} and HCO_3^- as expected of streams receiving alkaline mine drainage in the Appalachian coalfield region. I could not evaluate the influence of TDS composition on biotic condition, because there was not enough difference in TDS composition among sites for statistical analysis.

I found the relationship between VASCI score and TDS/SC/SO_4^{2-} to be similar using two linear regression approaches. Resultant models and empirical data provided evidence to support a range of observed effect concentrations (OECs) that vary in degree of aquatic life effect probability.

I was able to effectively isolate the dissolved solids variable in a survey of biological response to elevated TDS in headwater streams in Virginia's Central Appalachian coalfield region. Family-level richness of the benthic macroinvertebrate orders Ephemeroptera, Plecoptera, and Trichoptera declined with increasing TDS, as did overall richness, Ephemeroptera abundance, scraper abundance, and VASCI score. The streams in this study were similarly dominated by SO_4^{2-} and HCO_3^-, such that no evaluation could be made of the influence of ionic composition on relationships between water quality measures and biological condition. Biological effects, as defined by VASCI score < 60, were associated with TDS, with an increasing probability of effects from 0% at ≤ 190 mg/L TDS to 100% at ≥ 1,108 mg/L TDS, with 50% probability of effects observed at 422 mg/L TDS. Effect probabilities of 0, 50, and 100% were associated with specific conductance values of 332, 625, and 1,366 μS/cm, respectively. Sulfate (SO_4^{2-}) concentrations of 70, 219, and 849 mg/L were associated with 0, 50, and 100% probabilities of effect, respectively.

Interpretation of these results is limited by the statistically biased nature of the study design. In addition, OECs are not equivalent to TDS toxicities, because it is unknown whether the benthic macroinvertebrate community was exposed to a higher, biotic effect-inducing level of TDS at some time prior to sampling. Despite those limitations, these results provide strong support for the use of the OECs for TDS and/or a highly correlated measure such as SC or SO_4^{2-} that can be interpreted as a level of dissolved solids above which aquatic life effects are increasingly probable in headwater streams of Virginia's Central Appalachian coalfield region.

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CHAPTER 4. FIELD SENSITIVITY DISTRIBUTIONS FOR DISSOLVED SOLIDS IN COALFIELD STREAMS

Introduction

Elevated levels of total dissolved solids (TDS) have been suggested as stressors to aquatic life in Central Appalachian streams influenced by coal mining (Pond et al. 2008). In such streams, TDS is most often dominated by the dissolved ions SO_4^{2-} and HCO_3^-, with elevated concentrations (relative to natural background) of Ca^{2+}, Mg^{2+}, Na^+, K^+, and Cl^- also common (Mount et al., 1997, Pond et al., 2008). At present there are no aquatic life water quality criteria for TDS or dominant ions for streams in the primary coal-producing Central Appalachian states (KY, VA, WV). In all three states, aquatic life conditions in streams are assessed for compliance with the Clean Water Act using measures of benthic macroinvertebrate community composition.

Water quality criteria for many pollutants other than TDS have been developed by the U.S. Environmental Protection Agency (EPA) using laboratory toxicity data, but field data are not at present used to derive water quality criteria. The established approach uses laboratory toxicity data to construct species sensitivity distributions (SSDs), which identify pollutant levels that do not cause toxic effects to most (95%) species (Stephan et al. 1985, Posthuma et al. 2002). However, the concept of using field data for criteria development is gaining interest, as evidenced by U.S EPA's recent application of the SSD approach to field data, which was designed to determine the level of specific conductance associated with observance of 95% of reference-site benthic macroinvertebrate genera in Central Appalachian streams influenced by coal-mining (USEPA 2010).

Using field data with the SSD approach to derive criteria can have several benefits over using traditional laboratory tests. First, a wider range of organisms may be incorporated into the SSD because it is not feasible to test all species in the laboratory. Second, a field-data approach allows criteria development using data from the indigenous organisms targeted for protection, rather than the fewer, laboratory-adaptable indicator organisms used in toxicity testing. Third, field data represent effects of long-term, or life-cycle exposure to stressors – an exposure duration not easily achieved in the laboratory for all test species. These benefits support the use of field data with the SSD approach (Suter 2002). Use of field data also has disadvantages. One is the difficulty of controlling for effects by other stressors, so as to ensure that the effects being observed are those of the stressor in question. The variety of environmental factors that influence species occurrences in natural environments can also cause difficulties, especially as they affect the field distributions of sensitive and rare species.

This study applied the SSD concept using field-based genus-level data to construct taxa sensitivity distributions that could be used to determine levels of dissolved solids that are associated with low observation frequency of benthic macroinvertebrate genera in headwater streams of Virginia's Central Appalachian coalfield region.

Methods

Site Selection
The goal in choosing test sites was to identify streams with elevated TDS, but where all other observable factors were comparable to reference streams that represent "…the biological condition in places with a minimal amount of human disturbance." (USEPA 2006).

First- and second-order streams (Strahler 1957) within the Virginia portion of Ecoregion 69 (Omernik 1987) were selected for study. I endeavored to located elevated TDS, or "test" sites, meeting non-biological reference criteria (Table 4.1) used for Virginia Clean Water Act implementation studies (Burton and Gerritsen 2003, VDEQ 2006a).

Table 4.1. Abiotic criteria for stream selection.

Parameter or Condition (units or range)	Selection Criterion[1]
Dissolved Oxygen (mg/L)	≥ 6.0
pH	≥ 6.0 & ≤ 9.0
Epifaunal substrate score (0-20)[2]	≥ 11
Channel alteration score (0-20)[2]	≥ 11
Sediment deposition score (0-20)[2]	≥ 11
Bank disruptive pressure score (0-20)[2]	≥ 11
Riparian vegetation zone width score, per bank (0-10)[2]	≥ 6
Total RBP habitat score (0-200)[2]	≥ 140
Residential land use immediately upstream	None

[1]Parameters and numeric selection criteria from Burton and Gerritsen (2003)
[2]RBP habitat, high gradient streams (Barbour et al. 1999)

Streams were chosen by examining a variety of available data using a GIS, augmented by consultation with mine operators, consultants, and regulators with specific knowledge of stream conditions within the study area. Virginia Department of Mines, Minerals, and Energy (VDMME) provided data on water quality, mine permits, and historical surface-mining site locations.

Candidate sites were visited to assess suitability for study. Site reconnaissance allowed verification of current land uses and confirmed minimal catchment disturbance, as per study design. Physicochemical water parameters, including pH and specific conductance, were measured. Physical habitat was evaluated using the qualitative visual estimate approach for high-gradient streams as specified in U.S. EPA's Rapid Bioassessment Protocols (RBP) (Barbour et al. 1999).

In addition to meeting the physicochemical and habitat criteria, all test sites also had to be free from obvious influence from residential land use upstream. This criterion was important to avoid the unpredictable influence of failing septic systems (*e.g.*, dissolved N and P enrichment) or direct stream discharges of household waste (*e.g.*, particulate organic matter, toxics). Other potential sources of non-point source pollution were avoided, including road crossings, bridges, culverts, active logging, non-coal industrial operations or infrastructure (*e.g.*, rail beds), and commercial activity. Finally, accessibility was a practical criterion that had to be met to allow the site to be included in the study. Access permission was obtained for each study site from private landowners and/or mine permittees where necessary.

Field Methods
At each study site, benthic macroinvertebrate and water quality samples were collected up to four times during the study period. Samples were collected during the Spring (March through May) of 2009 and 2010, and Fall (September through November) of 2008 and 2009 biological index periods (VDEQ 2008). Benthic macroinvertebrate collections followed the single-habitat (riffle-run) approach (VDEQ 2008). Approximately 2 m^2 of riffle substrate were sampled using a 0.3 m wide D-frame kicknet with 500 µm mesh. A single composite sample was collected at each site, preserved in 95% ethanol, and returned to the laboratory for sorting and identification.

Water temperature, dissolved oxygen (DO), specific conductance (at 25 °C; henceforth referred to as SC), and pH were measured *in situ* with a calibrated handheld multi-probe meter (Hydrolab Quanta, Hach Hydromet, Loveland, Colorado). Single grab samples of water were collected using vacuum hand pumps and reusable polyethylene filter assemblies (VDEQ 2006b). All samples were stored in acid-rinsed polypropylene Nalgene bottles. Samples for dissolved metals, TDS, alkalinity, and major ions were filtered in the field immediately following collection using acid-rinsed cellulose ester filters with a nominal pore size of 0.45µm. Samples for metals analysis were preserved to pH < 2 with 1+1 concentrated nitric acid. All samples were transported on ice and stored at 4 °C. At each site, all biological and water samples were collected concurrently at base flow. All water quality sampling was conducted upstream of and/or immediately prior to biological sampling. In-stream and riparian habitat quality were assessed during each sample collection using RBP methods (Barbour et al. 1999).

Laboratory Methods
Biological sample processing followed modified VDEQ Biomonitoring Standard Operation Procedures (VDEQ 2008). Each sample was sub-sampled to obtain a 200 (±10%) organism count following RBP methods (Barbour et al. 1999). Benthic macroinvertebrates were identified to the family/lowest practicable taxonomic level.

An inductively coupled plasma - optical emission spectrometer (Varian Vista MPX ICP-OES w/ICP Expert software, Varian Instruments, Walnut Creek, California) was used to measure dissolved Ca^{2+}, Mg^{2+}, K^+, Na^+, and Al, Cu, Fe, Mn, Se, and Zn (APHA 2005). An ion chromatograph (Dionex DX500, Dionex Corp., Sunnyvale, California) was used to measure Cl^- and SO_4^{2-} (APHA 2005); TDS was measured via filtration of known volumes followed by drying at 180°C (APHA 2005), with modifications (0.45 micron cellulose ester filter, field filtration); total alkalinity was measured in an aliquot of filtered sample by titration with standard acid (APHA 2005); and CO_3^{2-}/HCO_3^- were calculated from alkalinity and pH measurements (APHA 2005). Samples were stored at 4 °C and analyzed within holding times of 7 days (TDS), 14 days (alkalinity), 28 days (anions), or 6 months (metals) (APHA 2005). Water chemistry data were examined to determine if trace metals levels exceeding criteria continuous concentrations (CCC) (USEPA 2011, IEPA 2001) were correlated with biological response.

Data Analysis

Statistical Analyses

Water quality and habitat were compared between site categories using Welch's t-test and Mann-Whitney test for normal and non-normal data, respectively. All analyses were conducted using JMP 8 (SAS Institute, Cary, North Carolina, USA) and R 2.12 statistical software (R Development Core Team) with test level of $\alpha = 0.05$.

Biological Data Filters

All organisms were identified to the genus level, except Chironomidae and Oligochaeta. Specimens in the family Chironomidae were identified to family level but Chironomidae was treated as if it were a genus for analysis. Aquatic earthworms were identified to class Oligochaeta, which was treated as a genus as well. These two taxa were not identified to lower taxonomic levels because of the specialized techniques and training required for reliable identification. They were included in analysis because they were common constituents in samples.

Although the SSD approach can be sensitive to the number of taxa included, a determination must be made as to which taxa to include in the analysis (Wheeler et al. 2002). For this study, any genera that were not observed in at least one reference sample were excluded from analysis to ensure that the analysis represented salt-sensitive taxa that would be expected to occur at reference sites, rather than salt-tolerant taxa occurring only at TDS-influenced sites. In addition, genera observed in fewer than four samples (5% of the 81 samples) were excluded from analysis to limit influence from the rarest taxa. Because they are unlikely to appear in a sample, it is not clear whether the absence of rare taxa in a sample is due to elevated TDS or natural scarcity. For this reason, estimated salt tolerance values for rare taxa would likely be underestimated compared to salt tolerance values of more-common taxa (Kefford et al. 2004a). That is, the difference between field-based salt tolerance values and laboratory-derived salt toxicities would be greater for rare taxa than for more-common taxa (Kefford et al. 2004a). Therefore, the rarest taxa were excluded because their inclusion would limit the utility of the analysis as an indicator of TDS sensitivity.

Field Sensitivity Distribution

The SSD approach is applied to laboratory toxicity data to derive water quality criteria (Posthuma et al. 2002). The SSD approach was used here with genus-level field data (with noted exceptions) to derive field sensitivity distributions in order to determine levels of dissolved solids that are associated with absence of benthic macroinvertebrate taxa at study sites. This approach was called a field sensitivity distribution (FSD) and FSDs were created for SC, TDS, and SO_4^{2-}, because those are the water quality measures found to be most highly correlated with biological condition in the study region (Timpano et al. 2010, Timpano 2011). Although the SSD approach uses species-level toxicity data (Posthuma et al. 2002), the conceptual approach translates to the use of genus-level field data, in that the focus remains on identifying stressor effect levels associated with absence of distinct taxa. Genus-level data were used because genus is a readily achievable level of taxonomic resolution for rapid bioassessment purposes (Barbour et al. 1999).

Maximum Field Concentration
The highest water quality parameter concentration at which a taxon was observed in a sample was defined as the Maximum Field Concentration (MFC). This is conceptually similar to the Maximum Field Distribution (MFD) approach employed by Kefford et al. (2004a) in examining salinity tolerance of freshwater benthic macroinvertebrates in Australian waters. The MFC values for each taxon were then used to construct an FSD for each water quality parameter.

Field Sensitivity Distribution and Observed Effect Concentration
An FSD is a cumulative distribution function (CDF) of the MFC values for a given water quality measure. The FSD curve describes the proportion of taxa with an MFC less than or equal to a given concentration. FSDs were constructed using R statistical software. The FSD was used to determine the water quality measure concentration above which different proportions of taxa were not observed. This was defined as the observed effect concentration – X%, or OEC_X. The CDF linear interpolation form of the quantile function in R was used to calculate the OEC_X for SC, TDS, and SO_4^{2-} for proportions of taxa not observed. The OEC_X represents the level of water quality measure at which (100-X)% of taxa were observed at study sites.

Seasonal Models
Maximum field concentrations, FSDs, and OEC_X values were calculated three ways based on sample seasons. One model used data from samples collected in Spring (42 taxa from 48 samples), one model used data from Fall samples (41 taxa from 33 samples), and an All-Year model used data from all samples (60 taxa from 81 samples). This was done to evaluate seasonal differences in OEC_X values. For each model's development, the individual samples were treated independently.

Results

Site Selection

The site selection process yielded 229 candidate sites. Of these, 185 were visited within Wise, Dickenson, Lee, Buchanan, and Russell Counties to verify land uses, habitat quality, and water quality. Twenty-eight sites in first- and second-order headwater streams were selected for study (Tables 4.2 and 4.3). Poor habitat quality was the primary reason for not selecting streams for study. Because of the scarcity of sites satisfying selection criteria, site selection was continued and new sites were added throughout the study period (Table 4.3).

Table 4.2. Site selection summary.

	Reference	Test	Total
Candidate Sites	48	181	229
Sites Visited	36	149	185
Study Sites Selected	6	22	28

The site selection process yielded six reference sites (Figure 4.1). Three, in the Jefferson National Forest (Wise County), approached "natural" or "undisturbed" condition free from significant human disturbance, but with dominant geology different from that of the test sites (Table 4.3). Therefore, three additional reference sites were selected near the Dickenson/Buchanan County border, where dominant geology was similar to test sites. The three additional sites were distributed to nearly encompass the latitudinal extents of the coalfields (Figure 4.1). Twenty-two test sites were located that met selection criteria.

