

EFFECTIVE MONITORING AND ASSESSMENT OF TOTAL DISSOLVED SOLIDS AS A BIOTIC STRESSOR IN MINING-INFLUENCED STREAMS

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EXECUTIVE SUMMARY

With total dissolved solids (TDS) increasingly identified as a candidate stressor to aquatic life in mining-influenced streams, there is an immediate need for a scientifically sound method for monitoring TDS and assessing its effects on biota in streams influenced by coal-mining activities. Our research goal is to improve industry- and agency capability to monitor and assess TDS for the purpose of characterizing biotic effects of TDS in streams influenced by coal-mining activities.

To accomplish our goal, we sought to characterize how temporal variability of TDS affects the biological community. We selected headwater streams with elevated TDS where non-TDS stressors were not evident, where we measured monthly TDS and component ions, along with specific conductance (SC or “conductivity”; a TDS surrogate) at 15-minute intervals for up to 36 months. We measured benthic macroinvertebrate community structure seasonally and quantified associations between biological and conductivity metrics.

A rigorous and extensive site selection effort enabled us to characterize biological response to TDS independent of significant influence from covariate stressors. Analyses provided no evidence that non-TDS stressors significantly influenced SC – biota correlations at our study sites. Test sites were comparable to reference sites with respect to water quality and physical habitat.

Continuous conductivity monitoring allowed us to characterize the temporal variability of TDS at our study sites. Specific conductance varied temporally over the study period, exhibiting a seasonal pattern of highest SC in fall and lowest SC in spring, with inter-annual consistency. Dilution spikes associated with precipitation events frequently lowered SC greatly for short durations throughout the year. Stream water grab-samples collected at multiple times during the study period revealed that test-site waters were composed primarily of the anions SO_4^{2-} and HCO_3^- and the cations Ca^{2+} and Mg^{2+} . Use of SC as a surrogate for TDS is reasonable given the strong relationship we observed between the two parameters, provided the ion matrix being described by SC is consistent among sites.

Changes in benthic macroinvertebrate community structure were significantly and often strongly correlated with increased SC, characterized by declines in taxa richness and relative abundance of sensitive taxa, with mayfly taxa exhibiting the strongest responses. These SC-biota associations were consistent in consecutive years. Spring data produced stronger and more frequently significant correlations with SC than did Fall data.

Our findings suggest that an effective plan for monitoring and assessing TDS as a biotic stressor will take the following approach:

- Sample the benthic macroinvertebrate community in the spring for maximum SC sensitivity.
- Measure SC at multiple times during the year for a more accurate accounting of stressor levels influencing biota.

- Maintain consistency of chemical and biological sample timing when making comparisons through space or time.

Such an approach may enhance the ability of resource managers and regulators to assess, predict, and control biological impacts from TDS.

INTRODUCTION

This final technical report covers the original study period (February 1, 2011 through January 31, 2013), as well as the follow-on study period (January 1, 2013 through December 31, 2014, including a 12-month no-cost time extension).

Background

With salinization (i.e., elevated major ions) increasingly identified as a candidate stressor to aquatic life in mining-influenced streams, there is an immediate need for a scientifically-sound method for monitoring salinity and assessing its effects on biota in streams influenced by coal-mining activities. Our studies to date have indicated that salinity in coalfield streams can exhibit high seasonal variability, the nature of which is not currently captured by the one or two samples per year that have been used by most other studies. Aquatic organisms are exposed to saline conditions throughout their life cycle and integrate effects of salinity over time. Hence, it is reasonable to expect that biological condition, at the point in time when sampled, is dependent upon prior exposure rather than salinity measured at the time of biological sampling. Therefore, to better predict and manage aquatic-life impacts from salinization, it is necessary to understand the temporal dynamics of salinity and its biological effects. Such understanding is essential to defining methods of salinity measurement that best predict biological response.

Water salinity is often measured as “total dissolved solids” (TDS), which is a measure of the mass of salts contained in a known volume of water after filtration and evaporation and reported in mg/L. Because TDS is resource-intensive to measure, electrical conductivity of the water is used often as an efficient surrogate measure of TDS. Higher levels of TDS occur when more dissociated salt ions are in solution, which yields greater electrical conductivity. Because temperature also influences electrical conductivity, data are often standardized to 25°C, a measure known as specific conductance (SC) but often referred to simply as conductivity.

Appalachian coalfield streams are often influenced by both TDS from mining activity and by non-TDS stressors, making TDS effects difficult to characterize in isolation from effects by confounding stressors. To understand the effects of salinity on biota, influence from salinity-covariate stressors must be minimized. Isolation of salinity as a biotic stressor through rigorous site selection, as done in this study, allows effects of salinity on aquatic community structure to be characterized and understood.

There are questions concerning how TDS levels in mining effluents and streams should be monitored, assessed, and controlled, as is necessary to effectively limit impacts of these constituents on aquatic communities. Specifically, it is not known which of the following parameters, or combination of parameters, would be the most effective and appropriate predictor of biotic response:

- TDS concentration, measured using the conventional method
- ionic strength
- concentration of specific TDS components (such as bicarbonate or sulfate ions)
- specific conductance, a proxy for TDS

It is necessary to know which water quality parameter(s) is/are most influential to biota so that those effects can be managed, controlled, or limited effectively.

The issue of how and when to measure these parameters has not been adequately studied. Most field studies of mining-origin TDS effects have relied upon water quality measurements taken infrequently, usually just one measurement at the time of biological sampling. Data collected in this manner do not characterize the temporal variability of salinity. If salinity is a biotic stressor, it is logical to expect that salinity measurements in a water body prior to the time of biotic sampling would be a more reliable indicator of salinity influence in that biological sample than the common means of salinity effect assessment, which is a TDS or SC measurement at the time of biological sampling. It is not clear, however, how salinity should be measured, and how those data should be interpreted to best predict biological influence by salinity. It is critical to know what measure or pattern of dissolved ion exposure drives biotic response. It is also important to characterize relationships between TDS, ionic composition, and SC if the latter is to be used as a surrogate for TDS.

Recent scientific findings concerning effects of elevated TDS of mining origin as a water-borne stressor have created a complex set of challenges for agencies that regulate coal mining, including OSM. Patterns and mechanisms underlying mining-origin TDS effects on aquatic communities are complex. It is essential to development of effective management and control strategies that these patterns and mechanisms be better understood.

Objectives

Our research goal is to improve industry- and agency capability to monitor and assess salinity for the purpose of characterizing biotic effects of salinity in streams influenced by coal-mining activities.

In pursuit of this overall goal we sought to meet the following specific objectives:

- 1) ***Isolate the salinity variable*** by identifying study streams that have a range of salinity levels and are of least-disturbed reference quality with respect to all other potential sources of stress to aquatic biota except elevated salinity.
- 2) ***Characterize the temporal variability in salinity*** by measuring SC, TDS, and component ions throughout the year.
- 3) ***Characterize biological response to salinity*** by measuring benthic macroinvertebrate community structure in association with salinity measured throughout the year.
- 4) ***Determine how salinity should be monitored and assessed*** for the purpose of predicting biotic response to salinity most accurately and precisely.

Achievement of these objectives will aid in improving the accuracy of methods for measuring TDS and predicting biological condition in mining-influenced streams with elevated TDS. Improved assessment of this water quality-biota relationship will enhance the ability of resource managers and regulators to 1) predict the biotic impacts of TDS, 2) develop strategies and

methods for limiting TDS in mining runoff and effluents as needed to protect stream biota, and
3) establish or modify TDS water quality criteria and methods of assessment.

METHODS

Conceptual Approach

To isolate the salinity variable, we selected headwater streams with elevated salinity where non-salinity stressors were not evident. To characterize the temporal variability of salinity, we 1) installed dataloggers to measure SC at 15-minute intervals (“continuously”) at each site during the study period, and 2) measured water chemistry of grab-samples (including TDS and component ions) multiple times during the study period. To assess the biological response to elevated salinity, we measured benthic macroinvertebrate community structure seasonally and quantified associations between a variety of biological and salinity metrics.

Site Selection

First- and second-order streams (Strahler 1957) within the Virginia and West Virginia portion of Ecoregion 69 (Omernik 1987) were selected such that all observable factors other than salinity were comparable to reference streams in the region (as described by USEPA 2006). In selecting sampling reaches, we attempted to avoid influence from major upstream tributaries through examination of the National Hydrography Dataset (U. S. Geological Survey) and site reconnaissance to ensure our study reaches had no perennial or intermittent tributaries immediately upstream.

We selected elevated-salinity, or “test” sites, meeting all abiotic reference criteria (with the exception of SC) used for Virginia Clean Water Act implementation studies (Burton and Gerritsen 2003, VDEQ 2006) (Table 1). Candidate streams were chosen by examining a variety of available water-quality and land-use data using a geographic information system (ArcGIS, ESRI Inc., Redlands, California USA), augmented by consultation with mine operators, consultants, and regulators with specific knowledge of stream conditions within the study area, and by analysis of data concerning water quality, mine permits, and historical surface-mining site locations provided by Virginia Department of Mines, Minerals and Energy and West Virginia Department of Environmental Protection. The eastern coalfield region of Kentucky was also searched for suitable sites, but we were not able to locate sites that met our selection criteria. The search for Kentucky sites included consultation with knowledgeable parties (U.S. EPA and University of Kentucky), and travel to the University of Kentucky’s Robinson Forest area, where five candidate streams were assessed for potential inclusion in the study.

Table 1. Abiotic criteria for selection of reference and test streams.

Parameter or Condition (units or range)	Selection Criterion ¹
Dissolved Oxygen (mg/L)	≥ 6.0
pH	≥ 6.0 and ≤ 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 habitat score (0-200) ²	≥ 140
Residential land use immediately upstream	None

¹reference-stream criteria from Burton and Gerritsen (2003)

²Rapid Bioassessment Protocols habitat assessment, high-gradient streams (Barbour et al. 1999)

Virginia sites identified through a prior study (Timpano 2011) that remained in a condition that satisfies the above criteria were included in the current study. We visited 18 candidate sites in Kentucky and 63 candidate sites in West Virginia to identify additional streams for study. More than 260 candidate sites in three states were visited to assess suitability for study, a number that includes those visited to select the Virginia sites also used for the Timpano (2011) study.

Site reconnaissance allowed verification of current land uses and confirmation of reference-quality conditions, as per study design. Physicochemical water parameters, including pH and SC, were measured to ensure non-acidic conditions and that a gradient of salinity among test sites was achieved. Reference-quality habitat was assured by conducting qualitative visual estimates of habitat parameters using the high-gradient stream method as specified in U.S. EPA's Rapid Bioassessment Protocols (RBP) (Barbour et al. 1999). In addition, potential sources of non-point source pollution were avoided, including upstream residential land use, road crossings, bridges, culverts, active logging, non-mining industrial operations or infrastructure (e.g., railbeds), and commercial activity.

We selected 21 test sites and six reference sites for initial study, retaining 20 and five of each type, respectively, for data analysis (Table 2). Each salinized study site was an independent stream segment, with no other study sites upstream or downstream. Reference site data were used to establish reference-quality habitat for ensuring test sites were comparable to reference sites in that respect. Reference sites represent minimally disturbed sites for the region of study.

Field Methods

Biological condition was characterized by measurements of benthic macroinvertebrate community structure for up to seven concurrent index periods (Spring 2011, Fall 2011, Spring 2012, Fall 2012, Spring 2013, Fall 2013, and Spring 2014). The Spring index period was from March through May (± 1 week), and the Fall index period was from September through November (± 1 week), but most samples were collected in April and October. The index period is the ecologically-based time of year selected for monitoring. We used the Virginia index period following Virginia Department of Environmental Quality protocol (Burton and Gerritsen 2003), but most of our samples were also collected during the West Virginia index period, which runs

mid-April through mid-October (WVDEP 2013). Note the correspondence of the Virginia three-month index periods with generally recognized seasons in relation to annual onset and decline of maximum biological activity. We followed the single-habitat method for high-gradient streams found in U.S. EPA's RBP (Barbour et al 1999). Using a 0.3-m D-frame kicknet with 500- μ m mesh, a single composite sample (approximately 2 m²) composed of six 1 x 0.3-m kicks was collected along a 100-m reach at each site. Because of the presence of Endangered Species Act-listed crustaceans and mollusks in the region, all specimens from those groups were returned to the stream unharmed. Samples were preserved in 95% ethanol and returned to the laboratory for sorting and identification.

Habitat quality was assessed following U.S. EPA RBP methods for high-gradient streams (Barbour et al. 1999). Habitat assessment was conducted at each site at the time of biological sampling during the first sample collection (Spring 2011 or Fall 2011), and during Spring 2013.

Water quality measurements were of two types. The first was long-term, continuous measurement of SC and temperature using automated logging meters ("dataloggers") installed within the 100-m biological survey reach. The dataloggers were installed upon completion of site selection (between July and October 2011), and continued recording measurements at 15-minute intervals (barring malfunction) through the last (to date) biological sample collection in Spring 2014.

The second type of measurement was of water chemistry grab-samples, collected approximately monthly at each site (flow permitting) from Spring 2011 through Spring 2013 and concurrently with biological sampling in Fall 2013 and Spring 2014. Water temperature, dissolved oxygen (DO), SC, and pH were measured *in situ* with a calibrated handheld multi-probe meter (Hanna HI-9828 - Hanna Instruments, Inc., Woonsocket, Rhode Island, USA; or YSI Professional Plus – YSI, Inc., Yellow Springs, Ohio, USA). Single grab-samples of streamwater for analysis of TDS, cations, anions, alkalinity, and trace elements were filtered immediately after collection using PVDF syringe filters with a nominal pore size of 0.45 μ m and stored in sterile polyethylene sample bags. Filtered aliquots for analysis of cations and trace elements were preserved to pH < 2 with 1+1 concentrated ultrapure nitric acid. All samples were transported to the laboratory on ice and stored at 4°C until analysis. Biological- and water quality samples were collected concurrently at base flow.

Laboratory Methods

Biological samples were sub-sampled randomly to obtain a 200 (\pm 10%) organism count following Virginia Department of Environmental Quality methods (VDEQ 2008), which are adapted from RBP methods (Barbour et al. 1999). Specimens were identified to genus/lowest practicable level using standard keys (Stewart et al. 1993, Wiggins 1996, Smith 2001, Merritt et al. 2008), except individuals in family Chironomidae and sub-class Oligochaeta, which were identified at those levels.

For water samples, an inductively coupled plasma-optical emission spectrometer (Varian Vista MPX ICP-OES w/ICP Expert software, Varian Instruments, Walnut Creek, California USA) was used to measure Ca²⁺, Mg²⁺, K⁺, Na⁺, and dissolved Al, Cu, Fe, Mn, Se, and Zn (APHA 2005)

for samples collected through Spring 2013; samples collected in Fall 2013 and Spring 2014, were analyzed for the same suite of ions using an inductively coupled plasma-mass spectrometer (Thermo Electron X-Series ICP-MS, Thermo Fisher Scientific, Waltham, Massachusetts USA). An ion chromatograph (Dionex DX500, Dionex Corp., Sunnyvale, California USA) was used to measure Cl^- and SO_4^{2-} (APHA 2005). Total dissolved solids were measured by drying of known volumes at 180 °C (APHA 2005), with modifications (0.45- μm filter, field filtration). Total alkalinity was measured for an aliquot of filtered sample by titration with standard acid (APHA 2005) using a potentiometric auto-titrator (TitraLab 865, Radiometer Analytical, Lyon, France). CO_3^{2-} and HCO_3^- were calculated from alkalinity and pH measurements (APHA, 2005). Water chemistry data were examined to determine if selected trace elements were present at chronically toxic levels (exceeding criteria continuous concentrations [CCC]) (USEPA 2012a, ILEPA 2001).

Data Analysis

We classified salinity data three ways for data summary and analysis: 1) Data from water samples collected concurrently with seasonal biological sampling (probe physicochemical data or grab-sample water chemistry) were considered “snapshot” data, because they represent salinity at a single point in time. This seasonal snapshot approach to salinity measurement and analysis is comparable to our prior work (Timpano et al. 2015) and research by most others (e.g., Pond et al. 2004, 2008, Gerritsen et al. 2010, Bernhardt et al., 2012). 2) Data from monthly (Spring 2011 – Spring 2013) and seasonal (Fall 2013 and Spring 2014) stream water chemistry were classified as “grab-sample” data. 3) Specific conductance data obtained every 15-minutes from dataloggers were termed “continuous” data and were considered a high-resolution representation of the long-term salinity influencing development of biota in the stream prior to biological sampling.

For general characterization of water quality, grab-sample data for the entire study period (up to 21 samples per site) were used to calculate summary statistics for physicochemistry, major ions, and trace elements. We also used grab-sample data to calculate mean relative proportions of component ions by site type for the entire study period. For evaluation of biota-salinity relationships, only biological samples from Fall 2012, Spring 2013, Fall 2013, and Spring 2014 were used, each associated with their respective snapshot SC and with prior 12-months of continuous water quality data.

Using SC as a surrogate for salinity is desirable in our analysis, because it allows comparisons between snapshot and continuous salinity measures as biological response predictors. We evaluated suitability of SC as a salinity surrogate using grab-sample data to calculate Pearson correlations between SC and TDS, as well as between SC and sum of the concentrations (mg/L) of the eight major ions (Ca^{2+} , Cl^- , CO_3^{2-} , HCO_3^- , K^+ , Mg^{2+} , Na^+ , SO_4^{2-} ; referred to as “Sum8”). The Sum8 metric was used because, for very dilute samples, gravimetric TDS was often below the method detection limit (MDL) of 42.8 mg/L, yielding inaccurate values. The analytical techniques we used for major ion measurement all had MDLs < 1 mg/L.

Time-series plots of continuous SC data for each site (Appendix A) were examined qualitatively for evidence of potential temporal patterns. Quantitative detection of seasonal differences in SC was accomplished by comparing Spring to prior Fall snapshot SC using Welch’s paired t-test

(i.e, Spring 2014 vs. Fall 2013, and Spring 2013 vs. Fall 2012). Continuous SC data by site and summary statistics by site type were calculated for each prior year of continuous data associated with the Fall 2012, Spring 2013, Fall 2013, and Spring 2014 biological data (Appendix B).

Characterization of inter-annual variation of continuous SC allows for determination of when during the year SC is high or low, and how it varies throughout the year. This was accomplished by first converting each continuous SC measurement for a site into a quantile of that site's range of SC measurements. This process standardizes a site's relative SC magnitude across all sites, be they high-SC test sites or low-SC reference sites, thus allowing averaging across sites to determine when during the year SC at a site in the region is relatively high and when SC is relatively low. Monthly mean quantiles were then calculated for each site and used to compare months using one-way ANOVA. Tukey's Honest Significant Difference was used to make pairwise comparisons of mean SC quantiles among months.

We used Spearman correlation analysis to evaluate associations between biota and snapshot or continuous SC data. As a complement to correlation analysis, and to visualize non-linear salinity-biota relationships and strength of associations, we used segmented regression to fit selected biological metrics to snapshot SC. We used snapshot data as a baseline, representing the status quo of salinity-biota analyses. We then compared snapshot correlations to correlations using continuous SC metrics. The method of SC characterization that yielded the strongest correlation with biological metrics was selected as the better method of SC measurement and summary for predicting biological response. A variety of common benthic macroinvertebrate community metrics were calculated that represented a range of community attributes. Those metrics were then correlated with snapshot SC and 12 summary measures of prior 12-month continuous SC: mean and quantiles (5,10,20,30,40,50,60,70,80,90,95). From the 11 quantiles examined, we selected the "best" quantile as the quantile yielding the highest absolute value of the correlation coefficient.

For each trace element measured by ICP-OES, we calculated three method detection limits (MDLs), one for each batch analysis of samples, which were used for determination of analyte detection. For trace elements measured by ICP-MS, one batch was run, and we used the minimum reporting limit (MRL) for quantitation of analyte concentration. Measurements less than batch MDL or MRL were treated as $\frac{1}{2}$ MDL or $\frac{1}{2}$ MRL for calculation of summary statistics, respectively. Raw data are reported as "less than" MDL or MRL in Appendix C.

All statistical analyses were conducted using R statistical software (R Core Team 2014) and interpreted at the $\alpha = 0.05$ level of statistical significance, except where other significance levels are stated.

Analyses Not Reported

We had proposed to characterize how salinity patterns were influenced by upstream mining landuse/structures. We conducted an analysis of mining-related structures considered as likely to be responsible for the elevated salinity in a subset of 13 Virginia test sites included in this study. For each of the 13 Virginia sites, we quantified watershed area and presence within that area of each of the following land uses: proportional area of active surface mining, proportional area of active and abandoned underground mining, proportional area of valley fills, proportional area of

ponds, and gas/oil well density. We also calculated mean daily SC and mean weekly CV from the continuous SC data collected through April 2012. We analyzed the resulting data in an effort to characterize associations of measured SC levels with mining landuse/structures.

Results of the exercise were inconclusive; statistically significant associations were not found. We determined that each watershed contained a mixture of historical and contemporary mining landuses, such that landuse variables were highly confounded with one another. The analysis was complex and time consuming. Because it did not yield conclusive results for the Virginia segment of the study area, where most of our sites are located, we did not extend it to the West Virginia sites.

Because the analysis was complex and inconclusive, we have not attempted to summarize that work in manuscript form for this report. We reported those results as a presentation at the American Society of Mining and Reclamation National Meeting in 2012. An abstract of that presentation (Timpano et al. 2012), and a summary table of catchment mining land use proportions are attached to this report as Appendix E. We have the ASMR presentation file available, and will provide that file to OSM upon request.

Analyses Not Conducted

We had proposed to analyze water samples for dissolved organic carbon (DOC). No samples were collected for DOC analysis because the value of such data was extremely reduced compared to our expectation at the time of submission of the study proposal. Our intent was to incorporate DOC measurements to account for reduced bioavailability of toxic trace metals that complex with DOC. However, several months after submitting the project proposal we completed analysis of trace metal samples from prior work in a large subset of the streams studied here (Timpano 2011). In that study, most trace metal values were below toxic levels (per U.S. EPA CCCs) or below detection limits, a pattern that held for the present study as well. We found that in nearly all samples for the present study 1) Al, Fe, and Zn were below U.S. EPA CCCs (100, 99, 98% of samples, respectively), and 2) Cu and Se were below detection limits (91 and 84% of samples, respectively). As such, trace metals in our streams were either below levels of toxic concern, or unquantifiable. Therefore, incorporating DOC-modified trace metal bioavailability data into our analysis was either unnecessary or not possible.

We had also proposed to link stream-water salinity to mineral composition of mine spoil material if our companion proposal to OSM (Daniels et al. 2010) was funded. The companion proposal was funded by OSM with an altered scope of work that included collection of mine spoils only from the state of Tennessee. Because we have no water samples from Tennessee, we did not link our work with Daniels et al. (2010).

RESULTS & DISCUSSION

Objective 1: Isolate the Salinity Variable

Site Selection

Twenty seven sites (7 reference and 20 test) were initially selected for study. After initiating study of all selected sites, one reference site and one test site were omitted from data analysis. The reference site, Clear Creek (CLE), was excluded from final analysis because it had only a partial record of continuous SC data during the study period. Dataloggers in Clear Creek were damaged on two occasions during high flows and the decision was made not to risk losing further hardware at that site. The very dilute SC signature (median SC = 18 $\mu\text{S}/\text{cm}$) of Clear Creek was still represented in the dataset by its tributary, Eastland Creek (EAS, median SC = 21 $\mu\text{S}/\text{cm}$).

Coal Fork (COA) was omitted from the dataset because analysis of its ion matrix revealed substantial differences in ionic composition relative to the other test sites. Because ion toxicity is influenced by ionic composition (Mount et al. 1997, Soucek and Kennedy 2005), use of SC as a surrogate for TDS is predicated on a comparable ion matrix among sites. Coal Fork was discovered to have significantly higher levels of Na^+ and Cl^- than other test sites, rendering its SC values incomparable to SC of other test sites with regard to ionic composition.

All of the 25 sites retained for analysis (Figure 1) were first-order streams, with mean (\pm SE) watershed area of 3.31 (\pm 0.5) km^2 . Mean watershed area was not significantly different between reference and test sites (Welch's t-test $p = 0.3805$).

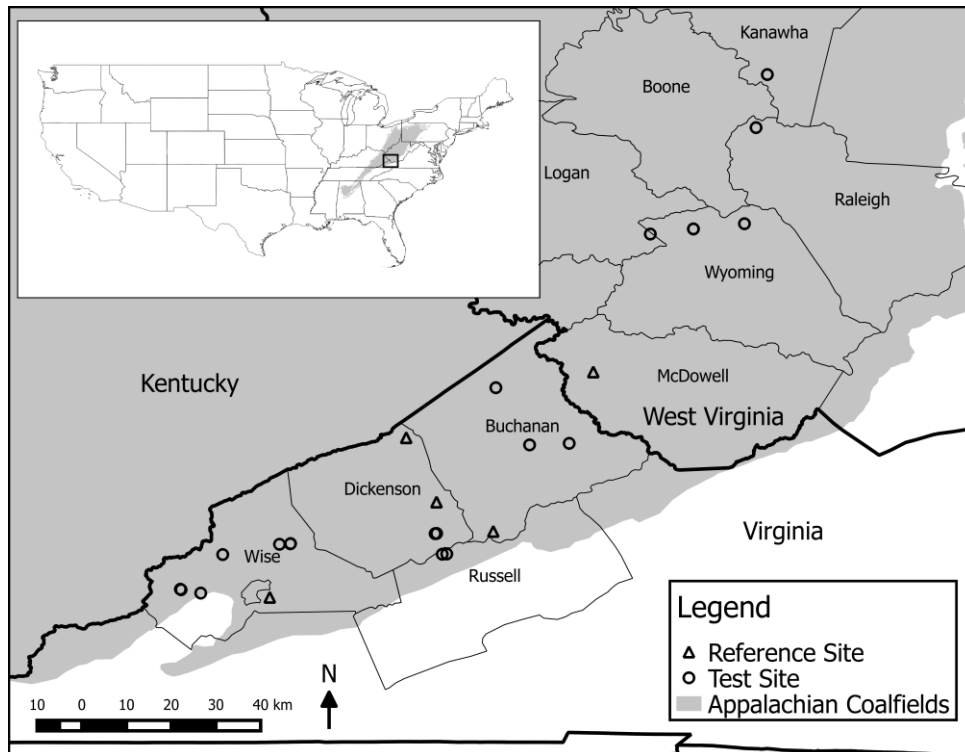


Figure 1. Map of 25 study sites retained for analysis

Table 2. Site attributes.

Stream	Site ID	Site Type	Stream Order ¹	County, ST	Latitude	Longitude	Watershed Area ¹ (km ²)
Birchfield Creek	BIR	Test	1	Wise, VA	37.03605	-82.57016	3.49
Clear Creek ²	CLE	Ref	2	Wise, VA	36.92765	-82.59113	12.26
Coal Fork ²	COA	Test	2	Kanawha, WV	38.09264	-81.48742	12.98
Copperhead Branch	COP	Ref	1	Dickenson, VA	37.06471	-82.09067	0.81
Crane Fork	CRA	Test	1	Wyoming, WV	37.75127	-81.52721	9.49
Crooked Branch	CRO	Ref	1	Dickenson, VA	37.13013	-82.21794	2.27
Dave Branch	DAV	Test	1	Logan, WV	37.72819	-81.73839	1.53
Eastland Creek	EAS	Ref	1	Wise, VA	36.91764	-82.59196	2.38
Fryingpan Creek	FRY	Test	1	Dickenson, VA	37.06021	-82.21774	5.73
Fryingpan Creek Right Fork	RFF	Test	1	Dickenson, VA	37.05981	-82.22114	4.56
Grape Branch	GRA	Test	1	Buchanan, VA	37.25776	-82.00918	4.07
Hurricane Fork (VA)	HUR	Test	1	Buchanan, VA	37.38540	-82.08481	1.22
Hurricane Fork (WV)	HCN	Ref	1	McDowell, WV	37.42042	-81.86627	5.93
Kelly Branch	KEL	Test	1	Wise, VA	36.93472	-82.79085	2.63
Kelly Branch UT ³	KUT	Test	1	Wise, VA	36.93575	-82.79250	1.09
Laurel Branch	LAB	Test	1	Russell, VA	37.01393	-82.20517	2.69
Left Fk/Laurel Fk/Coal Fk	LLC	Test	1	Kanawha, WV	38.08404	-81.47592	4.17
Longlick Branch East Fork	LLE	Test	1	Wyoming, WV	37.73959	-81.64158	0.67
Longlick Branch West Fork	LLW	Test	1	Wyoming, WV	37.73965	-81.64186	1.98
Middle Camp Branch	MCB	Ref	1	Dickenson, VA	37.27375	-82.28591	1.27
Mill Branch West Fork	MIL	Test	1	Wise, VA	36.92717	-82.74680	2.74
Powell River	POW	Test	1	Wise, VA	37.01310	-82.69751	2.68
Rickey Branch	RIC	Test	1	Wise, VA	37.03710	-82.54583	4.22
Rickey Branch UT ³	RUT	Test	1	Wise, VA	37.03763	-82.54536	1.92
Rockhouse Creek	ROC	Test	1	Raleigh, WV	37.96569	-81.50123	7.21
Roll Pone Branch	ROL	Test	1	Russell, VA	37.01446	-82.19490	1.30
Spruce Pine Creek	SPC	Test	1	Buchanan, VA	37.26124	-81.92038	6.71

¹determined using data from NHDPlus database (USEPA 2012b).²sites omitted from final data analysis.³UT – unnamed tributary.