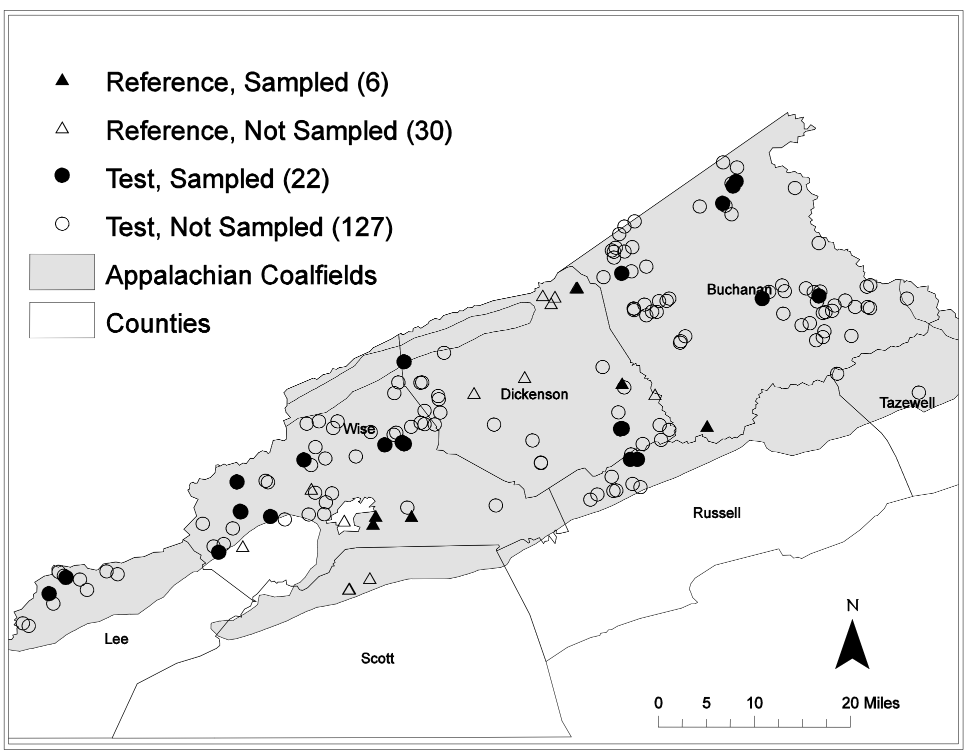

Figure 4.1. Map of visited and selected reference and test site locations in southwestern Virginia.

Table 4.3. Study site information.

Stream	Station ID	Type	Order	Dominant Geologic Formation	County[1]	Lat	Long	Sampled Fall 2008	Spring 2009	Fall 2009	Spring 2010
Burns Creek	BUR	Ref	2	Lee	Wise	36.929	-82.535	x	x	x	x
Clear Creek	CLE	Ref	2	Undivided Mississippian	Wise	36.929	-82.589	x	x	x	x
Copperhead Branch	COP	Ref	1	Norton	Dickenson	37.064	-82.090				x
Crooked Branch	CRO	Ref	2	Norton	Dickenson	37.130	-82.218				x
Eastland Creek	EAS	Ref	1	Undivided Mississippian	Wise	36.917	-82.593	x	x	x	x
Middle Camp Branch	MCB	Ref	1	Norton	Dickenson	37.274	-82.286				x
Birchfield Creek	BIR	Test	2	Wise	Wise	37.036	-82.575	x	x	x	x
Callahan Creek West Fork	CAW	Test	1	Wise	Wise	36.980	-82.797		x	x	x
Cane Branch	CAN	Test	1	Wise	Dickenson	37.160	-82.547			x	x
Fawn Branch	FAW	Test	1	Wise	Lee	36.811	-83.080		x	x	x
Fryingpan Creek	FRY	Test	2	Norton	Dickenson	37.060	-82.218		x	x	x
Fryingpan Creek Right Fork	RFF	Test	2	Norton	Dickenson	37.060	-82.220		x	x	x
Gin Creek	GIN	Test	2	Wise	Lee	36.836	-83.055		x	x	x
Grape Branch	GRA	Test	2	Norton	Buchanan	37.257	-82.007	x	x	x	x
Hurricane Fork	HUR	Test	2	Norton	Buchanan	37.400	-82.067		x	x	x
Jess Fork	JES	Test	2	Wise	Buchanan	37.295	-82.219		x	x	x
Kelly Branch	KEL	Test	2	Wise	Wise	36.935	-82.792			x	x
Kelly Branch UT[2]	KUT	Test	1	Wise	Wise	36.936	-82.792			x	x
Laurel Branch	LAB	Test	2	Norton	Russell	37.014	-82.205		x	x	x
Laurel Fork	LAU	Test	2	Wise	Wise	36.874	-82.825		x	x	x
Mill Branch Left Fork	MIL	Test	2	Wise	Wise	36.927	-82.747	x	x	x	x
Powell River	POW	Test	1	Wise	Wise	37.013	-82.697	x	x	x	x
Race Fork UT[2]	RAC	Test	1	Norton	Buchanan	37.427	-82.050		x	x	x
Richey Branch	RIC	Test	2	Wise	Wise	37.036	-82.546			x	x
Richey Branch UT[2]	RUT	Test	1	Wise	Wise	37.037	-82.544			x	x
Roll Pone Branch	ROL	Test	1	Norton	Russell	37.014	-82.195		x	x	x
Spring Branch	SPR	Test	1	Norton	Buchanan	37.434	-82.046		x	x	x
Spruce Pine Creek	SPC	Test	2	Norton	Buchanan	37.261	-81.922	x	x	x	x

[1]All sites located in southwestern Virginia; [2]Unnamed Tributary

Habitat
Mean total habitat scores, of 177 and 169 for reference and test sites, respectively (Figure 4.2, Table 4.4), were not significantly different ($p = 0.07$). All habitat parameter means were nominally lower for test sites than for reference sites (Table 4.4), with the largest nominal differences recorded for embeddedness, sediment deposition, and bank stability in that order. However, only mean riparian vegetated width scores were significantly different between reference and test sites ($p = 0.0002$), with higher values observed in reference sites. Habitat parameters were not significantly correlated to VASCI score. Water quality measures were not correlated to habitat parameters, except bank stability, which was moderately correlated with TDS ($\rho = -0.36$), SC ($\rho = -0.37$), and SO_4^{2-} ($\rho = -0.46$). Test site means were > 85% of reference mean for all habitat parameters and for total score, indicating that test site habitat was comparable to reference (Barbour et al. 1999).

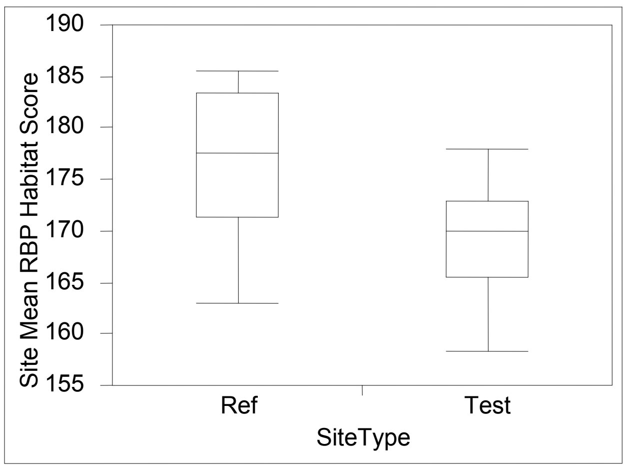

Figure 4.2. Box plot of mean total habitat scores for reference sites (n=6) and test sites (n=21). Mean total habitat scores were not significantly different between site types. Box plots represent 5th, 25th, 50th, 75th, and 95th percentiles.

Table 4.4. Site mean habitat summary data for study sites.

	Substrate/Cover	Embeddedness	Velocity/Depth	Sediment Dep.	Flow	Channel Alt.	Riffle Freq.	Bank Stability L+R	Bank Veg. Protection L+R	Rip. Veg. Width L+R	Total
Reference Sites[1]											
Mean	18.3	15.4	16.0	13.8	18.7	20.0	18.3	17.3	19.2	20.0	176.7
SD	1.1	2.0	3.1	1.6	1.2	0.0	1.1	2.3	0.8	0.1	8.2
Min	17	13	10	12	17	20	17	13	18	20	163
Max	20	17	19	16	20	20	20	20	20	20	186
Test Sites[2]											
Mean	17.4	14.2	15.7	12.6	18.2	19.9	18.1	15.5	18.4	18.9*	169.0
SD	0.8	0.9	1.2	0.7	0.9	0.2	0.8	1.8	1.0	1.0	5.4
Min	16	12	13	11	17	19	16	11	17	17	158
Max	19	16	19	14	20	20	19	18	20	20	178

[1]Six sites; [2]21 sites; *Mean is significantly different from reference ($\alpha = 0.05$).

Streamwater Chemistry

Physicochemical Properties

Streamwater temperature, pH, DO, and SC values ranged from 1.7 to 17.5 °C, 6.11 to 8.49, 7.7 to 12.3 mg/L, and 16 to 1,670 µS/cm, respectively across all samples (Table 4.5). Mean test site pH (7.71) and SC (593 µS/cm) were significantly different from reference sites (pH of 7.02, and SC of 31 µS/cm), but streamwater temperature and DO were not significantly different between reference and test sites (Table 4.5).

Table 4.5. Physicochemical summary statistics for study sites.

		Temp °C	pH SU	DO[1] mg/L	SC[2] µS/cm
Reference Sites[3]					
	Mean	11.0	7.02	9.6	31
	SD	4.0	0.45	1.1	27
	Min	1.7	6.11	7.7	16
	Max	14.4	7.80	11.8	116
Test Sites[4]					
	Mean	12.1	7.71*	9.4	593*
	SD	3.3	0.39	0.9	349
	Min	2.5	6.57	7.8	20
	Max	17.5	8.49	12.3	1,670

[1]Dissolved oxygen; [2]Specific conductance; [3]Six sites, 15 samples; [4]21 sites, 63 samples; *Mean is significantly different from reference (α = 0.05).

Major Ions and Trace Metals

Mean TDS and major ion concentrations at test sites were significantly higher than at reference sites (Table 4.6). Test site mean TDS was 406 mg/L, whereas reference site mean TDS was 21 mg/L (Table 4.6).

Table 4.6. Total dissolved solids (TDS) and major ion summary statistics for study sites.

		TDS	SO_4^{2-}	HCO_3^-	Ca^{2+}	Mg^{2+}	Na^+	K^+	Cl^-
					mg/L				
Reference Sites[1]									
	Mean	21	5.9	10.8	2.6	0.8	1.2	1.5	0.6
	SD	19	5.1	10.4	2.7	0.6	0.9	1.4	0.4
	Min	5	2.8	0.7	0.4	0.5	0.4	0.4	0.3
	Max	76	22.1	44.1	12.0	2.6	3.7	5.5	1.4
Test Sites[2]									
	Mean	406*	231.4*	117.6*	61.1*	36.3*	24.8*	3.5*	3.2*
	SD	284	187.2	69.7	41.4	29.7	28.0	3.4	1.5
	Min	16	4.2	5.1	1.5	1.1	0.7	0.3	0.6
	Max	1,378	849.0	301.7	183.9	160.6	135.9	15.1	7.6

[1]Six sites and 15 samples; [2]21 sites and 63 samples; *Mean is significantly different from reference (α = 0.05);

Median trace metal concentrations were nominally higher in test sites than in reference sites for most metals (Table 4.7). Streamwater dissolved metal concentrations were below method detection limits in 257 of 396 (65%) test site samples (Table 4.7). No measurements exceeded CCC for Al, Cu, Fe, or Mn. Two of 63 samples (3.2%) exceeded hardness-adjusted CCC for Zn. Nine of 63 samples (14.3 %) exceeded CCC for Se (Table 4.7). No significant correlations were found between metal concentrations > CCC and biological condition (Timpano 2011).

Table 4.7. Trace metals summary data for study sites.

		Al	Cu	Fe	Mn	Se	Zn
				μg/L			
Reference Sites[1]							
	Median	< 12.6	< 17.7	< 32.3	< 6.7	< 17.1	10.9
	Max	41.9	< 22.8	< 64.9	< 15.7	< 24.1	< 37.3
	# > MDL[2]	7	0	1	6	6	3
	# > CCC[3]					3	3
Test Sites[4]							
	Median	11.9	< 17.7	60.3	15.0	< 17.1	11.4
	Max	50.5	< 22.8	410.9	787.9	28.3	116.0
	# > MDL	37	1	23	44	9	25
	# > CCC					9	2
MDL							
	Mean	8.5	15.6	39.7	7.2	15.6	16.2
	Min	2.8	8.9	22.2	1.7	4.9	4.0
	Max	12.6	22.8	64.9	15.7	24.1	37.3

[1]Six sites, 15 samples; [2]Method Detection Limit, mean of four sample season batches, values below batch MDL reported as "< [MDL value]"; [3]Criteria Continuous Concentration, hardness-adjusted for Cu, Mn, Zn; [4]21 sites, 63 samples;

Benthic Macroinvertebrate Taxa

Benthic macroinvertebrate sampling yielded 97 taxa (95 genera, plus Chironomidae and Oligochaeta) from 81 samples during two years (Table 4.8). Of the 97 taxa, nine were unique to reference sites, whereas 26 were found only at test sites. Taxa observed only in Spring numbered 20, with 21 taxa found only in Fall. Data filters were applied, which resulted in 37 taxa that did not meet criteria for FSD inclusion. Two taxa (*Atrichopogon* and *Cheumatopsyche*) with more than four observations were excluded because they were not observed in reference samples. The remaining 35 excluded taxa had fewer than four observations. A total of 60 taxa were retained for FSD analysis (Table 4.8).

Table 4.8. Taxa sampled by season and site type.

Taxon	Obs	Season	Site Type	Excluded[1]
Acentrella	20	Both	Both	
Acroneuria	41	Both	Both	
Agapetus	7	Spring	Both	
Allocapnia	30	Fall	Both	
Allognosta	1	Spring	Test	x
Ameletus	33	Both	Both	
Amphinemura	48	Both	Both	
Antocha	4	Both	Both	
Arigomphus	39	Both	Both	
Atherix	1	Fall	Test	x
Atrichopogon	5	Fall	Test	x
Attenella	8	Both	Both	
Baetis	56	Both	Both	
Beloneuria	1	Fall	Test	x
Boyeria	2	Both	Test	x
Ceratopsyche	28	Both	Both	
Chelifera	34	Both	Both	
Cheumatopsyche	28	Both	Test	x
Chimarra	7	Both	Test	x
Chironomidae[2]	80	Both	Both	
Cinygmula	4	Spring	Both	
Clinocera	4	Both	Both	
Cordulegaster	3	Spring	Test	x
Cyrnellus	5	Both	Both	
Dicranota	13	Both	Both	
Diphetor	1	Fall	Test	x
Diplectrona	75	Both	Both	
Diploperla	1	Fall	Test	x
Dixa	13	Both	Test	x
Dolophilodes	38	Both	Both	
Drunella	12	Spring	Both	
Ectopria	37	Both	Both	
Epeorus	35	Both	Both	
Ephemera	4	Both	Both	
Ephemerella	27	Both	Both	
Eurylophella	2	Fall	Reference	x
Glossosoma	3	Spring	Test	x
Haploperla	17	Both	Both	
Helichus	4	Both	Both	
Hemerodromia	10	Both	Both	
Heptagenia	1	Fall	Test	x
Hexatoma	26	Both	Both	
Homoplectra	1	Spring	Test	x
Hydatophylax	6	Fall	Both	
Hydracarina	1	Fall	Reference	x
Hydrochus	1	Spring	Test	x
Hydropsyche	12	Both	Both	
Hydroptila	2	Spring	Test	x
Isonychia	3	Spring	Both	x

[1]Taxa with fewer than four observations or not found at a reference site were excluded from analysis. [2]Taxa not identified to genus, but treated as such for analysis.

Table 4.8, cont'd.

Taxon	Obs	Season	Site Type	Excluded
Isoperla	24	Both	Both	
Lepidostoma	9	Both	Both	
Leuctra	67	Both	Both	
Limnophila	25	Both	Both	
Limonia	2	Both	Reference	x
Lype	1	Fall	Reference	x
Maccaffertium	17	Both	Both	
Macronychus	2	Spring	Test	x
Mayatrichia	1	Spring	Test	x
Molophilus	8	Both	Both	
Neophylax	20	Both	Both	
Neotrichia	6	Both	Test	x
Nigronia	18	Both	Both	
Oemopteryx	1	Fall	Reference	x
Oligochaeta[2]	38	Both	Both	
Optioservus	10	Both	Both	
Ormosia	2	Spring	Test	x
Oulimnius	43	Both	Both	
Palpomyia	11	Both	Both	
Paracapnia	16	Fall	Both	
Paraleptophlebia	22	Both	Both	
Peltoperla	20	Both	Both	
Perlesta	21	Spring	Both	
Polycentropus	32	Both	Both	
Prosimulium	1	Spring	Reference	x
Psephenus	13	Both	Both	
Pseudolimnophila	1	Fall	Test	x
Pteronarcys	19	Both	Both	
Pycnopsyche	6	Both	Both	
Remenus	2	Spring	Reference	x
Rhyacophila	69	Both	Both	
Sialis	2	Fall	Test	x
Simulium	28	Both	Both	
Soyedina	4	Fall	Both	
Stenacron	5	Both	Both	
Stenelmis	3	Spring	Both	x
Stenonema	4	Fall	Both	
Stilobezzia	3	Fall	Reference	x
Stylogomphus	1	Fall	Test	x
Sweltsa	15	Both	Both	
Tabanus	1	Spring	Test	x
Taeniopteryx	11	Fall	Both	
Tallaperla	4	Both	Test	x
Timpanoga	1	Spring	Test	x
Tipula	40	Both	Both	
Viehoperla	1	Spring	Reference	x
Wormaldia	17	Fall	Both	
Yugus	23	Both	Both	

[1]Taxa with fewer than four observations or not found at a reference site were excluded from analysis. [2]Taxa not identified to genus, but treated as such for analysis.

Data Analysis

Maximum Field Concentrations

Specific conductance MFCs ranged from 357 to 1,335 µS/cm for the Spring model, 402 to 1,670 µS/cm for the Fall model, and 25 to 1,670 µS/cm for the All-Year model (Table 4.9). Total dissolved solids MFCs ranged from 298 to 1,070 mg/L for the Spring model, 263 to 1,378 mg/L for the Fall model, and 28 to 1,378 mg/L for the All-Year model (Table 4.10). The SO_4^{2-} MFCs ranged from 90 to 769 mg/L for the Spring model, 108 to 849 mg/L for the Fall model, and 5 to 849 mg/L for the All-Year model (Table 4.11).

Table 4.9. Maximum field concentrations (MFC) for specific conductance (SC) using three seasonal models.

Taxon	All-Year	Model Spring	Fall	Taxon	All-Year	Model Spring	Fall
	SC MFC (µS/cm)				SC MFC (µS/cm)		
Acentrella	1061	1061		*Isoperla*	842	842	546
Acroneuria	1670	1335	1670	*Lepidostoma*	594	594	
Agapetus	594	594		*Leuctra*	1335	1335	1183
Allocapnia	1670		1670	*Limnophila*	1335	1335	1183
Ameletus	1183	706	1183	*Maccaffertium*	784	357	784
Amphinemura	1335	1335		*Molophilus*	1061	1061	
Antocha[1]	25			*Neophylax*	607	607	402
Arigomphus	1670	1061	1670	*Nigronia*	1462	1282	1462
Attenella	656		656	Oligochaeta	1670	1282	1670
Baetis	1366	1335	1366	*Optioservus*	1670		1670
Ceratopsyche	1670	842	1670	*Oulimnius*	1366	1335	1366
Chelifera	1366	1335		*Palpomyia*	656		656
Chironomidae	1670	1335	1670	*Paracapnia*	682		682
Cinygmula	462	462		*Paraleptophlebia*	652	594	652
Clinocera[1]	607			*Peltoperla*	1087	757	1087
Cyrnellus[1]	842			*Perlesta*	1335	1335	
Dicranota	842	842	450	*Polycentropus*	970	970	652
Diplectrona	1670	1335	1670	*Psephenus*	706	706	
Dolophilodes	1670	1335	1670	*Pteronarcys*	970	970	450
Drunella	490	490		*Pycnopsyche*	1670		1670
Ectopria	1670	970	1670	*Rhyacophila*	1670	1335	1670
Epeorus	970	970	546	*Simulium*	1462	1335	1462
Ephemera[1]	263			*Soyedina*	1366		1366
Ephemerella	706	706		*Stenacron*[1]	462		
Haploperla	546	490		*Stenonema*	468		468
Helichus[1]	652			*Sweltsa*	784		784
Hemerodromia	1462	757	1462	*Taeniopteryx*	1366		1366
Hexatoma	1335	1335	865	*Tipula*	1670	1282	1670
Hydatophylax	682		682	*Wormaldia*	1087		1087
Hydropsyche	1282	1282	1183	*Yugus*	1335	1335	

[1]Taxa that did not meet minimum observation frequency requirements for separate Spring or Fall models.

Table 4.10. Maximum Field Concentrations (MFC) for total dissolved solids (TDS) using three seasonal models.

Taxon	Model All-Year	Model Spring	Model Fall	Taxon	Model All-Year	Model Spring	Model Fall
	TDS MFC (mg/L)				TDS MFC (mg/L)		
Acentrella	792	792		*Isoperla*	558	558	352
Acroneuria	1378	1070	1378	*Lepidostoma*	389	389	
Agapetus	389	389		*Leuctra*	1070	1070	862
Allocapnia	1378		1378	*Limnophila*	1070	1070	862
Ameletus	862	470	862	*Maccaffertium*	553	228	553
Amphinemura	1070	1070		*Molophilus*	784	784	
Antocha[1]	28			*Neophylax*	361	361	263
Arigomphus	1378	792	1378	*Nigronia*	1108	970	1108
Attenella	411		411	Oligochaeta	1378	970	1378
Baetis	1070	1070	1021	*Optioservus*	1378		1378
Ceratopsyche	1378	558	1378	*Oulimnius*	1070	1070	1021
Chelifera	1070	1070		*Palpomyia*	411		411
Chironomidae	1378	1070	1378	*Paracapnia*	493		493
Cinygmula	298	298		*Paraleptophlebia*	462	389	462
Clinocera[1]	361			*Peltoperla*	751	567	751
Cyrnellus[1]	558			*Perlesta*	1070	1070	
Dicranota	558	558	273	*Polycentropus*	792	792	462
Diplectrona	1378	1070	1378	*Psephenus*	470	470	
Dolophilodes	1378	1070	1378	*Pteronarcys*	792	792	273
Drunella	298	298		*Pycnopsyche*	1378		1378
Ectopria	1378	792	1378	*Rhyacophila*	1378	1070	1378
Epeorus	792	792	352	*Simulium*	1108	1070	1108
Ephemera[1]	94			*Soyedina*	1021		1021
Ephemerella	470	470		*Stenacron*[1]	298		
Haploperla	352	290		*Stenonema*	281		281
Helichus[1]	462			*Sweltsa*	553		553
Hemerodromia	1108	567	1108	*Taeniopteryx*	1021		1021
Hexatoma	1070	1070	694	*Tipula*	1378	970	1378
Hydatophylax	493		493	*Wormaldia*	751		751
Hydropsyche	970	970	862	*Yugus*	1070	1070	

[1]Taxa that did not meet minimum observation frequency requirements for separate Spring or Fall models.

Table 4.11. Maximum Field Concentrations (MFC) for SO_4^{2-} using three seasonal models.

		Model				Model	
Taxon	All Year	Spring	Fall	Taxon	All Year	Spring	Fall
	SO_4^{2-} MFC (mg/L)				SO_4^{2-} MFC (mg/L)		
Acentrella	531	531		*Isoperla*	311	311	205
Acroneuria	849	769	849	*Lepidostoma*	250	250	
Agapetus	250	250		*Leuctra*	769	769	477
Allocapnia	849		849	*Limnophila*	769	769	272
Ameletus	340	250	340	*Maccaffertium*	283	90	283
Amphinemura	769	769		*Molophilus*	494	494	
Antocha[1]	5			*Neophylax*	192	192	120
Arigomphus	849	531	849	*Nigronia*	679	623	679
Attenella	128		128	Oligochaeta	849	623	849
Baetis	769	769	629	*Optioservus*	849		849
Ceratopsyche	849	311	849	*Oulimnius*	769	769	629
Chelifera	769	769		*Palpomyia*	167		167
Chironomidae	849	769	849	*Paracapnia*	340		340
Cinygmula	156	156		*Paraleptophlebia*	272	250	272
Clinocera[1]	178			*Peltoperla*	477	456	477
Cyrnellus[1]	311			*Perlesta*	769	769	
Dicranota	311	311	167	*Polycentropus*	531	531	272
Diplectrona	849	769	849	*Psephenus*	221	221	
Dolophilodes	849	769	849	*Pteronarcys*	531	531	163
Drunella	221	221		*Pycnopsyche*	849		849
Ectopria	849	531	849	*Rhyacophila*	849	769	849
Epeorus	531	531	205	*Simulium*	769	769	679
Ephemera[1]	59			*Soyedina*	629		629
Ephemerella	221	221		*Stenacron*[1]	156		
Haploperla	221	221		*Stenonema*	108		108
Helichus[1]	272			*Sweltsa*	283		283
Hemerodromia	679	456	679	*Taeniopteryx*	629		629
Hexatoma	769	769	250	*Tipula*	849	623	849
Hydatophylax	340		340	*Wormaldia*	477		477
Hydropsyche	623	623	233	*Yugus*	769	769	

[1]Taxa that did not meet minimum observation frequency requirements for separate Spring or Fall models.

Field Sensitivity Distributions

The seasonal FSDs for TDS were very similar, within the range from approximately 250 to 750 mg/L, with some divergence at TDS > 750 mg/L (Figure 4.3). At TDS < 250 mg/L the All-Year FSD is skewed downward by two genera, *Antocha* and *Ephemera*, with MFCs < 100 mg/L. FSDs for SC and SO_4^{2-} followed a similar pattern.

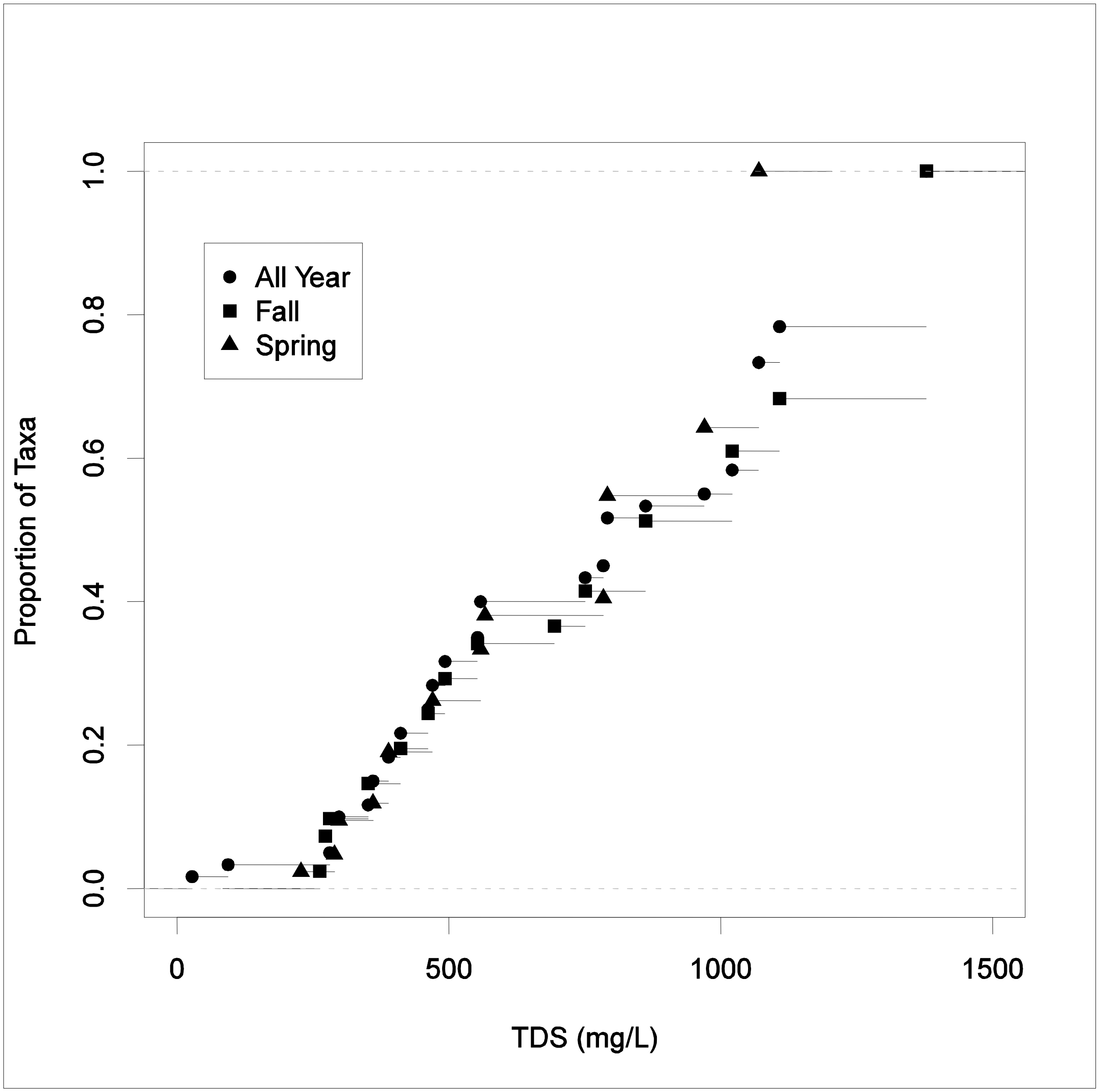

Figure 4.3. Field sensitivity distributions for total dissolved solids (TDS) for three seasonal models.

Observed Effect Concentrations

Observed effect concentrations were generally highest with the Spring model and lowest with the Fall model (Table 4.12). Mean OEC_{10} across all three seasonal models for SC, TDS, and SO_4^{2-} was 492 µS/cm, 299 mg/L, and 180 mg/L, respectively. The OEC_{05} and OEC_{10} were similar for

SC and TDS models, with a mean difference ($OEC_{10} – OEC_{05}$) of 7% for all models. The mean difference between OEC_{05} and OEC_{10} for SO_4^{2-} models was 40%. The OEC_{20} values were higher than OEC_{10} values for all water quality parameters and models, with a mean difference of 33%.

Table 4.12. Observed effect concentrations for each water quality parameter and model.