Reference-Quality Habitat

Total habitat scores (mean \pm 1 SE) were not significantly different between test (176.1 \pm 1.1) and reference (180.8 \pm 2.1) sites ($p = 0.0625$) (Table 3). In addition, all individual test-site total habitat scores were $> 85\%$ of the mean reference site habitat score (range: 90 – 102%), indicating that habitat for each of the test streams was comparable to reference (Barbour et al. 1999). All habitat scores were greater than the reference-site selection criteria used for Clean Water Act implementation studies in Virginia (Table 1).

Table 3. RBP habitat assessment summary statistics for Spring 2013 data.

Site Type	Statistic	Epifaunal Substrate	Substrate Embeddedness	Velocity/Depth Regime	Sediment Deposition	Channel Flow Status	Channel Alteration	Rifle Frequency	Bank Stability		Vegetative Protection		Riparian Vegetative Zone		Total Habitat Score
									Left	Right	Left	Right	Left	Right	
Ref	Min	17	13	15	12	18	20	18	8	8	10	10	8	10	169
	Max	20	17	20	17	20	20	19	9	9	10	10	10	10	187
	Median	19	16	18	14	20	20	19	9	9	10	10	10	10	184
	Mean	18.8	15.4	17.4	14.4	19.4	20.0	18.6	8.6	8.6	10.0	10.0	9.6	10.0	180.8
	SD	1.1	1.8	1.9	1.8	0.9	0.0	0.5	0.5	0.5	0.0	0.0	0.9	0.0	7.5
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Test	Min	17	13	16	12	16	20	16	6	7	6	6	8	8	162
	Max	20	17	18	15	20	20	19	10	9	10	10	10	10	184
	Median	18	15.5	17	14.5	20	20	18	8	8	10	10	10	10	176
	Mean	18.1	15.2	17.0	14.2	19.4	20.0	17.8	8.0	8.0	9.8	9.8	9.6	9.4	176.1
	SD	0.8	1.1	1.0	1.0	1.1	0.0	1.1	1.1	0.8	0.9	0.9	0.8	0.9	4.8
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20

Reference-Quality Water Chemistry

Study sites met criteria for reference-quality waters as applied to this study (Table 1). Dissolved oxygen was ≥ 6.0 mg/L for all but one reference and one test sample, and pH was in the range of 6 – 9 for all but one test sample (Table 4).

Table 4. Physicochemistry data summary by site type for grab-sample data.

Site Type	Statistic	Specific Conductance ($\mu\text{S}/\text{cm}$) ¹	pH ¹	Dissolved Oxygen ¹ (mg/L)	Temp ¹ (°C)	Total Dissolved Solids (mg/L)	Total Alkalinity (mg/L as CaCO ₃)	Total Hardness (mg/L as CaCO ₃)
Ref	Min	15	6.53	5.75	2.5	4.0	3.0	6.4
	Max	213	8.89	13.71	21.3	142.0	89.2	89.4
	Median	55	7.53	9.45	11.2	21.4	12.6	20.2
	Mean	68	7.60	9.42	11.6	36.9	19.9	26.4
	SD	43	0.47	1.86	4.7	25.8	18.7	18.9
	n	93	79	71	93	84	92	90
Test	Min	72	7.06	5.01	2.0	58.8	18.4	42.4
	Max	1,925	9.10	13.69	23.1	1,749.0	215.8	1,534.8
	Median	640	8.06	9.50	11.9	406.8	97.1	339.0
	Mean	721	8.03	9.58	12.1	501.2	99.8	408.6
	SD	385	0.31	1.49	4.7	329.6	46.6	277.8
	n	394	330	305	394	370	390	385

¹measured *in situ* with hand-held meter during monthly site visits.

Trace elements were below toxic levels or undetected in nearly all samples. Copper and Se were below ICP-MS MRLs in 100 and 84% of test site samples, respectively. The early samples (prior to Fall 2013) were analyzed on ICP-OES; however upon determination that MDLs were above chronic criteria, we analyzed the final batch (Fall 2013 and Spring 2014) on ICP-MS, where we obtained MDLs < CCC. Based on the more-precise ICP-MS data, we conclude that Se is not a strong influence on salinity-biota associations, because Se exceedances were not widespread, occurring at only three test sites. Aluminum, Fe, and Zn were below U.S. EPA aquatic-life CCCs in 100, 99, and 98% of samples, respectively. There is no U.S. EPA aquatic-life water-quality standard for Mn, but all values reported here were at least one order of magnitude lower than the proposed Illinois EPA aquatic-life CCC (Mn [dissolved] criterion = $0.9812(e(4.0635+(0.7467*\ln(\text{hardness})))$); B. Koch, Illinois EPA, personal communication), which is the only candidate aquatic-life criterion for Mn that we were able to identify. Median concentrations were very similar between reference and test sites, indicating that high values were not common at either site type (Table 5).

Table 5. Trace element concentration (µg/L) summary statistics by site type for monthly data.

Site Type	Statistic ¹	Al	Cu	Fe	Mn	Se	Zn
Ref	Min	1.3	0.5	3.6	0.5	2.5	0.9
	Max	43.4	0.5	498.3	55.4	2.5	309.8
	Median	17.4	0.5	34.7	4.7	2.5	16.2
	Mean	17.5	0.5	69.3	6.2	2.5	25.9
	SD	10.9	0.0	89.4	7.1	2.5	39.6
	n	90	9	90	90	9	90
	Test	Min	1.5	0.5	3.6	0.5	2.5
Max		75.1	0.5	1,725.0	286.4	15.7	272.7
Median		19.4	0.5	25.0	4.7	2.5	15.9
Mean		20.2	0.5	74.1	15.2	3.6	26.3
SD		14.3	0.0	166.5	29.7	2.8	34.0
n		385	38	385	385	38	385

¹values below batch MDL or MRL reported as ½ batch MDL or MRL.

Objective 2: Characterize Temporal Variability of Salinity

Specific Conductance as a Salinity Surrogate

We chose to use SC as a surrogate for TDS and major ions because SC was strongly correlated with both measures of salinity. Pearson correlations were strong between SC and Sum8 for reference ($r = 0.9640$, $p < 0.0001$, $n = 89$) and test ($r = 0.9814$, $p < 0.0001$, $n = 376$) sites. Correlations between SC and TDS were also strong for test sites ($r = 0.9671$, $p < 0.0001$, $n = 367$), but only moderate for reference sites ($r = 0.6855$, $p < 0.0001$, $n = 84$). The lower correlation between SC and TDS for reference sites is likely because 66% of reference samples measured below the detection limit for TDS (42.8 mg/L), which caused many of the dilute reference samples to register as $\frac{1}{2}$ MDL, rather than their true concentration.

The mean (95% confidence interval [CI]) ratio of TDS to SC for test sites was 0.6642 (0.6531 – 0.6754). That ratio was similar (0.6221), but more variable for reference sites (95% CI: 0.5514 – 0.6928). Mean (95% CI) ratios of Sum8 to SC were 0.7911 (0.7817 – 0.8005) for test sites and 0.7137 (0.6841 – 0.7432) for reference sites.

Continuous Conductivity

Dataloggers were installed in 17 streams by mid-July 2011, with the remaining eight dataloggers installed by mid-October 2011. The SC recorded at 15-minute intervals until early July 2014 has been used for this report. In some cases, SC data records suffered data loss from malfunction such as physical shock or burial by sediment. With potential for very low flow during the summer, dataloggers were positioned with the sensor near the stream bottom to avoid desiccation, which increased the likelihood of burial. We endeavored to visit each site monthly to download data and conduct datalogger maintenance. On occasions when the inter-visit period extended beyond 4-6 weeks (up to 3 months at some sites due to ice and snow inhibiting safe site access), we found greater accumulation of biofilms and sediment on the sensors. The monthly maintenance schedule was found to minimize biofilm and sediment accumulation, and ensure position in the deepest part of the stream channel with changing flow regimes. For time periods when burial caused erroneous SC readings, those data were removed from the record.

Several patterns were observed in the continuous conductivity data that were consistent among sites at all levels of salinity. Specific conductance varied widely over the study period, with mean CV of 26%. The mean range of SC for any given site was approximately 10-fold, generally ranging from approximately 20 to 200% of the mean SC for that site. Dilution spikes, associated with precipitation events, lowered SC frequently (Figure 2 scatterplot); magnitude of dilution was often high, but duration was short, as the bulk of SC values were confined to a relatively narrow range (Figure 2 boxplot). We also observed a general seasonal pattern of highest SC in fall and lowest SC in spring (Figure 3). The seasonal pattern was consistent across sites for the full study period, with highest SC values for a site (upper quantiles) generally occurring during the fall, and lowest SC values for a site (lower quantiles) generally occurring in the spring. This pattern is evident by calculating mean SC for a given day at each site, determining the quantile of that daily mean SC for that site relative to the range of SC during the study period, averaging across sites, and repeating for each day of the year (Figure 3).

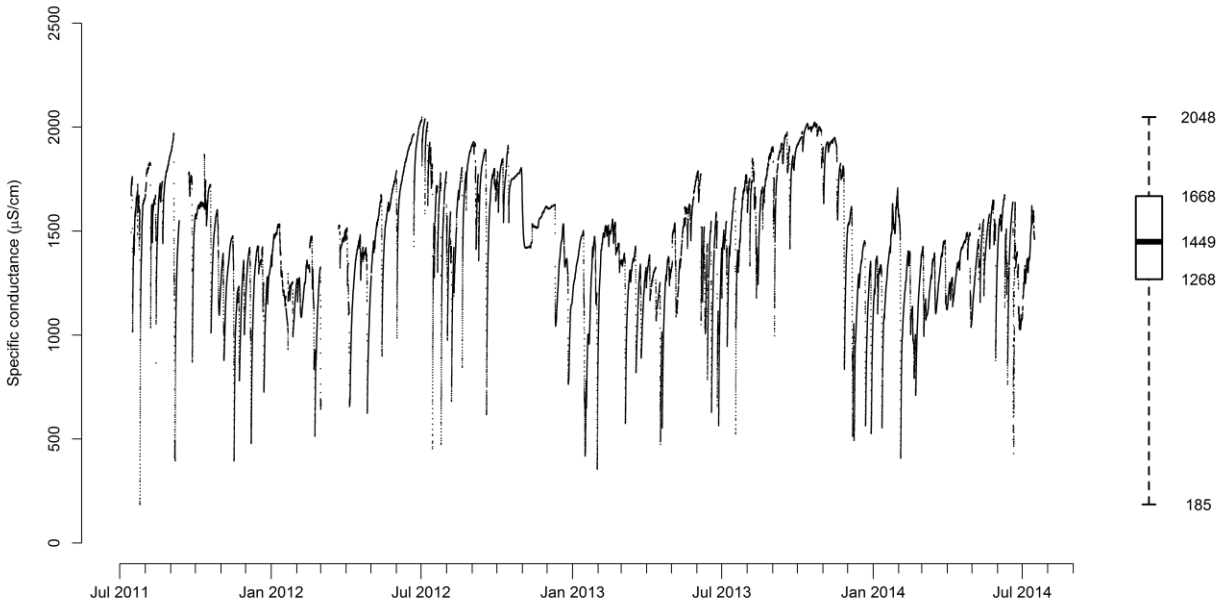


Figure 2. Typical specific conductance (SC) pattern of test sites. Scatterplot and boxplot of SC data measured at 15-minute intervals in Rickey Branch from mid-July 2011 through mid-July 2014. Box plot represents median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

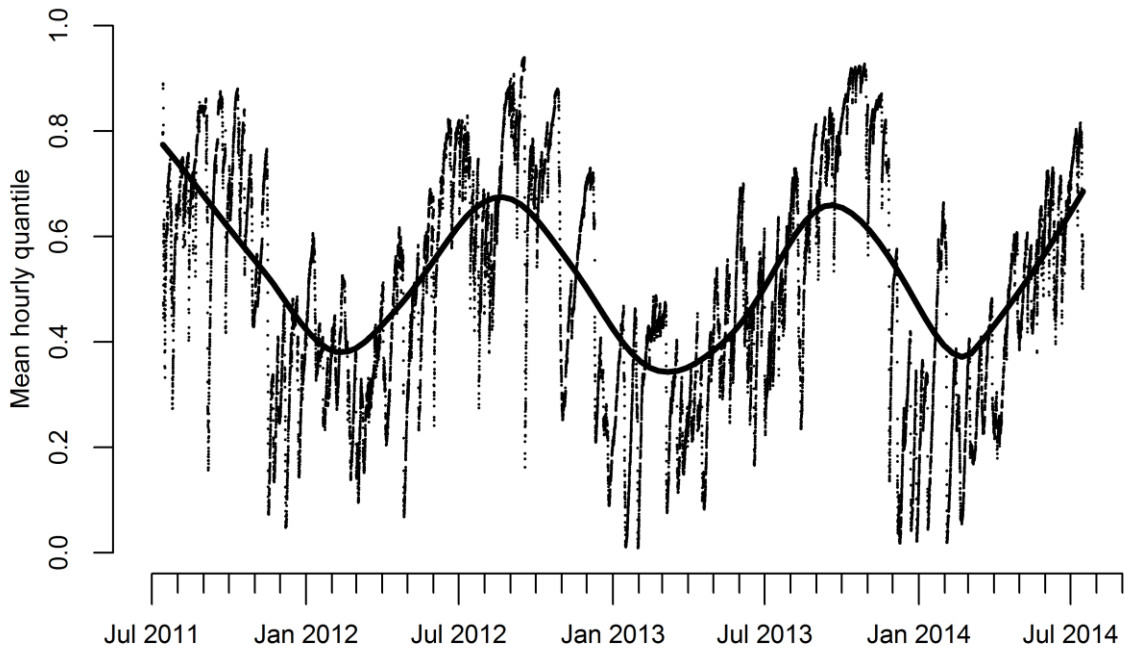


Figure 3. Mean hourly quantile of SC at 25 sites for the period July 2011 – July 2014. Black solid line is smoothed fit. High quantiles indicate period of year when SC is relatively high at a site, and low quantiles indicate when SC is relatively low at a site.

Mean SC quantiles were significantly different among months (ANOVA, $p < 0.0001$), but variance among months was similar (Bartlett's test for homogeneity of variance, $p = 0.1526$). Comparisons among mean monthly SC quantiles for the entire study period revealed that SC was significantly higher in summer/fall (months of Jul, Aug, Sep, Oct) than in winter/spring (months of Dec, Jan, Feb, Mar, Apr) (Tukey's HSD, $p < 0.05$), with October and March as the months with the maximum and minimum SC, respectively (Figure 4).

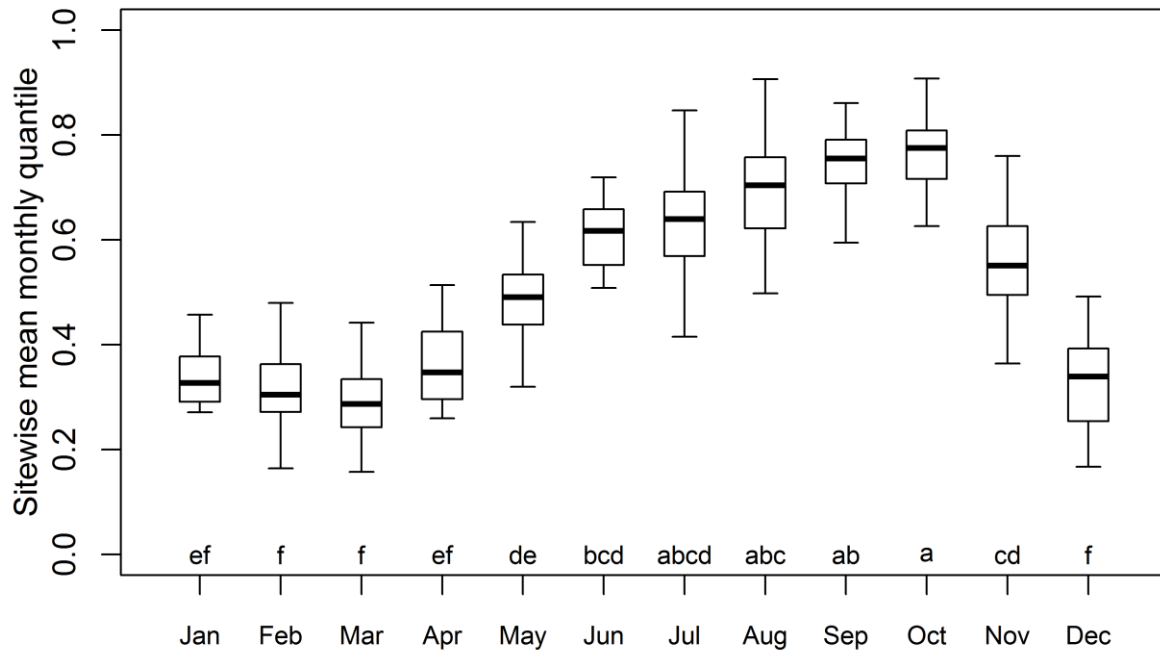


Figure 4. Boxplot of sitewise mean monthly quantiles of SC for the period July 2011 - July 2014. Months with different letters are significantly different from one another (Tukey's HSD, $p < 0.05$). $n = 25$ (5 reference sites, 20 test sites).

Major Ions

Mean concentrations of all major ions were significantly higher for test sites than for reference sites ($p < 0.05$), with SO_4^{2-} , Ca^{2+} , and Mg^{2+} exhibiting the greatest difference between site types (Table 6).

Table 6. Major ion concentration (mg/L) summary statistics by site type for monthly data.

Site Type	Statistic	Cl^-	SO_4^{2-}	CO_3^{2-}	HCO_3^-	Ca^{2+}	K^+	Mg^{2+}	Na^+
Ref	Min	0.21	0.55	0.00	3.67	1.73	0.30	0.51	0.46
	Max	11.80	22.58	2.03	108.88	24.60	2.64	6.76	18.82
	Median	1.88	9.09	0.00	15.08	4.45	1.37	2.17	2.49
	Mean	2.52	9.72	0.02	24.29	6.48	1.26	2.47	3.40
	SD	2.22	5.64	0.21	22.80	5.10	0.54	1.55	3.46
	n	91	91	92	92	90	90	90	90
Test	Min	0.19	0.43	0.00	22.43	9.80	1.19	4.28	3.96
	Max	73.88	1,060.74	21.86	260.88	231.35	16.84	232.14	78.16
	Median	4.72	227.81	0.00	118.07	69.12	3.76	39.52	14.69
	Mean	6.72	294.81	0.41	120.96	80.10	4.20	50.58	20.81
	SD	8.46	219.48	1.54	55.81	46.23	2.40	41.07	16.38
	n	388	390	390	390	385	385	385	385

We observed three general ion matrix types among study sites. Test site waters were primarily composed of SO_4^{2-} , Ca^{2+} , Mg^{2+} , and HCO_3^- (molar proportions), whereas reference site waters were primarily composed of HCO_3^- , Ca^{2+} , and Na^+ (molar proportions). A third matrix was represented by only one site, Coal Fork (COA), which was composed almost entirely of Na^+ and Cl^- (molar proportions) (Figure 5). Radar plots illustrate that the ion matrix was generally consistent among sites of a given site type, though there was some overlap in matrix composition, with some lower-salinity test sites having reference-like matrices (GRA, SPC, FRY, RFF, and DAV; mean monthly SC = 243, 374, 381, 433, and 479 $\mu\text{S}/\text{cm}$, respectively) (Figure 5). Considering column leaching data generated by our colleague, W. L. Daniels, with partial OSM support (Daniels et al. 2014): we interpret these differences to indicate a likelihood that the watershed mine spoils producing high- HCO_3^- leachates (GRA, SPC, FRY, RFF, and DAV) had a higher degree of weathering, on average, than those spoils producing leachates with SO_4^{2-} as the dominant anion. The fact that these sites are among the lowest (among test sites) in SC/TDS is consistent with this interpretation.

Considered together, test sites were dominated (mean molar proportion) by SO_4^{2-} (27.7%) and HCO_3^- (21.1%), followed by Ca^{2+} (19.3%) and Mg^{2+} (18.3%). Sodium comprised 10.3% by molar proportion, and Cl^- , K^+ , and CO_3^{2-} were negligible constituents (Figure 6). In contrast to test sites, reference sites were dominated (mean molar proportion) by HCO_3^- (35.7%) and Ca^{2+} (16.6%), followed by Na^+ , SO_4^{2-} , Mg^{2+} , and Cl^- , each contributing 13.5 – 8.0% and K^+ comprising 3.9% (Figure 7). Test site ion matrix composition was consistent with waters of similar streams in the region receiving alkaline mine drainage (Pond et al. 2008, Lindberg et al. 2011, Cormier et al. 2013, Timpano et al. 2015).

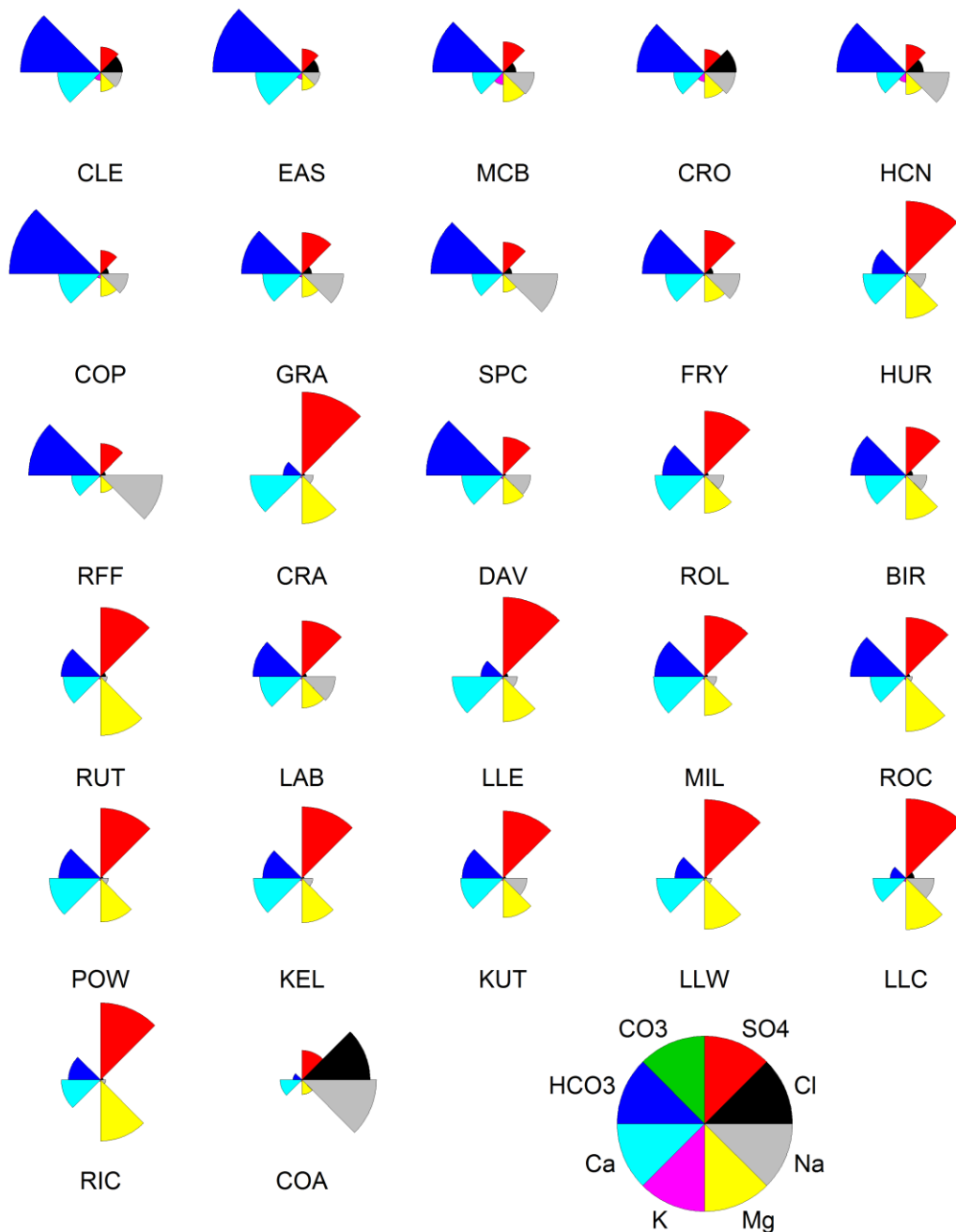


Figure 5. Radar plots of major ion molar proportions for each site , sorted by increasing mean monthly SC (left to right and down). The radius of each colored wedge represents the relative molar proportion of the ion indicated by the legend. The legend radius is equivalent to a molar proportion of 50% for scale. Reference sites: CLE, EAS, MCB, CRO, HCN, COP. Note: CLE and COA were excluded from analyses, but are shown here to illustrate 1) the ionic similarity between CLE and EAS, and 2) how the ion matrix of COA differs from the other test sites.

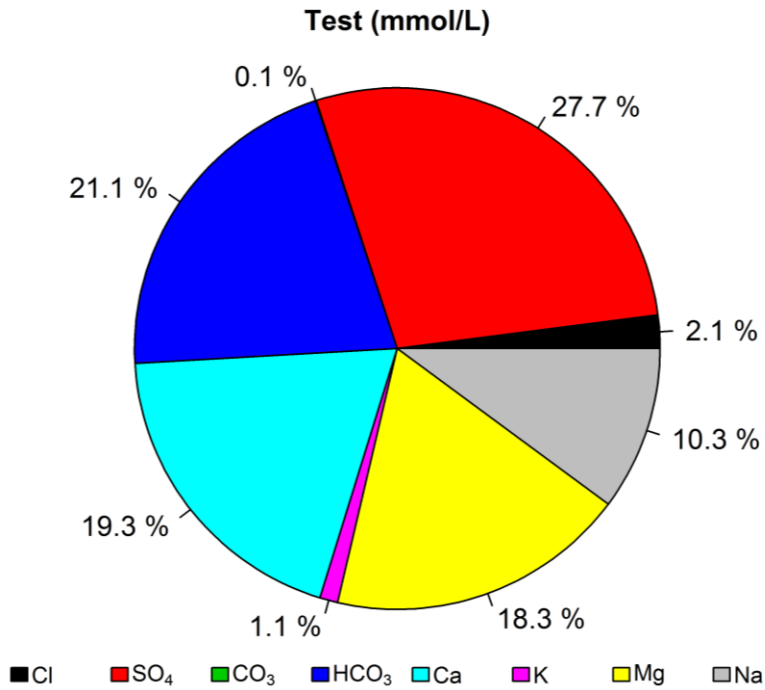


Figure 6. Mean molar proportions of major ions for test sites (n = 394 samples from 20 sites).

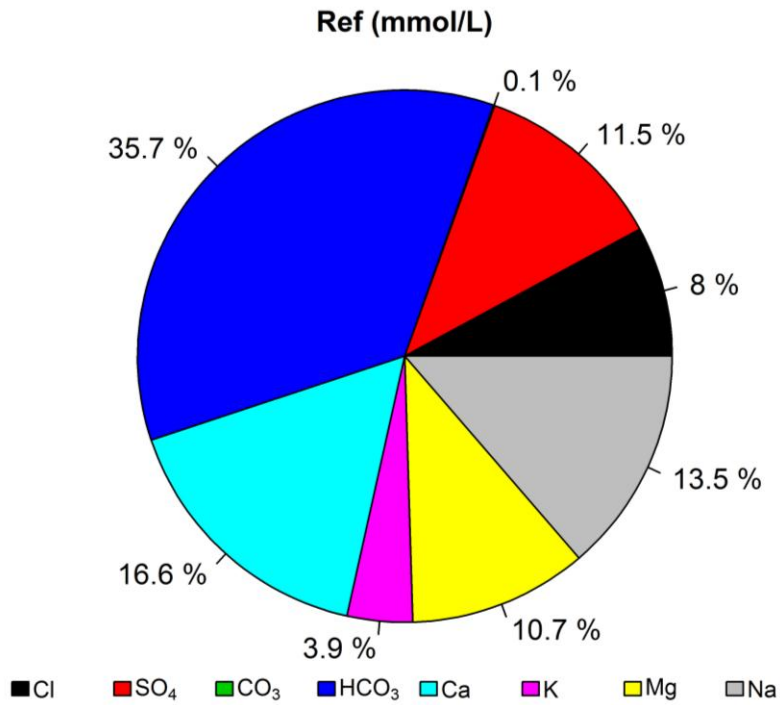


Figure 7. Mean molar proportions of major ions for reference sites (n = 104 samples from 5 sites).

Objective 3: Characterize Biological Response to Salinity

Salinity – Biota Associations

All 25 study sites were sampled for benthic macroinvertebrates in Spring 2014 and Spring 2013. Low stream flow prevented sampling the full complement of sites in Fall 2013 and Fall 2012. All sites except Roll Pone Branch (ROL, test), Middle Camp Branch (MCB, ref), and Eastland Creek (EAS, ref) were sampled in Fall 2013. All sites except Copperhead Branch (COP, ref) were sampled in Fall 2012. Macroinvertebrate samples collected in Spring and Fall, 2011, and Spring 2012 are not reported here because they were not preceded, consistently, by a full year of continuous SC data. Biological metrics for all seven seasons of sampling are included in Appendix D

For all biological samples, we calculated 26 biotic metrics that represent a variety of ecological categories (from Barbour et al. 1999), and we analyzed each metric for correlation with each of 13 SC summary statistics, three of which are reported here (Table 7). We focus on biological response to 1) snapshot SC, 2) mean of prior 12-month continuous SC, and 3) the “best” (i.e., strongest correlation) of the 11 quantiles of prior 12-month SC.

Spring data yielded generally stronger correlations (i.e., correlation coefficients with higher absolute values) and more statistically significant correlations than the Fall data. This finding was consistent among years for snapshot SC, mean of prior 12-month continuous SC, and quantiles of prior 12-month continuous SC. Therefore, we restrict discussion here to the most recent data, from Spring 2014.

Nearly all metrics exhibited moderate to strong correlations with SC in the direction expected from increasing perturbation – metrics expected to decrease in value with increasing SC did so, and those expected to increase in value with increasing SC did as well (Table 7). Richness, evenness, and composition metrics tended to have stronger correlations with SC, whereas functional feeding group, diversity, and habit metrics tended to have moderate or weak correlations with SC.

Correlation and segmented regression results for the pollution-sensitive taxa of mayflies (Order: Ephemeroptera), stoneflies (Order: Plecoptera) and caddisflies (Order: Trichoptera), referred to as EPT collectively, indicated that mayflies were considerably more sensitive to SC than stoneflies or caddisflies. Mayfly richness (No. Ephemeroptera Taxa) (Figure 8) and abundance (% Ephemeroptera) (Figure 9) were strongly correlated with snapshot SC, with Spearman’s correlation coefficients (ρ) of -0.83 and -0.85, respectively (Table 7). The strongest correlations with snapshot SC were with mayfly metrics that ignore the pollution-tolerant mayfly family Baetidae (No. Ephemeroptera Taxa less Baetidae: $\rho = -0.88$, Figure 10; and % Ephemeroptera less Baetidae: $\rho = -0.90$, Figure 11).

Table 7. Benthic macroinvertebrate community metrics, with expected response to perturbation and Spearman coefficients with SC values measured as single point-in time samples (“snapshot”), 12-month prior means of 15-minute interval data (“continuous”), and as a “best quantile” from prior 12-month records of 15-minute interval data.

Category	Metric	Expectation ¹	----- Fall 2012 -----				----- Spring 2013 -----			
			Snap-shot	Cont. mean	Cont. best quantile ²	Cont. best quantile used ²	Snap-shot	Cont. mean	Cont. best quantile ²	Cont. best quantile used ²
			--- Correlation coefficients ---				--- Correlation coefficients ---			
Richness	No. Total Taxa	D	-0.51**	-0.45**	-0.57**	5 th	-0.74**	-0.71**	-0.76**	20 th
	No. Total Taxa less Ephemeroptera	D	-0.27	-0.21	-0.34	5 th	-0.52**	-0.48**	-0.53**	20 th
	No. Ephemeroptera Taxa	D	-0.76**	-0.74**	-0.75**	95 th	-0.88**	-0.82**	-0.88**	20 th
	No. Ephemeroptera Taxa less Baetidae	D	-0.74**	-0.72**	-0.73**	10 th	-0.89**	-0.82**	-0.88**	20 th
	No. EPT Taxa	D	-0.63**	-0.62**	-0.68**	5 th	-0.79**	-0.77**	-0.80**	20 th
	No. Plecoptera & Trichoptera Taxa	D	-0.37*	-0.36*	-0.49**	5 th	-0.57**	-0.55**	-0.58**	20 th
	No. Plecoptera Taxa	D	-0.42**	-0.44**	-0.53**	40 th	-0.59**	-0.55**	-0.59**	20 th
	No. Trichoptera Taxa	D	-0.15	-0.09	-0.15	95 th	-0.36*	-0.38*	-0.40**	20 th
Evenness	% Top 2 Dominant Taxa	I	0.28	0.19	0.29	95 th	0.63**	0.62**	0.66**	5 th
	% Top 5 Dominant Taxa	I	0.38*	0.30	0.38*	95 th	0.71**	0.70**	0.73**	20 th
Diversity	Simpson Diversity	D	-0.36	-0.28	-0.36*	95 th	-0.55**	-0.53**	-0.60**	5 th
	Shannon Diversity	D	-0.41**	-0.33	-0.41**	95 th	-0.67**	-0.67**	-0.70**	20 th
Composition	% Ephemeroptera	D	-0.79**	-0.76**	-0.80**	95 th	-0.87**	-0.84**	-0.90**	5 th
	% Ephemeroptera less Baetidae	D	-0.74**	-0.71**	-0.72**	70 th	-0.90**	-0.85**	-0.91**	5 th
	% Plecoptera	D	0.01	-0.06	-0.10	40 th	0.55**	0.57**	0.61**	5 th
	% Trichoptera	D	0.37*	0.43**	0.43**	60 th	0.08	0.08	0.13	40 th
Functional feeding group	% Collector-Filterers	V	0.42**	0.49**	0.50**	40 th	0.51**	0.45**	0.51**	40 th
	% Collector-Gatherers	V	-0.24	-0.24	-0.31	95 th	-0.52**	-0.47**	-0.57**	5 th
	% Predators	V	-0.35*	-0.06*	-0.50**	5 th	-0.74**	-0.73**	-0.75**	20 th
	% Scrapers	D	-0.10	-0.10	-0.20	5 th	-0.53**	-0.49**	-0.57**	5 th
	% Shredders	D	0.10	0.05	0.13	95 th	0.56**	0.60**	0.62**	5 th
	No. Collector-Gatherer Taxa	D	-0.40*	-0.32	-0.39*	95 th	-0.58**	-0.60**	-0.63**	20 th
	No. Predator Taxa	D	-0.35*	-0.37*	-0.47**	5 th	-0.66**	-0.64**	-0.65**	20 th
	No. Scraper Taxa	D	-0.42**	-0.37*	-0.42**	40 th	-0.59**	-0.54**	-0.59**	20 th
Habit	% Clingers	D	0.26	0.26	0.33	95 th	-0.33	-0.38*	-0.41**	95 th
	No. Clinger Taxa	D	-0.45**	-0.41**	-0.54**	5 th	-0.72**	-0.69**	-0.73**	20 th

¹expected response to perturbation: D = decrease, I = increase, V = variable; from Barbour et al. (1999). ²“best” quantile is the quantile yielding the highest absolute value of the correlation coefficient among all quantiles tested. **statistically significant at $\alpha = 0.05$, *statistically significant at $\alpha = 0.1$.

Table 7 (cont'd). Benthic macroinvertebrate community metrics, with expected response to perturbation and Spearman coefficients with SC values measured as single point-in time samples (“snapshot”), 12-month prior means of 15-minute interval data (“continuous”), and as a “best quantile” from prior 12-month records of 15-minute interval data.

Category	Metric	Expectation ¹	----- Fall 2013 -----				----- Spring 2014 -----			
			Snap-shot	Cont. mean	Cont. best quantile ²	Cont. best quantile ² used ²	Snap-shot	Cont. mean	Cont. best quantile ²	Cont. best quantile ² used ²
			--- Correlation coefficients ---				--- Correlation coefficients ---			
Richness	No. Total Taxa	D	-0.79**	-0.74**	-0.79**	40 th	-0.74**	-0.69**	-0.73**	5 th
	No. Total Taxa less Ephemeroptera	D	-0.66**	-0.62**	-0.69**	20 th	-0.51**	-0.46**	-0.49**	5 th
	No. Ephemeroptera Taxa	D	-0.80**	-0.77**	-0.81**	90 th	-0.83**	-0.83**	-0.84**	5 th
	No. Ephemeroptera Taxa less Baetidae	D	-0.77**	-0.77**	-0.76**	40 th	-0.88**	-0.86**	-0.89**	5 th
	No. EPT Taxa	D	-0.72**	-0.70**	-0.74**	20 th	-0.81**	-0.79**	-0.80**	5 th
	No. Plecoptera & Trichoptera Taxa	D	-0.60**	-0.57**	-0.64**	20 th	-0.58**	-0.54**	-0.55**	95 th
	No. Plecoptera Taxa	D	-0.43**	-0.39*	-0.49**	20 th	-0.69**	-0.65**	-0.71**	10 th
No. Trichoptera Taxa	D	-0.65**	-0.64**	-0.67**	80 th	-0.23	-0.18	-0.25	90 th	
Evenness	% Top 2 Dominant Taxa	I	0.42*	0.32	0.38*	90 th	0.70**	0.72**	0.72**	50 th
	% Top 5 Dominant Taxa	I	0.59**	0.54**	0.60**	40 th	0.75**	0.75**	0.77**	5 th
Diversity	Simpson Diversity	D	-0.46**	-0.38*	-0.42*	90 th	-0.71**	-0.71**	-0.72**	50 th
	Shannon Diversity	D	-0.57**	-0.50**	-0.53**	40 th	-0.76**	-0.75**	-0.77**	5 th
Composition	% Ephemeroptera	D	-0.74**	-0.70**	-0.74**	90 th	-0.85**	-0.87**	-0.87**	50 th
	% Ephemeroptera less Baetidae	D	-0.77**	-0.76**	-0.76**	70 th	-0.90**	-0.89**	-0.91**	20 th
	% Plecoptera	D	0.23	0.16	0.21	90 th	0.66**	0.66**	0.67**	5 th
	% Trichoptera	D	0.33	0.39*	0.41*	5 th	-0.004	0.05	0.10	30 th
Functional feeding group	% Collector-Filterers	V	0.32	0.42*	0.47**	5 th	0.05	0.17	0.15	30 th
	% Collector-Gatherers	V	-0.54**	-0.45**	-0.50**	90 th	-0.62**	-0.64**	-0.65**	60 th
	% Predators	V	-0.50**	-0.53**	-0.62**	40 th	-0.68**	-0.68**	-0.75**	95 th
	% Scrapers	D	-0.33	-0.32	-0.41*	5 th	-0.56**	-0.53**	-0.66**	5 th
	% Shredders	D	0.26	0.18	0.22	40 th	0.70**	0.69**	0.70**	5 th
	No. Collector-Gatherer Taxa	D	-0.73**	-0.61**	-0.68**	90 th	-0.44**	-0.46**	-0.49**	30 th
	No. Predator Taxa	D	-0.55**	-0.58**	-0.69**	20 th	-0.50**	-0.47**	-0.53**	95 th
No. Scraper Taxa	D	-0.44**	-0.48**	-0.60**	5 th	-0.75**	-0.70**	-0.77**	5 th	
Habit	% Clingers	D	0.62**	0.56**	0.60**	90 th	-0.55**	-0.54**	-0.56**	95 th
	No. Clinger Taxa	D	-0.67**	-0.65**	-0.73**	5 th	-0.76**	-0.72**	-0.76**	5 th

¹expected response to perturbation: D = decrease, I = increase, V = variable; from Barbour et al. (1999). ²“best” quantile is the quantile yielding the highest absolute value of the correlation coefficient among all quantiles tested. **statistically significant at $\alpha = 0.05$, *statistically significant at $\alpha = 0.1$.

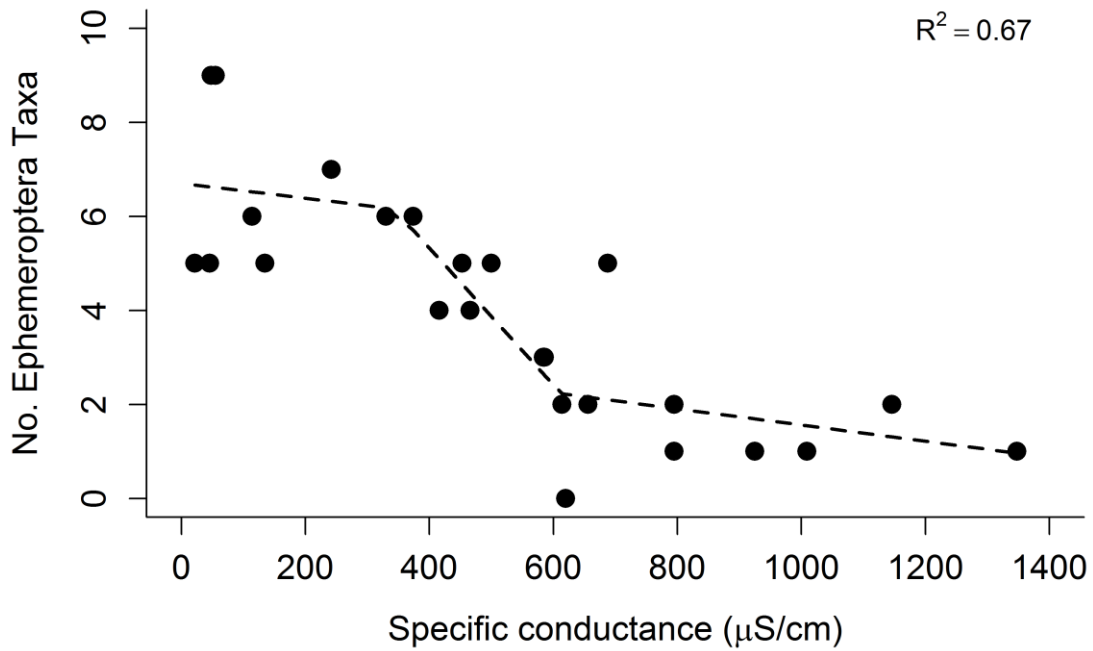


Figure 8. Scatterplot of Spring 2014 No. Ephemeroptera Taxa (mayfly richness) by snapshot SC. The dotted line is a fitted segmented regression line, with associated model fit (adjusted R^2) shown.

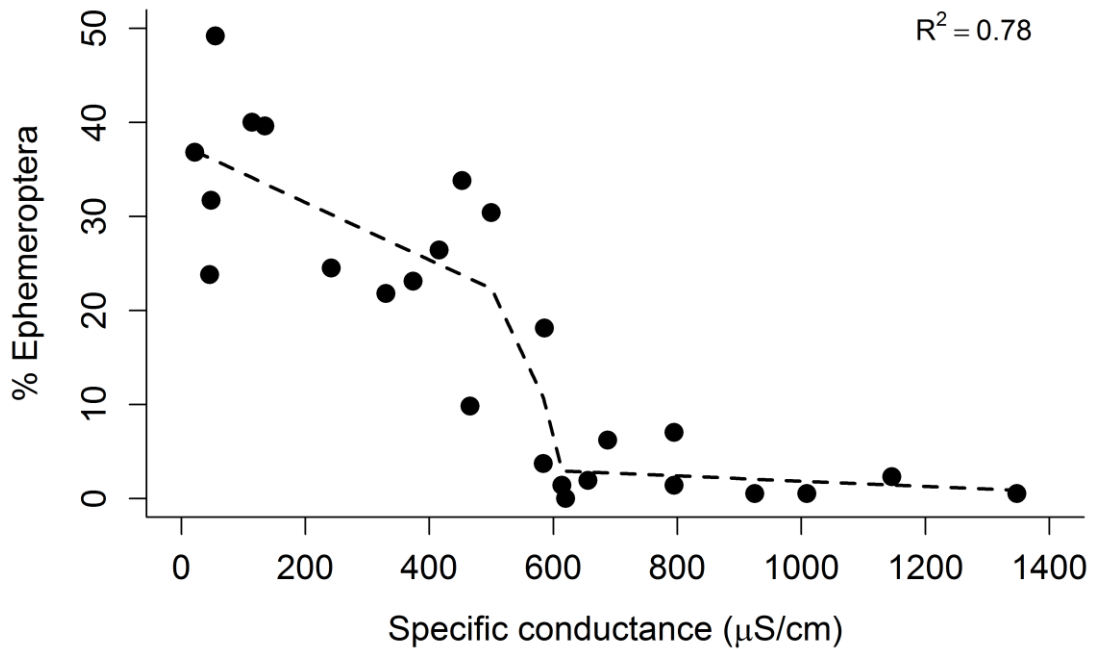


Figure 9. Scatterplot of Spring 2014 % Ephemeroptera individuals (mayfly abundance) by snapshot SC. The dotted line is a fitted segmented regression line, with associated model fit (adjusted R^2) shown.

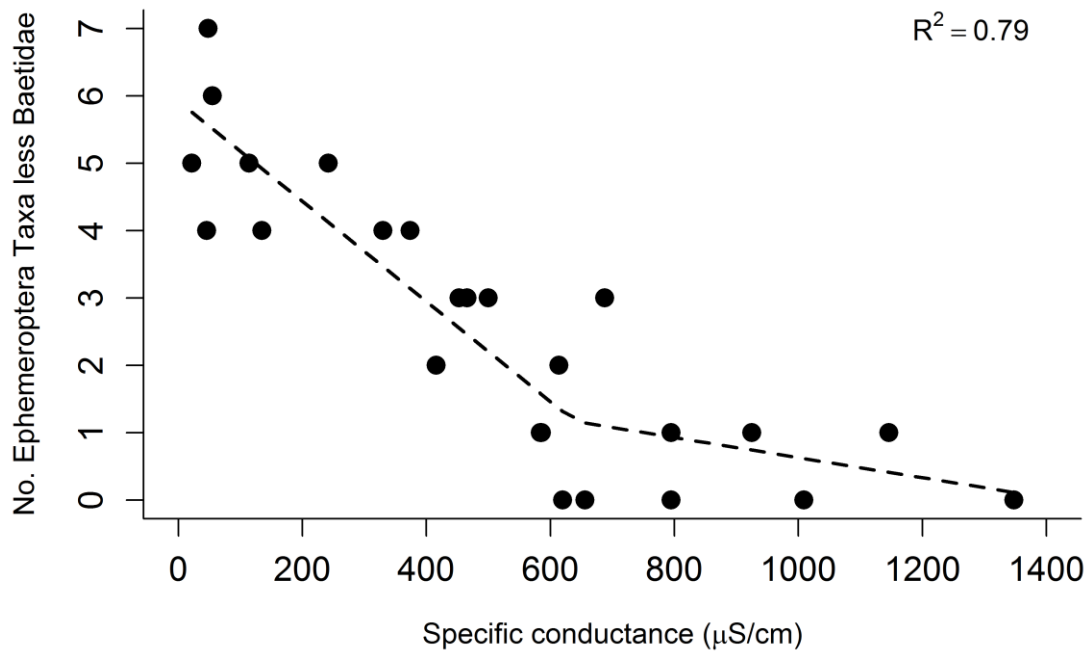


Figure 10. Scatterplot of Spring 2014 No. Ephemeroptera Taxa less Baetidae (richness of pollution-intolerant mayflies) by snapshot SC. The dotted line is a fitted segmented regression line, with associated model fit (adjusted R^2) shown.

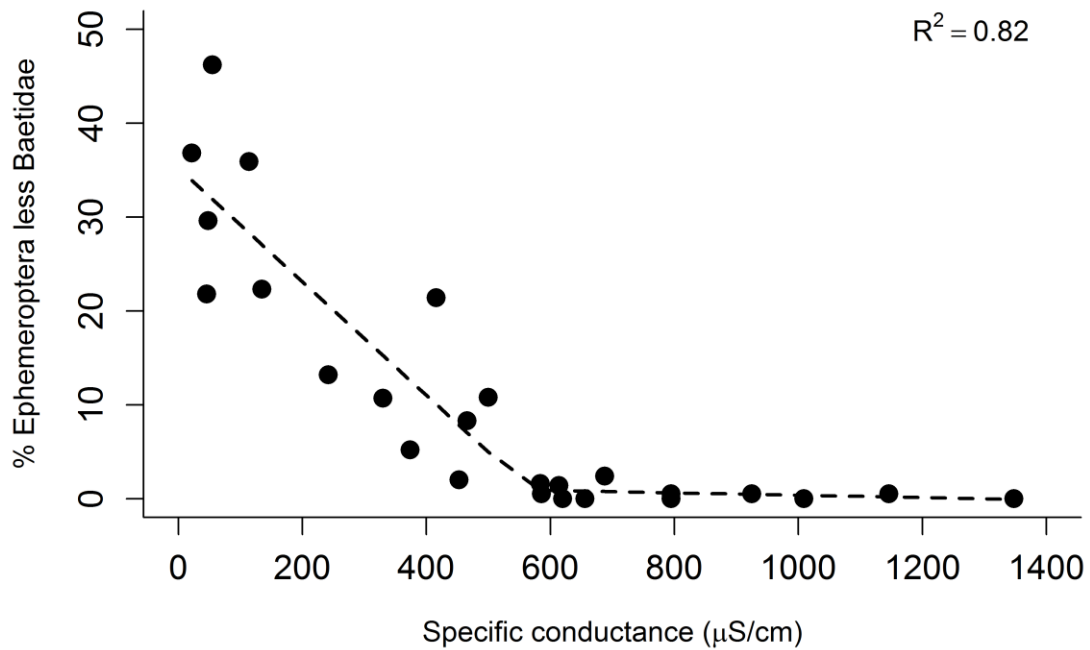


Figure 11. Scatterplot of Spring 2014 % Ephemeroptera less Baetidae (abundance of pollution-intolerant mayflies) by snapshot SC. The dotted line is a fitted segmented regression line, with associated model fit (adjusted R^2) shown.

Further evidence that community response to SC is driven by mayflies is found by comparing SC correlations between No. Total Taxa ($\rho = -0.74$, Figure 12) and No. Total Taxa less Ephemeroptera ($\rho = -0.51$, Figure 13). The considerably stronger correlation when taxa richness includes mayflies emphasizes the SC sensitivity of that group. Our prior work (Timpano et al. 2015) and work by others (Pond 2004, 2010) noted also that mayflies are the benthic macroinvertebrate group most affected in streams salinized from coal mining.

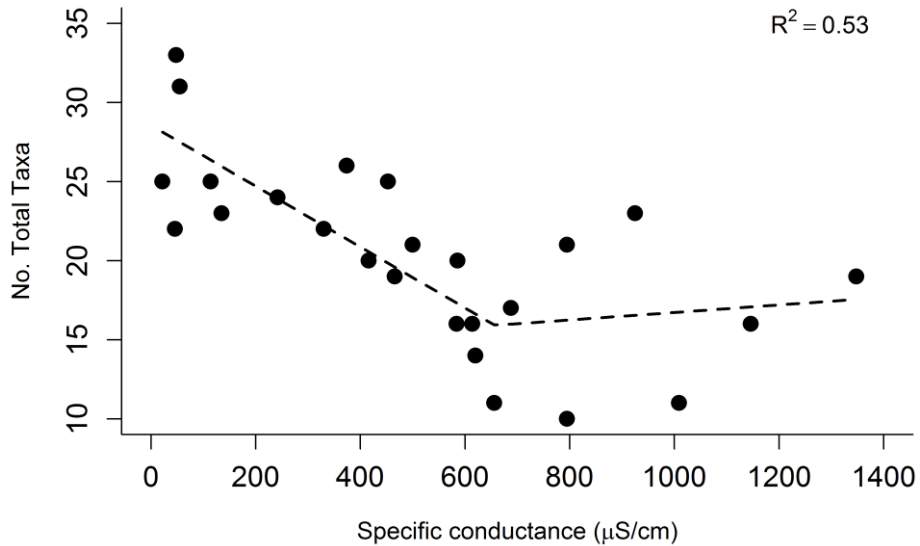


Figure 12. Scatterplot of Spring 2014 No. Total Taxa (total richness) by snapshot SC. The dotted line is a fitted segmented regression line, with associated model fit (adjusted R^2) shown.

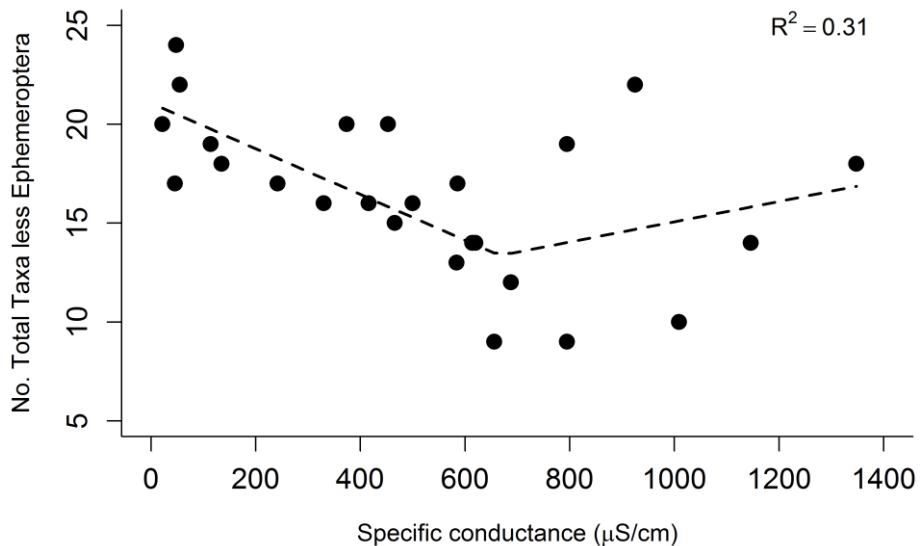


Figure 13. Scatterplot of Spring 2014 No. Total Taxa less Ephemeroptera (total richness less mayflies) by snapshot SC. The dotted line is a fitted segmented regression line, with associated model fit (adjusted R^2) shown.

Stoneflies and caddisflies had considerably weaker associations with SC than mayflies. Stonefly richness (No. Plecoptera Taxa) was moderately correlated with SC ($\rho = -0.69$), but the response by stonefly abundance (% Plecoptera) was opposite of that expected with increasing perturbation ($\rho = +0.66$).

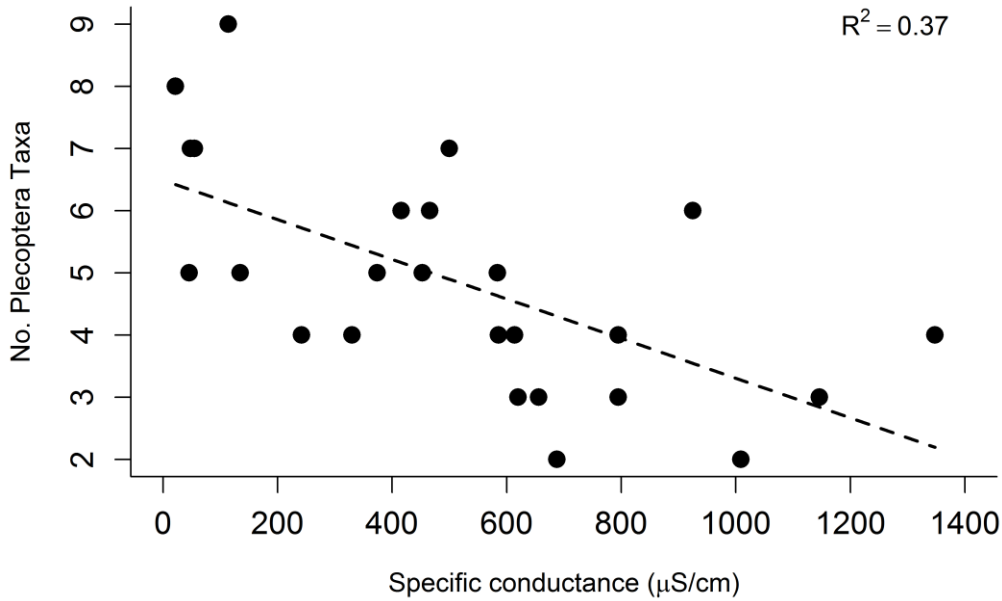


Figure 14. Scatterplot of Spring 2014 No. Plecoptera Taxa (stonefly richness) by snapshot SC. The dotted line is a fitted segmented regression line, with associated model fit (adjusted R^2) shown.

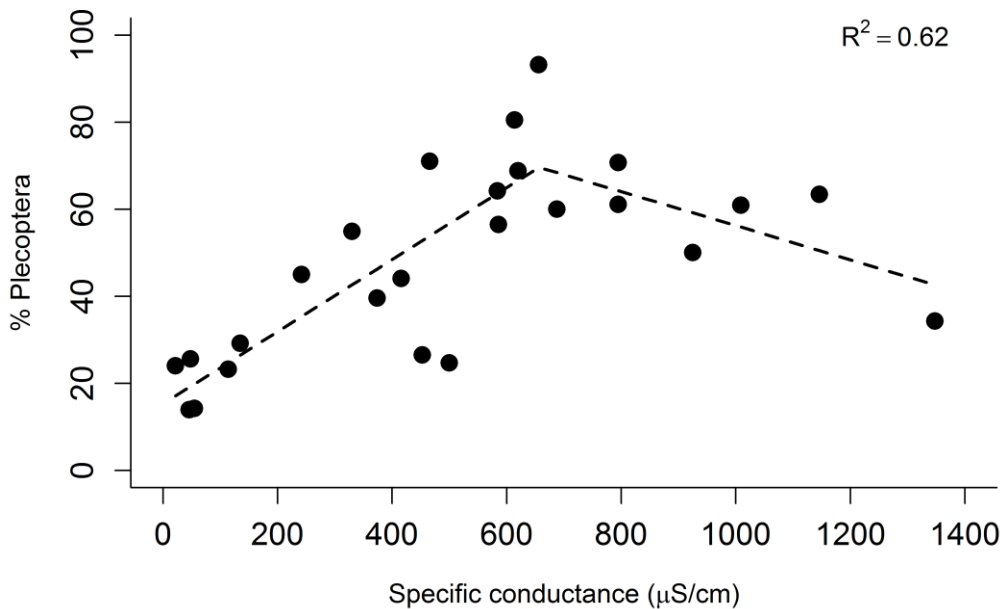


Figure 15. Scatterplot of Spring 2014 % Plecoptera (stonefly abundance) by snapshot SC. The dotted line is a fitted segmented regression line, with associated model fit (adjusted R^2) shown.

Though generally considered responsive to water quality changes, caddisflies did not respond to snapshot SC in our streams. Caddisfly richness (No. Trichoptera Taxa) and caddisfly abundance (% Trichoptera) were not significantly correlated with SC ($p = 0.2789$ and 0.9840 , respectively) (Figure 16 and Figure 17, respectively). The weak correlations of caddisfly metrics to SC could be an artifact of our sampling protocol, which captures caddisfly taxa living in erosional habitat (e.g., riffles and runs), but does not often capture many of the caddisfly taxa living in depositional habitat (e.g., pools and stream margins). Because we don't have a sample that fully represents the caddisfly community, we cannot conclude from these data that caddisflies in general are insensitive to SC at the levels measured in our study.