Water Quality Parameter	Proportion of Genera Not Observed	Model Fall	 Spring	 All-Year
Specific Conductance			(μS/cm)	
	5%	450	465	462
	10%	476	511	490
	20%	653	647	652
TDS			(mg/L)	
	5%	273	291	281
	10%	288	311	298
	20%	421	422	411
SO_4^{2-}			(mg/L)	
	5%	120	160	108
	10%	163	221	156
	20%	210	250	221

Salt Sensitivity by Taxonomic Group

Salt sensitivity varied among groups at multiple taxonomic levels. At the order level, genera of the typically salt-sensitive order Ephemeroptera exhibited lower MFCs (Figure 4.4) than genera from other orders. Trichoptera genera had the second lowest MFCs. Orders Plecoptera and Diptera were similarly sensitive to SC, and were observed at higher field concentrations than other orders.

Within orders, salt sensitivity varied among genera (Figure 4.5), with many taxa generally classified as pollution-tolerant (Barbour et al. 1999) located in the upper portion of the CDFs (*e.g.*, Ephemeroptera: genus *Baetis*; Trichoptera: family Hydropsychidae [genera *Hydropsyche*, *Ceratopsyche*, *Diplectrona*]).

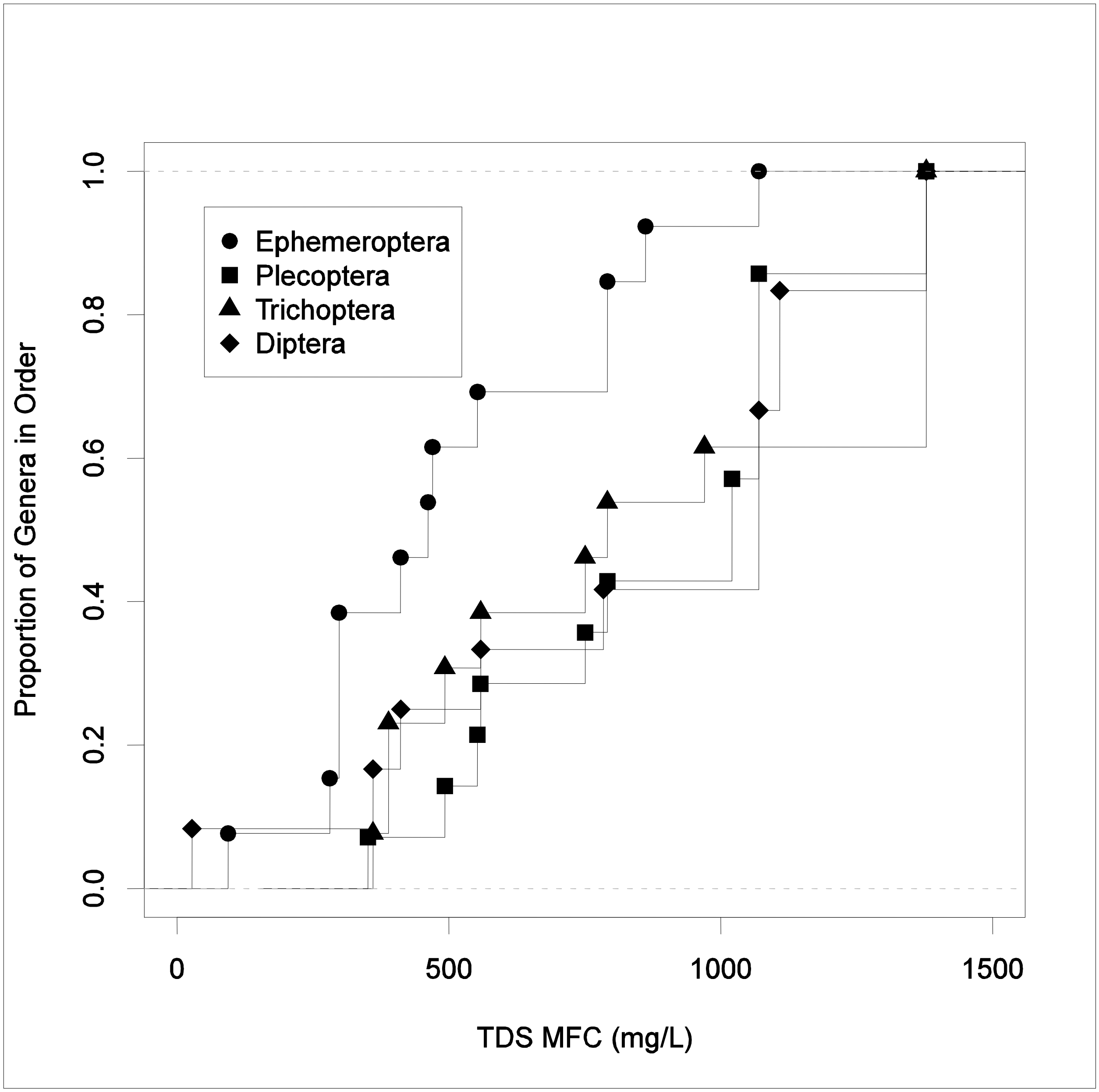

Figure 4.4. Plot of All-Year cumulative distribution functions for total dissolved solids (TDS) maximum field concentrations (MFCs) by taxonomic order for the four most abundant taxonomic orders.

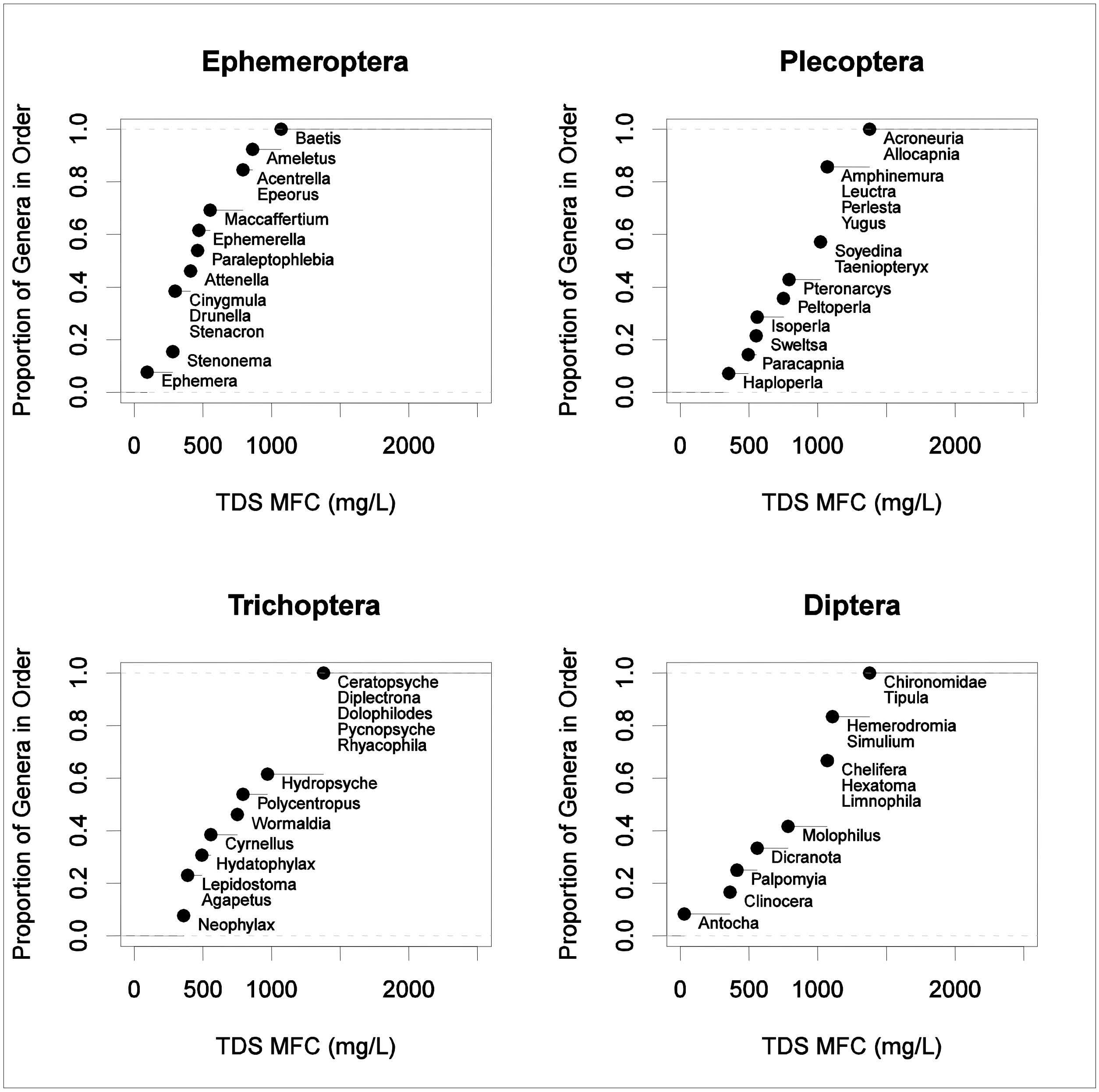

Figure 4.5. Plots of All-Year cumulative distribution functions of total dissolved solids maximum field concentrations (MFCs) with taxa names.

Discussion

Site Selection for Minimizing Influence of Non-TDS Stressors

Reference-quality streams with elevated TDS are rare in Virginia's Central Appalachian ecoregion, because most of the region's streams are influenced by landuses including agriculture, legacy mining, contemporary mining, infrastructure, and commercial, industrial, and/or residential development. Nonetheless, the extensive effort undertaken to locate test sites with abiotic conditions comparable to those of reference sites was successful in minimizing biotic influence from non-TDS stressors, including poor habitat quality (Figure 4.2). This was an important step toward defining TDS sensitivity, because other studies of TDS and effects of related measures in Appalachian coalfield streams have found that habitat quality is often positively correlated with biotic condition (Pond et al. 2008, Pond 2004, Howard et al. 2001).

Water Chemistry

Test site TDS was dominated by the anions SO_4^{2-} and HCO_3^-, which is typical of alkaline mine drainage in the region (Pond et al. 2008, Timpano et al. 2010, Timpano 2011). Reference site TDS, while significantly lower than TDS at test sites, had a higher relative proportion of the anion HCO_3^- (Timpano 2011). Test sites had significantly higher mean pH than did reference sites, a likely result of the higher HCO_3^- concentrations at the test sites (Table 4.6).

Benthic Macroinvertebrate Taxa Observed

The number of genera used for the All-Year model (60) is lower than the number of genera (128) from West Virginia's ecoregion 69 used by USEPA (2010) in developing a field-based SSD for specific conductance. However, the USEPA data included genus-level specimens in the family Chironomidae. The USEPA genera were also selected from 987 samples, with most sites yielding only a single sample, compared to the 81 samples from 28 sites used here. The USEPA genera were collected in Spring (March-June) and Summer (July-October), rather than Spring (March-May) and Fall (September-November) as was done here. The different sampling periods and number of samples may account for the difference in number of genera observed, whereas the lower taxonomic resolution of Chironomidae in this study contributed to the lower number of genera included here. Increasing the number of genera in the FSD analysis, particularly from the family Chironomidae, would be expected to result in higher OEC_X values. The USEPA (2010) study is the only known attempt to construct FSDs for benthic macroinvertebrates from this region using only field data. Further study of Virginia's coalfield streams would likely increase the number of taxa observed.

Maximum Field Concentrations

I am not aware of any other studies that have evaluated maximum field concentrations for individual taxa found in the Appalachian region, so comparisons to other values are not possible. Kefford et al. (2004a) examined maximum mean concentrations in Australian streams, and for different taxa than were observed here. In the present study some taxa were included in the All-Year model but were not included in one or both of the seasonal models. This is due to the requirement that a taxon be observed in a minimum of four samples to be used in analysis. For instance, if a taxon was observed in four Spring samples and one Fall sample, that taxon would be included in the Spring and All-Year models, but it would not be included in the Fall model (*e.g.*, *Acroneuria*). For taxa not included in both Spring and Fall models, MFCs for Fall tended to be higher. This is likely because the highest TDS levels were observed during the Fall season.

Where a taxon was included in only one of the Spring or Fall models, the All-Year model MFC was often equal to the seasonal model MFC.

Field Sensitivity Distributions
Field sensitivity distributions varied in differences between seasonal models. At the lower portion of the FSD curve, which represents the more-sensitive taxa, the Spring model tended to have higher OEC_X values. However, at upper quantiles of the FSD curves, Fall models tended to have higher OEC_X values. This phenomenon occurred because of the presence of salt-sensitive Ephemeroptera taxa being present in Spring samples and not in Fall samples, coupled with the nominally higher TDS levels in the Fall.

Observed Effect Concentrations
In the present study, the specific conductance OEC_{05} differed from findings of USEPA (2010) in two ways. First, this analysis observed negligible differences among seasonal models. Fall, Spring, and All-Year models had OEC_{05} values for SC of 450, 465, and 462 μS/cm, respectively. This is in contrast to the USEPA (2010) finding of greater variation among seasonal models, with Spring, Summer, and All-Year models indicating specific SC of 322, 479, and 297 μS/cm, respectively, in that study. Second, the present analysis found generally higher SC values associated with biotic effects than did USEPA (2010). The methods of the two studies differed, making direct comparisons of OEC_X values difficult, but the disparate results support observations that the SSD approach is sensitive to data quality, quantity, and summary method (Wheeler et al. 2002).

Results presented here differ from those derived by USEPA (2010) for several reasons, including differences in number of genera used as discussed above. Also, the decision to group Chironomidae and Oligochaeta as individual taxa, as an alternative to genus-level taxonomic identification, likely influenced the FSDs. In addition, because the FSDs here were constructed using non-independent data (81 samples from 28 sites), interpretation of results of this study as a precise analog to results of by USEPA (2010) is not appropriate.

Seasonal Models
The All-Year model, which uses data from both fall- and spring-season samples, is a reasonable choice among seasonal model options for two reasons. First, the models yielded similar results between seasons, with a mean seasonal difference of 7%, suggesting that all of the models are similar. Second, the All-Year model incorporated 60 taxa, whereas the separate Spring and Fall models used only 42 and 41 taxa, respectively. With more taxa accounted for, the All-Year FSD better represents the diversity of benthic macroinvertebrate communities found in the study streams (USEPA 2010).

Maximum Field Concentration vs. Toxicity
Other researchers working outside of the Central Appalachian region of the U.S. have evaluated salt sensitivity of freshwater biota by measuring maximum dissolved salt levels associated with their occurrence *in situ* (Piscart et al. 2006, Kefford et al. 2004a, Leland and Fend 1998, Hart et al. 1991). Although results of such studies could be interpreted to indicate toxicity levels, that is not the interpretation applied here. As noted by Kefford et al. (2004a), the MFC for commonly occurring species often does serve as an indicator of salt toxicity, but MFCs may be

underestimated for relatively rare species because of their inherently low probability of occurrence at a site, regardless of TDS level. Their results suggest that factors in addition to salt concentration influence distributions of the relatively rare taxa (Kefford et al. 2004a).

Findings here are similar to those of Kefford et al. (2004a) in that most of the taxa recorded here were observed infrequently at salt concentrations below the taxon's respective MFC. The median taxon observation frequency at TDS levels < MFC was 27% for the 60 taxa in the All-Year model, with a median taxon observation frequency < MFC of only 15% for taxa with the lowest 20% of MFCs. The relative rarity of taxa occurrence below the MFC suggests that environmental conditions other than salt concentration are influencing distributions of rare taxa. For this reason, the distribution of MFCs over the range of TDS levels are interpreted here as indicating that the recorded taxa vary in salt sensitivity, but MFCs are not interpreted as being equivalent to TDS toxicities. Supplemental analyses revealed that the lower portions of FSD curves, and OEC_X values derived from those curves, are influenced by the minimum-number-of-observations threshold used to determine which rare genera should be included in the FSD (Table A.5).