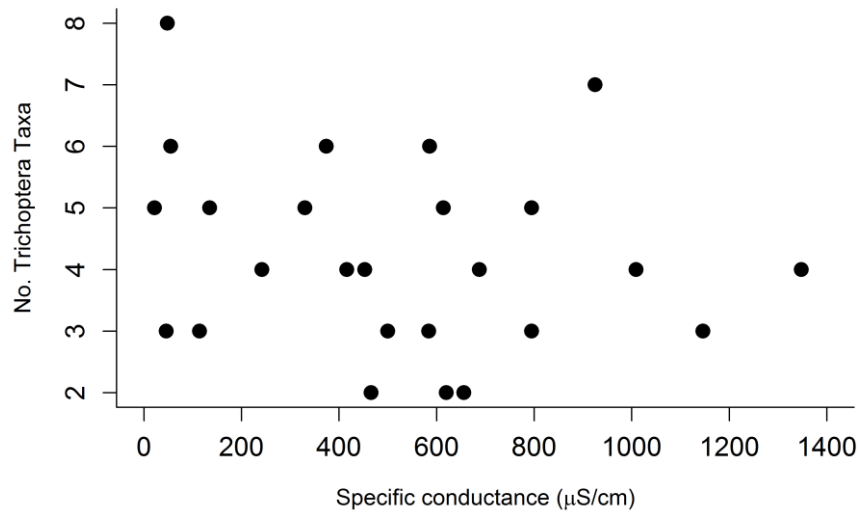


Figure 16. Scatterplot of Spring 2014 No. Trichoptera Taxa (caddisfly richness) by snapshot SC. No significant segmented regression or correlation (Spearman's rho, $p = 0.2789$).

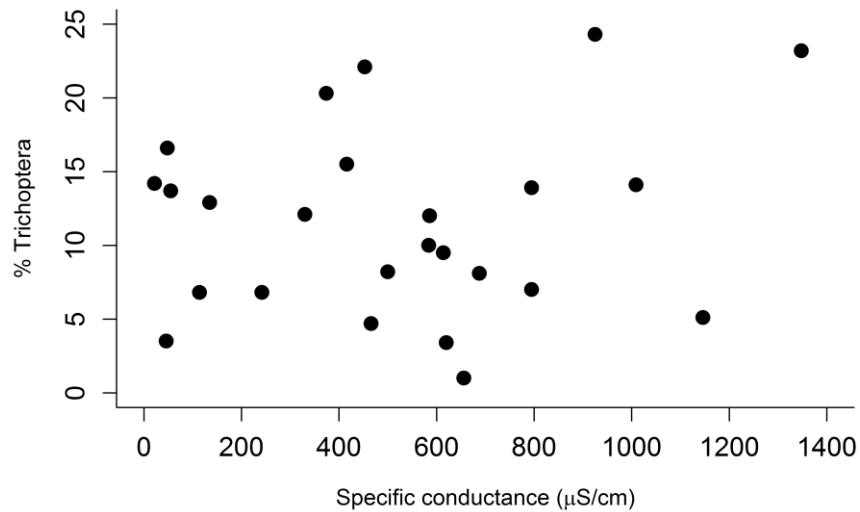


Figure 17. Scatterplot of Spring 2014 % Trichoptera (caddisfly abundance) by snapshot SC. No significant segmented regression or correlation (Spearman's rho, $p = 0.9840$).

Using Continuous SC to Characterize Salinity – Biota Associations

We examined how SC metrics derived from continuous data might affect salinity – biota correlations relative to the snapshot SC approach that is used typically and commonly. We found that correlations with the mean of prior 12-month SC were qualitatively similar to the snapshot-SC correlations (moderate to strong, with biological condition declining with increasing SC), but differed slightly in magnitude. Of the 23 metrics having statistically significant correlations with mean-SC, 14 had slightly weaker correlations, and 4 had slightly stronger correlations compared with snapshot-SC correlations. In contrast to mean-SC, the best quantile of prior 12-month SC yielded slightly stronger correlations compared to snapshot-SC. Best quantile correlations were slightly stronger for 17 of 23 metrics and slightly weaker for 4 metrics. Further, the best quantiles tended to be low quantiles, with 13 of 23 metrics having the strongest correlation with the 5th or 10th quantile, with 18 correlations having best quantiles of 50 or below.

Stronger correlations with low quantiles suggest that biota are responding to chronically elevated SC levels that occur routinely and continually (excepting dilution spikes) in the mining-influenced streams, rather than responding to temporally discrete SC levels such as short-term elevated concentrations that may result from unusual events and releases. Low quantiles can be considered a proxy for chronic SC exposure levels, because they represent values that SC exceeds for the majority of the 12-month period. High values of low quantiles indicate that SC remains elevated for most of the year, which in turn represents high chronic SC exposure levels. Of the nine metrics that showed the strongest correlations ($\rho \geq 0.73$) with SC (No. Total Taxa, No. Ephemeroptera Taxa, No. Ephemeroptera Taxa less Baetidae, No. EPT Taxa, % Top 5 Dominant Taxa, Shannon Diversity, % Predators, No. Scraper Taxa, No. Clinger Taxa), eight had the strongest correlation with the 5th quantile of prior 12-month SC (Table 7). The finding of generally stronger correlations for lower quantiles applies to both of the full study years, and is especially prevalent for the spring sampling results (Table 7).

The marginal increase in SC-biota correlation strength using continuous data as compared to the snapshot approach is likely attributable to the strong correlations among SC summary metrics (Table 8). In the case of test site data from Spring 2014 (n = 20 sites), snapshot SC was strongly correlated with low SC quantiles of prior 12-month SC (e.g., 10th quantile Pearson's $r = 0.96$) (Table 8). Although using continuous data yields only a marginal increase in predictive power, the approach better accounts for long-term patterns, thus allowing more accurate characterization of the salinity influencing biota.

Table 8. Pearson correlations for Spring 2014 Test site SC summary measures.

		Snap -shot ¹	Mean ²	-----Quantile ³ -----										
				5	10	20	30	40	50	60	70	80	90	
----- Quantile -----	Mean	0.95												
	5	0.94	0.92											
	10	0.96	0.95	1.00										
	20	0.97	0.97	0.98	0.99									
	30	0.97	0.98	0.97	0.98	1.00								
	40	0.97	0.99	0.95	0.97	0.99	1.00							
	50	0.95	0.99	0.93	0.95	0.98	0.99	1.00						
	60	0.94	1.00	0.91	0.93	0.96	0.98	0.99	1.00					
	70	0.93	0.99	0.87	0.90	0.94	0.95	0.97	0.99	0.99				
	80	0.90	0.98	0.84	0.88	0.91	0.93	0.95	0.97	0.98	0.99			
	90	0.88	0.96	0.80	0.83	0.87	0.89	0.91	0.93	0.95	0.98	0.99		
	95	0.86	0.95	0.79	0.82	0.85	0.87	0.89	0.92	0.94	0.97	0.99	1.00	

¹seasonal snapshot SC measured at time of biological sampling, Spring 2014 (April 2014)

²mean of continuous SC data from 12 months prior to Spring 2014 biological sampling (April 2013 – April 2014)

³quantile of continuous SC data from 12 months prior to Spring 2014 biological sampling (April 2013 – April 2014)

Seasonality of Salinity – Biota Associations

For both snapshot and continuous SC measurement methods, analyses revealed significant associations of SC with most biotic metrics, but those associations occurred more consistently for biological samples collected in the Spring, compared to those collected in the Fall. Because mayflies exhibited the strongest correlations with SC, natural seasonal differences in mayfly presence could explain seasonal differences in salinity-biota correlations. Most mayfly genera that we observed in our streams have one seasonal life cycle per year (Poff et al. 2006), with adults emerging, mating, and laying eggs in spring/summer (Merritt et al. 2008). As a result of such seasonal life histories, mayfly nymph abundance in samples is generally highest in spring, prior to emergence, and lowest in fall, as early instar nymphs are too small to be captured by our 500 µm-mesh net, or are not present in the swift current of the riffle habitat we sampled. Our seasonal samples exhibited the expected pattern, with mayfly richness and abundance highest in spring and lowest in fall for most years.

Mayfly richness was lower in reference (Welch’s paired t-test; $p = 0.0069$) and test ($p < 0.0001$) sites in Fall 2012 compared to Spring 2013. The same pattern for test sites was observed a year later, with mayfly richness lower in test sites ($p = 0.0006$) in Fall 2013 compared to Spring 2014. Only three of five reference sites had sufficient flow to enable sampling in Fall 2013; there was no significant difference in mayfly richness ($p = 0.1914$) between Fall 2013 and Spring 2014. Mayfly abundance was lower in Fall 2012 than in Spring 2013 for reference (Welch’s paired t-test; $p = 0.0420$) and test ($p = 0.0032$) sites. The following year showed the same pattern, with mayfly abundance lower in Fall 2013 than in Spring 2014 for reference ($p = 0.0312$) and test sites ($p = 0.0007$). Such seasonal variation suggests that timing of biological sampling could significantly influence how biotic effects of salinity are detected and interpreted.

Inter-Annual Consistency of Salinity – Biota Associations

For the majority of biological metrics, correlations with salinity measures were of consistent magnitude, direction, and significance from year to year. Comparing like seasons (i.e., Fall 2013 vs. Fall 2012, and Spring 2014 vs. Spring 2013), revealed that correlations using Spring data are more consistent than those using Fall data. We found that seven of 26 metrics had at least one salinity measure (snapshot, mean, or quantile) that was differently significant between Fall datasets (No. Total Taxa less Ephemeroptera, No. Trichoptera Taxa, % Top 2 Dominant Taxa, Simpson Diversity, % Collector-Gatherers, % Scrapers, and % Clingers). Spring data were more consistent, with only two of 26 metrics having different significance between Spring datasets (No. Trichoptera Taxa and % Collector-Filterers). With only two years for comparison, it is not clear which differences between years are anomalous. Additional years of data should allow for identification of the salinity-biota correlation expected in the long term. However, the greater consistency of correlations using Spring than Fall data suggest that assessing salinity-biota relationships would be accomplished more reliably in Spring than in Fall.

Objective 4: Determine How Salinity Should Be Monitored and Assessed

Our findings provide strong evidence that the monitoring status quo (seasonal snapshot sampling of water chemistry concurrent with biological sampling, with no expectation of seasonal or time-of-year consistency) is inadequate to accurately characterize the true nature of stream salinity and its association with biological condition. Though the exact monitoring approach will vary with agency needs, we offer several recommendations that can improve accuracy of monitoring and assessment of salinity and its impact on aquatic life in mining-influenced headwater streams.

Biological sampling during the spring, rather than fall, should allow for greater sensitivity to detect changes in biological condition where salinity is the primary stressor. Because mayflies are the benthic macroinvertebrate group most sensitive to salinity, an accurate accounting of their presence or absence is important for accurate determination of salinity effects; spring samples accomplish such accounting better than do fall samples.

Accurate characterization of salinity levels affecting biota requires measurement of salinity at multiple times of year. Salinity measured at the time of biological sampling does not represent the salinity levels to which organisms are exposed during their life cycle. Because most benthic macroinvertebrate taxa observed in this study, including the salt-sensitive mayflies, require approximately 12 months to mature (Poff et al. 2006), most invertebrates will be exposed to the full annual range of salinity. Furthermore, our data suggest that chronic salt exposure over extended periods of the year may be driving biological response. Therefore, salinity measured at multiple times during the year should increase accuracy of characterizing biologically-influential patterns of salt exposure.

Foremost among considerations when developing monitoring plans is to maintain consistency when making comparisons among sites or through time. Because neither biology nor salinity is constant throughout the year, annual timing of both must be consistent for maximum comparability between samples. However, both factors appear to follow a roughly annual cycle that is generally consistent from year to year (assuming consistent site and climatic conditions),

thereby allowing for establishment of a regular sampling schedule. An associated study (Boehme et al. 2015), conducted with non-OSM sponsorship, provides further detail concerning temporal variability of the benthic macroinvertebrate communities in a subset of the streams sampled for this study.

SUMMARY AND CONCLUSIONS

We accomplished our objectives to isolate the salinity variable, characterize temporal variability of salinity, and characterize the biological response to salinity. Although a single “best practice” for monitoring and assessing salinity effects on aquatic life is not apparent from our data, we provide several recommendations that should improve the accuracy of such efforts. Our findings for each of the four study objectives are summarized briefly below.

Objective 1: A rigorous and extensive site selection effort enabled us to characterize biological response to salinity independent of significant influence from covariate stressors. Analyses provided no evidence that non-salinity stressors significantly influenced salinity – biota correlations at our study sites. Test sites were comparable to reference sites with respect to water quality and physical habitat.

Objective 2: Continuous conductivity monitoring allowed us to characterize the temporal variability of salinity at our study sites. Specific conductance varied temporally over the study period, exhibiting a seasonal pattern of highest SC in fall and lowest SC in spring. Dilution spikes associated with precipitation events frequently lowered SC greatly for short durations throughout the year. Stream water grab-samples collected at multiple times during the study period revealed that test site waters were composed primarily of the anions SO_4^{2-} and HCO_3^- , and the cations Ca^{2+} and Mg^{2+} . Use of SC as a surrogate for TDS/major ions is reasonable given the strong relationship we observed between the two parameters, provided the ion matrix being described by SC is consistent among sites. A second consecutive year of study confirmed similar temporal patterns of conductivity and ionic composition.

Objective 3: Changes in benthic macroinvertebrate community structure were significantly and often strongly correlated with increased salinity (as indicated by our SC measurements), with declines in taxa richness and abundance generally responding more strongly than functional metrics. Spring data produced stronger and more frequently significant correlations with salinity than did Fall data. Mayflies were the benthic macroinvertebrate group most strongly responsive to salinity, an observation consistent among studies in similar streams. Observed salinity-biota associations were similar in a second consecutive year of study, indicating such associations are reliable and characteristic of the system studied.

Objective 4: Continuous conductivity data show potential for slight improvements in predicting biological condition as compared with discrete seasonal measurements. Although the strength of prediction using continuous data is marginally improved in our analysis, it cannot be overstated that models accounting for long-term conductivity trends have greater potential for accurately characterizing biologically-influential salinity patterns, with commensurate benefits in accuracy of related assessment efforts.

Toward the goal of improved efficacy of salinity-biota monitoring and assessment, our findings suggest several recommendations. 1) Sample the benthic macroinvertebrate community in the spring for maximum salinity sensitivity. 2) Measure salinity at multiple times during the year for a more accurate accounting of stressor levels influencing biota. Finally, 3) maintain consistency of chemical and biological sample timing when making comparisons through space or time.

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RELEASE FROM OBLIGATIONS

Virginia Polytechnic Institute and State University considers the cooperative agreement “Effective Monitoring and Assessment of Total Dissolved Solids as a Biotic Stressor in Mining-Influenced Streams” S11AC20004 and S12AC20023 to be complete, and payments from OSMRE for all allowable costs have been made, and OSMRE is released from all obligations under or arising from the cooperative agreement, pending any subsequent audit.

APPENDIX A – TIME SERIES PLOTS OF CONTINUOUS SC BY SITE

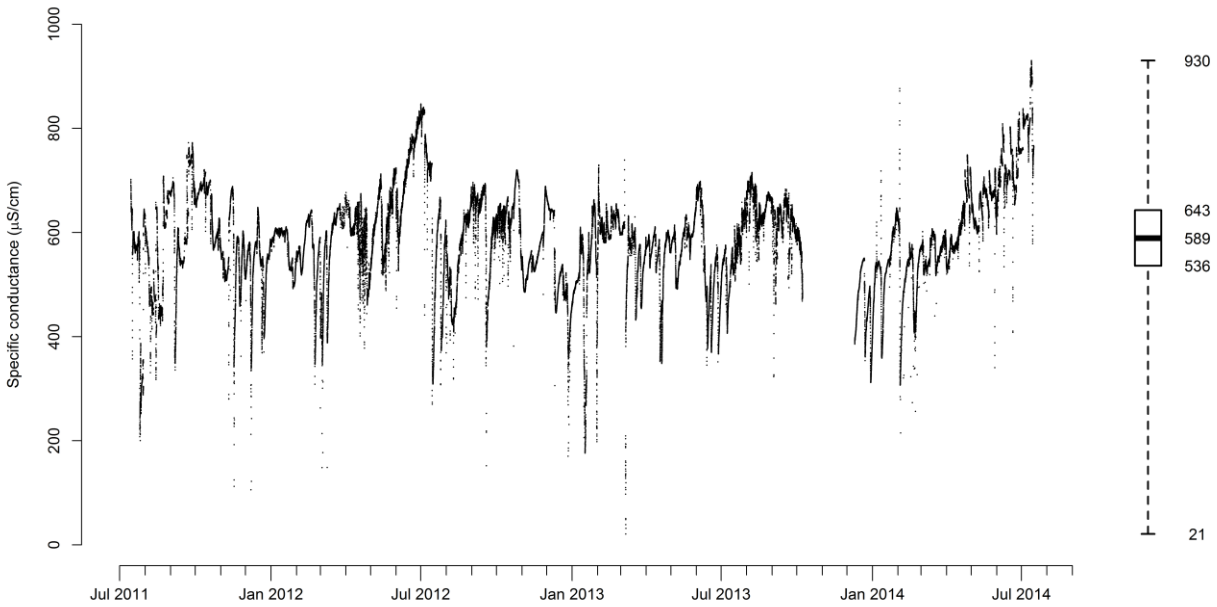


Figure A - 1. Birchfield Cr. (BIR, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

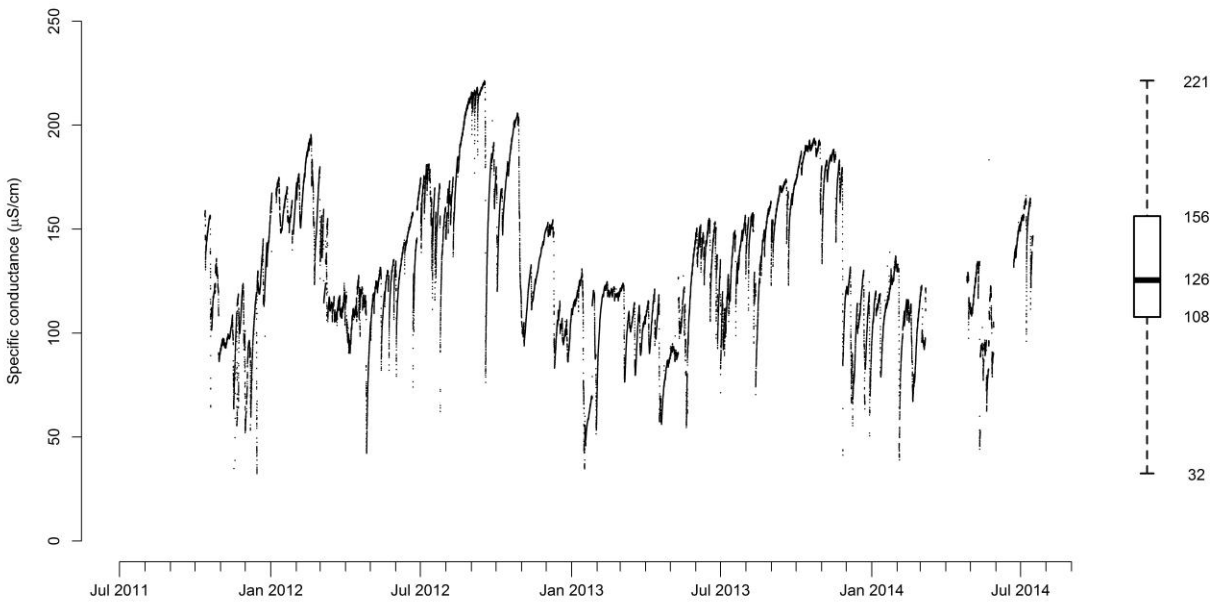


Figure A - 2. Copperhead Branch (COP, reference site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

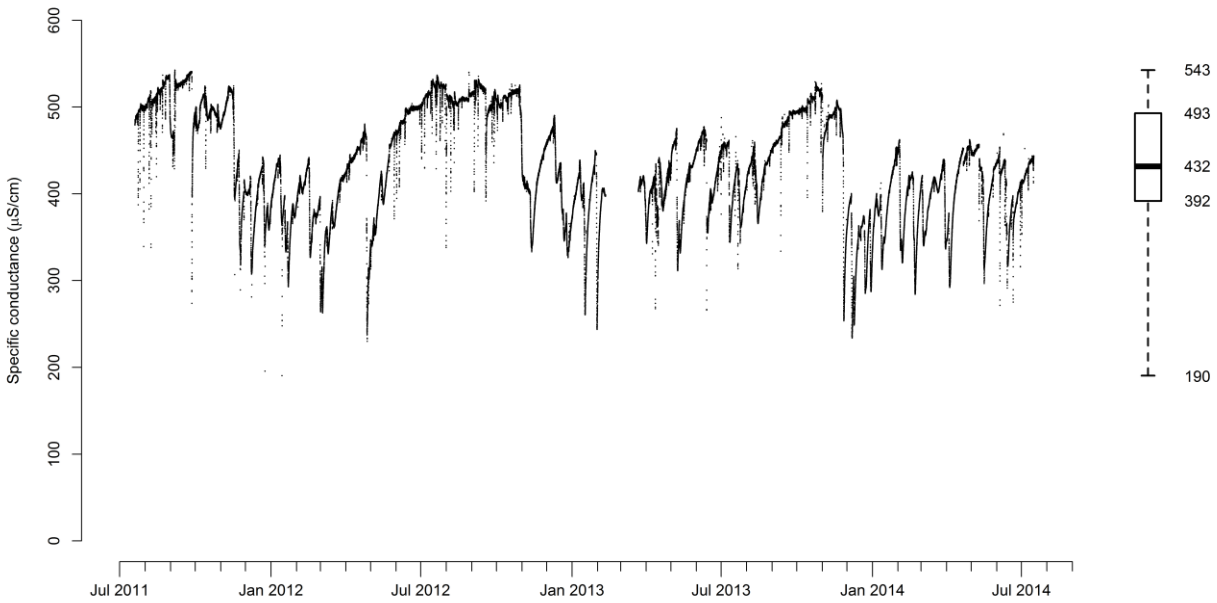


Figure A - 3. Crane Fork(CRA, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

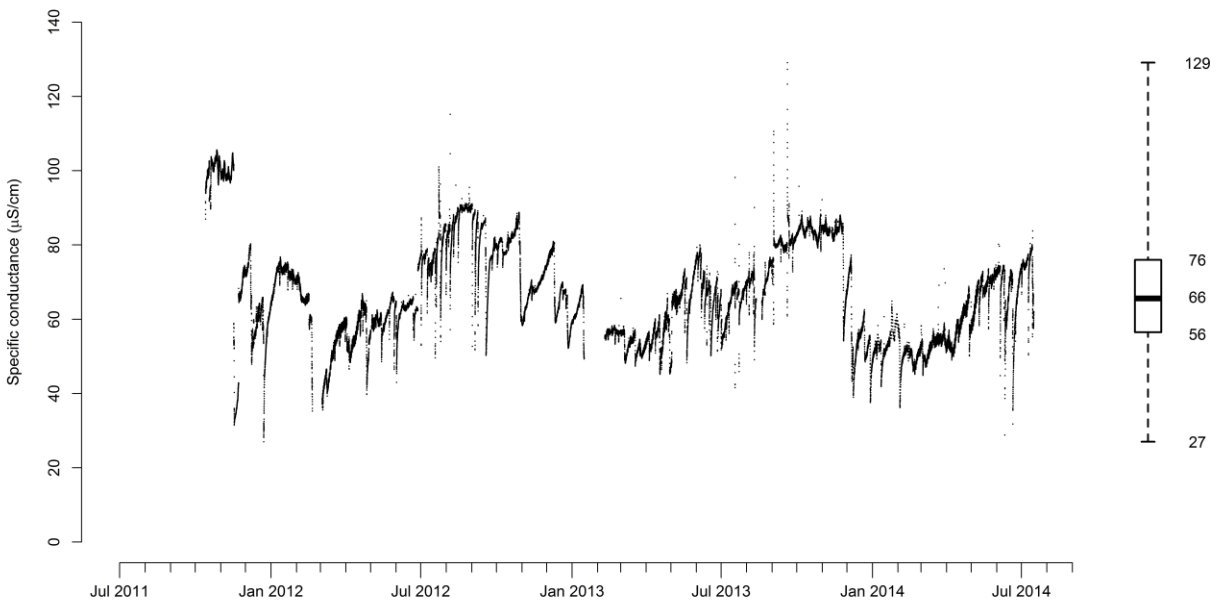


Figure A - 4. Crooked Branch(CRO, reference site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

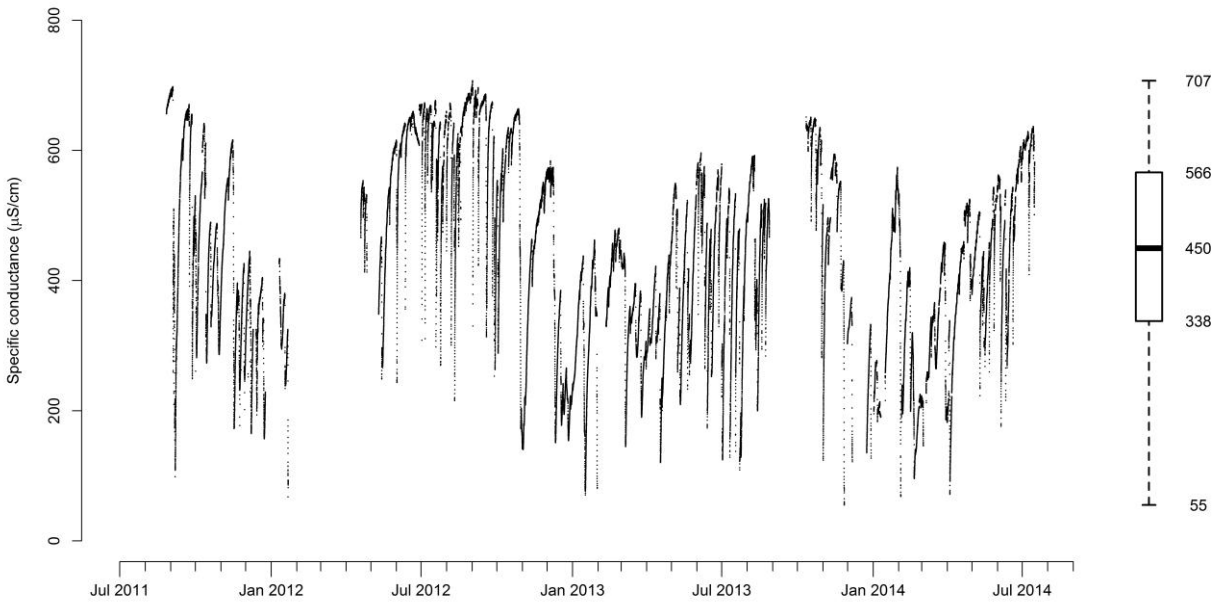


Figure A - 5. Dave Branch (DAV, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

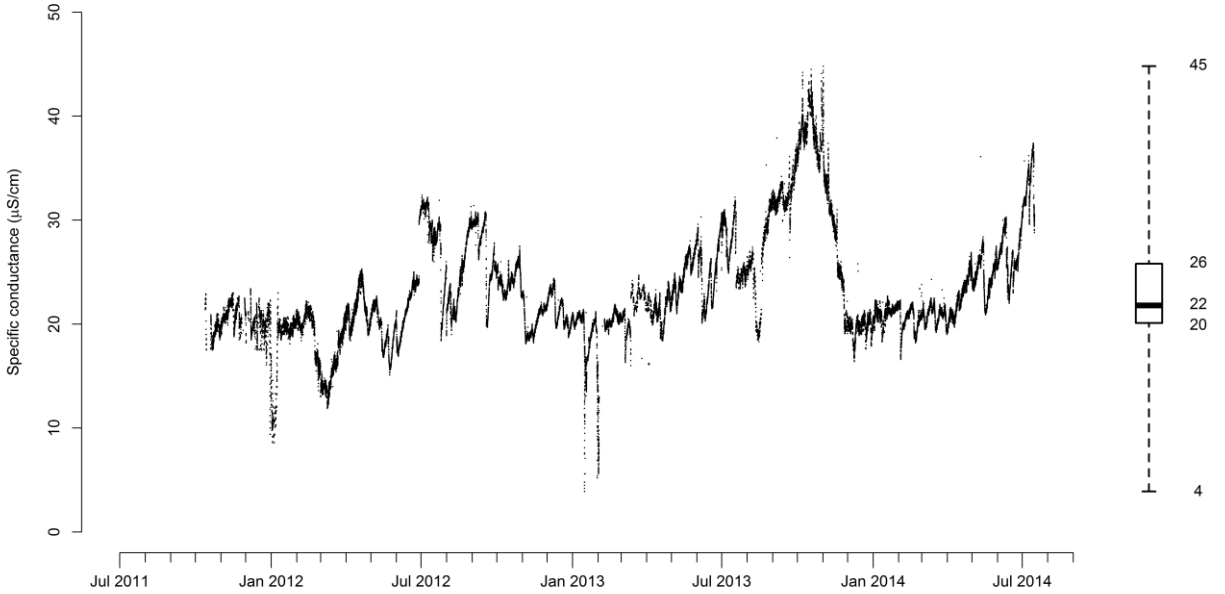


Figure A - 6. Eastland Creek (EAS, reference site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

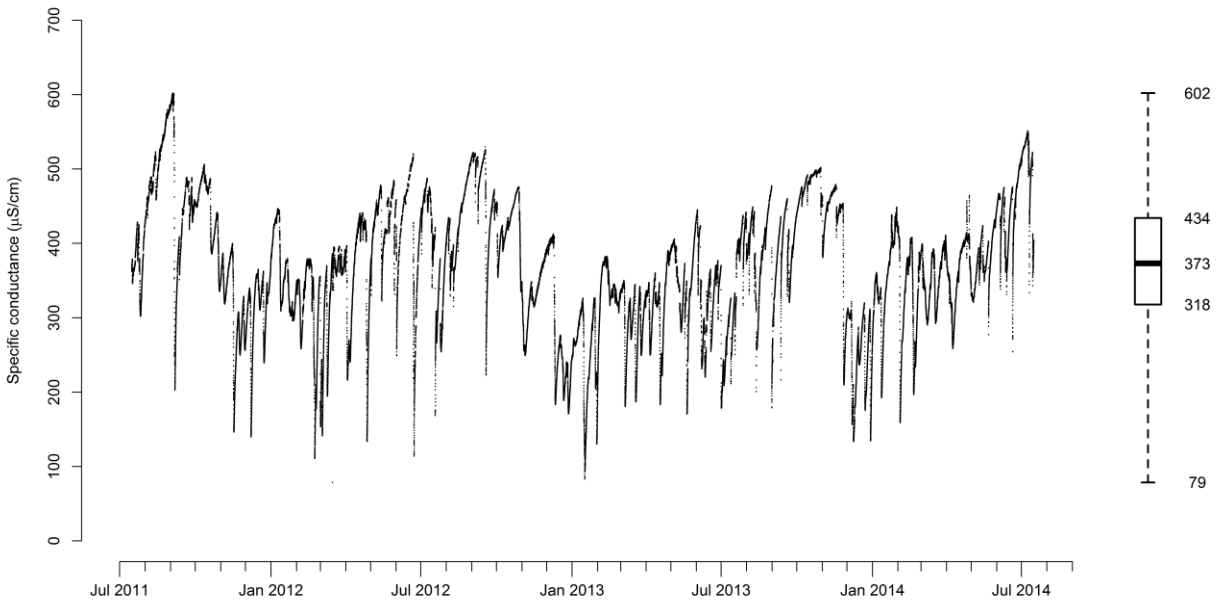


Figure A - 7. Fryingpan Creek (FRY, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

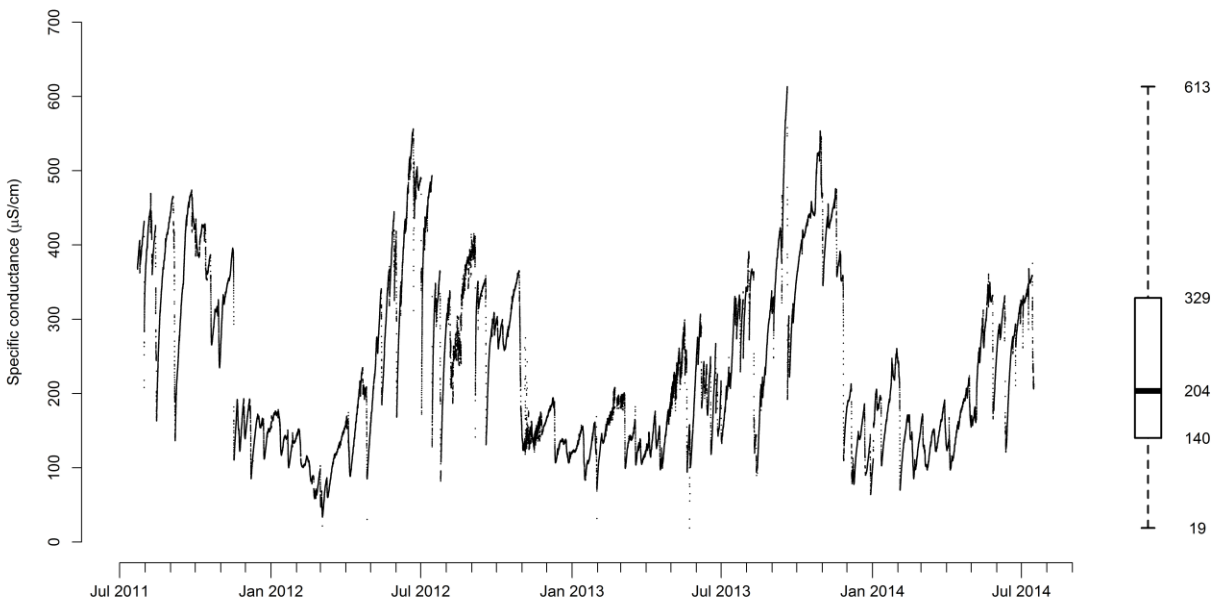


Figure A - 8. Grape Branch (GRA, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

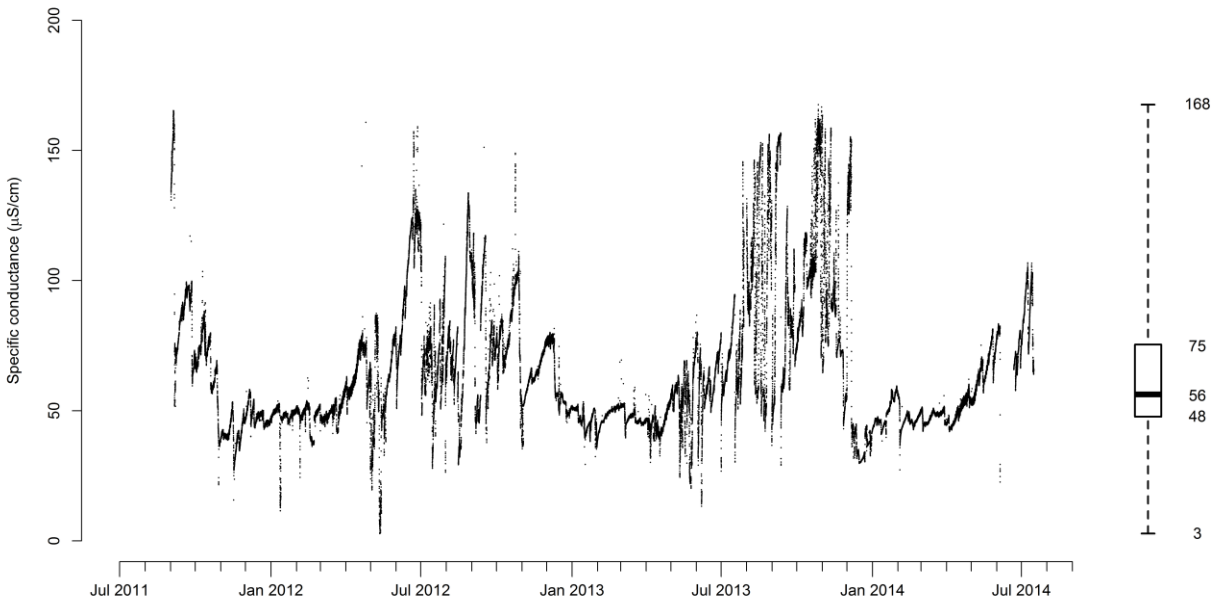


Figure A - 9. Hurricane Fork, WV (HCN, reference site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

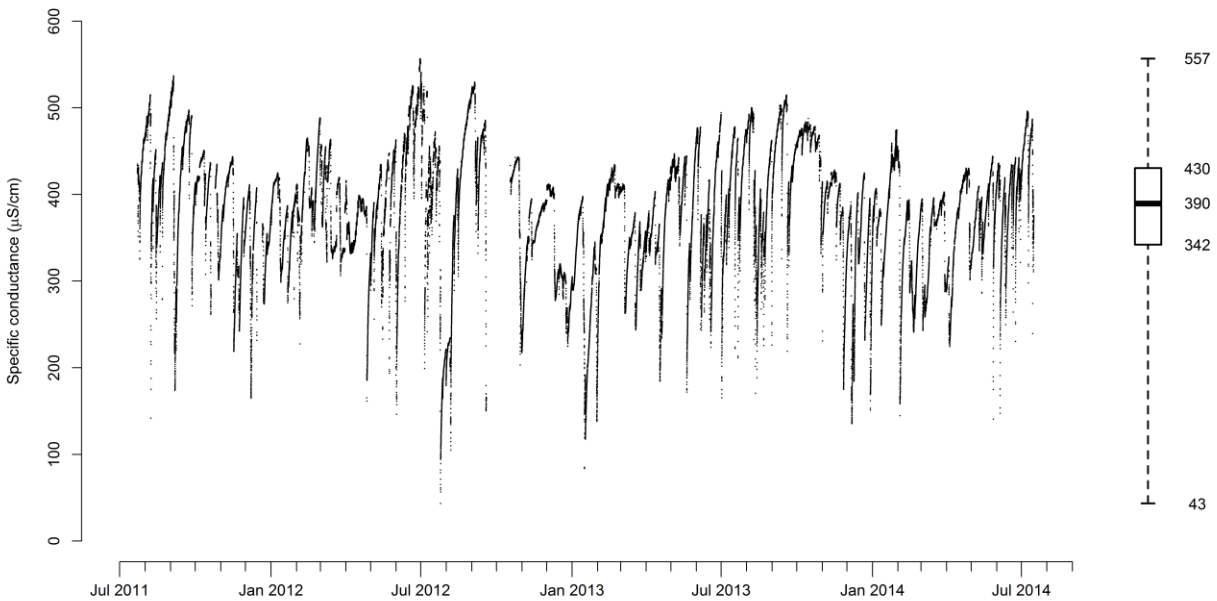


Figure A - 10. Hurricane Fork, VA (HUR, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

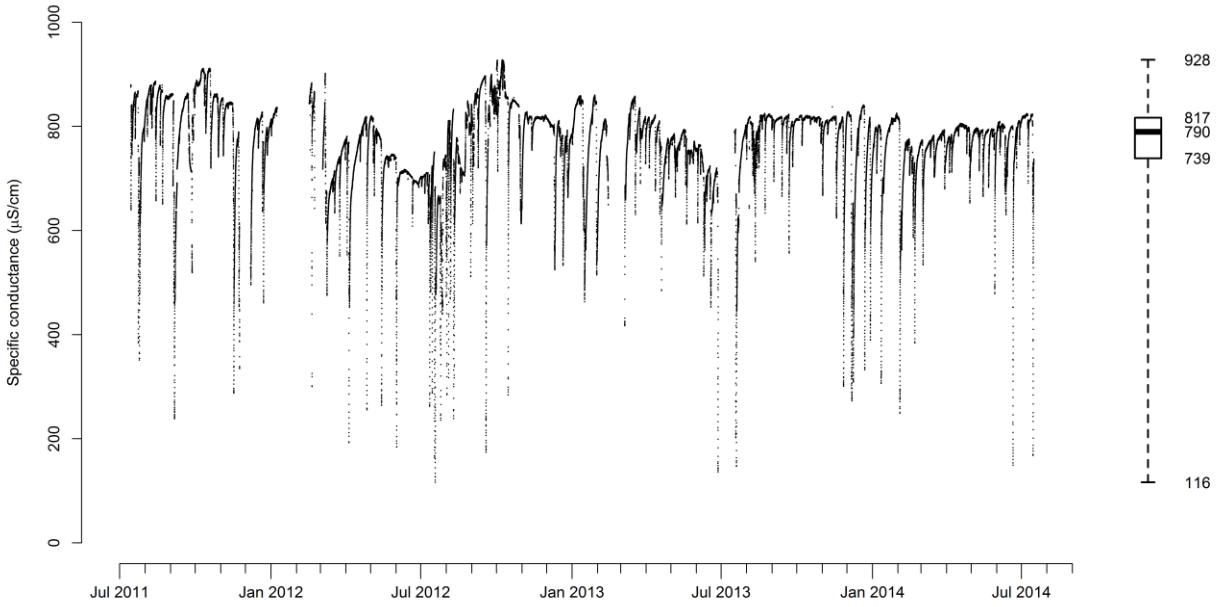


Figure A - 11. Kelly Branch (KEL, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

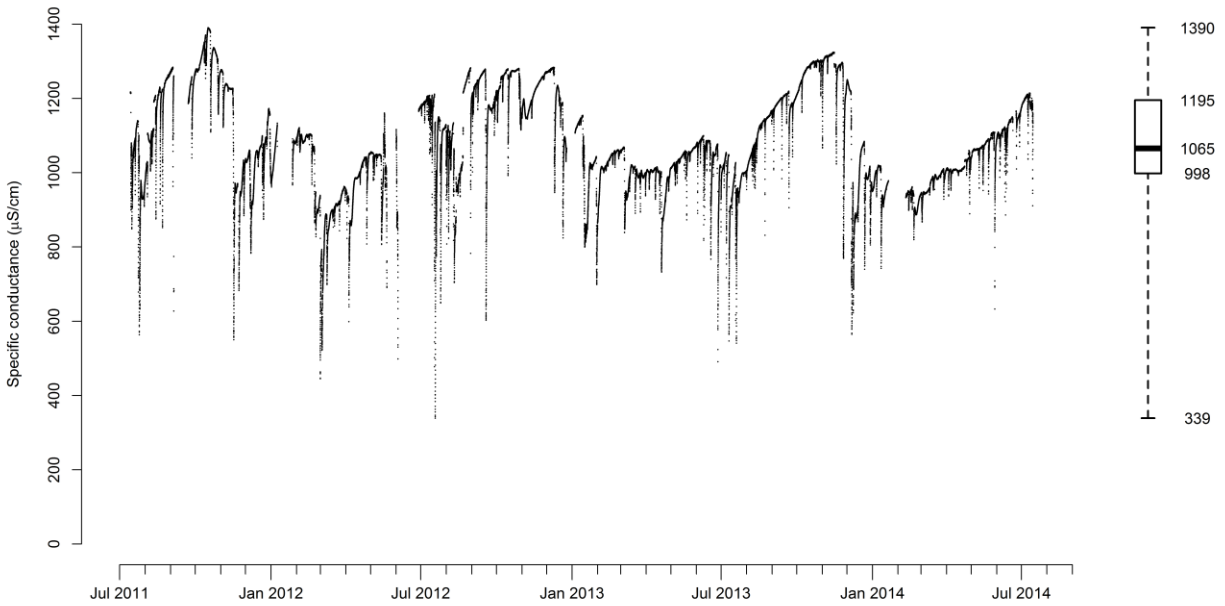


Figure A - 12. Kelly Branch Unnamed Tributary (KUT, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

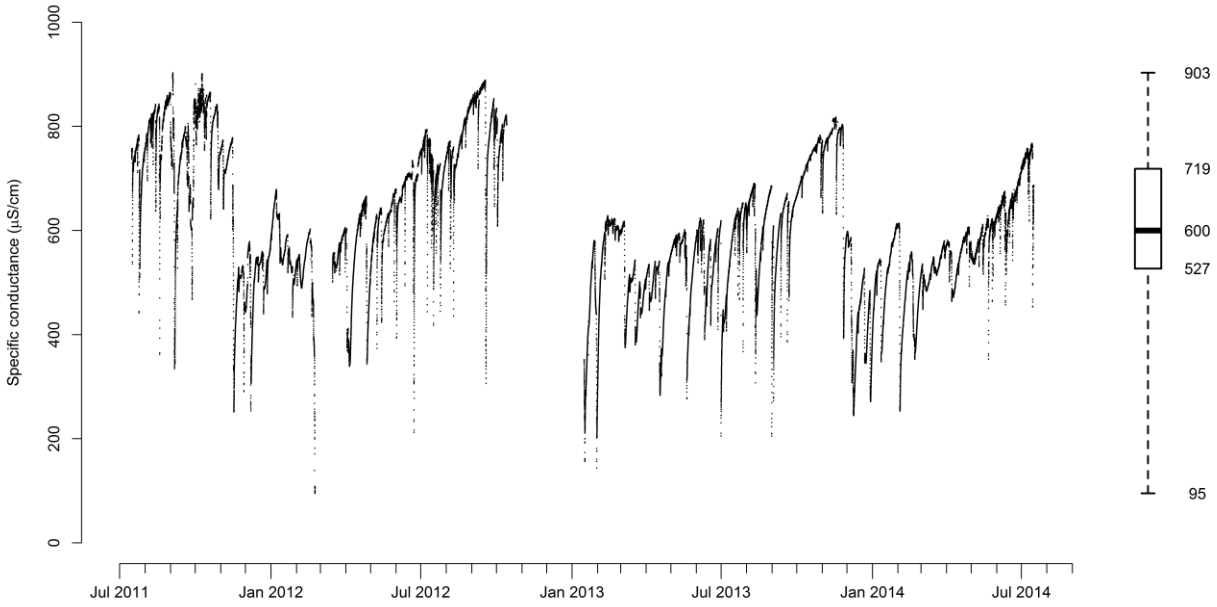


Figure A - 13. Laurel Branch (LAB, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

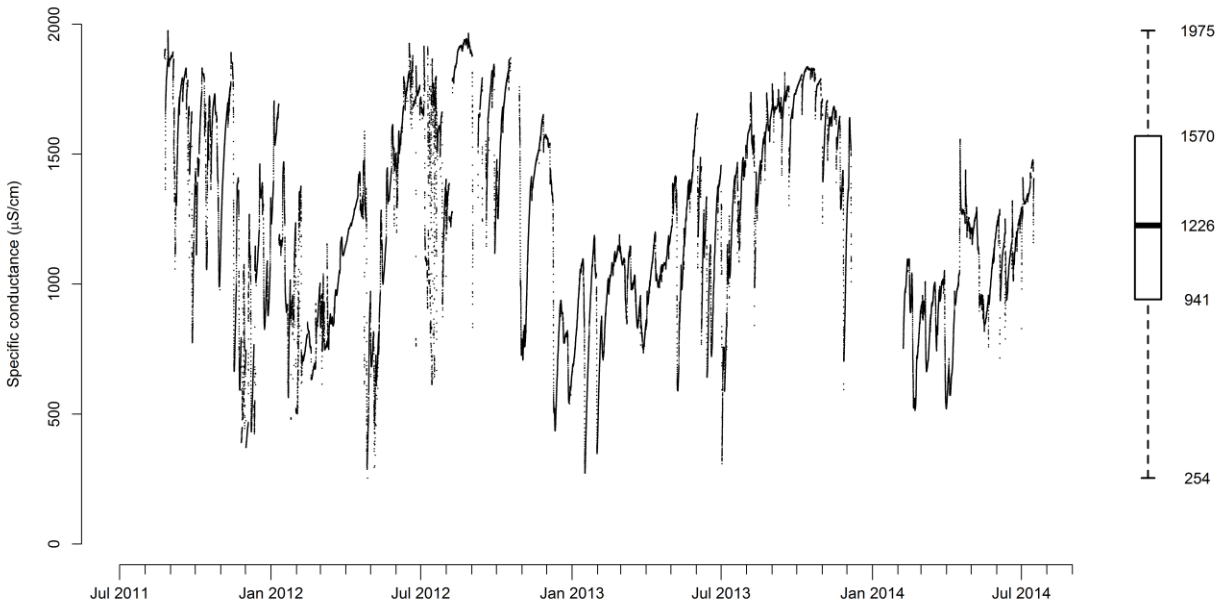


Figure A-14. Left Fork of Laurel Fork of Coal Fork (LLC, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

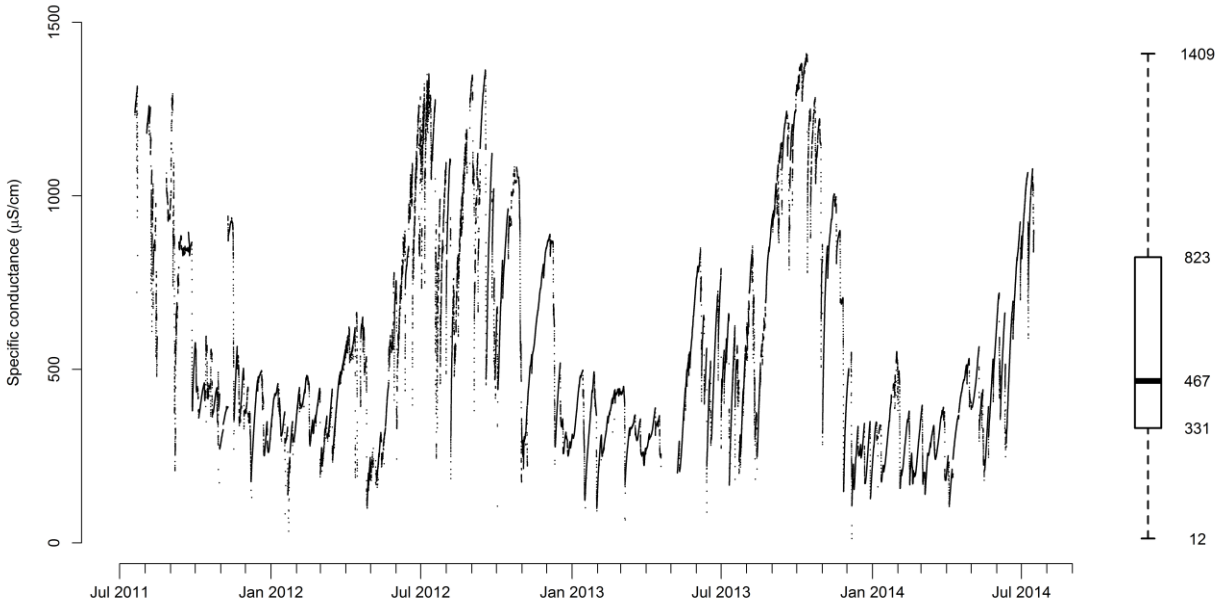


Figure A - 15. Longlick Branch East Fork (LLE, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

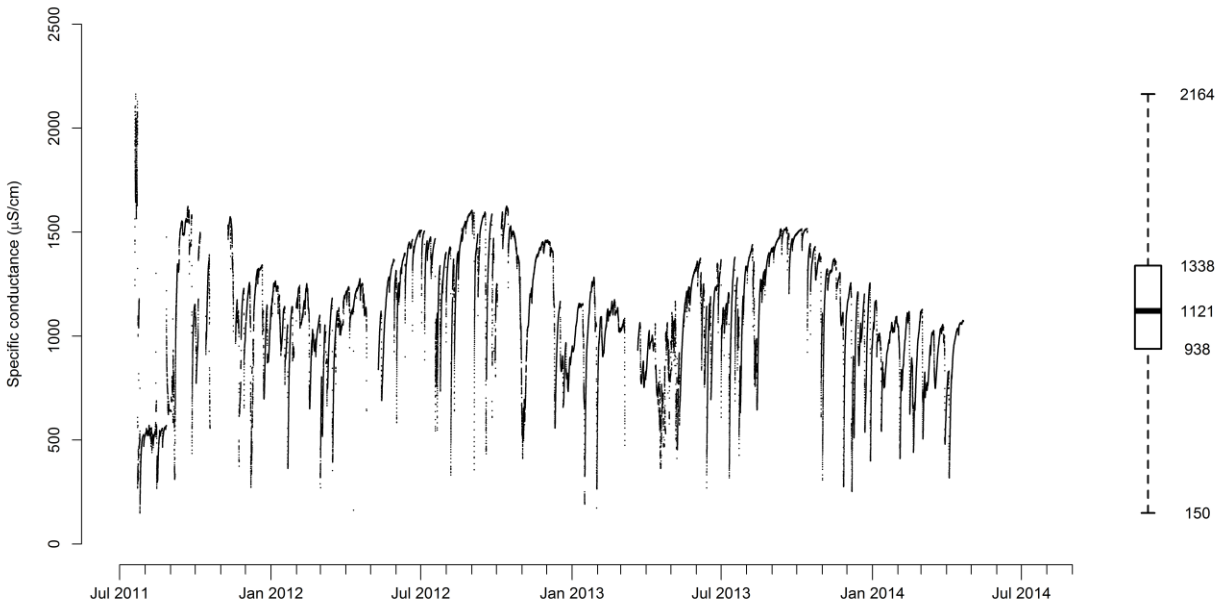


Figure A - 16. Longlick Branch West Fork (LLW, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

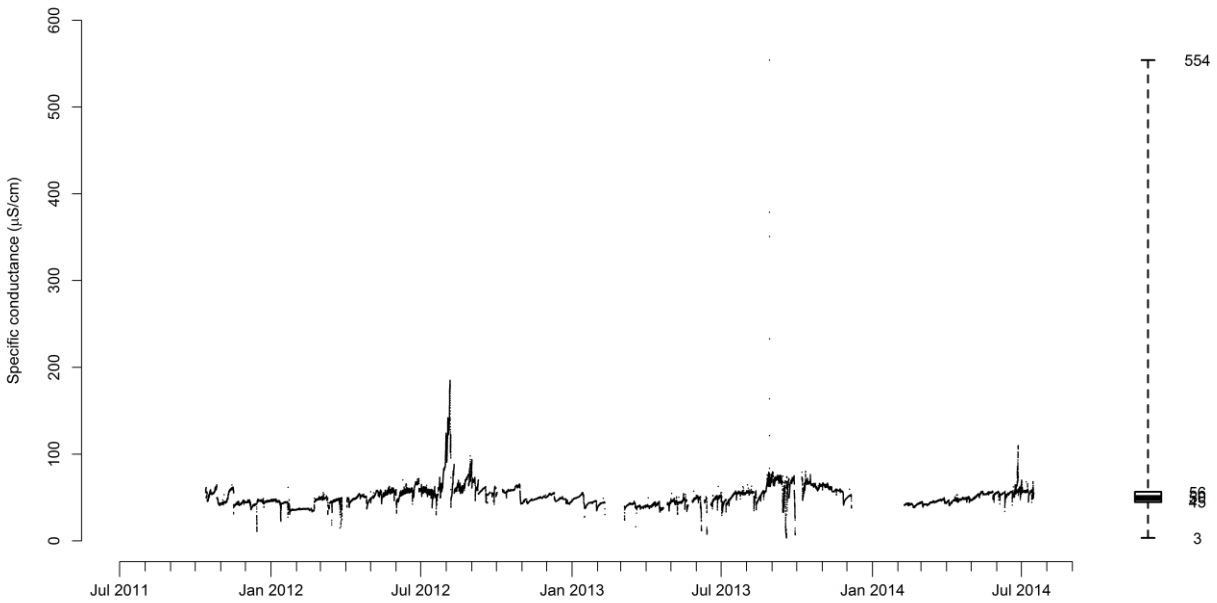


Figure A - 17. Middle Camp Branch (MCB, reference site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

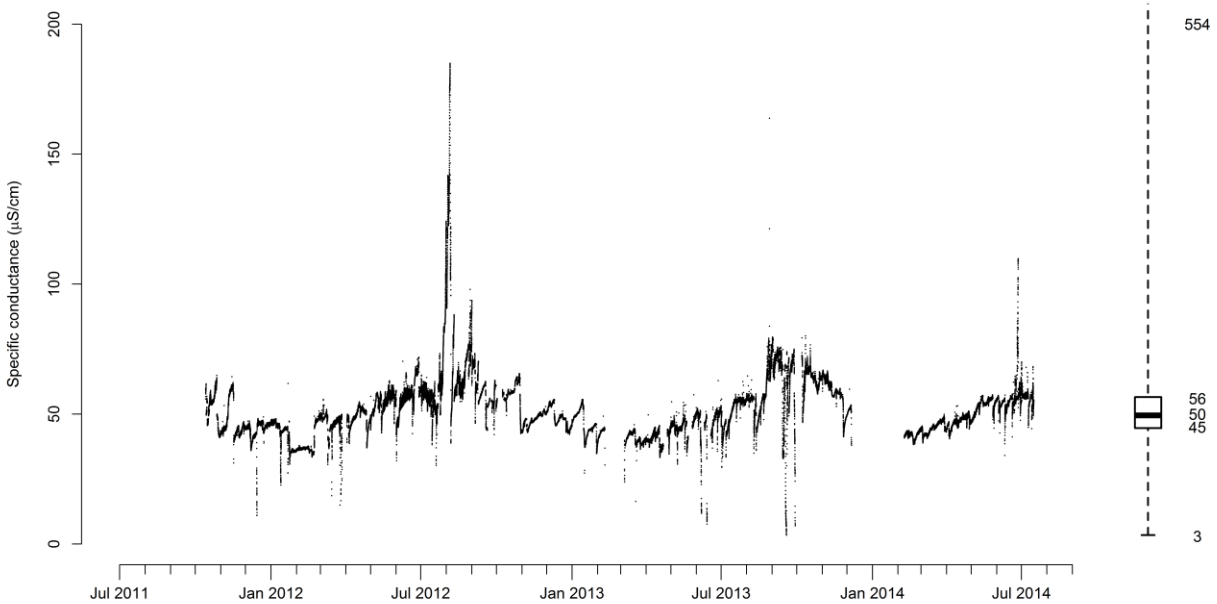


Figure A - 18. Detail plot for Middle Camp Branch (MCB, reference site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