Salt-Sensitivity by Taxonomic Group

The FSD results were as expected, in that they placed typically salt-sensitive taxa (*e.g.*, Ephemeropteran genera) in the lower portion of the FSDs, whereas the typically less-sensitive taxa (*e.g.*, Plecoptera, Trichoptera, Diptera) occupied upper portions of the FSDs (Figure 4.4). Five of the 10 lowest MFCs in the All-Year model were for Ephemeroptera genera. These findings are consistent with other studies that found Ephemeroptera to be a relatively salt-sensitive group, responding to elevated TDS/SC with decreased relative abundance and decreased richness (Merricks 2007, Pond 2004, Pond and McMurray 2002, Green et al. 2000), shifts toward more facultative genera (Pond 2010, Pond et al. 2008), lower maximum field concentrations (Kefford et al. 2004a), and lower laboratory survival (Kefford 2003, Kennedy 2004) than macroinvertebrates from other groups. This indicates that the FSD approach is useful in identifying biotic-effect levels of TDS for salt-sensitive taxa in coalfield streams.

Variability of TDS

Frequency, duration, and intensity of exposure can affect the biotic impact of a stressor (USEPA 2000). The benthic macroinvertebrate community is a good bioindicator because it integrates effects of stressors over the life cycle of each taxon (Barbour et al. 1999). Early life stages (eggs and hatchlings) of benthic insects can be ≤ 50% as tolerant of salinity as their older counterparts (Kefford et al. 2004b). Because it is the older, more mature specimens that are collected during sampling (mature specimens facilitate reliable identification), it is possible that the TDS observed at the time of collection may not represent the TDS to which the more-sensitive eggs and hatchlings were exposed. Therefore, more-frequent TDS monitoring over the course of one or more years may be necessary to characterize the pattern of TDS exposure throughout life cycles of benthic macroinvertebrates, such that biota-limiting levels of TDS may be more accurately determined.

By measuring TDS only twice per year, it cannot be determined whether the benthic macroinvertebrate community was exposed to a higher, biological effect-inducing level of TDS at some time prior to sampling. Therefore, the OEC_X values derived here were not interpreted as toxicities in the sense that biological effects are ensured at the OEC_X.

Interpreting Results

This analysis was not based on a probabilistic, spatially balanced dataset as used by other investigators to derive stressor effect levels from field data (*e.g.*, Paul and McDonald 2005). For this reason, the OEC_X values are not interpreted as salt concentrations that are biotic effect thresholds for any stream in the region. Rather, the OEC_X values represent TDS levels that are tolerable, or not effect-inducing, to most of the reference-site taxa; it is unknown whether a higher TDS level may also be tolerated by the taxa, because of the uncertainty surrounding temporal variability and exposure patterns of TDS and because of the prominence of relatively rare taxa in lower portions of the FSDs. In addition, because this study was similar to a controlled laboratory experiment in that influence from non-TDS stressors was minimized, the OEC_X values reported are reasonable estimates of TDS levels below which most taxa could be expected to occur in coalfield streams where non-TDS stressors are minimized (Paul and McDonald 2005).

Conclusions

I have shown how the SSD approach can be used with field data to create and FSD to identify TDS levels that are associated with low observation frequency of benthic macroinvertebrate genera in headwater streams of Virginia’s Central Appalachian coalfield region. My results indicate that the observed FSD reflects salt sensitivity of benthic macroinvertebrate genera. However, I do not interpret observed effect concentrations as toxic levels for respective water quality measures because many TDS-sensitive genera occurred infrequently in samples with salt concentrations below the MFC for that genus. The fact that taxa were not observed at concentrations above the MFC indicates salt sensitivity, although likely not at the precise concentration defined by the MFC.

I observed no seasonal differences in observed effect concentrations (OECs) and interpret the All-Year model comprised of data from both fall and spring sampling seasons as a better representation of benthic macroinvertebrate diversity in elevated-TDS coalfield streams, compared to the Spring and Fall models alone. These data indicate that concentrations of TDS from 411 to 281 mg/L are associated with observance in test sites of 80 to 95%, respectively, of genera observed at reference sites. However the OEC_X values derived here should be considered as estimates of salt tolerance, because there were relatively uncommon taxa included in the analysis. The small number of sites sampled (28) limited the scope of my findings and suggests that further study of additional streams in the region would likely increase the number of genera observed, as well as better determine which genera are rare, thus improving the accuracy of any OEC_X determination.

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CHAPTER 5. SUMMARY AND CONCLUSIONS

Summary

Modern societies face decisions and questions about how to limit environmental impacts so as to allow essential economic activity, while also protecting a socially desirable level of environmental quality. Currently, such questions are in the public eye in the Appalachian region of the U. S., where coal mining is an important industry, but also produces environmental disturbances. Recent studies have found that benthic macroinvertebrate communities in streams below Appalachian surface coal mines often differ from communities found in streams draining non-mined catchments (Green et al. 2000, Paybins et al. 2000, Pond 2004, Hartman et al. 2005, Merricks et al. 2007, Pond et al. 2008). Elevated levels of total dissolved solids (TDS) have been suggested as a primary aquatic life stressor in streams influenced by coal mining (*e.g.*, Green et al. 2000, Pond 2004, Pond et al. 2008). Although field studies have found altered aquatic communities in streams affected by coal mining, much remains unknown about how benthic macroinvertebrate communities respond to specific TDS concentrations and compositions. In studies conducted to date, both non-TDS stressors and elevated TDS have been present as potential influences on biota in the streams assessed (Pond et al. 2008, Hartman et al. 2005, Howard et al. 2001).

Research reported here was conducted to characterize the biotic response to elevated TDS by surveying streams with a range of TDS concentrations where non-TDS stressors were minimized. I evaluated TDS-biological condition associations of headwater streams in Virginia's Central Appalachian coalfields using family- and genus-level benthic macroinvertebrate data. I first determined TDS, specific conductance (SC), and sulfate (SO_4^{2-}) levels associated with biological effects as defined using the Virginia Stream Condition Index (VASCI), a family-level multimetric index of benthic macroinvertebrate community composition that is used for Clean Water Act enforcement in Virginia's non-coastal streams (Burton and Gerritsen 2003, VDEQ 2010). Then, I used an approach similar to that of USEPA (2010), applying the traditionally laboratory-based species sensitivity distribution (SSD) method to construct field sensitivity distributions (FSDs) using genus-level benthic macroinvertebrate field data. The FSDs were used to determine maximum field concentrations (MFCs) of TDS, SC, and SO_4^{2-} associated with absence of specific proportions of reference taxa.

With the study population constrained to headwater streams of Virginia's Central Appalachian coalfield region, I addressed the following research questions:

1) Can streams be located where non-TDS stressors are minimized such that the influence of dissolved solids on biological condition can be more accurately measured?

2) Is there an association between benthic macroinvertebrate community composition and TDS/component ion/SC level?

3) What is the ionic composition of TDS in streams of this region?

4) Does the ionic composition of TDS influence the association between TDS/component ion/SC and benthic macroinvertebrate community composition?

5) What level of TDS/component ion/SC is associated with benthic macroinvertebrate community composition effects as defined by VASCI score < 60?

6) What TDS/component ion/specific conductance levels are associated with absence of benthic macroinvertebrate genera using a taxa sensitivity distribution approach with field data?

Twenty-one first- and second-order streams (Strahler 1957) within the Virginia portion of Ecoregion 69 (Omernik 1987) were selected for study that had elevated TDS, where non-TDS factors were of reference-quality, with no detectable influence from poor habitat quality or toxic trace metals. Benthic macroinvertebrate and water quality samples were collected up to four times during the Spring (March through May) of 2009 and 2010, and Fall (September through November) of 2008 and 2009 biological index periods (VDEQ 2008). Benthic macroinvertebrate collections followed the single-habitat (riffle-run) approach (VDEQ 2008), which is based on U.S. EPA Rapid Bioassessment Protocols (Barbour et al. 1999).

Streamwater temperature, dissolved oxygen (DO), SC (at 25 °C), and pH were measured *in situ* with a calibrated handheld multi-probe meter. Single grab samples of streamwater were collected for measurement of TDS, alkalinity/HCO_3^-, dissolved SO_4^{2-}, Cl^-, Ca^{2+}, Mg^{2+}, K^+, Na^+, and all species of dissolved Al, Cu, Fe, Mn, Se, and Zn (APHA 2005).

Analyses of water quality-biota associations focused on SC, TDS, and SO_4^{2-}, because Spearman correlation analysis revealed that those are the water quality measures found to be most highly correlated with biological condition at the study sites. Ordinary least squares (OLS) and quantile linear regression analyses were conducted using VASCI scores versus transformed (natural log) values for water quality measurements. I created FSDs for SC, TDS, and SO_4^{2-} using the maximum field concentration at which each genus was observed. I then calculated observed effect concentrations (OEC_X), which were water quality concentrations above which X% of genera were not observed.

Mean relative ionic composition of streamwater at reference sites was dominated on a mass basis by HCO_3^- (43%) and SO_4^{2-} (26%), followed by Ca^{2+}, Cl^-, Na^+, Mg^{2+}, and K^+. Mean dissolved ion composition of streamwater at test sites was dominated on a mass basis by SO_4^{2-} (46%) and HCO_3^- (27%), followed by Ca^{2+}, Mg^{2+}, and Na^+. At test sites, Cl^- and K^+ each comprised approximately 1% of total ion concentration.

As TDS and associated water quality measures increased above reference levels, the probability of observing biological effects increased. Biological effects, as defined by VASCI score < 60, were observed with increasing probability from 0% at ≤ 190 mg/L TDS to 100% at ≥ 1,108 mg/L TDS, with 50% probability of effects observed at 422 mg/L TDS. Effect probabilities of 0, 50, and 100% were associated with SC values of 332, 625, and 1,366 µS/cm, respectively. Sulfate (SO_4^{2-}) concentrations of 70, 219, and 849 mg/L were associated with 0, 50, and 100%

probabilities of effect, respectively. Construction of genus-level SSD curves revealed similar OEC_X values regardless of season. Higher TDS levels were associated with observance of fewer taxa. Results were derived using taxa observed in ≥ 4 samples in order to limit influence from rare taxa, but the method is sensitive to the number of taxa included. Concentrations of TDS from 411 to 281 mg/L were associated with observance of 80 to 95%, respectively, of taxa present in reference samples. Specific conductance levels from 647 to 465 µS/cm were associated with observance of 80 to 95%, respectively, of reference taxa,. Sulfate concentrations from 250 to 160 mg/L were associated with observance of 80 to 95%, respectively, of reference taxa.

Salt sensitivity varied among groups at multiple taxonomic levels. At the order level, genera of the typically salt-sensitive order Ephemeroptera exhibited lower MFCs than genera from other orders. Trichoptera genera had the second lowest MFCs. Orders Plecoptera and Diptera were similarly sensitive to SC, being observed at higher MFCs than other orders.

Conclusions

I was able to effectively isolate the dissolved solids variable in a survey of biological response to elevated TDS in headwater streams in Virginia's Central Appalachian coalfield region. Family-level richness of the benthic macroinvertebrate orders Ephemeroptera, Plecoptera, and Trichoptera declined with increasing TDS, as did overall richness, Ephemeroptera abundance, scraper abundance, and VASCI score. The test streams in this study were similarly dominated by SO_4^{2-} and HCO_3^-, such that no evaluation could be made of the influence of ionic composition on relationships between water quality measures and biological condition. Biological effects, as defined by VASCI score < 60, were associated with TDS, with an increasing probability of effects as TDS concentration increased. However, associations between these water quality measures and VASCI score were variable, with approximately 47% of the variance explained by ordinary least squares regression. It is not evident from the data whether the biological condition observed was the result of concurrent water quality or whether organisms were influenced by higher levels of dissolved solids at some time prior to sampling, potentially during more-sensitive early life stages. More-frequent water quality monitoring could be employed to answer this question.

I have shown how the SSD approach can be used with field data to identify TDS levels that are associated with low observation frequency of benthic macroinvertebrate genera in headwater streams of Virginia's Central Appalachian coalfield region. Results observed here suggest that the FSD reflects salt sensitivity of benthic macroinvertebrate genera, but I do not interpret OEC_X values as toxic levels because many TDS-sensitive taxa occurred infrequently in samples with salt concentrations below the MFC field concentration for that genus. The fact that taxa were not observed at concentrations above the MFC indicates salt sensitivity, although likely not at the precise concentration defined by the MFC. However, the OEC_X values derived here should be considered as estimates, because there were relatively uncommon taxa included in the analysis. The general applicability of these findings is also limited by the small number of sites sampled (28). This suggests that further study of additional streams in the region would likely increase the number of genera included, and allow better determination of which genera are rare, thus improving the accuracy of the FSD method for defining TDS sensitivities.

It is important to note factors that should be considered when interpreting these results. Interpretation of these results is limited because the study design was not statistically unbiased in the manner by which sites were selected. I employed a strict targeted approach to site selection in order to isolate TDS effects. In that way, this study was less like a spatially-balanced probabilistic survey and more similar to a laboratory toxicity test where a gradient of treatment levels are assigned to experimental units free from influence by confounding factors. However, I did not have control of the specific treatment levels in this study and thus the frequency distribution of observations is not even across the gradient of TDS. I made multiple sampling visits to a changing number of sites across sample seasons; thus, the data set is not seasonally balanced. Despite these limitations, these results provide strong support for the use of the OECs for TDS and/or a highly correlated measure such as SC or SO_4^{2-} as a water quality measure that can be interpreted as a level of dissolved solids above which aquatic life effects are increasingly probable in headwater streams of Virginia's Central Appalachian coalfield region.

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APPENDIX A – RAW DATA

Table A.1. Sample information by sample ID.