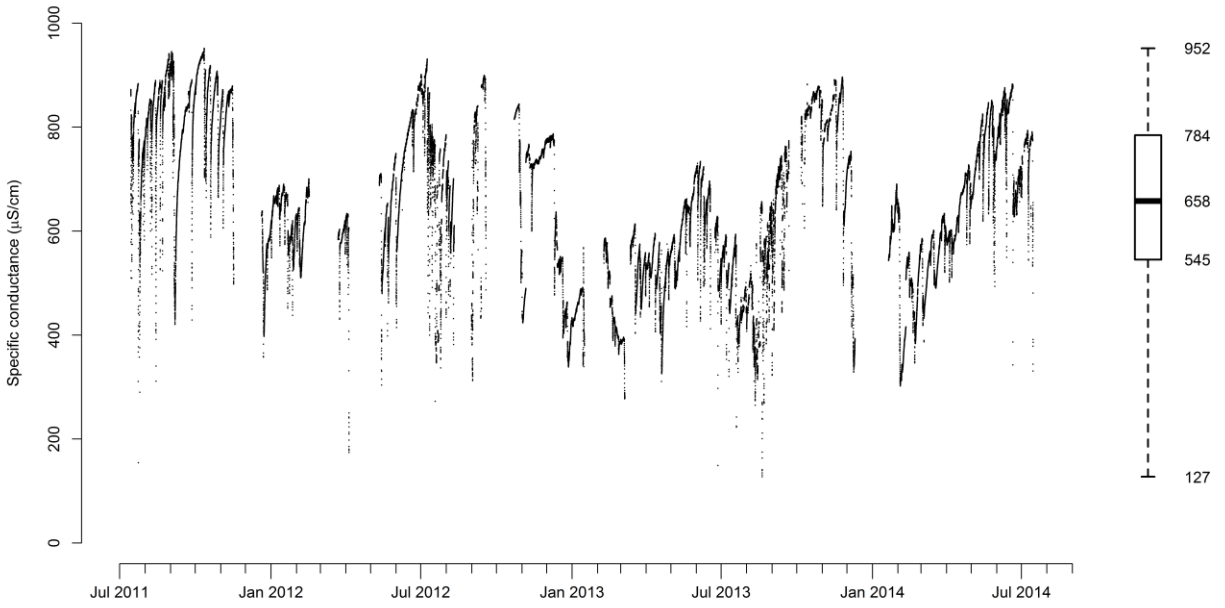


Figure A - 19. Mill Branch West Fork (MIL, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

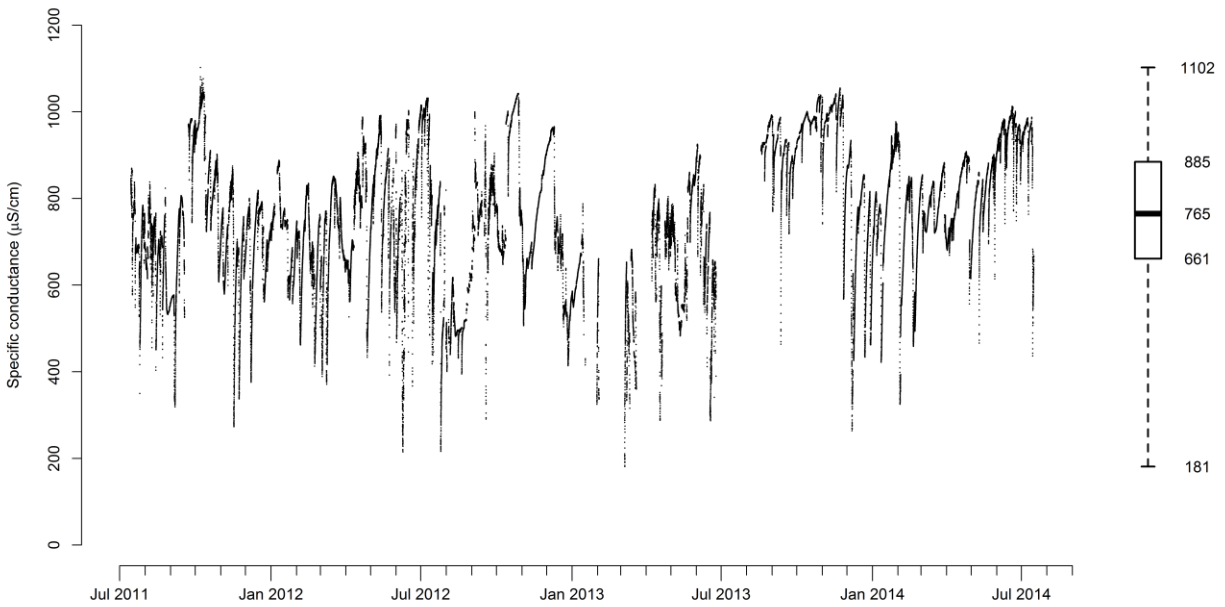


Figure A - 20. Powell River headwaters (POW, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

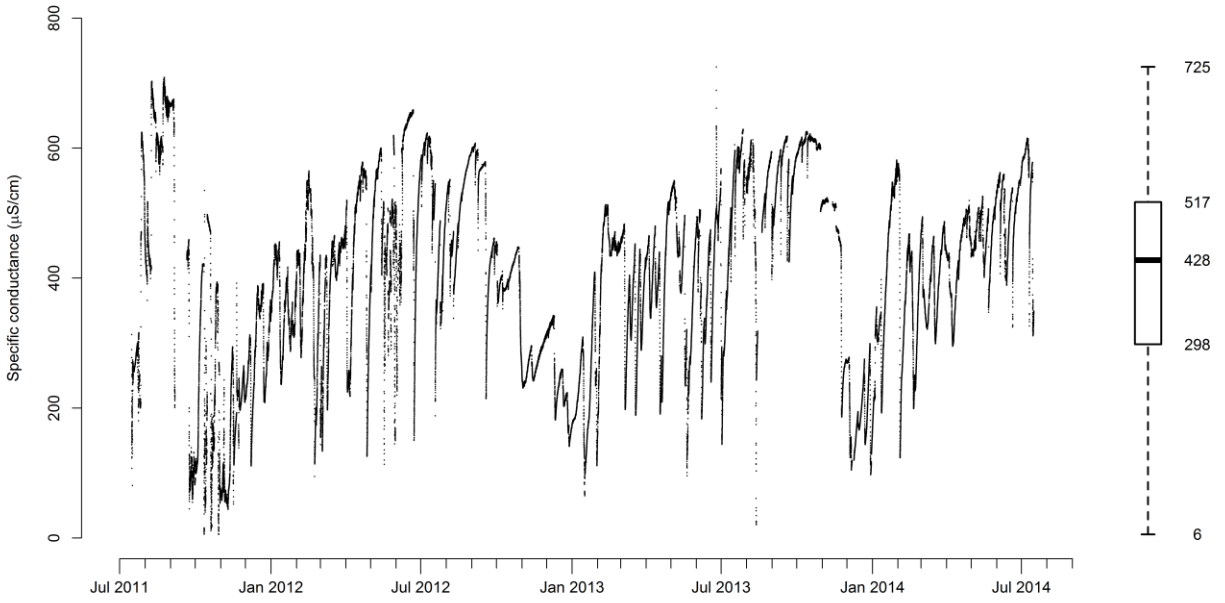


Figure A - 21. Fryingpan Creek Right Fork (RFF, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

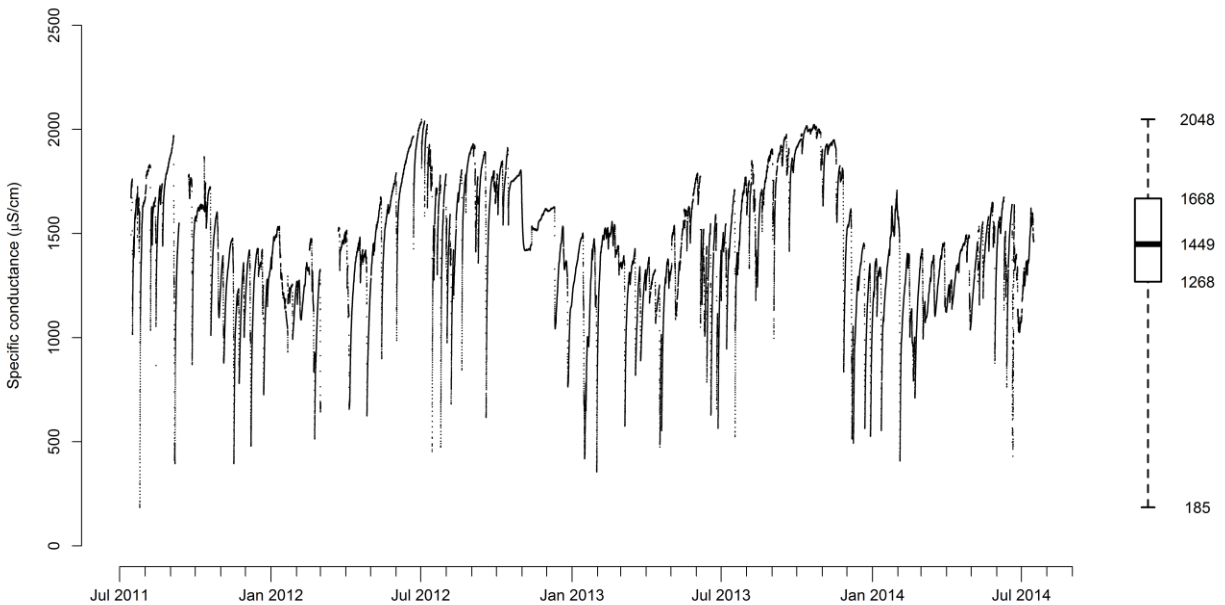


Figure A - 22. Rickey Branch, (RIC, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

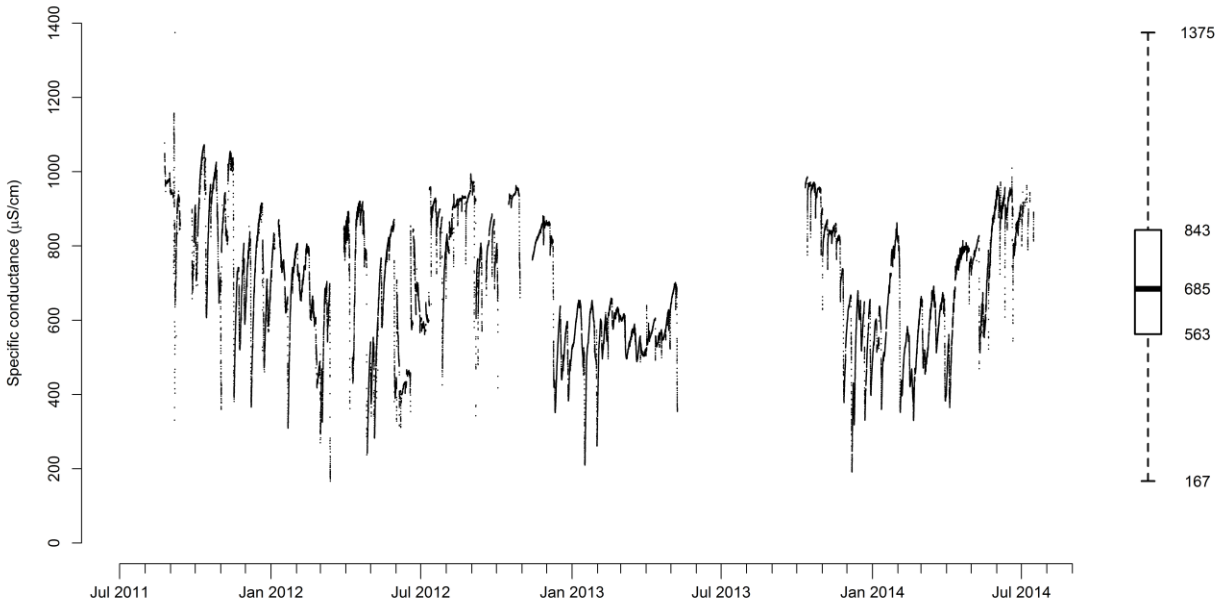


Figure A - 23. Rockhouse Fork (ROC, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

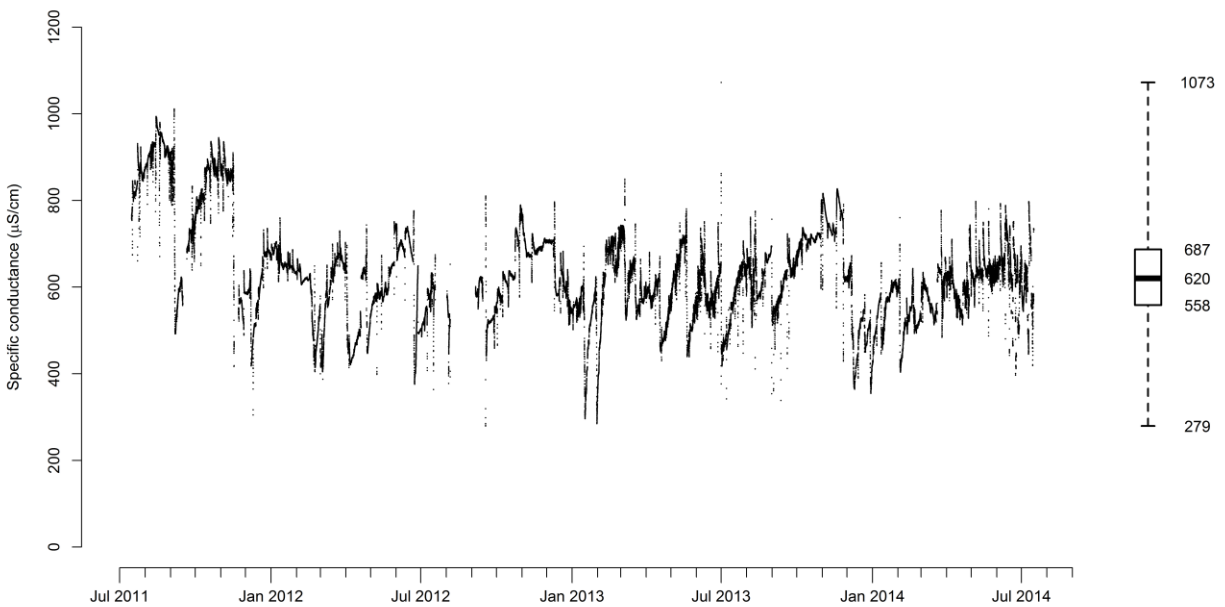


Figure A - 24. Roll Pone Branch (ROL, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

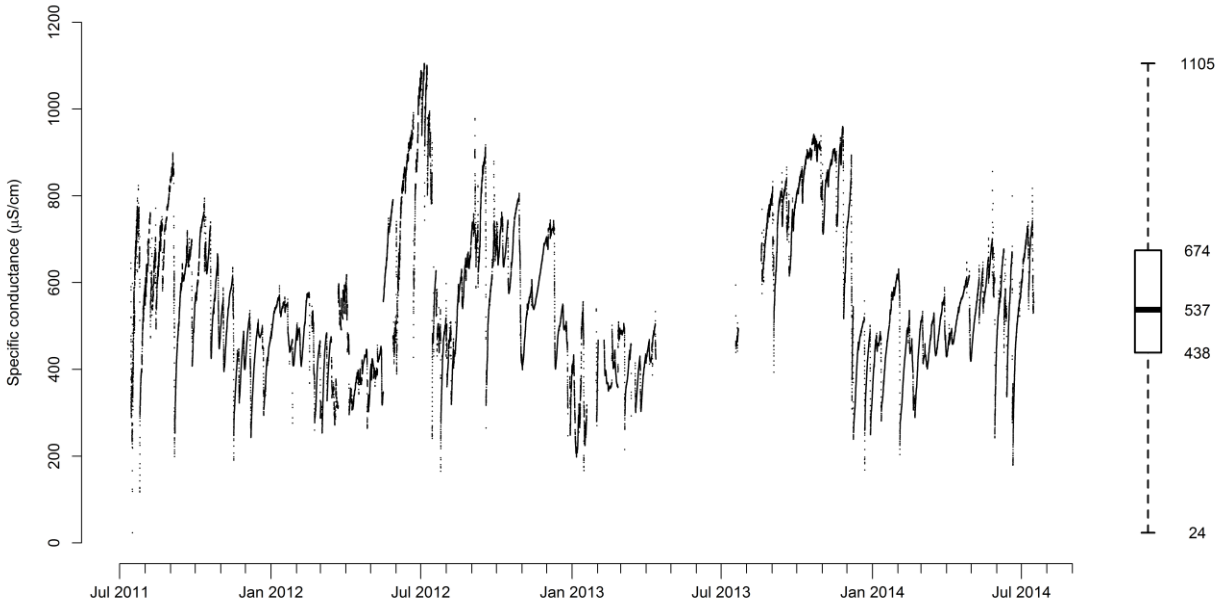


Figure A - 25. Rickey Branch Unnamed Tributary (RUT, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

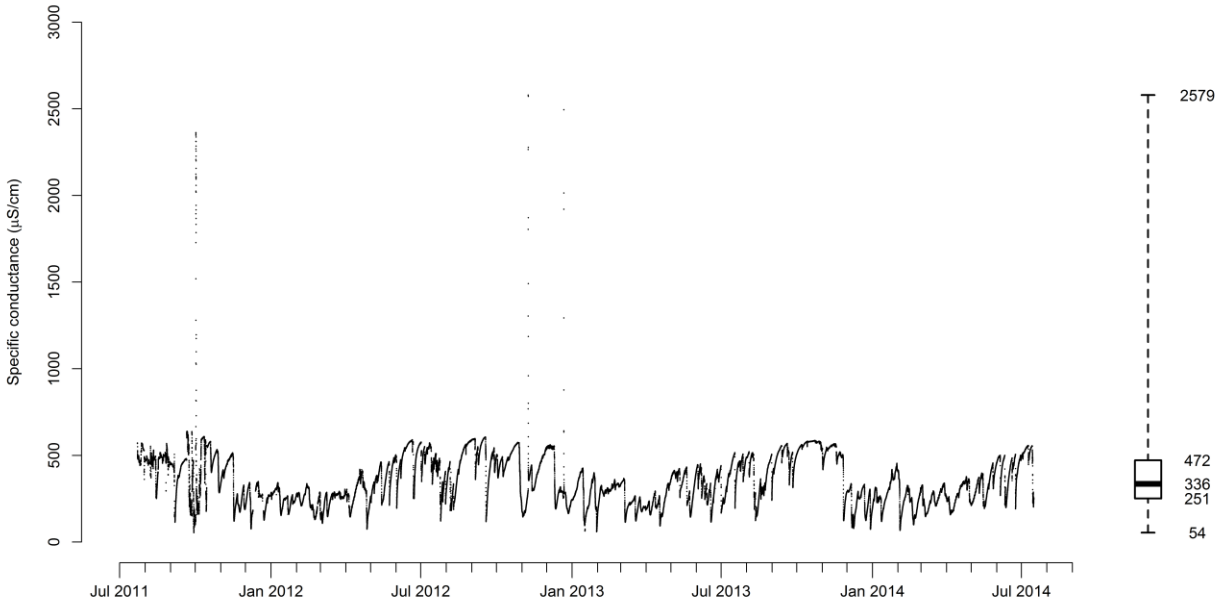


Figure A - 26. Spruce Pine Creek (SPC, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

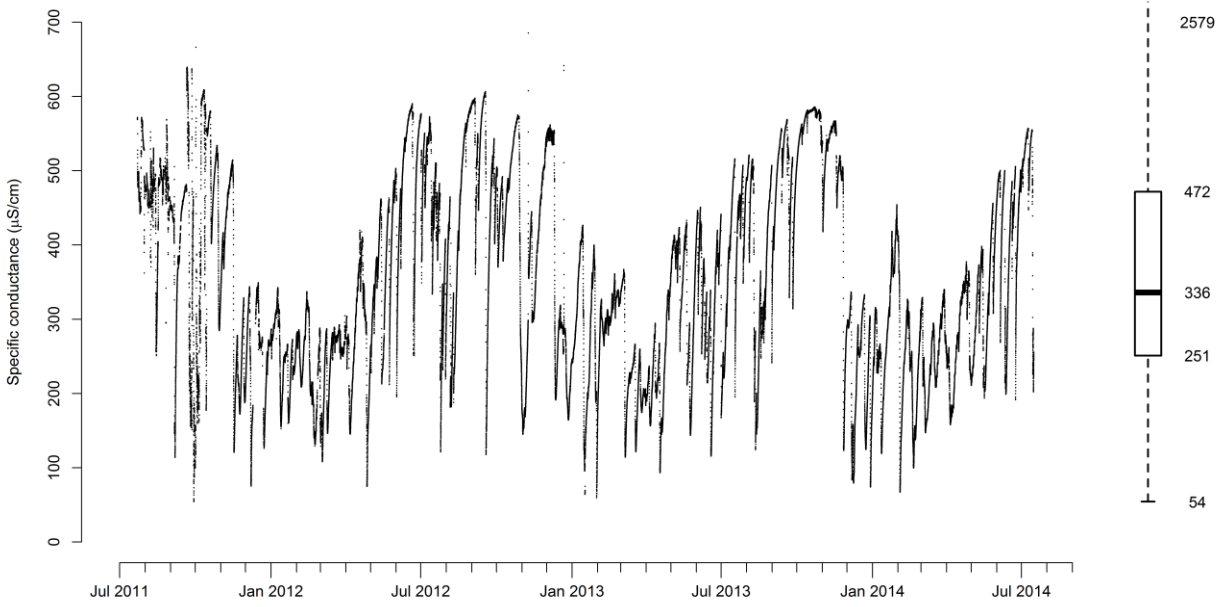


Figure A - 27. Detail plot for Spruce Pine Creek (SPC, test site) - 15-minute specific conductance (SC). Box plot represents 15-minute SC median value (centerline), 25th and 75th percentiles (upper and lower bounds of box), and minimum and maximum (box whiskers).

APPENDIX B – SC SUMMARY BY SITE

Table B - 1. Fall 2012 SC ($\mu\text{S}/\text{cm}$) metrics by site

Site ID	Site Type	Snap-shot	----- Quantile -----												Max	Mean	SD	CV
			Min	5	10	20	30	40	50	60	70	80	90	95				
COP	Ref		No sample															
CRO	Ref	78	27	49	54	58	61	64	70	74	78	85	91	109	126	72	17	0.23
EAS	Ref	23	8	14	16	18	20	20	21	22	23	25	29	30	50	22	5	0.22
HCN	Ref	85	3	38	42	47	49	51	58	66	72	77	95	120	168	64	25	0.38
MCB	Ref	48	23	36	40	44	46	48	50	54	57	59	63	69	131	52	11	0.21
BIR	Test	658	106	436	486	532	558	580	599	617	635	663	699	755	847	597	89	0.15
CRA	Test	512	190	338	359	384	407	429	462	490	500	509	516	523	540	447	64	0.14
DAV	Test	616	53	246	289	355	412	486	552	598	625	646	668	678	794	507	149	0.29
FRY	Test	271	79	245	273	320	346	367	388	414	434	455	479	499	530	383	79	0.21
GRA	Test	282	22	84	104	132	152	174	238	284	324	360	415	469	556	247	121	0.49
HUR	Test	418	43	210	247	310	339	364	384	401	419	439	466	500	557	373	82	0.22
KEL	Test	855	116	584	660	699	714	740	769	798	827	854	888	909	1118	767	112	0.15
KUT	Test	1270	339	846	895	952	1004	1047	1079	1116	1183	1229	1273	1313	1390	1081	154	0.14
LAB	Test	791	139	447	497	538	566	607	652	705	745	780	831	852	901	654	130	0.20
LLC	Test	1828	254	639	731	866	1020	1170	1325	1470	1655	1775	1905	2040	3406	1333	473	0.35
LLE	Test	941	100	252	298	356	419	484	584	775	864	934	1079	1216	1363	655	307	0.47
LLW	Test	1505	163	629	737	920	1036	1109	1193	1248	1347	1432	1523	1579	1798	1160	290	0.25
MIL	Test	810	209	380	428	503	564	600	642	677	736	808	884	919	1524	654	184	0.28
POW	Test	972	193	495	557	618	664	704	748	787	829	869	931	988	1132	743	150	0.20
RFF	Test	399	36	152	216	280	347	384	423	451	499	549	590	614	783	409	143	0.35
RIC	Test	1795	411	980	1105	1256	1349	1399	1461	1561	1668	1748	1848	1918	2048	1480	287	0.19
ROC	Test	901	94	451	510	614	679	740	780	828	879	916	1065	1133	1427	780	206	0.26
ROL	Test	677	85	455	490	534	578	597	624	644	671	703	807	852	925	631	112	0.18
RUT	Test	743	111	348	394	440	474	503	536	568	629	694	789	875	1034	563	156	0.28
SPC	Test	483	53	157	191	240	268	290	338	402	456	502	557	581	2498	365	153	0.42

Table B - 2. Fall 2012 SC ($\mu\text{S/cm}$) summary statistics by site type

Site Type	Statistic	Snapshot	Min	----- Quantile -----											Max	Mean	SD	CV
				5	10	20	30	40	50	60	70	80	90	95				
Ref	Min	23	3	14	16	18	20	20	21	22	23	25	29	30	50	22	5	0.21
	Max	85	27	49	54	58	61	64	70	74	78	85	95	120	168	72	25	0.38
	Median	63	16	37	41	46	48	50	54	60	65	68	77	89	129	58	14	0.23
	Mean	59	15	34	38	42	44	46	50	54	58	62	70	82	119	53	14	0.26
	SD	29	12	15	16	17	17	19	21	23	25	27	31	41	50	22	8	0.08
	n	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Test	Min	271	22	84	104	132	152	174	238	284	324	360	415	469	530	247	64	0.14
	Max	1828	411	980	1105	1256	1349	1399	1461	1561	1668	1775	1905	2040	3406	1480	473	0.49
	Median	767	109	408	457	518	561	589	612	661	704	742	819	864	1076	643	149	0.24
	Mean	836	140	419	473	542	595	639	689	742	796	843	911	961	1259	691	172	0.26
	SD	454	102	232	253	281	303	320	335	355	388	409	434	457	731	335	99	0.11
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20

Table B - 3. Spring 2013 SC ($\mu\text{S}/\text{cm}$) metrics by site

Site ID	Site Type	Snap-shot	Min	----- Quantile -----											Max	Mean	SD	CV
				5	10	20	30	40	50	60	70	80	90	95				
COP	Ref	96	35	84	95	105	114	119	124	139	153	169	194	211	221	135	38	0.28
CRO	Ref	53	40	52	54	57	60	62	65	70	77	81	86	89	115	68	12	0.17
EAS	Ref	21	7	18	19	20	20	21	22	23	24	25	29	30	44	23	4	0.16
HCN	Ref	45	3	42	46	48	51	56	63	69	75	79	102	121	168	68	24	0.36
MCB	Ref	40	27	40	42	44	48	50	52	54	57	59	64	69	131	53	10	0.19
BIR	Test	566	78	438	478	518	547	576	597	618	638	659	709	760	847	592	96	0.16
CRA	Test	402	230	343	364	400	416	439	464	494	503	511	518	524	540	453	61	0.13
DAV	Test	309	53	194	232	297	365	435	506	564	611	639	663	674	794	473	165	0.35
FRY	Test	278	83	213	247	284	320	342	374	403	425	450	474	500	530	366	88	0.24
GRA	Test	114	69	108	119	133	144	164	204	265	301	342	401	472	556	238	114	0.48
HUR	Test	352	31	211	242	286	317	346	368	390	408	431	465	500	557	360	85	0.24
KEL	Test	811	116	601	663	704	726	753	782	800	814	828	851	870	928	762	90	0.12
KUT	Test	1004	339	880	952	998	1019	1048	1091	1154	1191	1232	1267	1274	1284	1100	126	0.11
LAB	Test	472	144	393	447	511	582	612	638	691	726	768	818	849	889	639	139	0.22
LLC	Test	1188	254	617	739	845	990	1086	1242	1413	1575	1768	1912	2046	3406	1297	477	0.37
LLE	Test	305	66	237	271	323	410	482	579	719	846	976	1145	1271	1486	651	332	0.51
LLW	Test	958	163	661	780	932	1050	1152	1240	1318	1390	1443	1502	1557	1626	1186	282	0.24
MIL	Test	540	209	349	407	482	536	586	636	694	744	795	879	945	1524	646	195	0.30
POW	Test	748	156	447	526	597	638	687	733	775	825	889	983	1027	1375	736	175	0.24
RFF	Test	363	65	185	223	269	322	383	420	448	485	544	587	608	659	407	134	0.33
RIC	Test	1270	356	974	1141	1300	1376	1435	1514	1598	1682	1762	1861	1919	2048	1501	288	0.19
ROC	Test	557	94	406	488	526	573	616	715	791	852	896	960	1092	1427	719	218	0.30
ROL	Test	576	85	451	490	534	561	582	598	626	656	688	708	729	849	602	87	0.14
RUT	Test	433	169	332	366	416	457	499	539	595	665	724	806	884	1034	569	171	0.30
SPC	Test	161	60	171	197	240	288	319	362	412	464	512	554	574	2579	372	136	0.36

Table B - 4. Spring 2013 SC ($\mu\text{S}/\text{cm}$) summary statistics by site type

Site Type	Statistic	Snapshot	Min	----- Quantile -----											Max	Mean	SD	CV
				5	10	20	30	40	50	60	70	80	90	95				
Ref	Min	21	3	18	19	20	20	21	22	23	24	25	29	30	44	23	4	0.16
	Max	96	40	84	95	105	114	119	124	139	153	169	194	211	221	135	38	0.36
	Median	45	27	42	46	48	51	56	63	69	75	79	86	89	131	68	12	0.19
	Mean	51	22	47	51	55	59	62	65	71	77	83	95	104	136	69	18	0.23
	SD	28	17	24	28	31	34	36	37	42	47	53	62	68	66	41	14	0.09
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Test	Min	114	31	108	119	133	144	164	204	265	301	342	401	472	530	238	61	0.11
	Max	1270	356	974	1141	1300	1376	1435	1514	1598	1682	1768	1912	2046	3406	1501	477	0.51
	Median	506	105	371	427	497	542	579	598	659	696	746	812	860	981	621	137	0.24
	Mean	570	141	411	469	530	582	627	680	738	790	843	903	954	1247	683	173	0.27
	SD	328	94	235	269	296	311	323	342	363	386	413	438	459	744	339	103	0.11
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20