Chemical Sample ID	Biological Sample ID	Date Collected	Sample Season	Site Type	Station ID	Stream Name
C20081116A	G200B20081116A	11/16/2008	Fall 2008	Test	BIR	Birchfield Creek
C20081121A	G200B20081121A	11/21/2008	Fall 2008	Test	MIL	Mill Branch Left Fork
C20081121B	G200B20081121B	11/21/2008	Fall 2008	Test	POW	Powell River
C20081121C	G200B20081121C	11/21/2008	Fall 2008	Ref	BUR	Burns Creek
C20081126B	G200B20081126BD	11/26/2008	Fall 2008	Test	GRA	Grape Branch
C20081126D	G200B20081126D	11/26/2008	Fall 2008	Test	SPC	Spruce Pine Creek
C20081128A	G200B20081128A	11/28/2008	Fall 2008	Ref	CLE	Clear Creek
C20081128B	G200B20081128B	11/28/2008	Fall 2008	Ref	EAS	Eastland Creek
C20090512A	G200B20090512A	05/12/2009	Spring 2009	Test	SPC	Spruce Pine Creek
C20090512B	G200B20090512B	05/12/2009	Spring 2009	Test	GRA	Grape Branch
C20090513A	G200B20090513A	05/13/2009	Spring 2009	Test	HUR	Hurricane Fork
C20090513B	G200B20090513B	05/13/2009	Spring 2009	Test	RAC	Race Fork UT
C20090513C	G200B20090513C	05/13/2009	Spring 2009	Test	SPR	Spring Branch
C20090513D	G200B20090513D	05/13/2009	Spring 2009	Test	JES	Jess Fork
C20090514A	G200B20090514A	05/14/2009	Spring 2009	Test	RFF	Fryingpan Creek Right Fork
C20090514B	G200B20090514B	05/14/2009	Spring 2009	Test	FRY	Fryingpan Creek
C20090520A	G200B20090520A	05/20/2009	Spring 2009	Test	ROL	Roll Pone Branch
C20090520B	G200B20090520B	05/20/2009	Spring 2009	Test	GIN	Gin Creek
C20090520C	G200B20090520C	05/20/2009	Spring 2009	Test	FAW	Fawn Branch
C20090521A	G200B20090521A	05/21/2009	Spring 2009	Test	CAW	Callahan Creek West Fork
C20090521B	G200B20090521B	05/21/2009	Spring 2009	Test	MIL	Mill Branch Left Fork
C20090521C	G200B20090521C	05/21/2009	Spring 2009	Test	POW	Powell River
C20090522A	G200B20090522A	05/22/2009	Spring 2009	Test	BIR	Birchfield Creek
C20090522B	G200B20090522B	05/22/2009	Spring 2009	Ref	CLE	Clear Creek
C20090522C	G200B20090522C	05/22/2009	Spring 2009	Ref	EAS	Eastland Creek
C20090522D	G200B20090522D	05/22/2009	Spring 2009	Ref	BUR	Burns Creek
C20090602B	G200B20090602A	06/02/2009	Spring 2009	Test	LAU	Laurel Fork
C20090610B	G200B20090610B	06/10/2009	Spring 2009	Test	LAB	Laurel Branch
C20091009A	G200B20091009A	10/09/2009	Fall 2009	Test	MIL	Mill Branch Left Fork
C20091009B	G200B20091009B	10/09/2009	Fall 2009	Test	POW	Powell River
C20091009C	G200B20091009C	10/09/2009	Fall 2009	Ref	CLE	Clear Creek
C20091009D	G200B20091009D	10/09/2009	Fall 2009	Ref	EAS	Eastland Creek
C20091009E	G200B20091009E	10/09/2009	Fall 2009	Ref	BUR	Burns Creek
C20091030A	G200B20091030A	10/30/2009	Fall 2009	Test	KEL	Kelly Branch
C20091030B	G200B20091030B	10/30/2009	Fall 2009	Test	KUT	Kelly Branch UT
C20091030C	G200B20091030C	10/30/2009	Fall 2009	Test	BIR	Birchfield Creek
C20091030D	G200B20091030D	10/30/2009	Fall 2009	Test	RIC	Richey Branch
C20091030E	G200B20091030E	10/30/2009	Fall 2009	Test	RUT	Richey Branch UT
C20091031A	G200B20091031A	10/31/2009	Fall 2009	Test	ROL	Roll Pone Branch
C20091031B	G200B20091031B	10/31/2009	Fall 2009	Test	LAB	Laurel Branch
C20091031C	G200B20091031C	10/31/2009	Fall 2009	Test	RFF	Fryingpan Creek Right Fork
C20091031D	G200B20091031D	10/31/2009	Fall 2009	Test	FRY	Fryingpan Creek
C20091106A	G200B20091106A	11/06/2009	Fall 2009	Test	CAW	Callahan Creek West Fork
C20091106B	G200B20091106B	11/06/2009	Fall 2009	Test	LAU	Laurel Fork
C20091106C	G200B20091106C	11/06/2009	Fall 2009	Test	GIN	Gin Creek
C20091106D	G200B20091106D	11/06/2009	Fall 2009	Test	FAW	Fawn Branch
C20091106E	G200B20091106E	11/06/2009	Fall 2009	Test	CAN	Cane Branch
C20091107A	G200B20091107A	11/07/2009	Fall 2009	Test	JES	Jess Fork
C20091107B	G200B20091107B	11/07/2009	Fall 2009	Test	HUR	Hurricane Fork
C20091107C	G200B20091107C	11/07/2009	Fall 2009	Test	RAC	Race Fork UT
C20091107D	G200B20091107D	11/07/2009	Fall 2009	Test	SPR	Spring Branch
C20091107E	G200B20091107E	11/07/2009	Fall 2009	Test	GRA	Grape Branch
C20091107F	G200B20091107F	11/07/2009	Fall 2009	Test	SPC	Spruce Pine Creek
C20100519A	G200B20100519A	05/19/2010	Spring 2010	Test	MIL	Mill Branch Left Fork
C20100519B	G200B20100519B	05/19/2010	Spring 2010	Test	KEL	Kelly Branch
C20100519C	G200B20100519C	05/19/2010	Spring 2010	Test	KUT	Kelly Branch UT
C20100519D	G200B20100519D	05/19/2010	Spring 2010	Test	LAU	Laurel Fork
C20100519E	G200B20100519E	05/19/2010	Spring 2010	Test	POW	Powell River
C20100520A	G200B20100520A	05/20/2010	Spring 2010	Test	BIR	Birchfield Creek
C20100520B	G200B20100520B	05/20/2010	Spring 2010	Test	RIC	Richey Branch
C20100520C	G200B20100520C	05/20/2010	Spring 2010	Test	RUT	Richey Branch UT
C20100520D	G200B20100520D	05/20/2010	Spring 2010	Test	FRY	Fryingpan Creek
C20100520E	G200B20100520E	05/20/2010	Spring 2010	Test	RFF	Fryingpan Creek Right Fork
C20100520F	G200B20100520F	05/20/2010	Spring 2010	Test	LAB	Laurel Branch
C20100521A	G200B20100521A	05/21/2010	Spring 2010	Ref	CLE	Clear Creek
C20100521B	G200B20100521B	05/21/2010	Spring 2010	Ref	EAS	Eastland Creek
C20100521C	G200B20100521C	05/21/2010	Spring 2010	Ref	BUR	Burns Creek
C20100521D	G200B20100521D	05/21/2010	Spring 2010	Ref	COP	Copperhead Branch
C20100521E	G200B20100521E	05/21/2010	Spring 2010	Ref	CRO	Crooked Branch
C20100521F	G200B20100521F	05/21/2010	Spring 2010	Ref	MCB	Middle Camp Branch
C20100521G	G200B20100521G	05/21/2010	Spring 2010	Test	JES	Jess Fork
C20100524A	G200B20100524A	05/24/2010	Spring 2010	Test	SPC	Spruce Pine Creek
C20100524B	G200B20100524B	05/24/2010	Spring 2010	Test	GRA	Grape Branch
C20100524C	G200B20100524C	05/24/2010	Spring 2010	Test	HUR	Hurricane Fork
C20100524D	G200B20100524D	05/24/2010	Spring 2010	Test	RAC	Race Fork UT
C20100524E	G200B20100524E	05/24/2010	Spring 2010	Test	SPR	Spring Branch
C20100525A	G200B20100525A	05/25/2010	Spring 2010	Test	GIN	Gin Creek
C20100525B	G200B20100525B	05/25/2010	Spring 2010	Test	FAW	Fawn Branch
C20100525C	G200B20100525C	05/25/2010	Spring 2010	Test	CAW	Callahan Creek West Fork
C20100526A	G200B20100526A	05/26/2010	Spring 2010	Test	CAN	Cane Branch
C20100526B	G200B20100526B	05/26/2010	Spring 2010	Test	ROL	Roll Pone Branch

Table A.2. Chemical data by sample ID.

Sample ID	Temp	pH	DO	Specific Conductance	TDS	Cl^-	SO_4^{2-}	Total Alkalinity	HCO_3^-	Ca^{2+}	K^+	Mg^{2+}	Na^+	Al	Cu	Fe	Mn	Se	Zn
	°C		mg/L	µS/cm	mg/L	mg/L	mg/L	mg/L as CaCO3	mg/L	mg/L	mg/L	mg/L	mg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
C20081116A	7.04	7.61	11.56	755	556	8.4	233.3	153.2	178.1	75.3	4.9	55.0	27.9	1.4	4.4	52.4	0.8	2.5	18.7
C20081121A	2.53	7.68	12.25	1183	862	2.0	213.1	165.6	187.5	158.7	5.4	66.9	36.4	1.4	4.4	410.9	160.3	7.5	18.7
C20081121B	2.75	7.54	10.89	865	694	1.0	249.9	127.0	152.0	120.9	3.5	67.3	12.7	7.5	4.4	19.7	2.6	5.3	18.7
C20081121C	1.74	7.26	10.96	24	5	2.3	5.4	9.9	12.1	0.4	0.4	0.5	3.7	1.4	4.4	19.7	0.8	6.4	18.7
C20081126B	4.16	7.63	10.80	546	352	6.0	204.6	63.3	77.3	53.5	2.3	21.5	45.3	1.4	4.4	19.7	0.8	2.5	18.7
C20081126D	3.17	8.49	10.97	575	364	3.8	128.4	207.1	239.8	40.2	1.8	17.3	84.9	1.4	4.4	19.7	0.8	2.5	18.7
C20081128A	3.98	6.64	11.81	21	5	0.9	4.3	9.3	11.4	2.0	0.6	0.7	1.0	1.4	4.4	19.7	0.8	6.3	18.7
C20081128B	5.16	6.66	9.67	17	11	0.8	3.6	8.3	10.1	1.9	0.4	0.6	0.6	1.4	4.4	19.7	0.8	2.5	18.7
C20090512A	14.99	7.73	9.75	332	174	5.7	109.3	45.6	55.6	29.0	1.9	14.9	18.5	40.8	11.4	24.3	61.5	12.0	8.0
C20090512B	13.00	7.56	9.16	143	63	4.2	39.4	22.0	26.9	13.4	1.6	5.8	5.0	4.9	11.4	11.1	7.8	12.0	8.0
C20090513A	12.10	7.26	9.68	490	290	1.4	220.9	32.1	39.1	45.2	2.9	33.4	9.4	4.9	11.4	11.1	7.8	12.0	8.0
C20090513B	12.64	7.67	9.36	340	218	1.2	114.4	79.9	97.5	36.5	3.2	18.4	21.5	4.9	11.4	11.1	7.8	12.0	8.0
C20090513C	13.21	7.51	7.81	339	205	1.1	138.0	38.8	47.3	36.0	2.2	24.0	3.3	4.9	11.4	11.1	7.8	12.0	8.0
C20090513D	12.14	6.57	10.17	757	567	1.4	456.2	4.2	5.1	85.1	3.1	50.5	10.8	22.1	11.4	74.8	787.9	12.0	116.0
C20090514A	13.02	8.45	10.22	607	361	7.7	152.1	158.5	192.6	46.2	2.8	19.9	67.0	4.9	11.4	11.1	7.8	12.0	8.0
C20090514B	13.38	8.15	9.03	462	298	9.8	156.0	70.7	86.2	46.4	3.9	24.6	20.3	4.9	11.4	11.1	7.8	12.0	8.0
C20090520A	12.24	7.62	9.83	594	389	4.5	249.6	78.6	95.8	65.3	2.9	33.4	18.5	4.9	11.4	11.1	7.8	12.0	8.0
C20090520B	14.80	8.39	9.55	706	470	8.5	155.0	251.6	301.7	32.4	4.6	14.4	135.9	4.9	11.4	11.1	7.8	12.0	8.0
C20090520C	13.50	8.02		265	168	1.1	68.0	73.7	89.9	28.5	2.3	14.3	10.4	4.9	11.4	11.1	7.8	12.0	8.0
C20090521A	10.85	7.93	9.36	304	205	0.9	100.1	65.9	80.4	35.6	2.2	17.0	7.9	4.9	11.4	11.1	7.8	12.0	8.0
C20090521B	14.06	8.25	8.69	878	633	1.9	396.8	131.3	160.2	107.3	5.0	54.9	19.5	34.9	11.4	100.2	37.9	12.0	8.0
C20090521C	13.99	7.95	8.55	970	792	1.0	531.4	115.6	141.0	119.9	4.2	75.4	10.0	15.6	11.4	72.0	7.8	12.0	8.0
C20090522A	17.54	7.90	8.09	736	538	3.2	378.2	107.2	130.8	71.7	3.8	54.8	20.1	4.9	11.4	51.5	44.7	12.0	8.0
C20090522B	11.47	7.80	9.93	16	25	0.8	3.7	2.9	3.5	1.7	0.3	0.6	0.4	41.9	11.4	11.1	7.8	12.0	8.0
C20090522C	11.67	6.85	10.21	21	26	0.8	3.2	5.7	7.0	2.7	0.4	0.7	0.5	4.9	11.4	11.1	7.8	12.0	8.0
C20090522D	11.96	6.11	10.11	22	37	2.7	4.6	0.6	0.7	1.3	0.4	0.6	1.9	4.9	11.4	11.1	7.8	12.0	8.0
C20090602B	15.03	6.90	8.59	25	28	0.9	4.2	7.2	8.8	2.2	1.4	1.5	1.0	4.9	11.4	319.9	7.8	12.0	8.0
C20090610B	14.16	7.90	8.27	842	558	8.8	311.1	109.4	133.5	87.9	4.3	45.3	42.3	4.9	11.4	11.1	7.8	12.0	8.0
C20091009A	15.00	7.37	8.42	845	588	1.1	350.8	133.6	163.0	104.4	5.1	49.6	15.2	15.3	6.5	71.3	93.0	17.2	13.5
C20091009B	15.08	7.46	8.93	1087	751	0.5	477.2	126.7	154.6	122.7	4.5	72.0	9.7	11.4	6.5	32.5	14.1	16.6	17.4
C20091009C	13.81	7.25	8.39	20	14	0.5	3.1	6.3	7.7	2.1	0.4	0.6	0.6	35.1	6.5	32.5	12.2	8.1	10.9
C20091009D	13.79	7.15	7.69	22	10	0.4	2.8	8.0	9.7	2.8	0.4	0.7	0.6	4.3	6.5	32.5	5.1	8.1	10.3
C20091009E	13.62	6.50	8.36	21	12	2.0	4.2	1.2	1.5	1.2	0.3	0.5	1.7	11.3	6.5	32.5	6.7	8.1	18.1
C20091030A	11.67	7.30	9.34	873	615	1.8	412.7	88.0	107.4	100.3	4.9	59.6	14.1	4.3	6.5	32.5	9.5	8.1	10.6
C20091030B	12.71	7.96	9.69	1366	1021	1.9	629.3	173.1	211.2	151.8	7.6	82.4	55.3	17.2	6.5	32.5	12.2	22.9	11.3
C20091030C	14.38	7.67	8.45	647	410	8.7	220.7	120.1	146.6	63.2	4.2	46.3	19.4	4.3	6.5	32.5	32.9	8.1	12.0
C20091030D	13.49	7.93	8.49	1670	1378	5.8	849.0	190.8	232.8	183.9	6.5	160.6	14.6	36.4	6.5	32.5	19.7	8.1	10.2
C20091030E	12.85	7.63	8.77	545	388	4.6	219.4	75.1	91.6	46.4	4.2	50.1	5.5	9.9	6.5	32.5	11.7	8.1	10.5
C20091031A	12.97	7.20	8.77	652	462	3.2	272.4	83.4	101.8	76.5	3.9	39.6	16.0	4.3	6.5	32.5	5.8	8.1	11.1
C20091031B	13.01	7.63	9.19	784	553	3.9	282.7	124.7	152.2	82.0	4.3	41.9	46.5	4.3	6.5	32.5	11.2	8.1	11.1
C20091031C	12.90	7.41	8.37	340	218	5.9	76.7	90.5	110.4	29.0	2.4	11.9	29.1	4.3	6.5	32.5	10.4	8.1	10.2
C20091031D	13.08	7.62	9.05	402	263	11.2	100.9	93.1	113.6	37.2	2.4	18.0	27.0	4.3	6.5	32.5	6.6	8.1	10.0
C20091106A	7.81	7.31	10.75	292	187	0.7	88.2	68.4	83.4	33.1	2.1	14.9	6.8	9.4	6.5	32.5	6.3	8.1	12.6

Table A.2 (cont'd). Chemical data by sample ID.

Sample ID	Temp °C	pH	DO mg/L	Specific Conductance µS/cm	TDS mg/L	Cl^- mg/L	SO_4^{2-} mg/L	Total Alkalinity mg/L as CaCO3	HCO_3^- mg/L	Ca^{2+} mg/L	K^+ mg/L	Mg^{2+} mg/L	Na^+ mg/L	Al µg/L	Cu µg/L	Fe µg/L	Mn µg/L	Se µg/L	Zn µg/L
C20091106B	7.04	7.23	10.15	20	33	0.5	4.3	6.0	7.3	1.5	0.6	1.1	0.7	12.4	6.5	80.8	10.6	8.1	15.9
C20091106C	11.14	8.07	9.21	656	411	8.5	112.9	232.9	280.0	28.7	3.7	11.8	117.1	4.3	6.5	32.5	7.4	8.1	13.7
C20091106D	9.55	7.49	8.52	281	164	2.2	67.4	81.5	99.4	29.9	2.2	13.7	10.9	4.3	6.5	32.5	8.1	8.1	12.8
C20091106E	10.01	7.96	9.13	1462	1108	5.1	679.4	202.0	246.4	141.8	7.4	97.0	76.8	25.8	6.5	32.5	86.7	8.1	10.3
C20091107A	6.74	7.22	11.35	682	493	1.1	340.4	49.3	60.2	81.6	3.3	45.2	9.9	20.0	6.5	32.5	12.7	8.1	16.7
C20091107B	7.31	7.21	11.06	383	258	1.1	166.5	34.2	41.7	36.0	2.5	27.3	7.7	8.7	6.5	32.5	17.2	8.1	11.6
C20091107C	9.31	7.25	9.69	450	273	1.1	163.0	81.4	99.3	46.4	2.5	23.8	20.7	4.3	6.5	32.5	6.5	8.1	11.3
C20091107D	7.68	7.30	10.69	274	156	0.8	92.3	53.3	65.1	27.6	1.9	18.3	3.8	30.7	6.5	69.9	8.7	8.1	10.8
C20091107E	8.35	7.27	10.12	339	202	4.2	119.7	51.6	62.9	32.1	2.0	13.3	21.0	4.3	6.5	32.5	6.3	8.1	11.4
C20091107F	9.01	7.79	10.05	468	281	3.4	108.2	142.9	174.3	37.0	1.9	15.7	53.8	4.3	6.5	32.5	14.7	8.1	10.7
C20100519A	13.16	8.04	9.07	597	398	1.4	221.2	112.1	136.8	70.3	3.7	39.8	8.8	24.5	8.9	40.0	56.8	8.5	3.7
C20100519B	12.74	7.87	8.93	769	546	1.7	356.2	86.9	106.0	88.1	4.3	56.5	10.2	23.5	8.9	16.1	19.0	8.5	3.7
C20100519C	13.51	8.25	9.02	1061	784	2.1	494.2	140.2	171.1	120.2	6.2	72.6	35.4	39.3	18.0	85.5	22.1	28.3	7.8
C20100519D	13.11	7.78	8.76	25	16	0.5	5.2	6.9	8.4	2.0	0.7	1.4	0.8	20.3	8.9	83.8	15.2	8.5	3.7
C20100519E	12.41	7.95		707	492	1.0	305.7	93.8	114.5	83.2	3.2	51.4	5.9	28.6	8.9	16.1	12.7	8.5	3.7
C20100520A	15.11	7.91	8.65	588	380	1.3	167.9	115.8	141.3	56.5	2.9	43.3	15.9	26.0	8.9	68.1	253.1	8.5	3.7
C20100520B	12.27	8.23	9.54	1335	1070	15.1	768.6	163.0	198.9	137.1	4.4	112.2	9.9	23.0	8.9	16.1	19.3	8.5	3.7
C20100520C	13.08	8.10	9.40	485	316			60.6	73.9	39.7	2.3	44.9	2.8	20.2	8.9	16.1	7.0	8.5	3.7
C20100520D	14.24	8.03	9.12	300	184	5.5	89.5	57.7	70.4	28.7	1.7	12.8	12.5	15.8	8.9	16.1	3.3	8.5	3.7
C20100520E	14.33	8.25	9.33	357	228	3.9	90.3	88.7	108.2	28.6	1.9	9.9	33.8	16.8	8.9	16.1	3.3	8.5	3.7
C20100520F	13.70	7.91	9.44	413	252	2.0	137.7	79.9	97.4	43.0	2.6	20.8	16.1	36.9	8.9	55.7	12.1	8.5	3.7
C20100521A	11.97	6.67	9.81	18	8	0.5	3.4	6.3	7.6	2.0	0.3	0.6	0.5	37.4	8.9	16.1	8.3	8.5	3.7
C20100521B	11.87	7.11	9.72	20	10	0.5	3.2	7.9	9.6	1.6	0.3	0.6	0.6	13.1	8.9	16.1	3.3	8.5	3.7
C20100521C	12.10	7.01	9.71	23	14	2.9	4.4	-2.2	-2.7	1.3	0.3	0.6	2.0	24.0	8.9	16.1	7.9	8.5	3.7
C20100521D	13.24	7.55	8.33	116	76	1.3	22.1	36.1	44.1	12.0	1.4	0.5	1.7	6.3	8.9	16.1	8.0	8.5	3.7
C20100521E	14.40	7.48	9.46	64	40	5.5	9.2	12.2	14.9	3.5	1.3	2.6	0.8	13.6	8.9	34.5	3.3	18.4	3.7
C20100521F	13.68	7.26	10.10	44	24	0.7	11.3	8.8	10.7	2.1	1.1	1.9	1.8	6.3	8.9	16.1	3.3	8.5	3.7
C20100521G	13.55	7.25	9.91	568	396	0.3	280.7	27.5	33.5	63.4	2.4	37.2	6.9	50.5	8.9	100.5	264.9	8.5	35.4
C20100524A	14.26	7.50	10.34	364	190	12.8	106.9	81.0	98.8	31.7	1.8	12.7	27.2	36.3	8.9	91.0	32.8	8.5	3.7
C20100524B	15.47	7.22	8.89	226	106	3.4	69.6	38.8	47.3	18.8	1.7	4.6	14.4	17.9	8.9	46.0	3.3	8.5	3.7
C20100524C	15.62	7.32	9.17	422	248	0.7	178.1	39.5	48.1	39.2	2.4	28.6	8.4	20.1	8.9	33.4	16.5	17.5	3.7
C20100524D	15.39	7.77	9.08	417	230	0.8	127.1	99.3	121.1	40.8	2.3	19.3	23.4	18.8	8.9	16.1	3.3	8.5	3.7
C20100524E	15.66	7.51	8.69	329	164	0.6	126.0	49.4	60.3	34.0	2.1	22.0	0.8	16.8	8.9	16.1	3.3	8.5	3.7
C20100525A	15.18	8.23	8.86	644	348	5.8	113.5	236.5	284.6	28.5	3.6	9.5	111.3	6.3	8.9	16.1	6.7	8.5	3.7
C20100525B	14.28	7.66	8.53	263	94	0.7	59.0	82.7	100.9	28.2	2.0	11.8	8.5	15.3	8.9	67.7	7.8	8.5	3.7
C20100525C	14.37	7.54	8.67	282	112	0.6	82.6	68.4	83.4	32.6	2.0	13.2	5.1	23.2	8.9	16.1	6.9	17.6	3.7
C20100526A	12.86	8.36	8.50	1282	970	0.7	623.4	192.0	234.3	133.4	6.4	98.5	52.6	27.3	8.9	42.7	90.1	24.1	3.7
C20100526B	16.01	8.04	8.26	476	278	7.7	191.7	72.1	87.9	55.8	2.5	29.7	12.9	27.2	8.9	47.7	3.3	8.5	3.7

Table A.3. Benthic macroinvertebrate number of individuals by sample ID.

Taxon Final ID	G200B20081116A	G200B20081121A	G200B20081121B	G200B20081121C	G200B20081126BD	G200B20081126D	G200B20081128A	G200B20081128B	G200B20090512A	G200B20090512B	G200B20090513A	G200B20090513B	G200B20090513C	G200B20090513D	G200B20090514A	G200B20090514B	G200B20090520A	G200B20090520B	G200B20090520C	G200B20090521A	G200B20090521B	G200B20090521C	G200B20090522A	G200B20090522B	G200B20090522C	G200B20090522D	G200B20090602A	G200B20090610B	G200B20091009A	G200B20091009B	G200B20091009C	G200B20091009D	G200B20091009E	G200B20091030A	G200B20091030B	G200B20091030C	G200B20091030D	G200B20091030E	G200B20091031A	G200B20091031B	G200B20091031C	G200B20091031D	G200B20091106A	G200B20091106B	G200B20091106C	G200B20091106D	G200B20091106E	G200B20091107A	G200B20091107B	G200B20091107C	G200B20091107D	G200B20091107E	G200B20091107F	G200B20100519A	G200B20100519B	G200B20100519C	G200B20100519D	G200B20100519E	G200B20100520A	G200B20100520B	G200B20100520C	G200B20100520D	G200B20100520E	G200B20100520F	G200B20100521A	G200B20100521B	G200B20100521C	G200B20100521D	G200B20100521E	G200B20100521F	G200B20100521G	G200B20100524A	G200B20100524B	G200B20100524C	G200B20100524D	G200B20100524E	G200B20100525A	G200B20100525B	G200B20100525C	G200B20100526A	G200B20100526B
Acentrella									10	9	8				28	17		8			1	4						1			1																									1					3	12	8	2	5								3	1				2	5		
Acroneuria				4	3	1	8	2		6	10					3			1	1				4	1	2	1				2	2	12	1			3	2			1	2	2	1					6			2					4			1				1	2	2	1	1	2	2			3	7			1	1	3		1
Agapetus										1							5		1					1																																												3		2											2
Allocapnia	158	144	100	8	63	60	5	8																					51	76				80	10	128	90	54	128	60	144	95	5	28	101	80	109	17	46	27	17	38	13																												
Allognosta																																																																1																	
Ameletus	1	3	1			1	1	2			1	1	4		2	4	11	1	1	11				1	4													1	2				8	1	1	11		2	3	1	1	2					2													1						4			5		1
Amphinemura									11	12	32	38	48	42	31	46	62	9	26	72	51	54	8	8	16	35	2							2																				26	10	4	8	29	10	5	22	5	15	7	17	8	23	3	3	2	55	3	2	11	16	24	6	9	17	1	11
Antocha								1																1			1																														1																								
Arigomphus		1		1	1		1		2	1	2			1							1	3	1	2		1	1	1	2		1		1	5		3	2	12		1						1		1	2					1	7	2	1	2	1		4						1	1	1		2	2	1								
Atherix																																								1																																									
Atrichopogon		1			1																									2																				4	1																														
Attenella				15		2	1																				1						5												1								1														1														
Baetidae								1																	2																								2																																
Baetis					1		1		4	3	1	33	37	31	5	21	14	12	11	2	11	34		22			10	20		2	2	1			1					10			5		1			2			1		3	19	12	54	20	48	3	6	6	5	3	65	23	3	4	28	8	4	30	28	2	2	2	4	41	12	49		11
Beloneuria																																																				1																													
Boyeria																																																					1							1																					
Ceratopogonidae																											1								1																																		2			1									
Ceratopsyche				3		3	3											1										2	13		12	17	40			2	6	1	1	7	1	4	23	4	7		4	1	2			10	2											10			2	3											3		
Chelifera							1		9		3		3	1				1				3	2					3							1															1				8	2	2	1	13	2	5	2	2	7	1				2	1	3	5	14	2	2		21	1		3	2	2
Cheumatopsyche	7	15			3	4			1									2			2							2	51					1		15	37	2		6		3		1	16		13					3	15	2						10				3								2					3		1	4	17
Chimarra	6					6																	11													16									1								4						13																						
Chironomidae (A)	6	5	6	119	7	16	41	25	23	2	13	1	2	4	3	3	3	24	7	8	16	2	32	4	8	49	50	8	17	14	26	6	31	13	28	5	5	19	7	25	14	12	14	18	15	11	20	48	25	39	27	23	8	20	8	19	52	6	14	33	16	14	17	8	17	22		37	34	40	7	25	6	5	8	7	12	17	12	30	23
Chloroperlidae																																										1							17																											1					
Cinygmula																1			3					1			1																																																						
Clinocera															1											2																									1																							1							
Cordulegaster												1		1																																											1																								
Curculionidae																																																																																	
Cyrnellus																												1			2											1		1																													1								
Dicranota								1																		1		3															2						9	1	6						1										1	1	3					2							1
Diphetor																																																			25																														
Diplectrona	8	4	36	5	44	52	5	7	7	36	14	5	5	17		12		14			7	16		3	15	6	9	12	34	28	43	79	37	42	109	11	24	68	46	94	10	34	17	35	30	12	27	89	25	20	10	52	68	5	23	53	17	13		24	11	1	4	20	12	20	6	19	7	2	61	12	27	15	31	17	10	4	9	3	6
Diploperla																																												2																																					
Dixa		1										1																	1					1	1			2	1	1			1			1				7	2																									1					
Dolichopodidae												1																	1																													1																			1					
Dolophilodes			3	7	2	9				2	1							4					9	6	6	1	18				11	5	5				4			4		1		15	2				1			4		1			8			8	6	2	3	1	8	14	35	8	32	2		1	16	2							
Drunella									11	17	1					14								4																																						6	11					1	3			7	4	1							
Ectopria				1	1			1	1	1	3								1	1		2		3	2		1	1		2	6		2	2	3		1				1					2				2		1	2		2								1	1	2		1	3		4			4		1		1	1	1		1
Elmidae									3																					2																																																			
Empididae																																																						1																1			2				1			1	
Epeorus					3		7			9	2	6	4		1	1		5	13	7		2		36	36	8					3												24	20		8			6		18	1					1	1				1			19	35		22	3	3					1	13	10	22	15		
Ephemera																											1				1																										1																					1			
Ephemerella									4	4	11	2	7		6	2		32	13	10				14	9		2																	2													10					1			8	8		18	1	4		2		2		1	18	2	14		
Ephemerellidae																																											1			2			2				3																												
Ephydridae																																																														1																			
Eurylophella				6																													1																																																
Glossosoma																																																															2									2					1				
Gomphidae																																																				1																													
Haploperla					2					1	10		13						2	1					2																																1							4	3	2	3	2	2						2	3			5		
Helichus															1									1															1		1																																								
Hemerodromia				1					1					1																																	1		1	2		1		1							1							1													
Heptagenia						1																																																																										
Heptageniidae				5	1																					1														1		1		1													5						2		3	3			1	3											
Hexatoma			2	3				1	2				2		1							1		4	3	7	6					1	3											1		3			1	2	1						5			1					2		3		2	6				1		2					
Homoplectra																																																																												1					
Hydatophylax																																	1					4				2						2	3			2																													
Hydracarina																																	1																																																
Hydrochus																												1																																																					
Hydrophilidae																																		1				1												1																															
Hydropsyche	2	1		1				3										2			3																					1												1					1																1		1			1	
Hydropsychidae																							3																																																										
Hydroptila																						1																																						1																					
Hydroptilidae																																																	1																															
Isonychia																											1																																						3				2												
Isoperla				7	1													6						1			4	1			3	5	1								2	1	17			4			7		5						4	2			1			3	1			2		1							1	1			
Lepidoptera																																																			2																														
Lepidostoma							1										1							3	1		5																																						3	1				2											1
Leptophlebiidae															1																												1						2																													2			
Leuctra		1	11		1	1	5	34	80	61	58	89	42	109	47	39	84	52	97	59	91	42	85	45	61	42	20	101	2	31	3	6	1	13				1		2			4					7	2	2			2	101	121	43	16	50	124	73	71	114	87	48	25	42	65	4	42	47	24	68	109	124	108	57	58	95	22	124	77
Limnephilidae					2		2	8																							1				3		1											1		7		1																															
Limnophila		2								2	2	1					2					1		3		1					2		1					5	1				1														2	1		2	1							1	5	3	2			1	1				1	1	
Limonia																										1						1																																																	
Lype																															1																																																		
Maccaffertium				1	2		3																			1	1				3	3	5							2						2							9				2						1		3	2		1		2											
Macronychus									1														1																																																										
Mayatrichia																																																																																	1
Molophilus								1			1	1												2																										1						1						1						1													
Nemouridae		2				9															1															5																																					1		4					1	
Neophylax							1	5				2	1		30	2			2					1	3		1															1									4	2											4					8		3		2				1			1		2

Table A.3 (cont'd). Benthic macroinvertebrate number of individuals by sample ID.