Table B - 5. Fall 2013 SC ($\mu\text{S}/\text{cm}$) metrics by site

Site ID	Site Type	Snapshot	----- Quantile -----												Max	Mean	SD	CV	
			Min	5	10	20	30	40	50	60	70	80	90	95					
COP	Ref	180	35	77	90	102	110	118	125	140	149	163	181	189	206	131	34	0.26	
CRO	Ref	82	42	52	54	57	61	65	68	70	76	80	83	85	129	68	11	0.16	
EAS	Ref								No sample										
HCN	Ref	90	13	42	45	48	51	54	59	66	75	86	108	136	168	69	28	0.40	
MCB	Ref								No sample										
BIR	Test	717	21	440	478	517	543	564	586	599	620	639	659	675	739	573	77	0.13	
CRA	Test	490	244	349	368	393	406	417	431	448	461	487	506	514	529	434	51	0.12	
DAV	Test	559	70	192	226	293	340	379	422	456	494	543	587	632	664	415	132	0.32	
FRY	Test	469	83	213	242	279	313	329	347	376	400	426	460	476	502	351	80	0.23	
GRA	Test	427	19	109	119	133	143	160	191	230	282	329	411	442	613	230	113	0.49	
HUR	Test	415	84	254	287	320	346	366	389	406	422	443	470	479	515	380	71	0.19	
KEL	Test	808	136	649	697	748	769	787	802	810	815	818	842	852	928	780	68	0.09	
KUT	Test	1298	491	920	972	1004	1021	1049	1075	1147	1194	1243	1278	1299	1325	1106	125	0.11	
LAB	Test	798	144	390	437	487	524	558	585	607	630	668	753	776	835	580	115	0.20	
LLC	Test	1612	273	651	760	891	1020	1097	1221	1389	1511	1631	1731	1787	1871	1242	367	0.30	
LLE	Test	894	66	253	274	315	364	436	504	621	773	903	1139	1233	1409	609	321	0.53	
LLW	Test	1314	174	669	777	926	1020	1096	1162	1257	1341	1415	1479	1508	1626	1149	268	0.23	
MIL	Test	921	127	388	418	458	501	536	565	613	685	741	813	839	891	594	143	0.24	
POW	Test	1008	181	501	548	614	676	721	788	876	925	966	990	1013	1042	783	175	0.22	
RFF	Test	511	21	185	223	270	316	380	420	451	496	539	594	610	725	407	134	0.33	
RIC	Test	1894	356	987	1137	1285	1360	1436	1515	1605	1705	1798	1919	1974	2024	1513	304	0.20	
ROC	Test	845	210	455	498	528	552	574	602	630	776	854	939	956	986	659	167	0.25	
ROL	Test								No sample										
RUT	Test	867	167	313	362	417	471	524	618	690	735	794	868	901	942	606	190	0.31	
SPC	Test	545	60	167	194	234	282	315	360	404	453	504	551	572	2579	366	134	0.37	

Table B - 6. Fall 2013 SC ($\mu\text{S}/\text{cm}$) summary statistics by site type

Site Type	Statistic	Snapshot	Min	----- Quantile -----											Max	Mean	SD	CV
				5	10	20	30	40	50	60	70	80	90	95				
Ref	Min	82	13	42	45	48	51	54	59	66	75	80	83	85	129	68	11	0.16
	Max	180	42	77	90	102	110	118	125	140	149	163	181	189	206	131	34	0.40
	Median	90	35	52	54	57	61	65	68	70	76	86	108	136	168	69	28	0.26
	Mean	117	30	57	63	69	74	79	84	92	100	110	124	137	168	89	24	0.27
	SD	54	15	18	24	29	32	34	36	42	42	46	51	52	39	36	12	0.12
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Test	Min	415	19	109	119	133	143	160	191	230	282	329	411	442	502	230	50.9	0.09
	Max	1894	491	987	1137	1285	1360	1436	1515	1605	1705	1798	1919	1974	2579	1513	367	0.53
	Median	808	136	388	418	458	501	536	585	613	685	741	813	839	928	594	134	0.23
	Mean	863	154	426	475	532	577	617	662	717	775	828	894	923	1092	672	160	0.26
	SD	413	122	251	279	307	322	334	350	377	398	418	438	450	576	348	92	0.12
	n	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19

Table B - 7. Spring 2014 SC ($\mu\text{S}/\text{cm}$) metrics by site

Site ID	Site Type	Snap-shot	Min	----- Quantile -----											Max	Mean	SD	CV
				5	10	20	30	40	50	60	70	80	90	95				
COP	Ref	114	39	79	88	99	110	116	126	139	149	164	181	188	194	130	34	0.26
CRO	Ref	55	36	48	50	52	55	57	64	67	71	80	84	85	129	65	13	0.19
EAS	Ref	22	16	19	20	20	21	22	23	25	27	31	35	38	45	25	6	0.23
HCN	Ref	46	13	37	42	45	47	50	56	62	73	89	115	142	168	67	31	0.46
MCB	Ref	48	3	40	42	43	45	47	48	52	56	60	66	70	554	51	11	0.21
BIR	Test	656	215	415	460	515	538	552	570	586	600	623	649	669	876	562	73	0.13
CRA	Test	453	234	323	346	370	390	405	418	432	451	467	494	501	529	418	55	0.13
DAV	Test	500	55	190	214	277	320	355	397	440	480	518	567	595	651	396	130	0.33
FRY	Test	330	134	221	251	294	316	337	358	382	400	428	459	479	502	356	77	0.22
GRA	Test	135	19	101	112	132	148	170	197	228	283	353	424	451	613	234	118	0.50
HUR	Test	416	136	260	288	329	354	373	388	405	424	448	470	479	515	384	68	0.18
KEL	Test	795	136	632	687	731	754	768	779	794	807	813	818	820	841	762	73	0.10
KUT	Test	1009	491	890	932	961	993	1007	1027	1061	1128	1201	1286	1300	1325	1065	131	0.12
LAB	Test	586	205	369	422	480	512	533	557	579	607	650	738	776	818	563	113	0.20
LLC	Test	925	309	657	762	929	1026	1145	1318	1423	1566	1660	1741	1792	1836	1277	365	0.29
LLE	Test	466	12	190	214	263	308	350	433	528	691	882	1152	1239	1409	556	344	0.62
LLW	Test	1146	253	620	728	846	960	1018	1069	1161	1254	1338	1429	1492	1523	1081	265	0.25
MIL	Test	620	127	386	432	492	522	552	578	607	649	713	824	857	896	597	137	0.23
POW	Test	795	264	515	571	685	732	768	810	850	905	943	985	1008	1055	798	153	0.19
RFF	Test	374	21	180	228	299	354	403	444	480	517	558	597	611	725	426	134	0.31
RIC	Test	1348	408	944	1084	1195	1282	1350	1436	1547	1684	1809	1925	1982	2024	1467	328	0.22
ROC	Test	688	192	401	442	510	544	566	601	639	674	811	859	955	986	633	159	0.25
ROL	Test	614	339	462	486	524	551	574	595	616	636	670	717	739	1073	597	87	0.15
RUT	Test	584	168	332	399	440	465	490	537	618	748	810	885	906	960	601	187	0.31
SPC	Test	242	67	150	176	223	253	287	320	378	424	494	549	571	586	345	134	0.39

Table B - 8. Spring 2014 SC ($\mu\text{S}/\text{cm}$) summary statistics by site type

Site Type	Statistic	Snapshot	Min	----- Quantile -----											Max	Mean	SD	CV
				5	10	20	30	40	50	60	70	80	90	95				
Ref	Min	22	3	19	20	20	21	22	23	25	27	31	35	38	45	25	6	0.19
	Max	114	39	79	88	99	110	116	126	139	149	164	181	188	554	130	34	0.46
	Median	48	16	40	42	45	47	50	56	62	71	80	84	85	168	65	13	0.23
	Mean	57	21	45	48	52	56	58	63	69	75	85	96	105	218	68	19	0.27
	SD	34	16	22	25	29	33	35	38	42	45	50	56	60	196	39	13	0.11
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Test	Min	135	12	101	112	132	148	170	197	228	283	353	424	451	502	234	55.4	0.10
	Max	1348	491	944	1084	1195	1282	1350	1436	1547	1684	1809	1925	1982	2024	1467	365	0.62
	Median	600	180	378	427	486	517	543	564	597	643	692	778	798	886	580	132	0.23
	Mean	634	189	412	462	525	566	600	642	688	746	809	878	911	987	656	157	0.26
	SD	303	130	237	262	283	301	315	335	355	384	405	428	442	436	331	95	0.13
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20

APPENDIX C – WATER CHEMISTRY

Table C-1 (cont'd). Water chemistry data

Date	Site	Specific ¹ Conductance (µS/cm)	pH ¹	Dissolved ¹ Oxygen (mg/L)	Temp ¹ (°C)	Total Dissolved Solids (mg/L)	Total Alkalinity (mg/L as CaCO ₃)	Total Hardness (mg/L as CaCO ₃)	Cl ⁻ (mg/L)	SO ₄ ²⁻ (mg/L)	CO ₃ ²⁻ (mg/L)	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)
4/21/2014	LLW	1,146		9.22	15.6	889.2	117.6	576.9	0.80	543.82	0.00	143.53	108.80	4.99	74.37	9.50	7.2	< 1.0	< 10.0	1.5	< 5.0	< 10.0
4/22/2014	HUR	416		9.84	12.0	254.0	40.3	166.1	0.53	163.84	0.00	49.22	31.67	2.23	21.20	9.28	5.5	< 1.0	< 10.0	3.6	< 5.0	< 10.0
4/22/2014	LAB	586		9.65	13.6	374.0	97.1	220.8	1.14	204.41	0.00	118.45	48.98	2.96	23.99	22.97	8.6	< 1.0	17.4	6.0	< 5.0	< 10.0
4/22/2014	MCB	48		9.34	12.7	25.2	6.7	13.5	0.48	10.74	0.00	8.22	2.52	1.07	1.74	1.80	6.2	< 1.0	10.3	1.1	< 5.0	< 10.0
4/22/2014	ROL	614		9.25	13.1	408.0	80.4	261.5	1.17	238.10	0.00	98.11	57.30	2.48	28.84	14.58	3.7	< 1.0	< 10.0	< 1.0	< 5.0	< 10.0
4/23/2014	BIR	656		9.09	12.3	416.2	114.0	284.2	5.84	125.90	0.00	139.10	52.24	3.21	37.46	16.39	2.8	< 1.0	33.2	18.2	< 5.0	< 10.0
4/23/2014	RUT	584		10.26	9.2	381.4	68.3	264.4	5.89	219.07	0.00	83.33	38.70	2.51	40.89	5.95	5.2	< 1.0	< 10.0	1.5	< 5.0	< 10.0

¹measured *in situ* with a handheld meter

*sample excluded from final analysis (see Results & Discussion for details)

APPENDIX D – BIOLOGICAL METRICS & LIST OF TAXA

Table D - 1. Biological Metrics

Year	Season	Date	Site ID	Site Type	Abundance	No. Total Taxa	No. Total Taxa less Ephemeroptera	No. Ephemeroptera Taxa	No. Ephemeroptera Taxa less Baetidae	No. EPT Taxa	No. Plecoptera & Trichoptera Taxa	No. Plecoptera Taxa	No. Trichoptera Taxa	% Top 2 Dominant Taxa	% Top 5 Dominant Taxa	Simpson Diversity	Shannon Diversity	% Ephemeroptera	% Ephemeroptera less Baetidae	% Plecoptera	% Trichoptera	% Collector-Filterers	% Collector-Gatherers	% Predators	% Scrapers	% Shredders	No. Collector-Gatherer Taxa	No. Predator Taxa	No. Scrapper Taxa	% Clingers	No. Clinger Taxa
2011	Spring	6/6/2011	BIR	Test	191	18	17	1	0	9	3	5	8	69.1	89.0	0.71	1.68	0.5	0.0	48.2	17.8	18.3	27.2	5.2	1.0	48.2	4	5	2	69.6	11
2011	Spring	5/22/2011	COP	Ref	196	31	25	6	5	20	8	6	14	30.6	57.7	0.91	2.80	31.6	16.8	27.0	13.8	10.7	33.2	10.2	22.4	23.5	6	11	6	64.8	20
2011	Spring	5/21/2011	CRO	Ref	212	28	19	9	7	21	6	6	12	29.2	56.6	0.92	2.80	33.5	14.6	16.0	20.3	18.9	40.6	11.3	15.6	13.7	6	6	9	52.8	20
2011	Spring	6/6/2011	EAS	Ref	182	27	20	7	6	21	6	8	14	33.0	63.7	0.90	2.67	33.0	20.9	25.8	12.6	6.6	28.6	12.6	29.1	23.1	5	6	8	67.6	20
2011	Spring	5/22/2011	FRY	Test	198	23	17	6	4	15	3	6	9	48.5	71.2	0.85	2.36	15.7	8.1	35.9	30.3	24.7	20.7	16.2	6.1	32.3	7	7	4	75.8	16
2011	Spring	5/20/2011	GRA	Test	220	24	19	5	3	15	4	6	10	54.5	74.5	0.76	2.13	17.3	7.3	49.5	18.6	15.5	18.2	12.3	6.8	47.3	5	5	7	80.0	16
2011	Spring	5/20/2011	HUR	Test	190	23	17	6	4	16	6	4	10	64.2	82.1	0.68	1.83	9.5	7.4	70.0	10.0	9.5	16.3	7.4	1.6	65.3	7	6	3	77.9	13
2011	Spring	6/7/2011	KEL	Test	182	11	10	1	0	6	3	2	5	86.3	94.0	0.33	0.86	1.6	0.0	82.4	6.6	7.7	7.7	1.6	0.5	82.4	3	1	1	90.1	6
2011	Spring	6/7/2011	KUT	Test	188	15	13	2	1	8	2	4	6	80.3	89.9	0.43	1.15	5.9	0.5	77.1	6.4	8.0	9.0	4.8	1.1	77.1	4	4	1	87.8	9
2011	Spring	5/22/2011	LAB	Test	211	15	13	2	0	9	4	3	7	58.3	82.9	0.79	1.91	26.1	0.0	44.1	10.4	10.0	43.6	3.8	0.5	42.2	4	5	1	49.3	7
2011	Spring	5/21/2011	MCB	Ref	220	31	22	9	8	21	7	5	12	33.2	56.4	0.91	2.83	42.3	23.6	27.3	6.8	3.6	46.4	13.2	15.9	20.9	6	9	10	60.0	22
2011	Spring	5/23/2011	MIL	Test	220	15	13	2	0	8	2	4	6	63.6	81.4	0.67	1.71	8.6	0.0	60.9	8.2	11.4	21.4	5.5	0.9	60.9	5	3	1	69.1	8
2011	Spring	5/23/2011	POW	Test	194	11	10	1	0	7	4	2	6	58.8	88.1	0.76	1.76	17.5	0.0	46.4	9.8	11.3	26.3	2.1	15.5	44.8	3	3	1	70.1	7
2011	Spring	5/22/2011	RFF	Test	205	23	17	6	4	16	6	4	10	57.1	72.2	0.74	2.10	13.7	8.3	65.9	6.3	3.9	15.1	17.1	6.3	57.6	6	7	5	77.6	16
2011	Spring	6/6/2011	RIC	Test	201	21	20	1	0	12	4	7	11	57.7	75.1	0.79	2.14	0.5	0.0	46.3	23.9	23.4	19.9	8.5	5.5	42.8	4	5	4	74.6	14
2011	Spring	5/22/2011	ROL	Test	200	13	12	1	0	8	4	3	7	80.0	94.0	0.46	1.13	4.0	0.0	83.5	3.5	1.5	11.5	8.5	1.0	77.5	3	4	2	83.5	7
2011	Spring	6/6/2011	RUT	Test	185	22	20	2	0	11	4	5	9	38.9	67.0	0.89	2.50	11.9	0.0	28.6	17.8	26.5	33.0	6.5	5.9	28.1	6	6	2	58.4	12
2011	Spring	5/20/2011	SPC	Test	220	19	16	3	1	12	4	5	9	66.4	85.9	0.70	1.77	17.3	0.5	55.9	6.4	5.9	34.1	3.6	2.3	54.1	6	4	2	62.7	12
2011	Fall	10/11/2011	BIR	Test	195	13	13	0	0	7	1	6	7	63.1	93.3	0.72	1.62	0.0	0.0	13.8	63.6	62.6	17.9	2.1	0.5	16.9	2	2	1	79.5	11
2011	Fall	10/12/2011	COP	Ref	195	31	25	6	5	21	9	6	15	33.3	52.3	0.91	2.89	11.3	9.7	21.5	33.8	32.3	18.5	18.5	7.2	23.6	6	9	5	66.2	20
2011	Fall	10/6/2011	CRA	Test	188	22	20	2	0	15	7	6	13	37.2	63.8	0.89	2.54	5.3	0.0	18.6	58.5	59.6	11.7	11.2	1.6	16.0	3	5	2	75.5	14
2011	Fall	10/13/2011	CRO	Ref	184	27	23	4	3	16	6	6	12	41.8	67.9	0.88	2.53	11.4	9.8	22.8	37.5	36.4	21.2	9.8	11.4	21.2	5	9	5	55.4	16
2011	Fall	10/4/2011	DAV	Test	190	15	14	1	0	8	3	4	7	57.4	85.3	0.78	1.89	17.4	0.0	2.6	39.5	39.5	23.7	1.1	31.1	4.7	3	2	3	73.2	10
2011	Fall	10/12/2011	EAS	Ref	182	28	21	7	6	22	8	7	15	34.6	57.7	0.91	2.78	17.0	15.9	21.4	36.8	26.9	24.2	19.8	13.2	15.9	4	9	6	68.7	20
2011	Fall	10/15/2011	FRY	Test	220	24	21	3	1	15	6	6	12	33.2	62.3	0.90	2.57	8.2	7.3	10.9	56.8	51.4	5.5	10.9	22.7	9.5	4	6	6	92.3	17
2011	Fall	10/15/2011	GRA	Test	206	18	17	1	1	13	6	6	12	51.9	72.8	0.84	2.25	2.9	2.9	38.3	42.2	40.3	11.2	8.7	4.4	35.4	2	5	2	78.2	13
2011	Fall	10/13/2011	HCN	Ref	190	24	21	3	3	13	2	8	10	46.8	75.8	0.85	2.30	6.8	6.8	27.9	21.1	18.9	34.2	3.7	13.2	30.0	4	5	6	51.1	14
2011	Fall	10/13/2011	HUR	Test	220	24	22	2	1	16	7	7	14	65.0	80.5	0.67	1.85	1.4	0.9	16.8	64.5	62.7	8.6	13.6	1.8	13.2	3	9	3	83.6	16
2011	Fall	10/16/2011	KEL	Test	185	15	15	0	0	9	3	6	9	59.5	82.7	0.80	1.95	0.0	0.0	35.7	43.8	41.6	9.7	10.3	2.2	36.2	2	4	2	83.2	11
2011	Fall	10/16/2011	KUT	Test	182	13	12	1	0	9	4	4	8	68.1	87.4	0.66	1.59	6.6	0.0	19.8	59.9	56.0	16.5	3.3	2.2	22.0	3	1	2	77.5	7
2011	Fall	10/15/2011	LAB	Test	190	20	18	2	1	12	4	6	10	55.3	79.5	0.79	2.06	1.1	0.5	22.1	52.1	50.5	17.9	3.7	3.7	24.2	3	5	4	77.9	14
2011	Fall	10/5/2011	LLC	Test	181	19	19	0	0	10	4	6	10	34.3	63.0	0.90	2.48	0.0	0.0	7.7	55.2	58.6	9.4	2.2	10.5	19.3	2	4	3	77.3	15
2011	Fall	10/4/2011	LLE	Test	180	15	15	0	0	11	6	5	11	73.3	86.7	0.70	1.68	0.0	0.0	38.3	50.6	49.4	2.8	7.2	1.1	39.4	1	6	1	90.0	12
2011	Fall	10/4/2011	LLW	Test	193	15	15	0	0	6	2	4	6	71.5	89.6	0.62	1.53	0.0	0.0	2.6	72.5	72.5	13.0	1.6	3.1	9.8	3	2	2	79.3	9
2011	Fall	10/15/2011	MCB	Ref	207	30	23	7	6	21	8	6	14	29.5	56.0	0.92	2.87	26.1	25.6	29.5	15.9	13.0	32.4	15.5	14.5	24.6	5	10	6	59.4	20

Table D - 1 (cont'd). Biological Metrics

Year	Season	Date	Site ID	Site Type	Abundance	No. Total Taxa	No. Total Taxa less Ephemeroptera	No. Ephemeroptera Taxa	No. Ephemeroptera Taxa less Baetidae	No. EPT Taxa	No. Plecoptera & Trichoptera Taxa	No. Plecoptera Taxa	No. Trichoptera Taxa	% Top 2 Dominant Taxa	% Top 5 Dominant Taxa	Simpson Diversity	Shannon Diversity	% Ephemeroptera	% Ephemeroptera less Baetidae	% Plecoptera	% Trichoptera	% Collector-Filterers	% Collector-Gatherers	% Predators	% Scrapers	% Shredders	No. Collector-Gatherer Taxa	No. Predator Taxa	No. Scraper Taxa	% Clingers	No. Clinger Taxa
2011	Fall	10/11/2011	MIL	Test	207	17	17	0	0	9	2	7	9	58.9	82.6	0.80	2.00	0.0	0.0	30.9	53.1	51.7	7.7	6.3	1.9	32.4	2	3	3	88.4	13
2011	Fall	10/16/2011	POW	Test	191	11	10	1	0	4	2	1	3	78.0	89.5	0.69	1.51	4.2	0.0	46.6	35.1	36.6	7.9	1.0	6.3	48.2	2	2	2	90.1	7
2011	Fall	10/15/2011	RFF	Test	189	26	23	3	2	16	6	7	13	35.4	66.7	0.89	2.58	3.2	1.1	21.7	52.4	50.3	14.3	11.1	4.2	20.1	4	5	7	79.9	20
2011	Fall	10/11/2011	RIC	Test	205	17	17	0	0	11	3	8	11	56.6	80.5	0.75	1.95	0.0	0.0	9.8	73.2	75.6	9.8	4.4	0.0	10.2	2	4	0	85.9	12
2011	Fall	10/5/2011	ROC	Test	181	19	18	1	0	13	3	9	12	43.6	78.5	0.85	2.26	1.1	0.0	5.5	65.2	65.7	3.3	4.4	23.2	3.3	2	4	4	95.0	15
2011	Fall	11/22/2011	ROL	Test	200	21	20	1	1	13	5	7	12	66.5	83.5	0.74	1.89	1.0	1.0	49.5	33.0	26.0	11.0	10.5	6.0	46.5	2	7	3	86.0	16
2011	Fall	10/11/2011	RUT	Test	184	19	19	0	0	11	3	8	11	56.5	75.0	0.75	2.06	0.0	0.0	12.5	62.5	61.4	8.7	8.2	8.2	13.6	2	5	2	84.8	14
2011	Fall	10/14/2011	SPC	Test	199	18	17	1	1	12	5	6	11	62.3	79.9	0.78	2.00	2.0	2.0	49.7	34.7	32.7	8.0	4.5	6.0	48.7	1	4	4	84.9	14
2012	Spring	3/22/2012	BIR	Test	220	13	12	1	0	7	2	4	6	43.6	83.6	0.84	2.03	17.3	0.0	43.6	11.4	19.1	33.2	3.6	0.5	43.6	3	3	1	41.8	8
2012	Spring	4/19/2012	COP	Ref	216	30	22	8	6	20	8	4	12	37.0	59.3	0.89	2.69	34.7	19.0	14.8	13.4	12.5	55.6	8.3	12.5	11.1	8	9	6	51.4	19
2012	Spring	4/18/2012	CRA	Test	205	18	14	4	2	13	6	3	9	68.8	87.3	0.73	1.76	47.8	2.0	31.2	9.3	9.3	56.6	5.4	0.5	28.3	4	6	1	19.5	13
2012	Spring	4/19/2012	CRO	Ref	205	24	16	8	7	17	5	4	9	38.0	59.5	0.88	2.61	49.8	22.0	7.3	16.6	18.0	51.7	10.7	14.6	4.9	6	6	6	42.4	15
2012	Spring	4/18/2012	DAV	Test	201	20	15	5	3	14	5	4	9	38.3	67.7	0.88	2.44	27.9	10.9	33.3	14.4	12.4	26.9	5.0	22.9	32.8	5	4	3	73.1	13
2012	Spring	4/19/2012	EAS	Ref	200	25	19	6	5	19	8	5	13	32.0	63.5	0.90	2.61	40.0	35.5	22.0	15.0	12.5	28.5	10.0	32.5	16.5	4	9	6	77.0	19
2012	Spring	3/22/2012	FRY	Test	195	28	22	6	4	16	6	4	10	48.7	72.3	0.83	2.35	24.6	16.4	45.1	7.2	7.7	17.9	17.4	13.8	43.1	7	7	6	47.2	20
2012	Spring	5/1/2012	GRA	Test	220	26	18	8	6	18	4	6	10	53.6	75.0	0.78	2.17	24.1	14.5	47.7	16.4	13.2	21.8	13.2	5.0	46.8	9	5	7	77.7	17
2012	Spring	3/23/2012	HCN	Ref	220	25	19	6	5	16	4	6	10	43.6	75.9	0.86	2.34	36.8	26.8	16.8	3.2	24.1	44.1	7.3	8.2	16.4	6	6	5	62.7	17
2012	Spring	3/22/2012	HUR	Test	219	28	22	6	4	18	6	6	12	44.7	73.5	0.85	2.38	20.1	18.7	57.1	11.9	9.6	20.5	26.9	5.5	37.4	6	10	5	59.8	17
2012	Spring	5/1/2012	KEL	Test	193	10	9	1	0	6	2	3	5	85.5	96.4	0.36	0.88	2.6	0.0	82.9	6.2	5.7	9.3	1.6	0.0	83.4	3	2	0	86.0	4
2012	Spring	5/1/2012	KUT	Test	196	9	8	1	0	5	2	2	4	79.6	96.9	0.60	1.23	21.9	0.0	61.2	2.0	2.0	34.7	2.0	0.0	61.2	2	3	0	60.7	4
2012	Spring	4/19/2012	LAB	Test	210	18	16	2	0	12	5	5	10	48.1	78.1	0.84	2.14	27.6	0.0	50.0	10.5	9.5	37.6	10.0	0.5	42.4	5	7	1	51.4	12
2012	Spring	4/17/2012	LLC	Test	193	19	17	2	1	13	3	8	11	43.5	71.0	0.87	2.33	3.6	3.1	20.2	26.9	26.4	34.7	10.9	9.8	18.1	3	6	2	43.5	13
2012	Spring	4/18/2012	LLE	Test	189	22	18	4	2	15	9	2	11	59.8	79.9	0.80	2.12	4.2	2.6	71.4	7.9	6.9	15.3	12.2	2.1	63.5	8	8	1	52.4	13
2012	Spring	4/18/2012	LLW	Test	220	15	14	1	0	8	3	4	7	62.3	87.7	0.77	1.83	1.8	0.0	27.7	15.9	17.3	43.6	0.5	10.9	27.7	4	1	2	31.4	10
2012	Spring	3/23/2012	MCB	Ref	209	29	20	9	8	18	4	5	9	31.1	54.5	0.92	2.83	42.1	39.7	15.8	5.7	3.8	45.0	13.4	24.4	13.4	9	7	7	59.8	17
2012	Spring	3/22/2012	MIL	Test	219	18	17	1	0	9	3	5	8	64.4	84.5	0.70	1.78	2.3	0.0	64.8	11.9	13.7	16.4	3.7	0.9	65.3	4	3	2	29.7	10
2012	Spring	4/20/2012	POW	Test	220	15	14	1	0	8	4	3	7	60.5	91.8	0.75	1.72	18.6	0.0	60.5	4.1	3.6	25.5	1.4	9.5	60.0	5	2	2	57.7	9
2012	Spring	4/19/2012	RFF	Test	196	21	15	6	4	14	4	4	8	49.5	75.5	0.84	2.24	56.6	7.1	11.2	20.4	18.4	60.7	3.1	6.6	11.2	6	3	5	38.8	15
2012	Spring	5/1/2012	RIC	Test	200	19	18	1	0	12	5	6	11	61.0	80.0	0.78	2.01	20.5	0.0	48.5	17.0	15.5	30.0	5.5	1.5	47.5	3	6	2	61.0	12
2012	Spring	4/17/2012	ROC	Test	200	20	18	2	1	12	4	6	10	48.5	78.5	0.84	2.22	11.5	0.5	14.5	25.5	22.5	46.5	5.5	12.0	13.5	4	7	3	40.0	12
2012	Spring	4/19/2012	ROL	Test	184	13	11	2	0	11	5	4	9	56.0	84.2	0.79	1.90	22.8	0.0	62.0	10.9	10.3	25.5	6.0	2.2	56.0	3	3	2	54.9	9
2012	Spring	3/22/2012	RUT	Test	194	19	16	3	1	10	2	5	7	69.1	84.0	0.62	1.65	5.2	1.5	66.0	6.7	10.3	10.8	1.5	10.3	67.0	6	3	2	27.3	9
2012	Spring	5/1/2012	SPC	Test	216	20	16	4	2	14	6	4	10	63.4	83.3	0.73	1.88	22.7	3.7	55.6	7.9	6.5	33.3	6.0	1.9	52.3	5	7	3	63.9	14
2012	Fall	10/22/2012	BIR	Test	196	15	15	0	0	8	2	6	8	85.2	92.3	0.35	0.96	0.0	0.0	80.6	11.2	11.2	3.1	3.1	1.5	81.1	1	4	2	94.4	10
2012	Fall	10/16/2012	CRA	Test	194	22	21	1	0	16	7	8	15	32.0	60.3	0.91	2.63	4.1	0.0	28.4	42.3	41.2	20.1	8.8	3.6	26.3	2	5	3	55.7	15