Taxon Final ID	G200B20081116A	G200B20081121A	G200B20081121B	G200B20081121C	G200B20081126BD	G200B20081126D	G200B20081128A	G200B20081128B	G200B20090512A	G200B20090512B	G200B20090513A	G200B20090513B	G200B20090513C	G200B20090513D	G200B20090514A	G200B20090514B	G200B20090520A	G200B20090520B	G200B20090520C	G200B20090521A	G200B20090521B	G200B20090521C	G200B20090522A	G200B20090522B	G200B20090522C	G200B20090522D	G200B20090602A	G200B20090610B	G200B20091009A	G200B20091009B	G200B20091009C	G200B20091009D	G200B20091009E	G200B20091030A	G200B20091030B	G200B20091030C	G200B20091030D	G200B20091030E	G200B20091031A	G200B20091031B
Neotrichia															1																									1
Nigronia	2					1				2	1					1							2									1								2
Oemopteryx							3																																	
Oligochaeta		2		1				3	2	7		1	1	1	20				1		5	2				1			3								1	1		
Optioservus						8		1																				6									2	21		3
Ormosia														1																										
Oulimnius			2	6		2	13	9	3						3	15				4		27		9	2		4			11	21	6	2		3					
Palpomyia				3		2	1	3																		3														
Paracapnia				28	32	2	6	3																							6	2	43							
Paraleptophlebia				3			41	3				1	2				9		4					5	11		9				33	20							1	
Peltoperla				1				1		1		1	6	1						2				2	3	3				1										
Peltoperlidae																											1													
Perlesta															24			2					5					14												
Perlidae											1					8	12									1													3	
Perlodidae									3																		3													
Phryganeidae																															1									
Polycentropodidae									1																	1														
Polycentropus				2	2		3			1	2								3			1		1		1		1			1		1					2	1	
Prosimulium																										4														
Psephenus						2			3	1	1							4	4																					
Pseudolimnophila																																								
Pteronarcys							1	4							7	3						2			5		1					3								
Pycnopsyche							1								1														2					1			1			
Pyralidae																																								
Remenus																								1																
Rhyacophila	1	2	2	8	4	3	5	1	1	4	2	5	2	3	6	3	1	3		1		4	1	3	3	12	8	2	4	6	2	14	10	9	12	2	2	12	3	11
Scirtidae																																								
Sialis																													1								1			
Simuliidae																										1														
Simulium							4	30		1												1	37	2	13		15		4	6			1	4		7		3		1
Soyedina																													1		1				2					
Stenacron				1												3								1			1				2									
Stenelmis											1																													
Stenonema																															8	8								
Stilobezzia				4			6	6																																
Stylogomphus																																			1					
Sweltsa					4			3																							2	3								1
Tabanus													1																											
Taeniopteryx		1				8	14	2																										1	1					
Tallaperla			2																											1										
Timpanoga																											2													
Tipula	1					1				2		1	1	5	1	1						1								6				1	6	1	2		1	8
Tipulidae																								2																
Viehoperla																																								
Wormaldia			1		1		1																							1		1	3					3	6	1
Yugus										1		1	10		3		4	6	9	13				1	5															1

Taxon Final ID	G200B20091031C	G200B20091031D	G200B20091106A	G200B20091106B	G200B20091106C	G200B20091106D	G200B20091106E	G200B20091107A	G200B20091107B	G200B20091107C	G200B20091107D	G200B20091107E	G200B20091107F	G200B20100519A	G200B20100519B	G200B20100519C	G200B20100519D	G200B20100519E	G200B20100520A	G200B20100520B	G200B20100520C	G200B20100520D	G200B20100520E	G200B20100520F	G200B20100521A	G200B20100521B	G200B20100521C	G200B20100521D	G200B20100521E	G200B20100521F	G200B20100521G	G200B20100524A	G200B20100524B	G200B20100524C	G200B20100524D	G200B20100524E	G200B20100525A	G200B20100525B	G200B20100525C	G200B20100526A	G200B20100526B
Neotrichia										1																								1			1			1	
Nigronia	1				1		1														1			1							1					1	2			1	1
Oemopteryx																																									
Oligochaeta	2					1	1	8	11	13	7	2	1	1		1		2				1	3	1							5		1	1	2	1			1	2	
Optioservus	2												2											2													1				
Ormosia																					1																				
Oulimnius	3	11	2	1	2	2							4			1	9	17		2	10	5	3		4	4	1	16	17	17		4				1	2	1	2		
Palpomyia					1	1			2				1								1																	1			
Paracapnia		6	10	27		2		3		19		30	10																												
Paraleptophlebia				14		9											10						1		1	9			9	2											1
Peltoperla			1					1		1	1							1							1	1	3											1			
Peltoperlidae																																									
Perlesta														2				1		6	6	5	5	6		1			1	1		3		4		9	3	2		1	12
Perlidae												2																													
Perlodidae							1																																		
Phryganeidae																																									
Polycentropodidae						1																																			
Polycentropus		2		1					1	3		1			2		1				3	1		1	1		1	1	1	1			2	1				1			
Prosimulium																																									
Psephenus		3											2									1							3			4	1				5				
Pseudolimnophila							1																																		
Pteronarcys	3	3								1							1	1				1	2		5	1		1	1												
Pycnopsyche																						1																			
Pyralidae																																									1
Remenus																														2											
Rhyacophila		5	33	4	3	4		4	2	5		2	7		1	5	3	3	3	5	2	2	5	3	7	2	5	1			3	6			1	3	1	2			3
Scirtidae																1																									
Sialis																																									
Simuliidae																																									
Simulium				2	3		4							1			2		23	3					1		6						2					1		6	4
Soyedina									1																																
Stenacron																																									
Stenelmis																													1	4											
Stenonema						3							1																												
Stilobezzia																																									
Stylogomphus																																									
Sweltsa			8		1	16			5	16	40	3															2			1					4						
Tabanus																																									
Taeniopteryx			1	11		2			1				7																												
Tallaperla															1																			4							
Timpanoga																																									
Tipula	2	1	2				4	3		4	1	2		1	1		1	1			1		1	1				1			7			2	1	1	2		1	1	1
Tipulidae																																									
Viehoperla																									1																
Wormaldia	1		1			3			1	1	3	3	1																												
Yugus																		1		1			3		5	2		2		3						4	4	2	12		1

Table A.4. Rapid bioassessment protocol habitat assessement data by sample ID.

	C20081116A	C20081121A	C20081121B	C20081121C	C20081126B	C20081126D	C20081128A	C20081128B	C20090512A	C20090512B	C20090513A	C20090513B	C20090513C	C20090513D	C20090514A	C20090514B	C20090520A	C20090520B	C20090520C	C20090521A	C20090521B	C20090521C	C20090522A	C20090522B	C20090522C	C20090522D	C20090602B	C20090610D	C20091009A	C20091009B	C20091009C	C20091009D	C20091009E	C20091030A	C20091030B	C20091030C	C20091030D	C20091030E	C20091031A	C20091031B	C20091031C
Substrate/Cover	17	16	17	19	18	18	20	18	19	15	16	17	18	18	18	19	17	18	18	18	18	20	18	20	20	20	15	17	17	19	19	19	19	17	18	16	17	17	17	16	18
Embeddedness	17	14	15	16	14	16	18	16	15	11	14	16	15	14	15	16	12	16	16	16	13	16	15	16	17	17	12	17	13	15	17	17	18	13	16	13	14	13	12	13	16
Velocity/Depth	15	15	15	15	10	15	18	16	15	20	15	15	15	15	16	15	15	16	15	20	15	15	16	18	15	20	15	15	13	15	19	17	17	18	17	15	17	17	15	15	16
Sediment Deposition	13	12	12	15	13	14	15	14	14	12	13	14	13	12	12	11	11	13	13	12	12	14	12	13	15	16	12	12	12	14	16	17	17	12	14	12	13	12	12	12	14
Flow Status	15	15	16	14	15	16	17	16	16	18	18	19	17	20	20	18	15	18	18	18	18	17	19	19	19	20	17	15	16	20	20	17	18	20	18	18	18	18	16	20	15
Channel Alteration	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	19	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Riffle Frequency	19	18	19	19	19	18	19	19	20	17	17	16	19	19	20	20	19	20	20	20	19	20	18	20	20	18	16	16	18	20	20	20	17	17	19	18	17	17	19	16	18
Bank Stability L	8	8	7	8	8	9	8	8	6	7	7	9	8	8	7	9	7	8	9	9	7	7	7	9	9	9	8	7	8	7	9	9	9	10	6	7	7	6	8	8	8
Bank Stability R	9	7	7	8	8	9	8	8	6	5	7	9	8	8	7	9	7	8	8	8	9	7	6	9	9	9	9	7	7	9	10	9	10	10	6	7	7	6	8	8	8
Bank Veg. Protection L	9	9	8	9	9	9	8	8	10	10	8	9	10	9	10	10	10	9	10	10	10	9	9	10	10	9	9	10	10	10	10	10	10	10	9	8	9	10	9	8	10
Bank Veg. Protection R	9	9	8	9	9	9	8	8	10	10	8	9	10	9	9	10	10	9	10	10	10	9	9	10	10	9	10	10	10	10	10	10	10	9	9	8	9	10	9	6	10
Riparian Veg. Width L	7	10	10	10	9	10	10	10	10	10	9	9	10	9	10	10	10	9	10	8	10	10	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Riparian Veg. Width R	9	8	10	10	10	9	10	10	10	10	6	10	10	9	6	10	10	10	9	10	10	10	9	10	10	10	9	10	9	10	10	10	10	10	10	8	10	10	10	6	9
Total	167	161	164	172	162	172	179	171	171	165	158	172	173	170	170	177	163	174	175	179	171	174	168	183	184	187	162	166	163	179	190	185	185	176	172	160	168	166	165	158	172

Table A.4 (cont'd). Rapid bioassessment protocol habitat assessement data by sample ID.

	C20091031D	C20091106A	C20091106B	C20091106C	C20091106D	C20091106E	C20091107A	C20091107B	C20091107C	C20091107D	C20091107E	C20091107F	C20100519A	C20100519B	C20100519C	C20100519D	C20100519E	C20100520A	C20100520B	C20100520C	C20100520D	C20100520E	C20100520F	C20100521A	C20100521B	C20100521C	C20100521D	C20100521E	C20100521F	C20100521G	C20100524A	C20100524B	C20100524C	C20100524D	C20100524E	C20100525A	C20100525B	C20100525C	C20100526A	C20100526B
Substrate/Cover	18	16	20	16	17	17	15	16	18	17	17	17	16	18	20	19	17	17	19	19	18	16	16	19	19	19	18	17	17	16	18	19	16	19	17	18	18	18	16	19
Embeddedness	15	13	15	12	13	14	12	11	13	13	14	15	14	15	16	14	14	14	13	15	14	13	13	18	18	17	15	13	13	12	16	16	12	17	12	13	14	15	14	16
Velocity/Depth	16	16	17	16	16	17	15	15	14	16	16	17	16	19	16	17	15	15	17	14	15	15	15	20	16	20	10	17	16	10	19	17	14	15	13	17	15	16	19	15
Sediment Deposition	14	13	14	12	13	13	11	11	12	12	13	13	13	13	14	14	13	12	11	12	13	12	13	15	15	14	12	13	12	12	12	14	9	12	12	13	13	13	14	13
Flow Status	17	18	20	18	18	18	18	15	16	17	18	17	19	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	17	20	20	20	17	16	18	20	20	20	20	20
Channel Alteration	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	17	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Riffle Frequency	19	18	19	18	18	17	17	18	18	17	16	18	17	19	19	19	17	19	20	19	18	18	16	19	18	18	19	17	17	17	18	19	16	19	17	18	19	19	16	18
Bank Stability L	9	9	9	8	9	7	7	8	8	7	9	10	8	8	6	9	8	7	7	6	9	9	8	9	10	9	10	8	6	7	10	8	7	8	7	8	10	8	7	7
Bank Stability R	9	9	9	8	9	7	7	8	8	8	9	10	8	8	6	9	8	8	8	4	10	10	8	10	8	10	10	9	7	6	9	10	6	9	7	7	8	8	9	9
Bank Veg. Protection L	10	10	10	8	9	8	7	9	9	7	9	10	9	10	10	10	9	9	10	9	10	9	8	10	10	10	10	10	9	9	10	10	10	9	9	10	9	9	8	9
Bank Veg. Protection R	10	10	10	8	9	8	7	9	9	8	9	10	9	10	9	10	9	9	10	8	10	10	8	10	10	10	10	10	9	9	10	10	10	9	9	9	8	10	10	10
Riparian Veg. Width L	10	10	10	10	10	10	9	10	10	10	10	10	10	10	10	10	10	10	8	10	10	6	8	10	10	10	10	10	10	10	10	10	10	10	10	10	10	6	10	10
Riparian Veg. Width R	10	10	10	7	9	10	7	10	10	10	10	10	9	10	9	10	10	9	10	8	10	10	8	10	10	10	10	10	10	8	7	10	10	10	10	7	8	10	10	10
Total	177	172	183	161	170	166	152	160	165	162	170	177	168	180	175	181	170	169	173	164	177	168	158	190	184	187	174	174	163	156	179	183	157	173	161	170	172	172	173	176

Table A.5. Sensitivity of observed effect concentration to minimum number of taxa included in field sensitivity distributions.

All-Year Model	Minimum Observations			
5% Genera Not Observed	1	2	4	8
	(μS/cm)			
Specific Conductance OEC[1]	21	24	462	565
	(mg/L)			
TDS[2] OEC	11	26	281	355
	(mg/L)			
SO_4^{2-} OEC	4	5	108	177

[1]observed effect concentration; [2]total dissolved solids