Table D - 1 (cont'd). Biological Metrics

Year	Season	Date	Site ID	Site Type	Abundance	No. Total Taxa	No. Total Taxa less Ephemeroptera	No. Ephemeroptera Taxa	No. Ephemeroptera Taxa less Baetidae	No. EPT Taxa	No. Plecoptera & Trichoptera Taxa	No. Plecoptera Taxa	No. Trichoptera Taxa	% Top 2 Dominant Taxa	% Top 5 Dominant Taxa	Simpson Diversity	Shannon Diversity	% Ephemeroptera	% Ephemeroptera less Baetidae	% Plecoptera	% Trichoptera	% Collector-Filterers	% Collector-Gatherers	% Predators	% Scrapers	% Shredders	No. Collector-Gatherer Taxa	No. Predator Taxa	No. Scrapper Taxa	% Clingers	No. Clinger Taxa
2012	Fall	10/13/2012	CRO	Ref	219	26	21	5	4	17	6	6	12	31.1	71.2	0.89	2.55	21.5	18.7	30.6	23.3	21.5	33.3	5.9	10.0	29.2	5	6	6	49.3	16
2012	Fall	10/16/2012	DAV	Test	220	19	15	4	3	11	3	4	7	56.8	80.9	0.81	2.08	10.0	3.6	4.1	41.4	39.1	11.8	2.3	41.8	5.0	6	1	5	90.0	14
2012	Fall	10/12/2012	EAS	Ref	185	29	22	7	6	20	7	6	13	41.1	65.4	0.88	2.62	20.0	19.5	10.3	25.9	17.8	33.5	14.6	24.9	9.2	8	7	5	58.4	17
2012	Fall	11/6/2012	FRY	Test	220	25	22	3	3	14	3	8	11	60.0	77.3	0.80	2.16	4.5	4.5	40.0	37.7	35.0	3.2	2.7	17.3	41.8	4	5	6	90.9	16
2012	Fall	10/14/2012	GRA	Test	206	27	24	3	2	18	7	8	15	61.2	80.6	0.80	2.15	2.9	2.4	37.9	48.1	45.1	7.3	3.9	7.3	36.4	3	6	7	85.0	19
2012	Fall	10/17/2012	HCN	Ref	182	16	12	4	4	10	2	4	6	63.2	80.2	0.78	1.95	11.0	11.0	46.2	8.2	4.9	27.5	6.0	15.4	46.2	3	4	5	62.1	10
2012	Fall	10/17/2012	HUR	Test	199	24	22	2	2	14	8	4	12	45.7	83.4	0.84	2.17	2.5	2.5	40.7	27.1	25.1	23.1	21.1	1.5	29.1	4	9	2	67.8	13
2012	Fall	10/12/2012	KEL	Test	201	17	17	0	0	7	3	4	7	35.3	72.6	0.88	2.27	0.0	0.0	19.9	32.8	42.8	25.9	1.5	2.5	27.4	5	3	2	65.7	9
2012	Fall	10/12/2012	KUT	Test	183	11	11	0	0	5	2	3	5	79.8	93.4	0.67	1.43	0.0	0.0	7.1	45.9	45.9	38.3	3.3	1.6	10.9	2	2	2	57.4	7
2012	Fall	10/13/2012	LAB	Test	183	16	15	1	0	9	1	7	8	62.8	83.6	0.72	1.81	1.6	0.0	12.6	64.5	58.5	15.3	3.3	4.4	18.6	2	4	3	77.6	10
2012	Fall	10/15/2012	LLC	Test	200	19	19	0	0	10	2	8	10	55.0	81.5	0.79	2.02	0.0	0.0	40.0	29.0	26.0	7.0	5.0	21.0	41.0	1	6	4	89.5	13
2012	Fall	10/16/2012	LLE	Test	182	22	22	0	0	12	7	5	12	64.3	81.9	0.77	1.97	0.0	0.0	47.3	34.6	26.9	6.0	14.3	6.6	46.2	2	7	4	87.4	14
2012	Fall	10/16/2012	LLW	Test	186	19	18	1	1	9	3	5	8	56.5	78.0	0.80	2.10	0.5	0.5	8.1	48.9	47.8	11.3	1.1	29.6	10.2	5	1	5	84.9	13
2012	Fall	11/6/2012	MCB	Ref	213	28	23	5	5	19	5	9	14	32.9	59.6	0.91	2.74	39.9	39.9	13.6	16.4	8.5	48.4	11.3	20.2	11.7	5	8	7	59.2	19
2012	Fall	10/22/2012	MIL	Test	183	19	17	2	2	9	2	5	7	60.7	83.6	0.76	1.92	1.1	1.1	44.3	32.2	31.1	13.1	8.7	1.1	45.9	4	5	2	82.0	12
2012	Fall	10/12/2012	POW	Test	209	15	15	0	0	8	3	5	8	76.1	90.9	0.65	1.51	0.0	0.0	58.9	25.8	23.9	8.1	3.3	4.8	59.8	1	5	3	89.0	11
2012	Fall	10/13/2012	RFF	Test	220	26	25	1	1	13	6	6	12	47.3	71.4	0.86	2.44	0.9	0.9	34.5	47.3	44.1	2.3	10.0	10.9	32.7	2	7	6	90.5	19
2012	Fall	10/12/2012	RIC	Test	213	24	23	1	1	14	4	9	13	45.5	69.0	0.86	2.40	0.9	0.9	36.2	43.2	39.0	6.6	4.2	13.6	36.6	2	6	5	85.4	16
2012	Fall	10/15/2012	ROC	Test	181	22	20	2	1	10	2	6	8	54.7	80.1	0.82	2.13	1.1	0.6	28.7	40.9	40.3	6.1	3.3	20.4	29.8	4	4	6	90.6	15
2012	Fall	11/6/2012	ROL	Test	183	15	14	1	1	9	2	6	8	74.9	91.8	0.62	1.46	6.0	6.0	60.1	22.4	19.1	15.3	2.7	2.2	60.7	4	3	2	82.0	8
2012	Fall	10/22/2012	RUT	Test	187	15	15	0	0	7	1	6	7	66.3	91.4	0.73	1.63	0.0	0.0	39.6	31.6	28.9	3.7	5.9	21.9	39.6	2	6	2	93.6	10
2012	Fall	10/14/2012	SPC	Test	215	16	14	2	2	11	4	5	9	78.1	88.8	0.49	1.29	3.3	3.3	78.6	12.1	11.2	2.8	1.9	5.6	78.6	1	3	4	89.3	13
2013	Spring	4/7/2013	BIR	Test	200	11	10	1	0	5	2	2	4	65.5	94.0	0.74	1.57	2.5	0.0	58.0	4.0	31.0	9.5	1.0	0.5	58.0	3	2	1	51.5	6
2013	Spring	4/6/2013	COP	Ref	216	24	18	6	5	17	8	3	11	55.1	69.4	0.74	2.16	61.1	60.2	14.8	6.9	5.6	57.4	12.0	14.4	10.6	6	9	3	83.8	15
2013	Spring	4/5/2013	CRA	Test	220	24	20	4	2	16	7	5	12	59.1	75.0	0.75	2.10	5.9	1.8	60.9	22.3	20.5	9.5	17.3	1.4	51.4	5	9	2	39.1	15
2013	Spring	4/6/2013	CRO	Ref	205	31	24	7	6	20	9	4	13	34.1	56.6	0.90	2.81	46.8	44.9	14.1	10.7	8.3	37.6	15.1	28.3	10.7	7	10	8	74.6	21
2013	Spring	4/5/2013	DAV	Test	205	16	13	3	2	10	4	3	7	41.0	72.7	0.87	2.28	18.0	7.8	33.7	11.7	16.1	30.7	4.4	15.6	33.2	4	3	3	48.3	12
2013	Spring	4/8/2013	EAS	Ref	182	27	19	8	7	23	9	6	15	27.5	59.3	0.92	2.77	34.1	31.9	25.8	10.4	6.0	25.3	11.0	35.7	22.0	5	8	6	73.6	22
2013	Spring	4/7/2013	FRY	Test	188	26	18	8	6	16	4	4	8	51.1	69.1	0.83	2.38	16.0	10.6	45.7	9.0	6.9	24.5	9.6	13.8	45.2	6	7	7	41.5	19
2013	Spring	4/6/2013	GRA	Test	190	26	20	6	5	19	7	6	13	50.5	74.7	0.84	2.32	20.0	19.5	56.8	9.5	7.9	18.4	16.8	4.7	52.1	5	9	4	65.3	18
2013	Spring	4/5/2013	HCN	Ref	201	26	20	6	6	18	7	5	12	50.2	78.6	0.83	2.24	32.8	32.8	34.8	4.5	2.5	45.8	10.4	8.5	32.8	4	8	6	49.3	18
2013	Spring	4/6/2013	HUR	Test	197	22	16	6	4	17	6	5	11	33.0	71.6	0.88	2.37	23.9	21.3	49.7	11.7	10.2	33.5	23.4	3.0	29.9	6	8	3	68.5	15
2013	Spring	4/8/2013	KEL	Test	183	10	9	1	0	5	2	2	4	74.3	95.1	0.64	1.38	0.5	0.0	74.3	3.3	13.1	12.6	0.0	0.0	74.3	5	0	0	68.3	4
2013	Spring	4/8/2013	KUT	Test	201	9	8	1	0	5	2	2	4	76.6	94.5	0.55	1.24	3.5	0.0	74.1	4.5	4.0	19.9	2.0	0.0	74.1	3	3	0	70.1	4
2013	Spring	4/7/2013	LAB	Test	219	23	20	3	1	13	4	6	10	46.1	79.9	0.84	2.22	10.0	0.5	52.5	13.2	11.4	29.7	10.5	2.7	45.7	5	7	5	39.7	15

Table D - 1 (cont'd). Biological Metrics

Year	Season	Date	Site ID	Site Type	Abundance	No. Total Taxa	No. Total Taxa less Ephemeroptera	No. Ephemeroptera Taxa	No. Ephemeroptera Taxa less Baetidae	No. EPT Taxa	No. Plecoptera & Trichoptera Taxa	No. Plecoptera Taxa	No. Trichoptera Taxa	% Top 2 Dominant Taxa	% Top 5 Dominant Taxa	Simpson Diversity	Shannon Diversity	% Ephemeroptera	% Ephemeroptera less Baetidae	% Plecoptera	% Trichoptera	% Collector-Filterers	% Collector-Gatherers	% Predators	% Scrapers	% Shredders	No. Collector-Gatherer Taxa	No. Predator Taxa	No. Scrapper Taxa	% Clingers	No. Clinger Taxa
2013	Spring	4/11/2013	LLC	Test	189	25	23	2	1	15	6	7	13	51.9	73.0	0.84	2.34	2.1	1.1	30.2	14.3	14.8	34.4	7.9	13.8	29.1	5	6	5	36.5	17
2013	Spring	4/5/2013	LLE	Test	192	19	15	4	3	14	8	2	10	57.8	81.2	0.78	2.02	6.2	4.7	70.3	5.7	5.7	17.7	10.4	2.1	64.1	4	8	1	40.1	13
2013	Spring	4/5/2013	LLW	Test	192	13	11	2	2	7	3	2	5	68.8	93.2	0.74	1.65	1.0	1.0	42.2	9.9	19.3	31.2	0.0	7.3	42.2	4	0	3	28.6	8
2013	Spring	4/6/2013	MCB	Ref	194	27	21	6	5	18	6	6	12	31.4	55.2	0.91	2.74	38.7	38.1	17.5	4.6	2.1	46.4	18.0	22.2	11.3	5	11	6	57.7	18
2013	Spring	4/8/2013	MIL	Test	180	13	11	2	1	6	2	2	4	60.6	88.3	0.78	1.82	2.8	1.1	60.6	5.0	18.3	17.2	2.2	1.7	60.6	4	2	2	47.8	7
2013	Spring	4/7/2013	POW	Test	217	12	11	1	0	7	4	2	6	62.2	89.4	0.78	1.79	1.4	0.0	65.4	9.7	12.0	9.2	3.2	12.0	63.6	3	2	2	63.1	8
2013	Spring	4/7/2013	RFF	Test	211	20	14	6	4	15	5	4	9	54.0	76.8	0.82	2.16	49.3	14.2	31.3	5.2	6.2	49.3	9.5	6.6	28.4	6	4	2	35.5	14
2013	Spring	4/7/2013	RIC	Test	180	18	17	1	0	11	5	5	10	50.0	78.9	0.84	2.14	2.8	0.0	52.2	18.3	23.9	16.1	3.3	6.1	50.6	3	5	3	57.8	13
2013	Spring	4/5/2013	ROC	Test	211	19	16	3	2	11	3	5	8	83.4	91.0	0.58	1.37	3.8	2.8	63.0	6.2	4.7	24.6	4.3	2.8	63.5	3	5	4	13.7	12
2013	Spring	4/7/2013	ROL	Test	220	10	9	1	1	7	4	2	6	88.2	97.3	0.52	1.07	0.9	0.9	89.1	3.2	2.7	7.3	1.4	0.0	88.6	3	3	0	27.3	5
2013	Spring	4/7/2013	RUT	Test	197	16	14	2	1	7	3	2	5	54.8	83.2	0.79	1.95	5.6	1.0	53.8	12.2	15.7	10.2	3.6	16.2	54.3	4	3	2	49.2	8
2013	Spring	4/6/2013	SPC	Test	213	19	11	8	7	14	2	4	6	51.2	82.6	0.82	2.05	31.9	28.6	51.2	3.3	2.3	36.2	4.7	5.6	51.2	6	2	6	54.5	14
2013	Fall	11/16/2013	BIR	Test	186	8	8	0	0	6	2	4	6	84.4	97.3	0.46	1.03	0.0	0.0	72.6	18.3	17.2	4.8	1.1	0.0	76.9	1	1	0	90.3	5
2013	Fall	11/12/2013	COP	Ref	191	31	24	7	7	23	8	8	16	32.5	57.6	0.92	2.85	27.2	27.2	24.1	25.7	22.5	33.5	10.5	15.2	18.3	4	9	8	76.4	23
2013	Fall	11/10/2013	CRA	Test	192	24	21	3	1	18	7	8	15	42.2	74.0	0.87	2.42	5.2	0.5	27.1	53.1	51.6	15.6	6.2	3.1	23.4	5	6	3	60.9	14
2013	Fall	11/12/2013	CRO	Ref	181	29	23	6	5	21	8	7	15	32.6	53.0	0.91	2.85	21.0	16.0	37.0	28.2	23.2	27.1	9.4	8.3	32.0	8	7	4	51.4	18
2013	Fall	11/10/2013	DAV	Test	192	23	20	3	2	15	6	6	12	37.5	72.9	0.88	2.45	25.0	3.6	6.2	32.3	27.1	25.5	5.7	36.5	5.2	4	4	6	75.0	18
2013	Fall	11/17/2013	FRY	Test	183	20	19	1	0	12	4	7	11	56.8	82.0	0.77	1.98	1.6	0.0	45.9	37.7	30.6	6.0	7.7	8.7	47.0	3	3	3	90.2	12
2013	Fall	11/11/2013	GRA	Test	188	24	19	5	4	19	6	8	14	49.5	77.7	0.85	2.31	6.9	5.9	39.9	42.6	37.2	13.3	6.9	3.7	38.8	4	7	4	61.7	17
2013	Fall	11/11/2013	HCN	Ref	207	26	23	3	3	14	3	8	11	67.6	80.7	0.71	1.92	9.2	9.2	20.3	8.7	2.9	55.6	6.8	11.6	23.2	3	7	6	36.7	16
2013	Fall	11/11/2013	HUR	Test	185	27	22	5	3	20	7	8	15	44.9	65.9	0.87	2.57	17.3	6.5	35.1	34.6	31.9	19.5	18.4	6.5	23.8	6	9	2	78.9	18
2013	Fall	11/15/2013	KEL	Test	188	22	20	2	0	13	4	7	11	48.4	81.9	0.84	2.21	1.6	0.0	42.6	39.4	38.8	9.0	3.7	4.3	44.1	4	3	3	84.0	13
2013	Fall	11/15/2013	KUT	Test	197	14	13	1	0	9	2	6	8	64.5	87.3	0.71	1.72	3.0	0.0	13.2	62.4	52.3	18.8	10.7	2.5	15.7	3	2	2	77.7	9
2013	Fall	11/13/2013	LAB	Test	216	22	20	2	1	15	5	8	13	55.1	81.9	0.81	2.09	9.7	2.3	37.5	33.3	26.9	23.1	5.6	4.6	39.8	3	4	4	72.2	14
2013	Fall	11/9/2013	LLC	Test	203	19	19	0	0	13	6	7	13	45.3	81.3	0.85	2.17	0.0	0.0	30.0	47.3	45.8	14.8	5.4	6.4	27.6	1	6	3	81.8	14
2013	Fall	11/11/2013	LLE	Test	180	23	23	0	0	15	9	6	15	60.0	76.1	0.81	2.18	0.0	0.0	46.1	39.4	30.0	8.3	16.1	4.4	41.1	2	10	3	87.8	16
2013	Fall	11/10/2013	LLW	Test	196	19	18	1	1	10	3	6	9	47.4	80.6	0.83	2.13	0.5	0.5	19.9	45.9	44.4	7.7	1.5	26.0	20.4	3	2	5	86.2	12
2013	Fall	11/15/2013	MIL	Test	192	14	13	1	0	10	2	7	9	78.6	92.2	0.58	1.36	0.5	0.0	62.5	29.2	24.5	4.2	5.2	1.0	65.1	2	4	1	90.6	7
2013	Fall	11/16/2013	POW	Test	211	14	13	1	0	10	5	4	9	80.1	93.8	0.52	1.23	1.4	0.0	71.6	16.6	14.2	10.0	3.3	1.4	71.1	2	3	2	89.6	11
2013	Fall	11/13/2013	RFF	Test	200	21	18	3	2	15	5	7	12	60.5	83.0	0.77	1.99	8.0	1.0	45.5	35.5	31.5	13.0	4.5	5.0	46.0	4	3	5	84.0	15
2013	Fall	11/16/2013	RIC	Test	189	12	12	0	0	9	4	5	9	71.4	91.5	0.67	1.53	0.0	0.0	55.6	38.6	37.6	2.1	2.1	3.7	54.5	1	2	2	95.8	10
2013	Fall	11/9/2013	ROC	Test	210	16	16	0	0	9	3	6	9	55.7	88.1	0.79	1.88	0.0	0.0	39.0	47.6	47.1	7.1	1.9	4.3	39.5	3	3	1	90.0	9
2013	Fall	11/16/2013	RUT	Test	214	15	15	0	0	10	3	7	10	75.7	92.1	0.56	1.35	0.0	0.0	69.6	16.8	12.1	2.8	4.2	11.2	69.6	2	3	4	95.8	11
2013	Fall	11/11/2013	SPC	Test	196	25	22	3	3	17	6	8	14	57.1	76.0	0.82	2.24	2.0	2.0	45.4	44.9	38.8	3.1	9.7	4.1	44.4	3	8	4	81.6	17
2014	Spring	4/23/2014	BIR	Test	206	11	9	2	0	7	3	2	5	90.3	96.1	0.58	1.12	1.9	0.0	93.2	1.0	0.5	3.4	5.8	0.0	90.3	4	4	0	41.7	5

Table D - 1 (cont'd). Biological Metrics

Year	Season	Date	Site ID	Site Type	Abundance	No. Total Taxa	No. Total Taxa less Ephemeroptera	No. Ephemeroptera Taxa	No. Ephemeroptera Taxa less Baetidae	No. EPT Taxa	No. Plecoptera & Trichoptera Taxa	No. Plecoptera Taxa	No. Trichoptera Taxa	% Top 2 Dominant Taxa	% Top 5 Dominant Taxa	Simpson Diversity	Shannon Diversity	% Ephemeroptera	% Ephemeroptera less Baetidae	% Plecoptera	% Trichoptera	% Collector-Filterers	% Collector-Gatherers	% Predators	% Scrapers	% Shredders	No. Collector-Gatherer Taxa	No. Predator Taxa	No. Scrapper Taxa	% Clingers	No. Clinger Taxa
2014	Spring	4/12/2014	COP	Ref	220	25	19	6	5	18	9	3	12	37.7	63.6	0.89	2.57	40.0	35.9	23.2	6.8	6.8	43.6	11.4	22.3	15.9	4	10	4	70.5	18
2014	Spring	4/21/2014	CRA	Test	204	25	20	5	3	14	5	4	9	42.6	72.5	0.87	2.39	33.8	2.0	26.5	22.1	22.5	42.6	8.3	3.4	23.0	6	9	3	34.8	14
2014	Spring	4/12/2014	CRO	Ref	197	31	22	9	6	22	7	6	13	34.5	58.4	0.90	2.76	49.2	46.2	14.2	13.7	11.2	42.6	14.2	21.8	10.2	8	10	5	75.6	20
2014	Spring	4/21/2014	DAV	Test	194	21	16	5	3	15	7	3	10	32.0	62.4	0.90	2.52	30.4	10.8	24.7	8.2	8.8	34.5	3.1	30.4	23.2	5	4	4	56.7	16
2014	Spring	4/12/2014	EAS	Ref	204	25	20	5	5	18	8	5	13	28.9	58.3	0.91	2.70	36.8	36.8	24.0	14.2	15.2	24.5	9.3	30.9	20.1	4	8	5	78.9	19
2014	Spring	4/12/2014	FRY	Test	206	22	16	6	4	15	4	5	9	51.5	71.8	0.79	2.21	21.8	10.7	54.9	12.1	11.2	21.8	7.8	5.8	53.4	6	4	4	39.3	16
2014	Spring	4/11/2014	GRA	Test	202	23	18	5	4	15	5	5	10	39.1	74.8	0.87	2.38	39.6	22.3	29.2	12.9	14.4	43.1	7.9	8.4	26.2	4	5	6	48.5	19
2014	Spring	4/11/2014	HCN	Ref	202	22	17	5	4	13	5	3	8	51.0	72.8	0.83	2.26	23.8	21.8	13.9	3.5	35.1	28.7	9.4	13.9	12.9	6	5	7	74.3	15
2014	Spring	4/22/2014	HUR	Test	220	20	16	4	2	14	6	4	10	35.5	72.3	0.88	2.35	26.4	21.4	44.1	15.5	15.0	37.3	17.7	2.7	27.3	5	5	4	70.9	13
2014	Spring	4/13/2014	KEL	Test	215	10	9	1	0	7	3	3	6	70.2	93.0	0.64	1.44	7.0	0.0	70.7	7.0	17.2	7.0	4.7	0.9	70.2	1	2	1	31.6	7
2014	Spring	4/13/2014	KUT	Test	220	11	10	1	0	7	2	4	6	62.7	95.0	0.73	1.56	0.5	0.0	60.9	14.1	10.9	25.0	2.7	0.5	60.9	5	1	1	56.4	6
2014	Spring	4/22/2014	LAB	Test	216	20	17	3	1	13	4	6	10	44.9	71.8	0.85	2.27	18.1	0.5	56.5	12.0	11.6	27.8	13.4	1.9	45.4	5	5	2	57.4	14
2014	Spring	4/11/2014	LLC	Test	206	23	22	1	1	14	6	7	13	61.2	83.5	0.75	1.94	0.5	0.5	50.0	24.3	28.2	17.5	5.3	1.9	47.1	3	7	4	35.9	16
2014	Spring	4/21/2014	LLE	Test	193	19	15	4	3	12	6	2	8	63.7	75.6	0.73	1.98	9.8	8.3	71.0	4.7	6.2	16.6	3.6	5.7	67.9	6	2	3	36.8	12
2014	Spring	4/21/2014	LLW	Test	216	16	14	2	1	8	3	3	6	69.9	85.2	0.63	1.60	2.3	0.5	63.4	5.1	7.4	12.0	4.2	13.4	63.0	5	3	2	27.3	10
2014	Spring	4/22/2014	MCB	Ref	199	33	24	9	7	24	7	8	15	32.7	59.3	0.91	2.84	31.7	29.6	25.6	16.6	12.1	39.2	17.1	11.1	20.6	7	10	8	53.8	23
2014	Spring	4/13/2014	MIL	Test	205	14	14	0	0	5	3	2	5	78.5	89.8	0.55	1.36	0.0	0.0	68.8	3.4	16.1	9.3	3.4	2.4	68.8	4	4	1	23.4	6
2014	Spring	4/12/2014	POW	Test	208	21	19	2	1	11	4	5	9	58.7	85.1	0.77	1.94	1.4	0.5	61.1	13.9	13.5	7.7	5.3	13.5	60.1	5	6	3	48.1	11
2014	Spring	4/12/2014	RFF	Test	212	26	20	6	4	17	5	6	11	46.2	66.5	0.86	2.49	23.1	5.2	39.6	20.3	18.9	26.9	8.0	9.9	36.3	5	6	7	42.5	20
2014	Spring	4/13/2014	RIC	Test	207	19	18	1	0	9	4	4	8	52.2	80.2	0.83	2.13	0.5	0.0	34.3	23.2	31.4	26.1	4.8	4.8	32.9	4	6	3	44.0	12
2014	Spring	4/11/2014	ROC	Test	210	17	12	5	3	11	2	4	6	72.4	89.0	0.62	1.54	6.2	2.4	60.0	8.1	21.4	12.9	1.9	3.3	60.5	4	2	3	26.7	10
2014	Spring	4/22/2014	ROL	Test	220	16	14	2	2	11	4	5	9	78.2	90.9	0.64	1.48	1.4	1.4	80.5	9.5	8.2	7.3	5.9	0.5	78.2	3	7	1	68.6	11
2014	Spring	4/23/2014	RUT	Test	190	16	13	3	1	11	5	3	8	61.6	90.5	0.77	1.83	3.7	1.6	64.2	10.0	17.9	4.7	3.2	12.1	62.1	4	4	2	56.8	10
2014	Spring	4/11/2014	SPC	Test	220	24	17	7	5	15	4	4	8	45.9	79.5	0.84	2.25	24.5	13.2	45.0	6.8	7.7	37.3	5.5	5.5	44.1	5	6	6	41.8	18

APPENDIX E – INFLUENCE OF MINING LANDUSE ON SALINITY

Note: The following abstract was prepared and submitted prior to the data analysis and the presentation. The presentation file can be provided to OSM, as a means of providing further information on the analyses conducted, upon request.

LINKING TEMPORAL PATTERNS OF DISSOLVED SOLIDS IN CENTRAL APPALACHIAN COALFIELD STREAMS TO MINING SOURCES¹

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Abstract: Headwater streams influenced by coal mining often exhibit elevated levels of dissolved solids relative to minimally disturbed, or reference, streams. Elevated levels of dissolved solids have been associated with aquatic life effects in mining-influenced streams. In addition, in-stream salt levels have been observed to vary over time, though the cause and consequence of such variability are not fully understood. Although some variability may be explained by the seasonal precipitation cycle, non-seasonal patterns may be explained by the nature of the source of dissolved solids. The extent to which aquatic life is affected by a stressor is a function of the magnitude, duration, and frequency of exposure to that stressor. To date, efforts to quantify the stressor of dissolved solids have typically focused on magnitude, measuring dissolved solids and/or specific conductance during discrete sampling events at fixed dates and times. Such efforts may fail to fully capture the temporal variability of dissolved solids, thus prohibiting full characterization of aquatic organism stressor exposure. To address these issues, we have employed in-stream conductivity monitors to record specific conductance continuously in Central Appalachian headwater streams with elevated dissolved solids where other stressors are not evident. Continuous conductivity data were supplemented with monthly measurements of total dissolved solids and constituent ions. For each stream, the nature and extent of the mining activity influencing the catchment was determined from reviewing permit information. Examination of these data reveals temporal variability of dissolved solids that can be used for high-resolution characterization of the magnitude, duration, and frequency of aquatic life salt exposure. In addition, distinct patterns of dissolved solids can be linked to mining type and operational patterns. Understanding the influence of mining practices on in-stream temporal patterns of dissolved solids will allow operators to better mitigate the aquatic life impacts from such practices.

Additional keywords: biomonitoring, benthic macroinvertebrates, water quality

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Table E-1. Proportional mining land uses in catchments of 13 test sites in Virginia.

Site	Site Type	Catchment Area (ha)	Prop. Active Surface Mining	Prop. Active Underground Mining	Prop. Abandoned Underground Mining	Prop. Filled Area	Prop. Pond Area	Gas/Oil Well Density (1/km ²)
FRY	Test	567	0.11	0.01	1.24*	0.00	0.00	3.2
GRA	Test	444	0.04	0.30	0.33	0.00	0.00	4.7
HUR	Test	150	0.03	0.41	0.40	0.00	0.00	1.3
KEL	Test	265	0.78	0.00	0.89	0.01	0.01	1.9
KUT	Test	109	0.82	0.00	1.28	0.09	0.01	0.0
LAB	Test	268	0.01	0.12	2.10	0.02	0.00	0.0
MIL	Test	271	0.66	0.00	1.25	0.00	0.00	2.2
POW	Test	270	0.97	0.00	2.07	0.00	0.01	0.0
RFF	Test	467	0.03	0.20	0.39	0.00	0.00	2.8
RIC	Test	424	0.00	0.00	0.49	0.03	0.00	1.2
ROL	Test	130	0.43	0.75	0.80	0.00	0.00	1.5
RUT	Test	192	0.00	0.00	0.18	0.00	0.00	0.0
SPC	Test	676	0.00	0.01	0.79	0.03	0.00	4.6

*Proportions > 1 result from multiple strata of abandoned underground mines beneath catchment